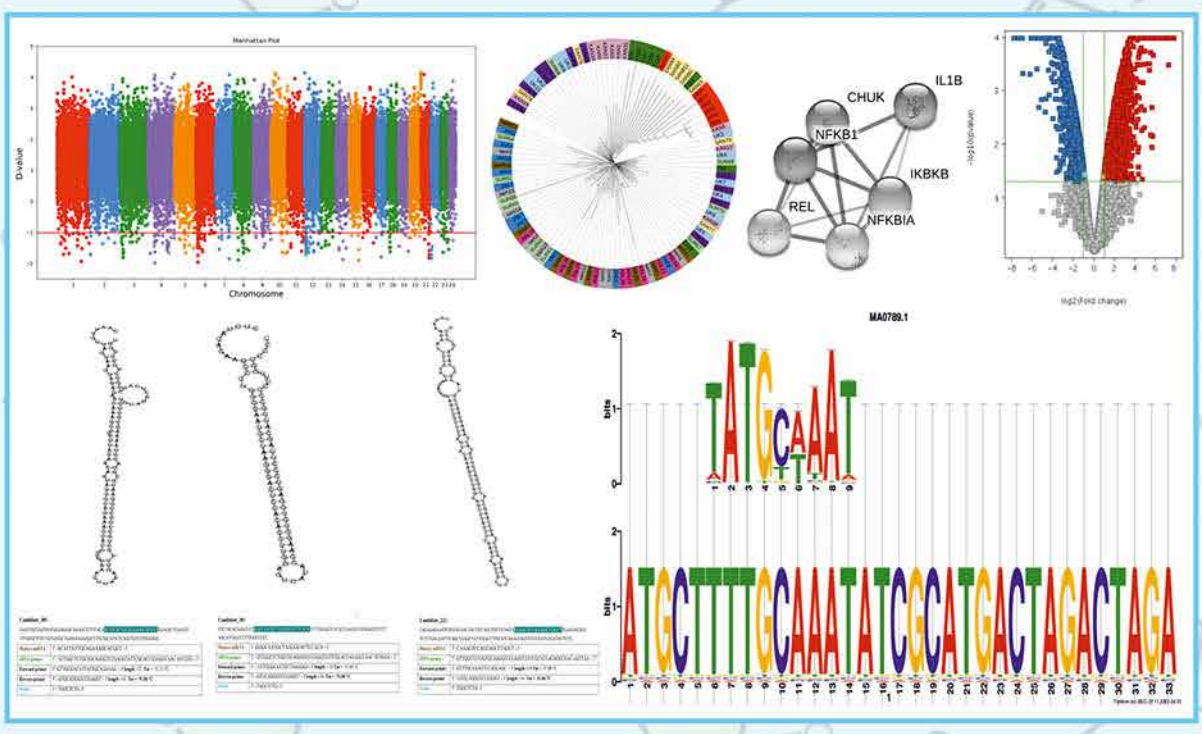
COMPENDIUM

National Conference
on
Advances in Genetics and Genomics for Sustainable Livestock Transformation
&
XVII Annual Convention of Indian Society of Animal Genetics & Breeding

November 16-17, 2023



ISAGB-2023

Organized by

Indian Society of Animal Genetics & Breeding
&
ICAR-National Bureau of Animal Genetic Resources
Karnal - 132001 (Haryana) India

National Conference

on

Advances in Genetics and Genomics for Sustainable Livestock Transformation

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ICAR-National Bureau of Animal Genetic Resources,

Karnal - 132001 (Haryana) India

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परशोत्तम रूपाला
PARSHOTTAM RUPALA

सत्यमेव जयते

मंत्री
मत्स्यपालन, पशुपालन एवं डेयरी
भारत सरकार
Minister
Fisheries, Animal Husbandry and Dairying
Government of India

D.O. No.2693.MIN(FAH&D)/20.23-24

14 NOV 2023

संदेश

भारतीय पशुधन अपनी विविधता, उत्पादन और जलवायु लचीलेपन के लिए दुनियाभर में जाना जाता है। हाल ही में, इसने पशु-आधारित उत्पादन में कई उपलब्धियां हासिल की हैं, और बिना किसी संदेह, यह भारतीय कृषि में आशा की किरण वाला क्षेत्र है। उच्च योग्यता वाले पशुओं के तेजी से चयन के लिए प्रौद्योगिकियों का उपयोग और प्रजनन कार्यक्रमों में उनका व्यापक प्रसार देश में पशुधन के उत्पादन स्तर में सुधार के लिए सबसे अधिक योगदान देने वाले कारक हैं। स्वदेशी पशुधन की क्षमता को देखते हुए, भविष्य में भी आनुवंशिक सुधार के अधिक अवसर हैं। हालांकि, जलवायु परिवर्तन के युग में पशुधन क्षेत्र के परिवर्तन के लिए अब अत्यधिक लचीले स्वदेशी पशुधन नस्लों की क्षमता का दोहन करने की ओर उन्मुख हैं। निश्चित रूप से, पशुधन क्षेत्र में नए परिवर्तन की आवश्यकता है, जिसका ध्यान न केवल बेहतर उत्पादन पर बल्कि भविष्य की जलवायु चुनौतियों के मद्देनजर स्वदेशी पशुधन के लचीलेपन को बढ़ाने पर भी होना चाहिए।

मुझे बहुत खुशी हुई कि इंडियन सोसायटी ऑफ एनिमल जेनेटिक्स एंड ब्रीडिंग 16-17 नवंबर, 2023 को करनाल (हरियाणा) में 'सतत पशुधन परिवर्तन के लिए जेनेटिक्स और जीनोमिक्स में प्रगति' पर एक राष्ट्रीय सम्मेलन का आयोजन कर रही है। मैं पशुधन क्षेत्र, विशेष रूप से स्वदेशी पशुधन के प्रजनन कार्यक्रमों में बदलाव के लिए आनुवंशिकी और जीनोमिक्स के क्षेत्र में अच्छे विचार-विमर्श की उम्मीद करता हूं।

मैं राष्ट्रीय सम्मेलन की सफलता की कामना करता हूं।

(परशोत्तम रुपाला)

परशोत्तम रूपाला
PARSHOTTAM RUPALA

सत्यमेव जयते

मंत्री
मत्स्यपालन, पशुपालन एवं डेयरी
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Minister
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Government of India

D.O. No.2693.MIN(FAH&D)/20..2.3.-2.4

14 NOV 2023

Message

Indian livestock are well acknowledged for its diversity and production and climate resilience, worldwide. In recent time, it has achieved many landmarks of animal-based production; and without any doubt, it is a silver lining sector in Indian agriculture. Use of technologies for faster selection of high merit animals and their wider dissemination in breeding programs are the most contributing factors for improving production levels of the livestock in the country. Looking in to the potential of indigenous livestock, still, there are greater opportunities for genetic improvement, in future also. However, the transformation of the livestock sector in the era of climate change is now required to be more relied upon highly resilient indigenous livestock. Many of the genetic improvement programs are now oriented towards harnessing the potential of native livestock breeds. Certainly, new transformation in livestock sector is required, which should be focused not only on improved production but also enhancing resilience of indigenous livestock in view of future climate challenges.

I am glad to know that Indian Society of Animal Genetics & Breeding is organizing a National Conference on 'Advances in Genetics and Genomics for Sustainable Livestock Transformation' on 16-17 November 2023 at Karnal (Haryana). I hope for good deliberations in the area of genetics and genomics for transforming the livestock sector, specially breeding programs of indigenous livestock.

I extend my best wishes to the Organizer and for success of the National conference.

(Parshottam Rupala)

Delhi Office : Room No. 234, 'B' Wing, Krishi Bhawan, Dr. Rajendra Prasad Road, New Delhi-110 001

डॉ. हिमांशु पाठक
DR. HIMANSHU PATHAK
सचिव (डेयर) एवं महानिदेशक (आईसीएआर)
Secretary (DARE) &
Director General (ICAR)

भारत सरकार
कृषि अनुसंधान और शिक्षा विभाग एवं
भारतीय कृषि अनुसंधान परिषद
कृषि एवं किसान कल्याण मंत्रालय, कृषि भवन, नई दिल्ली–110 001

GOVERNMENT OF INDIA
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Message

Livestock sector is a vital component of our agricultural system, providing nutritional sustenance and additional income to the country's population. In addition to providing milk, meat, eggs and fuel, animal husbandry also creates jobs, which drives economic growth. It is noteworthy that the contribution of livestock and poultry sector to the overall agriculture-based economy is increasing every year, and it is among the most promising ventures with tremendous potential for export as well. Therefore, we must harness the full potential of India's animal genetic resources to meet the growing demand for food and ensure sustainable utilization. To make progress towards livestock improvement, we need to strengthen the network between the researchers, academia, policymakers, and industry partners.

I am delighted to know that Indian Society of Animal Genetics and Breeding (ISAGB) in collaboration with ICAR-National bureau of Animal Genetic Resources, Karnal (Haryana) is organizing a National Conference on "*Advances in Genetics and Genomics for Sustainable Livestock Transformation*" at ICAR-NBAGR, Karnal on November 16-17, 2023. I hope this conference will foster scientific collaboration between diverse stakeholders and pave the way for sustainable livestock development.

I wish the National Conference a grand success!

(Himanshu Pathak)

27th October, 2023
New Delhi

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Dr. Joy Krushna Jena
Deputy Director General (Animal Science)

MESSAGE

I am glad to know that the ISAGB in collaboration with ICAR-National Bureau of Animal Genetic Resources, Karnal is organizing XVII Annual Convention of Indian Society of Animal Genetics and Breeding (ISAGB) and National Symposium on "Advances in Genetics and Genomics for Sustainable Livestock Transformation" at ICAR-NBAGR, Karnal on November 16-17, 2023.

Recent development in genomic technologies is playing a vital role in livestock and poultry industry by improving the overall production. These technologies impact significantly in increasing production potential and improving animal welfare. Therefore, adoption of latest techniques and technologies by the livestock holders, farmers, and stakeholders is of paramount importance to improve farm animal productivity and strengthen national food security system. I sincerely believe that the National Symposium will provide an unique opportunity to researchers and stakeholders across the country to discuss about the advancement of genetics and genomics for sustainable livestock production. Further, I hope that the recommendations of this Symposium will be invaluable for better management of India's animal genetic resources.

I congratulate organizers and all the delegates and I wish the Symposium a resounding success.

[J. K. Jena]

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Dr Gyanendra Kumar Gaur
Assistant Director General (AP& B)

MESSAGE

I am indeed happy to know about the efforts taken by the Indian Society of Animal Genetics and Breeding (ISAGB) and ICAR-National Bureau of Animal Genetic Resources (NBAGR), Karnal in organizing the XVII Annual Convention of ISAGB and National Symposium on "Advances in Genetics and Genomics for Sustainable Livestock Transformation" at ICAR-NBAGR, Karnal on November 16-17, 2023. I have gone through the list of invited speakers, technical sessions and the topics of two days technical event; and I am sure that it is going to be an enriching experience for the participants.

The symposium is very topical in today's context when recent developments in both genetics and genomics play an important role in taking decisions for the genetic improvement of the livestock and improving the overall production. Recent technological advances in the field of genomics also offer conservation managers and practitioners new tools to explore for conservation applications. Utilization of the new genomic technologies has the potential to transform animal breeding concepts. Adoption of these technologies by the livestock owners to improve the productivity and to ensure the national food security is therefore the need of hour.

I am sure this conference will bring researchers, scientists, academicians and students with such skills together to draw the future line of action for the overall genetic improvement of the livestock.

I thank ISAGB and NBAGR for managing this important mega event.

I offer my best wishes to all delegates and organizing committee to make this event a grand success.

[Gyanendra Kumar Gaur]

भाकृअनुप - राष्ट्रीय पशु आनुवंशिक संसाधन ब्यूरो
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डॉ बी. पी. मिश्रा
निदेशक
Dr. B. P. Mishra
Director

MESSAGE

It is a matter of immense pride that the Indian Society of Animal Genetics and Breeding (ISAGB) has provided responsibility to host XVII Annual Convention and the National Conference on "Advances in Genetics and Genomics for Sustainable Livestock Transformation" at ICAR-National Bureau of Animal Genetic Resources (NBAGR), Karnal (Haryana) during 16-17 November 2023.

India is one of the most Animal Genetic Resources (AnGR) rich and diversified regions in the world. Many of native livestock breeds have been evolved here through thousands of years of natural and man-made selection. Traditional breeds and their production systems have become most sustainable to cater the needs of the society in different climatic zones. These native breeds are well acknowledged for their production potential and climate resilience, worldwide. Our indigenous breeds have tremendous potential, which is still to harness in best way. The transformation in livestock sector is much required, focussing on their improved production with greater pace, particularly in light of climate change. Surely research on the genetics and genomics of all kind of AnGR is important for such transforming the livestock sector, so that it could be implemented in their breeding programs. Our Bureau team is deeply committed for overall AnGR management in the country, and also working towards their sustainable utilization. Recent advances in genomic, biotechnological, and computational tools aid in achieving our mandates and targets within a specified timeframe. Organizing this National Conference on the very thematic area would have a higher relevance and greater significance for the Bureau.

I warmly welcome all delegates and life members of the society from across the country to this national conference. The conference will provide an excellent opportunity for all delegates to network with each other and with leading national and international eminent speakers. I would like to appreciate the ICAR-NBAGR team, especially the organizing committee, for their dedicated planning and execution of this National Conference. I am sure the conference would provide a roadmap and sound strategies for sustainable livestock transformation and help animal stakeholders to achieve sustainable animal production in the country.

I wish a great success of the National Conference.

(B.P. Mishra)

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Dr. V. K. Saxena
Director Research
& Secretary, ISAGB

MESSAGE

It is a matter of great pleasure that Indian Society of Animal Genetics and Breeding (ISAGB) is organizing its XVII Annual Convention of Indian Society of Animal Genetics and Breeding (ISAGB) and National Conference on "Advances in Genetics and Genomics for Sustainable Livestock Transformation" at ICAR-NBAGR, Karnal during November 16-17, 2023.

The Indian Society of Animal Genetics and Breeding is founded in 1982 by eminent researchers in the discipline of animal genetics and breeding. The society has been established for benefiting researchers, students, policy makers and livestock stakeholders engaged in genetics, genomics and animal breeding besides providing a platform for sharing advance knowledge as well as exchanging the thoughts and ideas among researchers and entrepreneurs across the world.

The principles of quantitative genetics have helped in developing innovative selection and breeding techniques which brought in many-fold increase in animal production besides, development of high yielding animal varieties for faster genetic gains. The advance genomic technologies are also exhibiting great potential for significantly improving the production and welfare of livestock and poultry species. These advance technologies are also paving the way for transforming animal sector to achieve sustainable production. I hope this conference will help to bring together the stakeholders from different backgrounds for collaborating in their endeavors.

I wish the Annual Convention & National Conference a grand success.

(V.K. Saxena)

From The Organizers' Desk

Dr. SP Dixit
Principal Scientist &
Head, AG Division,
ICAR-NBAGR, Karnal

Dr Indrajit Ganguly
Principal Scientist,
ICAR-NBAGR, Karnal

Dr. Amod Kumar
Scientist,
ICAR-NBAGR, Karnal

We are honoured to host the XVII Annual Convention of the Indian Society of Animal Genetics and Breeding (ISAGB) and National Symposium on "Advances in Genetics and Genomics for Sustainable Livestock Transformation" at ICAR-NBAGR, Karnal during November 16-17, 2023. The livestock sector is a vital source of livelihood for the vast majority of the country's population, providing essential income and employment opportunities. The contribution of livestock in total agriculture and allied sector GVA (gross value added) has increased from 24.32 percent (2014-15) to 30.13 percent (2020-21). The theme of the National Symposium, "Advances in Genetics and Genomics for Sustainable Livestock Transformation," is highly relevant to improve the growth of the livestock and poultry sector, and its management for achieving sustainable livestock goals. This conference will bring together students, scientists, university professors, NGOs, industry entrepreneurs, and farmers to interact and learn about new techniques and technologies for sustainable livestock production.

We are grateful for the help and support from the Indian Council of Agricultural Research, New Delhi. We are deeply grateful to the Director, ICAR-NBAGR for his guidance and constant support throughout the organization of this National Conference. We are thankful to our sponsoring firms for their financial support for this event. We are grateful to the ICAR-NBAGR scientists and all committees for their cooperation in organizing the conference successfully. We thank the ISAGB for the opportunity to coordinate this National Conference and XVII Annual Convention. We believe that the thoughtful discussions and meaningful recommendations from this conference will provide a roadmap for future sustainable development of livestock genetic resources.

COMMITTEES FOR ORGANIZATION OF THE ISAGB-2023 CONFERENCE

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Dr Satpal Dixit : Organizing Secretary
Dr Indrajit Ganguly : Co-Organizing Secretary
Dr Amod Kumar : Co-organizing Secretary
Dr R K Pundir : Member
Dr Ravi Kumar Gandham : Member

Reception and Registration Committee

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Shri Deepak Rai
Shri Satbir

CONTENTS

LEAD PAPERS

ABSTRACTS

Technical Session-I
GENETICS AND GENOMICS IN LIVESTOCK AND POULTRY

Technical Session-II
ISAGB YOUNG SCIENTIST AWARD

Technical Session-III
ANIMAL BREEDING STRATEGIES IN THE ERA OF GENOMICS AND PHENOMICS

Technical Session-IV
FUNCTIONAL GENOMICS AND EPIGENETICS IN LIVESTOCK AND POULTRY

LEAD PAPERS

National Conference

Advances in Genetics and Genomics
for Sustainable Livestock Transformation

&

XVII Annual Convention of
Indian Society of Animal Genetics & Breeding

ICAR-NBAGR, Karnal || November 16-17, 2023

UNDERSTANDING THE NATIONAL ANIMAL GENETIC RESOURCES AND THEIR SUSTAINABLE UTILIZATION: CHALLENGES AND OPPORTUNITIES

B P Mishra and S K Niranjan
ICAR-National Bureau of Animal Genetic Resources, Karnal-132001 (Haryana) India

Animal Genetic Resources (AnGR) are important for the requirement for food, agriculture and livelihood across the globe. It includes more than 40 livestock species domesticated so far, however, only a few species like cattle, sheep, goat, pig and chicken are larger in numbers and distributed widely, whereas, many of the species are only region-specific. The AnGR is a big source of direct food, worldwide; however also contributes to the crops by providing agricultural and transport power, manure as bio-fertilizer and ecosystem services. A large part of the world is dependent on animal-based systems and at present, about one-fifth of the world's food is contributed by the AnGR. Globally, farm animals produced 804.8 million tons of milk, 334 million tons of meat, and 87 million tons of egg production in the year 2017. These marks have been more than doubled for milk, tripled for meat, and quadrupled for egg production during the last fifty years. Besides, milk, meat, eggs as food, the AnGR also contributes through draft and transport power, manure and energy to the masses as well as employment to the rural people.

AnGR of India: A silver lining of their utilization

India is one of the leading countries in the world, in the form of farm animal species and native breeds. The country possesses about 10 percent of the global livestock population and 4 percent of the total global breeds. There are 11 livestock species- cattle, buffalo, sheep, goat, pig, horse, donkey, camel, yak, Mithun rabbit and a few poultry species. These species are distributed over a large range of geographical and ecological regions. Some of the species like zebu cattle, riverine and swamp buffalo, chicken, etc. have been domesticated in the Indian subcontinent. The country possesses 536.8 million livestock and 851.8 million poultry (Livestock Census, 2019), with a continuous increase in population since independence. India possesses the largest number of dairy bovines in the world. The country leads in cattle and buffalo population, globally. Almost 46 percent of this total livestock population falls under the breed or a specific type category and is claimed as descript. There are 187 registered indigenous breeds of livestock, 22 poultry and 3 dogs in the country. which include 53 for cattle, 20 for buffalo, 37 for goat, 44 for sheep, 7 for horses & ponies, 9 for camel, 13 for pig, 3 for donkey, 3 for dog, 1 for yak, 19 for chicken, 2 for duck and 1 for geese have been registered so far. The livestock population has registered more or less a continuous increase since independence.

Presently, livestock contribution in India is 6.17 percent of the total Gross Value Added (GVA) and about 30 percent of the agricultural GVA. Total share of livestock in total agricultural economic output is also increasing consistently and has been doubled during last four decades. Since 1980s, the growth in livestock sector is recorded between 3 to 6 percent annually, that is much higher than core agriculture. Milk production is now 221 million tons, 129.6 billion eggs, and 9.3 million tons of meat production in 2021-22. Further, livestock sector is an important source of income for millions of rural families, providing a cushion to the entire agricultural system in the country. Livestock contributed about 16 percent to the income of small farmers households. Importantly, the livestock share is more than one-fourth of the total agricultural income in about 20 states in the country. Livestock also provide about two-thirds of the energy required for ploughing the cultivated area comes from animal power.

Challenges for AnGR utilization:

Despite of silver lining in livestock sector in the country, there are many of the constraints and challenges which are dragging behind the overall growth as well as sustainable use of AnGR in the country. Here, some of the important challenges have been discussed, which needs urgent attention by the policy makers.

Considering vast geographical and ecological regions, climatic conditions in the country, there is still a sizable undefined population. About 54 percent of total livestock is non-descript, as per recent breed survey (DAHD, 2022). Species-wise, this percentages are 52.0 of cattle, 45.4 of buffalo, 50.6 of sheep, 63.5 of goat and 56.3 of pig, to be regarded as non-descript. There are a number of states like Madhya Pradesh, Chhattisgarh,

Jharkhand, Bihar, Uttar Pradesh, West Bengal, Telangana etc. which are rich in AnGR diversity and have large proportion of non-descript population.

Population dynamics of various livestock species are also changing rapidly. The share of cattle population to total livestock population has been deceased from 53 to 37 percent since independence. On other side, buffalo population has been increased from 15 to 20 percent and goat population from 16 to 28 percent during same period. A radical change has been observed with significant decline in indigenous cattle population, after emergence of crossbreds, with very high growth rate. During 2012 -2019 the growth of crossbred cattle was about 30 percent against 6 percent decline in indigenous cattle. There is a fast shrinkage of populations of minor species including pigs, equines, camel, yak and mithun; the decline being so fast that they may lose some of best indigenous germplasm in near future.

Indigenous breeds of many livestock species have been also dwindled due to decline in their utilization as well as over emphasis on only some of the breeds. Among the registered livestock breeds, a number of breeds are declining very fast. Beside these registered breeds, there are many more unique populations which are not recognised yet and also having greater threat of dilution of germplasm due to breeding policies for non-descript animals. In recent breed-wise livestock survey (2022), about 20 per cent of the total indigenous livestock breeds in the country are under risk. The proportion of breeds under threat is much higher in minor species including camel and horses.

Although the country is leading in milk production in the world; however, the productivity per animal is almost half of the world average. About 97 percent of the milk is being produced by cattle and buffalo only, despite of having seven dairy species in the country. Nearly 50 percent of total bovines, as non-descript is contributing only 23 percent of total milk. Improvement for meat and egg production is also enormous and even future prospects are also more positive in the country due to wide variety of species available. Major species for meat in the region are, buffalo, goat, sheep and chicken, however, there is high disparity in meat production among the species. The country has progressed in food and nutritional security of the people in recent years. The level of malnourished-stunting in children below five years has declined from 48 to 38 percent from 2006 to 2016 (UN Report, http://in.one.un.org). But still there is a large population, which is undernourished in the country. Protein consumption is at critical levels in most of the population.

Opportunities for AnGR utilization

Our country has vast opportunities related to the AnGR diversity as well as its management framework. First and foremost, the diversity of AnGR at the level of farm animal species, their breeds, varieties and similar kinds of distinct populations. Further these species are also in much of the balanced proportion except minor species. There are more than 10 food species to provide nutritional support. Many of the species are reared in mixed form as well as in diverse production system. This wider group of genetic stocks makes the country more sustainable at regional level agro-ecological, economical and nutritional production system. It is obvious, this all makes Indian livestock system the most sustainable.

Indigenous livestock of India are well acknowledged, worldwide, for possessing unique attributes or combinations of characteristics like disease resistance, tolerance to climatic extremes particularly heat tolerance and better thriving ability. With changing global climate, these traits will become more and more important for sustainable animal production. Nevertheless, the world is now having a greater need of the AnGR possessing these traits in challenging or adverse conditions. Certainly, there is a need for effective management and conservation of these valued AnGR, particularly when they are facing serious threat in view of pressure of high production demand.

For inventorization and documentation of such a large AnGR pool, India has a unique system of breed registration and Gazette notification in place. Such framework is important for claiming sovereignty over our native germplasm and their unique traits. This is also important for recognizing local breeders and production system as well as indigenous knowledge pertains to local community. Among total 212 native breeds, 83 breeds were added in last 12 years only, especially more from North-East and Himalayan states. It is anticipated that about 100 more breeds may be registered in the country in coming years. It is another facet that our AnGR is monitored in every five years. The country possesses a unique model for monitoring AnGR

in every five years interval. All of the species and breeds are counted by head through conducting Breed-wise survey by the DAHD, Govt. of India. Based on the survey, breeds at risk could be assessed and monitored throughout the country. Bureau has also released the Breed Watchlist 2022, wherein 38 breeds were assessed at risk out of 164 registered breeds for which population data is available in 20th Livestock Census.

Harnessing production potential is another opportunity for our AnGR. Milk production has now reached to 221 million tons (2021-22), doubling in 15 years. Improvement for meat and egg production is also enormous and even prospects are also better in the country due to wide variety of species available. Egg production has been doubled only in ten years. Meat production, as of now, is more than two-fold production than that of 20 years back. Animal productivity of all indigenous bovines is also increasing rapidly; which shows tremendous opportunity of our AnGR specially of indigenous.

There is moderate to high change in demand for livestock products in terms of quantity and quality. Demand for all animal foods - milk, meat and egg has increased in view of burgeoning human population, increased purchasing power and increased awareness for requirement of animal based products. The trend is more prominent since last 3-4 decades, when a change of utility pattern of major species of AnGR has been observed. The utility of AnGR for food is likely to increase with a shift towards production of more milk and meat. Increased purchasing power has also resulted in demand of quality products.

There is large network of state and central agencies for AnGR improvement and conservation in the country. Central Government is making all efforts for strengthening the dairy sector through various Central sector Schemes dedicated for the genetic improvement and conservation of indigenous bovine breeds. Rashtriya Gokul Mission has been initiated with the aim to conserve and improve indigenous bovines and to upgrade nondescript cattle using elite indigenous breeds. National Livestock Mission focuses to ensure quantitative and qualitative improvement in livestock production systems and capacity building of all stakeholders. ICAR is also running various genetic improvement programmes for indigenous livestock breeds, under different Network Programmes for breed improvement at different locations, specifically in their native tracts in the country.

Various Acts, rules and regulations formulated by the Government of India, time to time, related to prevention of diseases, animal, regulating Exchange of animal germplasm as well as Intellectual Property Rights issues. Access to any biological material including Animal Genetic Resources of the country is regulated under Biological Diversity Act (BDA, 2002). Guidelines for germplasm exchange of livestock species have been laid down by the government.

Way forward for AnGR utilization

Approaches for AnGR management and their sustainable utilization may be multipronged; however, some of the strategies need to be prioritized. At present, primary focus should be documenting all kinds of AnGR in country, including cataloguing indigenous as well as their improved genetic stocks. Considering almost half of the livestock population is still non-descript, country-wide identification of new indigenous breeds is need of the hour. Many of the breeds with unique characters are being explored from remote and inaccessible regions of the country. ICAR-NBAGR has taken the initiative to characterize and document all non-descript animal genetic resources of the country. The task has been taken under Mission towards Zero Non-descript Animal Genetic Resources of India, in 2021. The Mission would encompass the activities in all states of the country in collaboration of the state Animal Husbandry departments/ SAUs/SVUs/NGOs. After the launch of the mission, several new populations of native livestock and poultry have already been identified that are being characterized in their respective breeding tracts falling in various states. It is important that to complete the inventory of all such breeds in the country as per FAO global plan of action. Besides breeds, improved synthetic breeds and varieties for various livestock species are also required to be inventoried at national level.

Secondly, much required efforts are needed for monitoring of the AnGR and further conservation efforts of all threatened breeds. For monitoring, breed-wise livestock census conducted by DAHD is the most important task, however it needs to be more accurate in terms of breed count. More efforts are required to

make the census more accurate breed-wise so that each of the breed may be monitored. DAHD may work in conjunction with ICAR-NBAGR to improve the accuracy. The breeds at risk are required to be conserved.

Cryopreservation of germplasm of all such breeds should be the priority. Germplasm of AnGR, especially native breeds/populations are being cryopreserved at National Gene Bank at ICAR-NBAGR for medium and long time conservation. At present, 63 breeds/populations have been cryopreserved in form of semen and 47 native breeds/populations in form of somatic cells. Cryopreservation of oocytes (vitrified) and DNA is also being done in ICAR-NBAGR.

Further, for *in situ* conservation, community-based programs are needed to be initiated. Initiatives to designate "Hot spots" for AnGR biodiversity in the country, to protect and preserve AnGR diversity in such regions. Declaring existing State Breeding Farms /Central breeding Farms as germplasm repositories. Breed Registers may be created and maintained with the information of local breeds and their breeders/communities at village /block levels. Breeders may be encouraged for their efforts for conservation and promotion of native indigenous breeds.

As, a number of breeds are fading their utilities, such breeds may be explored for alternate utility. Many native breeds are known to produce quality functional foods with nutraceutical or medicinal values in their food products. Focussed research efforts are required for identification and evaluation of such pharmaceutical and nutritional properties in animal produces/products. New findings about therapeutic properties of milk and milk products of different species are examples for creating a value chain for such products. Branding of indigenous animal produces and products and developing niche market, more awareness programmes among stakeholders are needed. Breed societies are known for their efforts in indigenous breed development. They may contribute in many facets like generation of elite animals, product formation and value chain creation. There are some model examples of Breed Society to best utilize the indigenous breeds in the country.

For augmenting the production of indigenous breeds, better infrastructure for production of bulls and semen is required. Policies may be framed for more use of indigenous breeds for breed improvement programmes of non-descript animals. Genomic selection may be technology for the future for early selection of bulls for semen production.

Specific legislations for breed conservation, farmers'/pastoralist's rights, grazing land etc. are needed to provide legal rights for AnGR management as well as benefit sharing for the communities.

In conclusion, a holistic approach is required for overall optimum and sustainable utilization of AnGR, oriented mainly towards increasing productivity, improve livelihood and inclusive economic growth. Surely, mainstreaming of the AnGR biodiversity with preserving natural habitat and production system would be utmost important for suitable utilization of our valuable AnGR for the posterity.

A SWOT ANALYSIS OF ANIMAL BREEDING PRACTICES IN INDIA AND WAY FORWARD

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Livestock sector has been an essential component of human life since the dawn of the civilization. It is a vital component of our agricultural system, providing nutritional sustenance and additional income to the country's population. The contribution of livestock in total agriculture and allied sector GVA (gross value added) has increased from 24.32 percent (2014-15) to 30.13 percent (2020-21). Livestock sector contributed 4.90 percent of total GVA in 2020-21. In addition to provide milk, meat, eggs and fuel; animal husbandry also creates jobs, which drives economic growth. It is worth mentioning that the India possesses vast animal genetic resources which includes 536.36 million livestock population, 851.81 million poultry and 212 registered indigenous livestock and poultry breeds. The species-wise population and registered breeds have been summarised in Table 1.

Table 1: Species-wise population and registered breeds of India

Sr. No.	Species	No. of breeds registered till now	Total Population (million)
1.	Cattle	53	193.46
2.	Buffalo	20	109.85
3.	Sheep	44	74.26
4.	Goat	37	148.88
5.	Pig	13	9.06
6.	Camel	09	0.25
7.	Horse	07	0.34
8.	Chicken	19	851.81
9.	Donkey	03	0.12
10.	Yak	01	0.058

Major breeding strategies and programs in India

Genetic improvement of Indian livestock species has been a central objective of all development programs since the beginning of planned development in India. The major development programs have been summarised below:

Cattle and buffalo development programs

Crossbreeding of cattle in India began in 1857 when Mr. Taylor, the then commissioner of Patna division, developed the 'Taylor breed' of cattle by crossing Taurus bulls (Ayrshire bulls from the U.K.) with native cows. Thereafter, Indian Council of Agricultural Research (ICAR) initiated a large crossbreeding programme of crossing low yielding non-descript animals in hilly and heavy rainfall areas with Jersey in 1955 at several locations in different states.

The Central Herd Registration Scheme (1949) was the first initiative to establish a one herd book for single breed in the country. Further, the Key Village Scheme (KVS) and the Intensive Cattle Development Programme (ICDP) have been initiated for covering of very large population of cattle and buffaloes. The All-India Coordinated Research Project (AICRP) on Cattle was launched in 1969 as a major initiative to develop new dairy breeds for tropical climates. Presently, AICRP on cattle has been operated at ICAR-CIRC, Meerut with the objectives; to develop a national milch breed of cattle 'Frieswal' using Holstein Friesian X Sahiwal base; conservation and genetic improvement of important indigenous cattle breeds; production of progeny tested crossbred bulls and genetic improvement of cattle under field conditions. For improvement of buffalo

germplasm, the Network Project on Buffalo Improvement was launched in 1993 at five Murrah centres, with the ICAR-Central Institute for Research on Buffalo, Hisar as the coordinating centre. The project was initiated with the goal of producing genetically superior bulls to improve buffaloes.

Recognizing that genetic improvement is a long-term activity, the Government of India launched the "National Project for Cattle and Buffalo Breeding" (NPCBB) in October 2000 for a period of ten years. The NPCBB aimed to improve the genetic makeup of indigenous breeds and conserve them. The NPCBB was later merged into the National Dairy Plan. The Rashtriya Gokul Mission (RGM), launched in December 2014, is a government program to develop and conserve indigenous bovine breeds. The program aims to increase milk production and productivity to meet the growing demand for milk and make dairying more profitable for rural farmers.

Sheep and goat development programs

The All-India Coordinated Research Project (AICRP) on Goat Breeding was launched in 1971 with the goal of improving the production performance of Indian goat breeds through crossbreeding with exotic breeds. Presently, the AICRP on Goat Improvement is operated from the ICAR-Central Institute for Research on Goats (CIRG), Mathura. It is a long-term and well-structured program aimed at achieving genetic improvement and conservation of goat genetic resources.

The All-India Coordinated Research Project on Sheep Breeding (AICRP SB) was launched in 1971 to crossbreed native breeds with exotic rams. In 1990, the AICRP SB centers were merged into the Network Project on Sheep Improvement (NWPSI), which focuses on evaluating and improving indigenous sheep genetic resources through selective breeding for mutton and wool production.

Poultry development program

The All-India Coordinated Research Project (AICRP) on Poultry began modestly during the Fourth Five-Year Plan period. The Indian Council of Agricultural Research (ICAR) approved two coordinated projects; the All-India Coordinated Research Project on Poultry for Egg and the All-India Coordinated Research Project on Poultry for Meat. These projects were merged into a single project called the All-India Coordinated Research Project on Poultry Breeding during the Fifth Five-Year Plan period, with no change in objectives.

SWOT Analysis

SWOT analysis is a simple but powerful strategic planning tool that has been in continuous use since the 1960s (Phadermrod et al., 2019). It helps countries to identify their strengths, weaknesses, opportunities, and threats in order to develop policies, programs, and institutional frameworks for the sustainable management of animal genetic resources (AnGR).

Strength for animal breeding practices in India

Genetic diversity: India has huge livestock and poultry genetic resources. Presently, 53 breeds of cattle, 20 breeds of buffalo, 37 breeds of goat, 44 breeds of sheep, 7 breeds of horses and ponies, 9 breeds of camel, 13 breeds of pigs, 1 breed of yak, 19 breeds of chicken, 2 breeds of duck, and 1 for geese have been registered till date. These resources and genetic diversity can be effectively used to achieve optimal animal production.

Population: In addition to have many breeds of each livestock species, India also has a large population of livestock (536.76 million) and poultry (851.81 million). This large population size is a strength for India's animal production system, as it allows for increased selection intensity and genetic gain.

Infrastructure: Many agencies, such as State Agriculture and Veterinary Universities, ICAR Institutes, the National Dairy Development Board (NDDB), the BAIF Development Research Foundation, Central Agriculture Universities, and Central and State livestock farms, are working for improving animal production systems. Their infrastructure can be used to implement refined animal breeding practices.

Human resources: As mentioned earlier, many researchers have been launched by universities and research institutes in the field of animal genetics and breeding. Presently, 25 Indian universities offer Master's and PhD degrees in Animal Genetics and Breeding, with a total of 182 seats. This human resource can be utilised in the research of animal breeding and management.

Market of animal products: Recently, India has been tagged as a country with the highest human population (approximately 142.8 crore) in the world. This itself created a huge market for the consumption of animal products. Presently, per-capita availability of milk, meat and eggs are 444 gram/day, 6.82 kg/annuam and 95/ annuam, respectively. However, it can be increased by implementing suitable region-specific breeding plans for India's growing population.

Weakness for animal breeding practices in India

Low productivity of animals: Indigenous livestock breeds have low productivity per animal in India. There is need to focus on selected animal breeds of different species to exploit their inherent potential of high productivity. The average annual milk production of cattle in India during 2019-20 was 1777 kg per animal, as compared to the world average of 2699 kg per animal per year during 2019 (Source, PIB, India).

Herd recording: A successful breeding program is totally based on the accuracy of phenotypic data collected from the farm animals. The implementation of latest technologies to collect precise data and pooling of data at common centre would be useful for selection of elite animals.

Unexplored animal genetic resources: Animal genetic resources of India have not been full characterised till now. Identification of newer germplasms with high production potential and their inclusion into the breeding programs would be helpful for increasing animal production.

Use of reproductive tools: Latest reproductive tools are not fully exploited yet. Therefore, application of these tools (Embryo Transfer Technology and Artificial Insemination) at the grass root level is required to increase genetic gain.

Extension services: Scientific rearing and breeding programs are essential for livestock keepers at the village level. Raising awareness among farmers about the latest technologies and their implementation would increase animal production.

Scarcity of feed and fodder: The scarcity of feed and fodder is a major constraint to livestock development in India. This is due to factors such as changing land use patterns, urbanization of agricultural land, reduced pasture productivity, diversion of land to commercial crops, and diversion of crop residues to other industrial uses. The ICAR-Indian Grassland and Fodder Research Institute (IGFRI) has estimated that there is deficit of 11.24, 23.4 and 28.9 per cent for green fodder, dry fodder and concentrates respectively in the country. Therefore, availability of high quality feed and fodder may be secured to exploit the full genetic potential of indigenous breeds.

Opportunities for animal breeding practices in India

Biodiversity/ Genetic variation availability: India has a vast genetic diversity, broad genetic base and low inbred populations. Genetic diversity allows flexibility in a population. Therefore, different genotypes may be selected as per the requirement. In addition, selection intensity may be applied to increase genetic gain.

Breeds with unique traits: Indigenous populations have been developed over a period of time with unique characteristics such as disease tolerance (tick resistance of Sahiwal cattle), climate resilient (heat tolerance in Tharparkar cattle), draft ability, survivability on low input system etc. These can be well exploited as per the breeding plans and schemes.

Statistical and Computational tools: In the era of computer science, various biocomputational tools, software's and algorithm have been developed for analysis of big data and making fruitful interpretation. These high-end technologies can be used for maintenance, analysis and interpretation of phenotypic and genomic data.

Data recording strength: Presently, only few agencies have been involved in phenotypic data recording in farm animals. Phenotypic data recording can be strengthened by using latest techniques and by including small animal holders.

Use of genomic technologies: Development of SNP chips and low-cost genotyping have opened newer opportunity to genotype large number of animals simultaneously. These developed genomic technologies can be utilised for genomic selection in India.

Export potential: India is the largest producer of milk in the world. Further, India's exports of Animal Products in 2022-23 was Rs. 32,597.39 Crores/ 4,062.15 USD Millions, which includes the major product like Buffalo Meat (Rs. 25648.10 Crores/ 3194.70 USD Millions), Sheep/ Goat Meat (Rs. 537.18 Crores/ 66.92 USD Millions), Other Meat (Rs. 16.93 Crores/ 2.18 USD Million), Poultry Products (Rs. 1081.62 Crores/ 134.04 USD Millions), Dairy Products (Rs. 2269.85 Crores/ 284.65 USD Millions), Animal Casing (Rs. 326.02 Crores/ 40.87 USD Millions), Processed Meat (Rs. 11.72 Crores/ 1.47 USD Millions), Casein (Rs. 816.32 Crores/ 101.19 USD Million), Albumin (Eggs & Milk (Rs. 266.88 Crores/ 33.06 USD Millions)) (https://apeda.gov.in/). This export can be increased by producing high quality animal products which competes international market.

Threats in animal breeding practices

Financial issues: A constant and substantial financial help is required to implement all breeding schemes. Further, to implement genomic selection, genotyping cost is additionally required. Therefore, any financial crunch may impact breeding scheme application at the grass root level. The Union Budget for FY22 has allocated Rs 6,407.31 crore to the Ministry of Fisheries, Animal Husbandry, and Dairying. However, funds allocated purely on breeding plan and scheme for improved production need to be reviewed.

Fodder requirement: Feed and fodder requirement of animals will be increased along with the productivity. Therefore, more cultivated land and perennial crops may be required for sustainable livestock production.

Quality of livestock produced: There is possibility of decrease in the quality of livestock produce as the quantity increases. Further, low quality will have negative impact on market acceptability. Therefore, checks may be required for maintenance of quality of livestock produce.

Disease outbreaks: Recent outbreak of lumpy skin disease (LSD) in India has affected over 2 million animals including 100,000 deaths (Kumar et al., 2022). Therefore, proper vaccination and animal health facilities are required for sustainable animal production.

Way forward of animal breeding practices in India

Smallholder production systems are characterized by small animal numbers per household, lack of systematic animal identification, absence of performance and pedigree recording, poor infrastructure and ill-functioning institutions. Therefore, establishment of functional breeding programmes under smallholder conditions remains a challenge. Following points may be considered for future breeding practices in India:

Strengthening of infrastructure: All the breeding schemes such as AICRPs, Networks projects are need to be strengthened to cover substantial numbers of animals for selection of elite animals. Further, all the government and private livestock farms may also be included in the various schemes.

Phenotypic data recording: The success of a breeding program depends on its efficiency in supplying genetically superior stock to customers on a continuous basis. This requires accurate and timely breeding decisions, which in turn relies on the availability of accurate data. Therefore, data collection and analysis are central to the profitability of commercial livestock breeding programs, and a phenotyping data recording and management system is essential for the successful implementation of any breeding program.

Creation of reference population: Phase-wise, reference population may be created for superior breeds within species by including large number of animals for implementation of genomic selection in India.

Use of genomic technologies: All the animals having complete phenotypic data should be genotyped using SNP chips. This data would be helpful for increasing number of animals in the reference population and development of prediction equation for genomic selection.

National Genetic Evaluation System: Genetic evaluation is a key tool for improving livestock production. Now, it is the need of hour to formulate the National Genetic Evaluation System in the country. It will perform routine evaluations for all livestock such as dairy cattle and buffalo, sheep, goat, pig and poultry. The robustness of National Genetic Evaluation System will be depend on amount of precise phenotypic data collected from the country, statistical models, pedigree information and genotypic data. This evaluation system is then used to make breeding decisions, which can lead to improvement in the livestock herds.

Genetic improvement in crossbred animals: In the past decades, crossbreeding has been commonly used to improve the milk production all over the world. The population of crossbred pigs in India is about 20 % of the total pig population. There are 51.36 million exotic/crossbred cattle in India. Therefore, a separate breeding plan need to be developed for the crossbred population to maintain milk/ meat production of the country.

Use of reproductive technologies: Reproductive biotechnologies are used to shorten generation interval and propagate genetic material among breeding animal populations. These technologies have been developed over the years, including artificial insemination (AI), embryo transfer (ET), and in vitro fertilization (IVF). The challenge for assisted reproductive technologies (ART) to become widely used in India is to match artificial insemination (AI) in terms of simplicity, cost-effectiveness, and success. The advantages of using genomic selection together with MOET are twofold; it enables the discrimination of the best heifers to be flushed and the early differentiation of the best prospective males obtained from flushings (Sørensen and Sørensen, 2009).

Integration of feeding and health parameters in breeding programs: As cattle and buffaloes have been bred for higher milk yield, and other parameters have been ignored such as health, feeding efficiency, and fertility problems. Therefore, we need to take into account all of the relevant factors during formulating breeding plan.

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GENOMIC DISSECTION OF FERTILISING SPERMATOZOA: TIME FOR A NEW EQUATION FOR MALE FERTILITY ASSESSMENT

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In spite of their optimum milk-producing abilities, bovine milch animals often suffer from several reproduction shortfalls restricting their lifetime productivity. Involvement of intensive dairy farming practices and aggressive human interventions in terms of breeding for increased milk yield have resulted in compromised fitness (reproductive) which has been witnessed worldwide. A dismally low percentage of bulls inducted in artificial insemination (AI) programs are finally used for semen production. The breeding bulls of high genetic merit exhibit unexplained male sub-fertility and end up resulting in below optimum conception rates. The 'sperm morphology and motility' criteria currently used as the benchmark for evaluation of a semen ejaculate requires to be revisited. The spermatozoa have hitherto been considered merely as a haploid genome transfer vehicle; however, recent findings indicate very conclusively that it is much more than just a 'DNA delivery boy'. In its thin cytoplasm it carries several coding, non-coding RNAs, like piRNAs and miRNAs and an assortment of diverse proteins that perform disparate functions. The epigenetic marks like methylation or acetylation patterns along with the quality of bimolecular cargo have recently been implicated in male fertility. The identification of a sub-fertile ejaculate is a necessity and of major economic value in a commercial farm set-up or semen production centres. To maximize the efficiency in modern livestock systems, novel ways to ascertain the fertilizing ability of the spermatozoa are the need of the hour. This is because, the predictive capacity of morphology-based and sperm number-based criteria to evaluate the fertilizing ability of the spermatozoa is limited. Thus, it is imperative to find novel means to assess the fertilizing to identify the competent, spermatozoa and to avoid the freezing of sub-fertile spermatozoa. Low conception rate, despite insemination with morphologically normal spermatozoa if fairly common in bovine animals and this can be ascribed to numerous factors that are known to govern male fertility e.g., the cargo carried by seminal extracellular vesicles, the epigenetic factors, the quantitative and qualitative aspects of biomolecular composition of sperm structural elements etc. It is imperative to explore and determine the factors that govern the survival and successful fertilization of spermatozoa and its regulation in the bovine model. Recently, immense interest has spawned in 'multi-omics' approaches for developing methods to augment the production of quality semen and to best utilize the available semen. Overall, it appears that the acquisition of high genetic merit bulls alone may not suffice to meet the demands for widespread implementation of a planned animal improvement plan. It will be imperative to pre-select and identify the finest individual ejaculates/spermatozoa by a system biology approach that can be used to improve selection for fertility traits in livestock species. It will be equally important to validate the identified molecular biomarkers in standardized field trials using random male to make certain the efficacy of these markers for predicting male fertility.

Moving further, from the conventional approaches to assess the fertilizing potential, a multitude of molecular analysis techniques and tools are now capable of evaluating the genome, transcriptome, proteome, metabolome, glycome, lectome and epigenetic factors to identify candidate genes, proteins and biomarkers primarily associated with the seminal plasma and the spermatozoa. These most advanced and high throughput approaches provide the holistic way to ascertain the fertility of dairy bulls. Assessing the male germ cell from the molecular point of view, its DNA is tightly packed around protamines and less histones within the head region. The structural arrangement and integrity of DNA are key determinants of sperm fertility. We still do not have a good understanding of many sperm attributes, including apparently simple characteristics such as sperm size. The fact that sperm have until now been presumed to only contribute DNA to the eggs, but the recent discovery of a complex sperm RNA carriage seems to be surprising and hard to explain. Although, the mature spermatozoa are transcriptionally inactive, yet they do contain numerous copies of mRNA, miRNA, iRNA, piRNA and antisense RNA and a study of human sperm detected more than 4000 different mRNAs alone. The spermatozoa from high-fertility bulls contained higher concentrations of transcripts for membrane and extracellular space protein locations, while spermatozoa from the low-fertility bulls were deficient of transcripts for transcriptional and translational factors. Lower expression of protamine 1, casein beta 2 and

a third transcript (thrombospondin receptor *CD36*) detected in low-fertility bulls. Differentially expressed miRNAs correlated with the number of testicular mature germ-cells and described the combined expression values of a panel of three miRNAs (miR-449a, miR-34c-5p and miR-122) as a predictive test for the presence of mature germ-cells in testicular biopsy of humans**.** Piwi-interacting RNAs (piRNAs), is another class of short non-coding RNAs which are strongly related with the process of spermatogenesis in testes and they are supposed to regulate spermatogenesis, thus, piRNAs vital for maintaining male fertility in animals and humans. In addition, high throughput proteomic studies largely contributed the more detailed descriptions underlying the cellular and molecular mechanism related to sperm functions. The findings from our lab and elsewhere elucidated that catalogue of proteins derived through LC-MS/MS approaches unravelled the valuable information related to sperm functions and fertilizing potential. In our studies, a comprehensive list of sperm and seminal plasma proteins in buffalo and Sahiwal bulls was generated. We highlighted fertility associated highly abundant proteins such as AKAP3 and SP17 proteins in spermatozoa of high fertile buffalo bulls. Identification of highly abundant proteins in contrasting fertility buffalo and cattle bulls delineated the role of candidate proteins regulating sperm fertilizing potential. Semen comprises an about 5% of spermatozoa fraction and 95% seminal plasma secretion from the accessory glands. The vast metabolite content of seminal plasma is largely neglected which are essential for the successful fertilization of spermatozoa. The proteomic signature of seminal plasma was evaluated for male infertility and a group of enzymes like prostaglandin-D synthase, estrogen sulpho transferase (EST) and SP-10 were strongly associated with sperm quality and fertility outcome in animals. Seminal plasma of bulls contains heterogenous population of nano sized bodies known which are known as extracellular vesicles (EVs). These seminal EVs harbour a plethora of signature cargoes like miRNA, mRNA, proteins and enzymes. The molecular carriages of seminal EVs have enormous capacity to modulate the sperm functional characteristics. Based on the recent findings of our group in distinct fertility buffalo and cattle bulls, it was found that higher abundance of fertility factors/enzymes such as PLA2, EST and PGDS were reported to be highly abundant in seminal EVs of high fertile bulls.

With the new emerging views on transfer of inheritable epigenetic processes, this field is being keenly followed up and the same has been found to be involved in several genetic disorders and diseases. Besides, with the availability high throughput sequencing technologies, an altogether new paradigm has been added to the study of epigenetics. Complexities in epigenome data are challenging as epigenetics features differ not only among individuals but also among different cells and tissues within the same individual. These differential features are the result of epigenetic processes mentioned above and has further highlighted the complex relationship between genotype and phenotype. Epigenetics impart changes in gene expression without any alteration in nucleotide sequence. It is a normal biological phenomenon influenced by nutritional, climatic and disease factors. A simplest example can be differentiation of brain, liver and other cellular features from the same epigenetics manifests itself under different processes like DNA methylation, histone modifications and RNA based gene regulation. For an orderly expression of genetic information, nature has provided for chromatin organization involving DNA and histones which in turn is influenced by epigenetic changes in them. Both of these are oppositely charged (Histones being positively charged containing positively charged amino acids, lysine and arginine while DNA is negatively charged) which allows them to hold together. It is these associations between histones and DNA which are regulated by epigenetic mechanisms resulting in activation or repression of cellular processes such as transcription as per cellular needs. Also, during meiosis, a compact heterochromatin in which DNA is tightly associated with histones is needed for chromosomes to align and recombine efficiently but on the contrary for transcriptional processes, euchromatin – a loose binding state between DNA and histones is required. Thus, a clear dynamism of changing epigenetic states throughout cellular functioning and development regulation tends to make sure that the happening of destined events takes place at destined time intervals. Several specific epigenetic modifications are established during spermatogenesis to form highly specialized mature spermatozoa, allowing significant reorganization of sperm chromatin structure, thus, spermatozoa are particularly vulnerable to epigenetic alterations.

DNA methylation is most predominantly concentrated in the CpG regions distributed throughout the genome. In promoter regions of the genome where the occurrence of CpG is especially high (known as CpG islands), methylation is usually absent which accounts for gene expression due to availability of the sites for binding of transcriptional machinery. Conversely, methylation of these promoter regions and also of differentially methylated regions (DMR's) of imprinted genes is associated with gene silencing as a result

of masking of sites for transcription initiation. DNA methylation is catalysed by DNA methyltransferases grouped into two categories: *de novo* methyltransferases (DNMT 3A and DNMT 3B) and maintenance methyltransferases (DNMT1). Recently a new methyltransferase DNMT3L has been discovered which has been shown to stimulate DNMT3A but probably not DNMT3B. One of the most intricately controlled and highly coordinated process is spermatogenesis leading to formation of mature spermatozoa. Foundation of epigenetic landscape establishes in primordial germ cells in the form global demethylation and subsequently establishment of methylation by DMNTs during spermatogenesis. Dysregulations in the DNA methylations process during spermatogenesis can result in abnormal expression of target genes, which may lead to infertility in males. In recent years, there has been a dramatic increase in our knowledge of the genetic & protein content of the spermatozoa, its structures, and the surrounding fluids (epididymal & vesicular) contributing to its function. This however, represents only but a starting point and detailed physiological experiments are required to fully understand the biological function of these in the mature cell and its interaction with its functional environment – the female tract and human egg. Growing evidences suggest that the number and motility characteristics of spermatozoa are more of a reflection of the sperm producing ability of a bull rather than its actual fertilising ability.

In view of the severe scarcity of quality frozen semen doses in India still required to cover a modest 50% of our breeding bovine population we need to consider both genomic and epigenomic/epigenetic criteria to design our strategies to maximally utilise the available semen resource from the outstanding cattle and buffalo bulls enhancing the reproductive performance of dairy bulls.

Interesting reads from the authors lab on the subject

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GENOME EDITING BY CRISPR/CAS FOR IMPROVING PRODUCTIVITY IN POULTRY INCLUDING LIVESTOCK

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Science was revolutionized consequent upon the discovery of DNA structure making it possible to understand the molecular mechanism of life, characters, and, its inheritance. Even though there is much more to discover and characterize DNA but genome editing has directed a leap further as it becomes the most discussed topic and overcoming many issues. However, Scientists have been trying to edit genes of interest for a long time, but a major constraint has been to devise a technique that should be simple, precise, and easy to handle because there are few techniques that need protein engineering to target a gene. But, from a ray of hope to huge light, one technique that has become a boon to researchers in the 20th century is CRISPR-Cas technology due to its simple editing technique with excellent precision. This technique is adapted as a genomic tool to overcome the difficulties in terms of solving problems of diseases and food security as the world population is expanding in a huge way. To overcome such cognitive difficulties, genome editing is being used as a master stroke by researchers across the globe and till recently, the most coveted Nobel prize has been conferred to its inventors. Even though for any new technique, advantages and controversies are like a twin sister, CRISPR is not an exception, but due to its versatility in application, many nations are adapting this technique in both invitro and invivo to edit the desired gene(s) or to add new gene of interest in the genome. In this context, the present review paper focuses on and discusses different genes edited in livestock to enhance productivity, disease resistance, animal welfare, etc.

Keywords: Livestock, CRISPR/Cas9, Genome editing, Single guide RNA (SgRNA), Proto adjacent motif (PAM).

Introduction

Genome-wide editing techniques are defined as procedures that alter DNA sequences by deletions, substitution, mRNA processing, and post-transcriptional alterations, resulting in altered gene expression and functional protein activity (Khan, et al.,2019). CRISPR/Cas(Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein), a new technology based on Streptococcus pyogenes has recently sparked researchers' attention when a virus infects a prokaryote, the spacer sequences in CRISPR arrays are translated into short CRISPR RNA (crRNA), which directs the Cas protein to cleave complementary DNA or RNA of virus genome (Pawluk et al.,.2018). CRISPR/Cas9 may be targeted to any DNA sequence of interest by altering the 20-nucleotide guide sequence. It is a viral defense mechanism in bacteria, which has turned to become a genome editing tool by the efforts of many researchers. Other emerging techniques for targeted genome editing including zinc-finger nucleases (ZFNs) and transcription-activator-like effector nucleases (TALENs), allow researchers to introduce permanent alterations by activating repair mechanisms by generating double-stranded breaks (Maeder et al.,2016). Because these methods are expensive, time-consuming to develop, and lack precision, CRISPR-Cas9 has become a strong alternative technique to edit DNA sequences with excellent precision and low cost (Caplan et al.,2015). The first existence of these sequences are identified accidentally by Ishino *et al.*,1987 when they were studying phosphate metabolism in Escherichia coli (*E.coli*). However, due to the lack of a whole genome sequence of bacteria, it became difficult to characterize these unknown sequences. Later, research on these unknown sequences is in the dusk. However, the discovery of these interspaced repeat sequences in more than 40% of sequenced bacteria and 90% of archaea by Mojica *et al.* (2000) lead to enter of the CRISPR era. Subsequently, spacer sequences confer immunity to bacteria (Mojica *et al.,* 2005; Barrangou *et al.*,2007; Sorek *et al.,* 2008). Further, a subsequent collaboration of the Charpentier and Doudna laboratories demonstrated the programmable dual-RNA-guided DNA endonuclease started the era of CRISPR/Cas9 genome editing (Jinek *et al.,* 2012 and Doudna and Charpentier, 2014). The demand for food is expected to rise significantly by the year 2050, and to meet this need, 70–100% more food must be produced (McKenzie and Williams, 2015). Genome editing could help increase food production efficiency and may meet the demand of the world (Ma et al.,2018:

Menchaca,2021). CRISPR/Cas technology is also used to understand complex diseases by producing animal models (Shrock and Guell, 2017) and also to improve animal welfare. Hence, the present review is to focus on applications of CRISPR/Cas technology in livestock and poultry because, it produces animals that are more resilient to diseases, has improved production efficiency, and produces animals that can serve as models for biomedical research.

Mechanisms of CRISPR/Cas9 genome editing

The mechanism of CRISPR/Cas9 genome editing can be generally divided into three steps: recognition, cleavage, and repair. Through its 5'crRNA complementary base pair component, the proposed sgRNA drives Cas9 and detects the target sequence in the gene of interest. In the absence of sgRNA, the Cas9 protein stays inactive. At a location of three base pairs upstream from the protospacer adjacent motif (PAM), the Cas9 nuclease generates double-stranded breaks (DSBs). The PAM is 2-6 nucleotides downstream of the guide RNA-targeted DNA sequence, while the Cas activity site is 3-4 nucleotides upstream. The widely used spCas9 recognizes a 5'-NGG-3'. The Cas9 initiates local DNA melting followed by the creation of an RNA-DNA hybrid once it has discovered a target spot with the right PAM. The Cas9 protein is then activated to cleave DNA(Figure 1). The HNH domain cleaves the complementary strand of target DNA, whereas, the RuvC domain cleaves the non-complementary strand, resulting in mostly blunt-ended DSBs. Finally, the host cellular machinery repairs the DSB. In the CRISPR/Cas9 system, the non-homologous end joining (NHEJ) and homology-directed repair (HDR) pathways are used to repair DSBs caused by Cas9 protein(Figure 2). NHEJ aids in the repair of DSBs by enzymatically connecting DNA fragments in the absence of external homologous DNA and is active throughout the cell cycle. It is the most common and effective cellular repair pathway in mammals (Mao *et al.,* 2008) but it is also the most error-prone, as it can result in minor random insertions or deletions (indels) at the cleavage site, resulting in frameshift mutations or premature stop codons (Bernheim *et al.,* 2017). HDR is the second DSB repair pathway. The fidelity of this mechanism is good, but the frequency is modest. Homologous donor DNA sequences from sister chromatids or foreign DNA are used in the HDR process. HDR is a less prevalent type of DSB repair than NHEJ, but it is extremely accurate and has a substantial impact on genome editing (Rothkamm *et al.,* 2003). In CRISPR gene editing, a large number of donor (exogenous) DNA templates bearing a sequence of interest is required. HDR performs exact gene insertion or replacement by introducing a donor DNA template with sequence homology at the predicted DSB site. The procedure of targeting the gene of interest is given in Figure no.3

An extensive study was conducted to knock in and knockout genes in livestock based on the purpose after the development of CRISPR/Cas technology. The literature on the use of CRISPR/Cas in various livestock species is scanty. Therefore, this review provides a general incite into the various livestock species and the genes altered in them.

In Chicken

Using CRISPR/Cas, the genes encoding for ovalbumin and ovomucoid have been knocked out in an effort to remove two major allergenic components from egg white. This could render eggs digestible for a wider range of consumers that could otherwise not consume chicken eggs (Oishi et al., 2016). By combining the CRISPR/Cas9 platform G0S2 knockout chickens are produced and results showed a dramatic reduction of abdominal fat deposition without affecting other economic traits. Additionally, G0S2 null KO chickens had altered fatty acid compositions in their blood and abdominal fat compared with wild-type chickens under normal dietary conditions.G0S2disruption in chickens would activate the adipose tissue-specific peroxisomal oxidation pathway, and enoyl-coenzyme A (CoA), hydratase/3-hydroxy acyl CoA dehydrogenase might be a target molecule in metabolic homeostasis in the chicken adipose tissue(Park et al.,2019). Myostatin-knockout (MSTN KO) chickens were created utilising primordial germ cells and D10A-Cas9 nickase, and they displayed skeletal muscle hypertrophy and hyperplasia along with decreased abdominal fat deposition (Kim *et al.,* 2020). The acidic leucine-rich nuclear phosphoprotein-32A (ANP32A) was found to play a key role in avian influenza virus replication in both chicken and waterfowl. It has been demonstrated in vitro that the deletion of a small region of chicken ANP32A can prevent the replication of the avian influenza virus (Long *et al.,* 2019). Although the functional consequence of edited ANP32A has yet to be demonstrated in vivo, such approaches offer exciting opportunities that have the potential to benefit both industry and

animal welfare. Avian leukosis virus infection results in inappetence, diarrhea, weight loss, and a reduction in eggs laid, and often causes tumor formation in the chicken. The cellular receptor of ALV-J was identified to be the chicken sodium/hydrogen exchanger 1 protein on the cell surface. Chicken somatic cell lines have been edited to introduce changes to this gene-conferring resistance to avian leucosis virus in vitro (Lee *et al.,* 2017). Zhang *et al.* (2019) first reported the successful and effective use of CRISPR technology to make targeted mutations insitu into the viral genome of Marek's disease virus (MDV) – transformed lymphoblastoid cell line (LCL) for studies of viral latency and interactions between a virus and its host. The same gRNAs were used for precise editing of the viral gene phosphoprotein 38 (pp38) in infected primary chick embryo fibroblast (CEF) and to insert the GFP gene into the viral pp38 locus in MDV transformed cell line (HP8 cells) by NHEJ pathway. Chickens can be made resistant to the J subgroup of the avian leukosis virus by precise CRISPR/Cas9 editing of the chicken Na+/H+ exchanger type 1 (chNHE) gene, which is a functional cellular receptor for replication of ALV-J (Koslová *et al.,* 2020; Hellmich *et al.,* 2020). The ability of altered Mareks disease virus (MDV) to deliver CRISPR/Cas9 to chickens both in vitro and in vivo was demonstrated by its effective resistance to the avian leukosis virus (ALV-J) (Liu *et al.,* 2020). Cas13a's promise as an antiviral method was demonstrated by the in vitro inhibition of the influenza virus by CRISPR/Cas13a in chicken cells, which resulted in decreased virus titers (Challagulla *et al.,* 2021). In the case of CSIRO genetically engineered chickens, CRISPR would paste a gene derived from a sea anemone into the male chromosome. That gene produces "red fluorescent protein" (RFP), which glows under laser light, causing the cells to give off a red-orange fluorescence. The fluorescence within the egg tells a handler (or machine) there's a male chick inside, and the incubation process is terminated. The Stable Stra8 gene knockdown was seen in domestic chicken cells after Stra8 gene knockout, which may be important in the differentiation of chicken embryonic stem cells (ESCs) into spermatogonial stem cells (SSCs) via CRISPR/Cas9 (Zhang et al., 2017).Similar to this, Deletion of C1EIS Inhibits Chicken Embryonic Stem Cell Differentiation Into Male Germ Cells (Gallus gallus) shown a critical role in encouraging the differentiation of avian ESCs to SSCs (Zuo et al., 2017). CRISPR/Cas based edited Nicobari chicken (Indian native chicken) was produced at ICAR-Directorate of Poultry Research, Hyderabad, which is laying around 260 eggs while control Nicobari chicken laid around 128 eggs up to 72 weeks of age (ICAR Annual Report 2022-23). The technical platform for CRISPR/Cas9 can enable accurate and effective targeted genome modification and may increase the scope of potential uses for poultry with altered genomes.

In Pig

Especially in swine, many researchers focussed to increase meat production and disease resistance eventually leading to CRISPR-edited swine. The protein level of myostatin precursor decreased dramatically in mutant cloned piglets and exhibited partial double-muscled phenotype, faster growth rate and showed myofibre hyperplasia such as prominent muscular protrusion, wider back and hip compared with wild-type piglets(Wang et al.,2017). *FBXO40* is a promising candidate gene for improving production traits in swine was targeted by combining CRISPR/Cas and somatic cell nuclear transfer (SCNT) technologies to generate *FBXO40* knockout pigs which lead to a 4% increase in muscle mass compared to control animals (Zou et al.,2018). *MSTN* biallelic knockout in Chinese Bama pigs, which was confirmed to have significantly faster growth rates showed myofibre hyperplasia when they reached sexual maturity (Zhu et al., 2020). Zou et al.,2019 demonstrated that CRISPR/Cpf1 system can be used efficiently to generate double-stranded breaks and also to mediate homologous recombination to introduce precise genomic modifications in pigs. However, it was reported that the concentration of Cas9 protein affected gene editing efficiency in embryos but not embryonic development, gene editing rate, and non-specific cleavage of off-target sites (Le et al.,2020). After transferring a blastocyst to a recipient gilt, MSTN gene-edited pigs was successfully generated using a novel lipofection-mediated RNP transfection strategy (Hirata et al., 2021). This technique produced no observable off-target events. This recently developed lipofection-based method still has to be developed, especially in terms of editing effectiveness. Wei et al.(2020) also generated GDSS pig single-cell colonies with biallelic mutations in the myostatin (MSTN) gene and insulin-like growth factor 2 (IGF2. These gene-edited single-cell colonies can be used in the future to generate gene-edited pigs using somatic cell nuclear transfer (SCNT).CD163 is a putative fusion receptor for the virus of the porcine reproductive and respiratory syndrome (PRRS). Tanihara et al.,2021 successfully developed a CD163-edited pig by electroporation of the CRISPR/Cas9 system into *in vitro*-fertilized zygotes. Similarly, attachment and internalization of PRRSV are

dependent on the interaction between sialic acid on the virion surface and the sialic acid binding domain of the SIGLEC1 gene. The knockout pig was created by removing part of exon 1 and all of exons 2 and 3 of the SIGLEC1 gene (Prather et al.,2013). However, it is suggested that gene-edited CD163 pigs are resistant to the PRRSV infection trials (Whitworth et al.2016). After infection, they showed no clinical signs (fever or respiratory signs), lung pathology, viremia, or antibody response and remained healthy for the 35 d after infection measured in this study. Similarly, Yang et al. (2018) generated CD163 knockout (KO) pig using CRISPR/Cas9 gene targeting and somatic cell nuclear transfer (SCNT) technologies. By comparison, wild-type (WT) controls displayed typical signs of PRRSV infection and died within 2 weeks, while gene edited pigs showed no adverse effects without impairing the biological function associated with the gene.

In Cattle

The successful use of CRISPR/Cas9 for targeting a specific genomic locus has been dramatically increased and most of the laboratories have switched to using CRISPR/ Cas9 as a genome editor for their purpose. Disease resistance was also demonstrated in cattle that became resistant to infection with Mycobacterium bovis (*M. bovis*) with the aid of DNA nuclease-mediated genetic modifications (Wu et al., 2015). The *M. bovis* has a wide host range and causes significant economic hardship for livestock farmers with estimates of >50 million cattle infected worldwide. The Cas9 nickase enzyme was employed to introduce the NRAMP1 gene into the bovine genome, where inserted NRAMP1 was correctly expressed and provided increased resistance to the *M. bovis* infection in animals (Gao et al., 2017). Cow prion protein gene (PRNP) exon 3 was targeted by CRISPR-Cas9 to have a deletion of 875 bp of exon3 in the gene (Bevacqua et al.,2016). Myostatin (MSTN), which is a negative regulator of the growth in animals was knocked out with CRISPR/Cas in Belgian Blue cattle and the animals were visually different with an obvious difference in muscle mass which was also observed during histological comparison of the muscles (Luo et al., 2014; Qian et al., 2015). The gene encoding the bovine whey protein β- lactoglobulin, which is a major milk protein and a dominant allergen was knocked out in cattle, where modified animal-produced milk having significantly altered milk protein composition and elevated casein levels, but lacking any β- lactoglobulin in milk. This approach has made bovine milk a modified one and more consumer friendly. The horns of cattle could constitute a significant risk for serious injuries and removal of the horn is painful and stressful for the animals. Several cattle breeds are naturally horn free due to a dominant trait referred to as polled. Using TALENs, the causative Celtic mutation (Pc) has been introgressed into the Holstein cattle genome resulting in a polled phenotype of the offspring (Carlson et al., 2016). Similar to this, using CRISPR/Cas12a system fibroblasts functioned as the donor cells for somatic cell nuclear transfer after having the Polled Celtic variant from the genome of an Angus cow knocked into it. On day 90 of gestation, one pregnancy was aborted, confirming the polled phenotype. The remaining calf with a polled phenotype, however, died away shortly after delivery (Schuster et al.,2020)

In Buffaloes

In comparison to other farm animals, the use of CRISPR/Cas technology in buffaloes is extremely limited. In a few experiments, 18s rDNA was removed from mammary epithelial cells using an adenovirus delivery system, demonstrating that this method is more effective than lipid-based or electroporation techniques for editing the target gene (Zhu et al.,2017). To confirm the functionality of the TSP1 gene in buffalo, Paul et al., 2019 used CRISPR/Cas9-mediated gene technique to edit TSP1 in cultured luteal cells obtained from late luteal stage CLs. According to their findings, thrombospondins (Tsp), which are induced by PGF2, are crucial in modulating the development of structural and functional luteolysis in buffalo. The Y-Chr-eGFP transgenic BFF cells and cloned buffalo embryos were successfully created in the *Bubalus bubalis* using CRISPR/Cas9-mediated gene editing in conjunction with the somatic cell nuclear transfer (SCNT) technique. It was determined that the eGFP reporter is suitable for the visualization of the sex of embryos by knocking-in in the eGFP the fluorescence gene using CRISPR technology in the Y chromosome (Zhao et al.,2020). The corpus leuteum in water buffalo has undergone functional validation of the EGR1 gene to examine its impact and modulate VEGF A and FGF 2 signaling. Therefore, the EGR1 gene is knocked out and externally activated by VEGF A and FGF 2. In contrast to the EGR 1 KO cells, the data demonstrated that wild-type luteal cells had increased angiogenesis, cell proliferation, and steroidogenesis (Punetha et al.,2020). It was demonstrated that CRISPR/Cas9 can be helpful for the construction of a new animal strain that can

yield more meat when the muscle-limiting gene such as the Myostatin (MSTN) receptor and GDF8 gene could be knocked out in fibroblasts and embryos of swamp buffalo (Su et al., 2018).

In Sheep and goat

Increasing body weight and accelerating growth rates of farm animals is an important aim in animal agriculture. The *MSTN* was among the first gene which was targeted through CRISPR/Cas9 in sheep for enhancing meat production. Crispo et al. (2015) reported the development of *MSTN*-disrupted sheep using CRISPR/Cas9, where lambs showed the mutation with heavier body weight as compared to their wild-type sheep. Sheep with biallelic modification in the BCO2 gene showed yellow fat compared to white fat and highlighting the role of BCO2 in fat color determination in sheep (Niu *et al.,* 2017). An earlier report described the application of CRISPR/Cas9 for the introduction of a point mutation within the suppressor of the cytokine signaling-2 (SOCS2) gene in sheep (Zhou *et al.,* 2019). This single nucleotide variant revealed profound effects on both body weight and size as well as milk production. Sheep and goats form a valuable source for the production of fibers. The genes associated with fiber quality and quantity are a source of attraction. The *FGF5* gene, which is a dominant inhibitor of fiber length and growth is an attractive target. In sheep, CRISPR/Cas9 has been applied to disrupt the normal function of the *FGF5* gene and found that the disruption in *FGF5* showed increased wool length (Hu *et al.,* 2017). Zhang *et al*. 2017 introduced targeted disruption of the *ASIP* gene by using CRISPR/Cas9. The result showed various coat color patterns highlighting the critical role of the *ASIP* gene in determining coat color in sheep. Manipulation of milk components and the expression of the desired transgenes in milk with the aim to enrich its components with valuable proteins are among the main aims of livestock genetic modification programs. CRISPR/Cas9 has been used to target the *BLG* gene in goat primary fibroblasts (Ni et al., 2014) gene-edited goats with disrupted *BLG* gene have been generated (Zhou *et al.,* 2017), which has shown decreased expression of *BLG* and abolished *BLG* protein production in milk. Another example of CRISPR/Cas9 application for the manipulation of milk components includes the generation of gene-edited sheep with an enriched production of melatonin in milk (Ma et al., 2017). Genes such as stearoyl-CoA desaturase1 in goat mammary epithelial cells (Tian *et al.,* 2018) and acetyl CoA acyltransferase-2 in sheep precursor adipocyte cells (Zhang et al., 2019) are investigated by CRISPR/Cas9 to explore the functional role of the gene in regulating milk quality traits. Improving reproductive performance is an important direction of livestock breeding. Desirable traits related to reproductivity such as litter size have been suggested as goals for introduction to farm animals using gene-editing tools. Mutations in the sheep BMPR-IB(FecB) gene has shown to be responsible for increased ovulation rate and consequently larger litter size (Fabre *et al.,* 2006). CRISPR/Cas9 has been reported to target sheep *BMPR-IB* resulting in gene-edited embryos that were characterized by the presence of indels at the *BMPR-IB/FecB* locus (Zhang *et al.,* 2017). CRISPR/Cas9 has also been applied to investigate the biological role of the glucocorticoid receptor *(NR3C1)* in sheep conceptus elongation using recovered elongating conceptuses (Brooks *et al.,* 2015). In goats, the growth differentiation factor 9 (*GDF9*) gene, exerts a large effect on both the ovulation rate and litter size (Niu *et al.,* 2018). The results highlighted the role of CRISPR/Cas9 in introducing reliable and defined point mutations. The CRISPR/Cas9 system has been applied in sheep and goats to improve the health and welfare of animals. Several disease-related genes have been disrupted using CRISPR/Cas9. The *PrPc* is directly associated with the pathogenesis of the transmissible spongiform encephalopathies, which occur in humans and a number of livestock species including sheep and goats. The *PrP*-resistant animals were produced by suppressing the expression of *PrP* where CRISPR/Cas9 was employed to target the *PrP* gene in goat fibroblasts with the aim to generate *PrP* knockout donor cells to produce of *PrP* resistant goats (Ni *et al.,* 2014; Hu *et al.,* 2015; Fan *et al.,* 2019). The targeting efficiency of *PrP* increased by 70% in goat fibroblasts. Moreover, of the nine dual-gene mutant colonies, five had mutations in all four alleles of both genes, which suggests the use of the CRISPR/Cas9 system to target genes to confer potential disease resistance in farm animals. Fan *et al.* (2017) reported the generation of *IFNAR*-knockout sheep by applying CRISPR/Cas9 in combination with somatic cell nuclear transfer (SCNT) to establish a highly susceptible ZIKA virus large animal model. Williams *et al.* (2018) have established an interesting sheep model, recapitulating human hypophosphatasia by applying CRISPR/Cas9. In this study, a single point mutation in the tissue-nonspecific alkaline phosphatase (TNSALP) gene has been introduced and generated gene-edited lambs accurately phenocopied the human HPP gene.

Conclusion

A ground-breaking method in the realm of genetic engineering is CRISPR-Cas9 among various gene-editing methods. The high degree of flexibility and accuracy in cutting and pasting DNA makes the CRISPR/Cas9 technology relatively more popular (Azimzadeh *et al.,* 2022). The way CRISPR differs from earlier genetic engineering methods is that it enables the addition or deletion of many genes simultaneously. It is now possible to quickly change many different genes in a cell line, plant, or animal, cutting the time required from years to only a few weeks. Due to its effectiveness and simplicity, it has a wide range of uses in livestock and poultry. However, off-target effects remain a serious concern in complex eukaryotic organisms, especially when used in *in vivo* for therapeutic purposes (Zhang *et al.,* 2015, Zischewski *et al.,* 2017). Targeting specificity is determined by Cas9 gRNA and PAM sequences, as well as off-target cleavage in the genome. Off-target effects can be considerably reduced by developing a well-optimized and tailored CRISPR system such as, eSpCas9 (Fan, *et al.,* 2020), HF-Cas9 (Murugan *et al.,* 2021), HypaCas9 (Ikeda, *et al.,* 2019), and Sniper Cas9 (Lee *et al.,* 2019) are some of the Cas proteins that have been modified to improve target selectivity. Another method is to use Cas9 nickases, in which one of the endonuclease domains was catalytically inactivated and the low off-target effect was investigated in the genome (Shen *et al.,* 2014, Gopalappa *et al.,* 2018). The development of base editors and prime editors as a result of Cas9 variant modifications is a major invention for application in the future. It offers new, commercially significant traits in species that have undergone genetic modification. By combining advanced animal genomics based on genome sequencing technology, genome editing and animal breeding can be combined with each other to generate novel animals with desirable traits such as heat tolerance or disease resistance. In addition, genome-edited poultry has potential as an alternative bioreactor platform for production of therapeutic proteins in eggs, as poultry bioreactors can overcome the limitations of mammalian cell culture systems related to *N*-glycosylation patterns and production costs. Thus, in the near future, CRISPR/Cas will take over as the primary editing tool in a variety of scientific study and effective tool for enhancing productivity in livestock and poultry. Collectively, rapidly developing genome-editing technology will accelerate progress in livestock opening up new opportunities for to contribute to various sectors.

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EXPLORING THE ROLE OF EPITRANSCRIPTOMICS IN LIVESTOCK AND POULTRY

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Overview of Epitranscriptomics:

Epitranscriptomics represents a repertoire of RNA modifications that can influence range of biological functions through regulation of different stages of mRNA life cycle (splicing, localization, stability and translation efficiency). This emerging field is also known as RNA epigenetics which can rival other well-known epigenetic mechanisms (DNA methylation) in terms of innovations and breakthrough results. Recently explored major RNA modifications include methylation at N6 adenosine (m6A), pseudouridine (Ψ), methylation of 5-cytosine (m5C), deamination of adenosine (A to I). Earlier, these modifications were detected in RNA molecules using laborious chemical methods. However, recent methodological advances (next generation sequencing and its variations) made it possible to the identify and map modified sites to reveal their topology and dynamics in a transcriptome-wide manner. This information together with functional analyses and the identification of proteins that are able to write, erase and read such modifications have led to the birth of a novel layer of gene regulations.

Wide range of biological functions are potentially affected through epitranscriptomic changes as cells can utilize dynamic (spatio-temporal/ context dependent) and reversible nature of these modifications as critical regulatory points. Major effects of epitranscriptomic changes are being investigated in the field of cancer, immune responses, metabolic functions and host-pathogen interactions. The significance of the research on RNA modifications is highlighted by their tight association with different conditions. For example, aberrations in RNA modification levels are linked to hepatocellular carcinoma (HCC), Hodgkin lymphoma and metabolic diseases. Viruses have been found to exploit RNA modification process against the host cells. The recent success of Nobel prize winning discovery involved one of the RNA modifications (N1-methylpseudouridine) that was found to significantly increase the translational efficiency of mRNA (mRNA vaccines against SARS-CoV2). Similar to human health and medicine, epitranscriptomics field holds promise for control animal diseases (infectious and non-infectious) and strategies to improve animal productivity.

Methods of Epitranscriptomics:

1) Methylated RNA immunoprecipation sequencing (MeRIP-seq)

Mapping m6A modifications in mRNA molecules has been taken up by combining the methods of RNA immunoprecipitation (RIP) and next generation RNA sequencing (RNAseq). Briefly, the purified RNA will be fragmented to ~100–150 nucleotide length and immunoprecipitated by an m6A-specific antibody. The enriched m6Acontaining RNA fragments are then subjected to library construction and high-throughput sequencing. The analysis of data taken up to identify peaks of m6A by comparing with the input control sequencing reads using Fisher's exact test with minimum peak size of 100bases.

2) Mapping of m5C modifications by bisulfite-sequencing

The standard bisulfite treatment used for DNA will degrade the fragile RNA fragments. The modified bisulfite treatment converts unmodified C into U, whereas m5C will remain as C. The input RNA will be fragmented and sequencing libraries constructed for high throughput sequencing. The reads from the bisulfite treated and untreated samples will be aligned to the reference transcriptome sequences using specific computational tools to identify m5C modified sites.

3) Mapping of adenosine to inosine (A to I) modification

Inosine represents an important RNA base modification created by deamination of adenosine residues. Inosine acts as guanosine in terms of base pairing property (pairs with cytosine instead of uracil) and hence important for changing coding profile of the mRNA. A chemical method has been developed for the detection of inosine called Inosine Chemical Erasing (ICE) technique (Sakurai et al., 2010). In this approach,

acrylonitrile will be used to selectively react with inosines in RNA, forming N1- cyanoethylinosine (ce1I). Because ce1I stalls reverse transcriptase (RT) and results in truncation of the cDNA, inosine-containing RNA is 'eliminated', while only unmodified RNA gives rise to full length cDNA. By comparing sequencing results of treated and untreated RNA samples in parallel, the A-to-I editing sites can be successfully detected.

4) Mapping of uridine to pseudouridine modifications

Pseudouridine (Ψ) is generated by isomerization of uridine (U). It is an abundant modification in rRNA and tRNA, however recently it was also found in mRNA. A chemical labelling method developed to distinguish Ψ from U. N-cyclohexyl-N'-b-(4methylmorpholinium) ethyl carbodi-imide metho-p-toluene-sulfonate (CMCT). After reaction with Ψ, CMC-Ψ adduct stalls RT and terminates the cDNA just before one nucleotide during primer extension. This phenomenon of truncation used for detection of Ψ. However, the primer extension assay relies on prior knowledge of candidate Ψ-containing regions and is more suited for Ψ detection at specific loci. Recently, several approaches have been developed to map Ψ sites in a transcriptome-wide manner by coupling this selective labelling reaction to high-throughput sequencing. In Pseudo-seq method, fragmented mRNA is reacted with CMCT, and the precise Ψ positions are identified in the transcriptome-wide manner.

Biological functions of RNA modifications

The presence of modifications in transcripts (mRNA) indicate the possibility of their critical functions. For example, m1A and m6A prominently found in the 5'UTR and near the start codon both in transcripts of human and mice showed their role in translation regulation (Li et al., 2016; Dominissini et al., 2016). The writer, eraser and reader proteins for some of these modifications have been identified. The knock down or overexpression models (widely-utilized cultured cells, animal models) for these proteins are being established. These systems could facilitate the investigations on physiological roles of a particular modification, such as its relations to fertility, differentiation, and pathogenicity (Wilkinson et al., 2022). The functional studies of m6 A modification on mRNA stability, translation efficiency, and exon inclusion, relied on manipulating the methyltransferases METTL3/METTL14/WTAP (Zhang et al., 2021). Molecular mechanisms and clinical therapeutic implications of RNA m6A modification in different cancerous conditions have been recently deliberated (Deng et al., 2022; Pan et al., 2023). The dynamic pattern of a particular modification may have the potential to serve as a biomarker to monitor the status of the disease by establishing the correlation between a certain disease and a modification involved (Zhu et al., 2023).

RNA modification in virus-host interactions

Viruses (including animal viral pathogens) were found to manipulate RNA modification pathways in the host cells. Apart from host origin transcripts, viral genomic (in RNA viruses) and their transcripts undergo these modifications. Among others, m6A epitranscriptomic mark is the most studied and has shown key functions in virus-host interactions. Multiple studies have confirmed the involvement of m6A during human immunodeficiency virus I (HIV-1) infection including the presence of m6A modification in the viral genome. Different flaviviruses (HCV, ZIKV, DENV, WNV, and YFV) shown to exploit host m6A modification process for their replication. For example, HCV infection induced m6A modification of PTEN mRNA that leads to disruption Interferon synthesis affecting host innate immunity (Kim *et al.,* 2020b). Influenza A virus (IAV) utilizes the m6A reader protein (YTHDF2) for its replication and infectious particle production (Courtney *et al.,* 2017). Vesicular stomatitis virus (VSV) that causes disease in different animals (cattle, horse and pigs) has been found to regulate m6A modification process. During VSV infection, m6A demethylase (ALKBH5) removes m6A from antiviral transcripts (reduce their translation) and facilitate virus replication. (Zheng et al., 2017). Our research group found the role of m6A modification in peste-des-petits ruminants (PPR) virus infection. The viral genomic RNA and viral transcripts were found to have m6A modification. Small molecule inhibitors and stable knock down host cells system indicated the involvement of m6A modification process on PPR virus-host interactions. Higher than basal level of host m6A modification facilitated the virus replication whereas inhibition of m6A modification showed negative impact on virus infection and gene expression (Khan et al., 2023). Recently, m6A epitranscriptomic profile of Newcastle disease virus (NDV) infected chicken macrophages showed its role in innate immune response (Li *et al.,* 2023).

Deciphering the role of RNA modifications in livestock and poultry

How m6A RNA modification process regulate heat stress response in cattle has been studied in bovine mammary epithelial cells. The heat stress induced gene expression regulations were found to involve m6A mediated mechanisms (Qi et al., 2022). The m6A modification proteins (METTL3, FTO) mediated molecular changes of heat stress response in sheep (Chen et al., 2023). Epitranscriptomic changes play an important role in regulating lipid production and energy metabolism, hence the potential role of m6A in growth rate and meat quality in different beef cattle breeds was identified. The correlation of m6A levels and transcript abundance indicated its involvement in steroid biosynthesis process, fatty acid metabolism, and galactose metabolism (Dang et al., 2022). RNA modification (m6A) was found to modulate gene expression during yak muscle development (Ma et al., 2022). Similar findings were reported for meat production phenotypes in pigs, goats and poultry (Dou et al., 2023; You et al., 2023; Gu et al., 2022). Further, m6A mediated mechanisms were analyzed in regulation of fatty acid synthesis and metabolism genes between fat and lean birds (Cheng et al., 2021). The key functions of gonadal sex differentiation in chicken embryo were found mediated through m6A RNA modification in the relevant genes (Li et al., 2022).

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GENETIC IMPROVEMENT OF BOS INDICUS IN BRAZIL

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History of *Bos indicus* Genetic improvement in Brazil

The first cattle arrived in Brazil in 1533, in the Expedition of Martin Alfonso de Souza, which resulted in the founding of the first Portuguese Captaincy on the Island of São Vicente. At the end of the 16th century, there was a great abundance of cattle on the Brazilian coast and in all Portuguese Captaincies. Especially cattle, which had a great predominance of breeds of Iberian origin (Portuguese and Spanish), impacted significantly the Brazilian development, were raised production for subsistence and to supply national urban centers (Figueiredo, *et al*., 1982; da Silva, 2012; Teixeira e Hespanhol, 2014; Macedo, 2019; Bastianetto *et al*., 2020; Cruz, 2022).

These animals contributed to Brazilian historical economic cycles, initially in the brazilwood's transport, following in sugar mills and, later, in the production of leather and beef jerky, which fueled the gold rush. Therefore, during the first three centuries after colonization cattle was raised for work, food and clothing in an extractive and empirical way (Euclides filho, 2009; Teixeira e Hespanhol, 2014; Medrado, 2018; Pineda, 2000; Bastianetto *et al.,* 2020).

The *Bos taurus* cattle was fundamental during the Colonial, and Imperial, Brazil. Over the years, the process of mixture and adaptation gave rise to different locally adapted breeds with increased ability to tolerate the high temperatures of the tropics (McManus *et al*., 2009), among which can be highlighted the Curraleiro (Pé-duro), the Franqueiro or Junqueiro, Caracu, Mocho Nacional, Crioulo Lageano and Pantaneiro breeds. However, at the end of the 19th century and beginning of the 20th century, with the Proclamation of the Republic, the demand for more productive animals increased (Alencar, 2004; Medrado, 2018).

In this historical context, imports of individuals of different Zebu breeds began and due to their greater heat tolerance and resistance to ectoparasites, greatly contributed to a revolution in Brazilian livestock farming. Between 1870 and 1962, a total ranging from only 2465 cattle animals (*Bos indicus*) (Bastianetto *et al*., 2020) to 7.000 animals (Brasil, 2002) were imported from India. The results of crossbreeding *Bos indicus* animals and the local breeds created great expectations around the Zebu, leading to an increase in the population raised in Brazil. However, possibly a large proportion of the success attributed to Zebu genetics was due to the heterosis obtained with the crossbreeding (Euclides filho, 2009; Medrado, 2018).

It was during that period, with the first large-scale imports of cattle from India that commercial production-oriented selection began. The selection process started empirical based on external traits, whose main objective was to establish characteristics linked to the animal's body shape and beauty. However, those breeders and researchers of the time, intelligently exercising an aesthetic vision, were able to establish, with persistence and understanding of functionality, the different Zebu breeds existing today in Brazil. This empirical approach persisted during the early 20th century. Only after the 50s did scientifically based genetic improvement programs begin (Euclides filho, 2009).

Nowadays, the prominent role of cattle genetic improvement is observed by the large number of genetic evaluation programs applied to different breeds, especially Zebu and synthetic breeds, with consequent recognition and valorization of animals carrying estimated genetic values, whether differences expected in progeny (DEPs) or Predicted Transmitting Ability (PTA). Furthermore, it is important to consider the advances made in other areas of science such molecular genetics, mathematical modeling, new statistical methods, which allied to a greater capacity computational and advances reproductive biotechniques allowed to increased annual genetic gains (Mariante *et al*., 2003; Bastianetto *et al*., 2020, Buzanskas *et al*., 2015).

All of this contributed to the improvement of animal husbandry and the consolidation of the livestock production chain, which favored Brazil's rise as a recognized major exporter of quality meat. On the other hand, the Brazilian cheese and dairy sector, traditionally focused on the domestic market, is experiencing a revolution. It participated on the "'6th Mondial du Fromage et des Produits Laitiers Tours" (World Cheese

and Dairy Products Tours) in 2023, which is organized every other year in the Loire Valley-France. Brazilian won 81 medals for its cheeses, 17 of which were gold, and many of them produced exclusively with Zebu (Gyr and Guzerat), cattle milk (Euclides Filho, 2009; https://www.cotentin-tourisme-normandie.fr/resultats-du-jeu-cotentin-tourisme-2023-liste-des-gagnants/).

THE ARRIVAL OF ZEBU IN BRAZIL

The first entrance of Zebu animals into Brazil occurred over two centuries ago, in 1813, when one sire and a cow (similar to the Ongole/Nellore) were imported and gave rise to the cattle named "Malabar" (Weiss, 1956). Other imports of Zebu animals, without productive purposes, occurred from 1813 onwards. Highlight for to the importation carried out in 1826 by the Emperor D. Pedro I to the Fazenda Real de Santa Cruz, in Rio de Janeiro, establishing in this way, the first Zebu herd in Brazil (Pineda, 2000). Those animals came from Egypt in Africa and not from India, and gave rise to the "China" cattle. In addition to those importations, a couple of Zebu were imported to Rio de Janeiro in 1875, coming from the London Zoo to the farm of breeder Acácio Americano de Azevedo (Machado, 2015; Pineda, 2000; Bastianetto *et al*., 2020).

According to Weiss (1956), Teófilo de Godoy, a farmer from Araguari-MG, began a new era for Brazilian livestock farming in 1898, being the first breeder to go to India with the specific purpose of acquiring Zebu cattle for production. In this import, six bulls and two cows were brought and sold to farmers from Uberaba-MG. Teófilo de Godoy organized two more imports of Zebu from India in 1903 and 1906, when a relatively larger number of animals was brought to Brazil. At least eight (1920, 1930, 1939, 1960, 1940, 1955, 1960 and 1962) more imports were released during following international health control protocols. Among the imports, one of the most important was made in 1962, when the main Nellore breed Genearchs and individuals of the Sindhi breed arrived, thus completing the major Zebu breeds of interest in Brazil (i.e. Nellore, Gyr, Guzerat, Indubrasil and Sindhi) (Santiago, 1960; Santiago, 1986; Machado, 2015; Bastianetto *et al*., 2020). Although its importance and impact on Brazilian development, between 1870 and 1962, it is estimated that only from 2.465 (Bastianetto *et al*., 2020) to 7.000 (Brasil, 1984) Zebu cattle animals were imported from India.

THE EPOCHS OF BRAZILIAN ZEBU BREEDING

Santos (2013) carried out a broad and detailed survey of facts and dates that marked the history of the Zebu breeds and divided them into 7 periods, presented below (Machado, 2015):

Phase 1 - Multiplication (1889-1925):

This phase begins with the first imports of Zebu with the specific purpose of increasing Brazilian livestock production. This was the "multiplication" period, as with only 171 animals imported until 1889, there was practically no disposal of imported pure Zebu (Santiago, 1986).

It was characterized by disordered crossings of different Zebu types and the *Bos taurus* locally adapted breeds (ex: Caracu and Mocho nacional). The World War I accelerated the importation and use of the Zebu in crossbreeding for meat exports, which rose from zero to more than 60,000 tons/year. This period ended with the 1920's imports when 1904 heads entered Brazil (Santiago, 1986). From 1919 onwards, the Herd Book of Zebu breeds were created, and had a predominance of the Guzerat animals. At the same time, milk control and fat content analysis began on some farms in Rio de Janeiro. During this period, Brazilian breeders chose the Gyr, Nellore and Guzerat as the best breed options for tropical farming, but disorderly crossings began the formation of the Indubrasil breed (Carneiro et al., 2009; Machado, 2015).

Phase 2- Miscegenation (1921-1945)

This period is marked by the Miscigenation of different *Bos indicus*, and in some cases, *Bos taurus* breeds. It led to the formation of the Indubrasil breed and the consequent discredit of other Zebu breeds. At this stage, the Indubrasil dual-purpose breed was consolidated, due to its high meat production and good milk production (Borges, 1946). As Indubrasil population growth increased, it threatened the continuity of the other Zebu breeds, especially Guzerat, at the end of the 1930s, as females served as the basis for the Indubrasil. Disorderly crossing and inbreeding weakened the Indubrasil causing a movement to return to purebred selection (Machado, 2015).

In the mid-30s, probably due to good results on milk control tests observed in Rio de Janeiro different breeders of Minas Gerais, Goiás e Sao Paulo elected Gyr as the best breed for milk, what led to a hegemony among milk breeds during the 40s. In 1934, the Rural Society of Triângulo Mineiro emerged (SRTM) and continued the genealogical record of Zebu breeds keep by private farmers, later becoming the Brazilian Association of Zebu Breeders (ABCZ). In 1939, the Ministry of Agriculture, together with researchers, technicians and professors, established the racial standards of the Zebu breeds, which are still used today (Santos, 2013).

In 1940, another war (WWII) accelerated the development of Brazilian livestock farming, when Brazil exported 120,000 tons/year. During that time, Zebu helped to incorporate and integrate new Brazilian territories. After the war, the need to improve livestock productivity led to the first Pasture Weight Gain Tests, with prominence of the Nellore breed. The crisis of the 1945s led to the search for rustic and resistant animals to be raised in the immense fields of Mato Grosso and Goiás. The cows should easily give birth to a vigorous calf capable of breastfeed alone. These characteristics, present in Nellore cows, and their performance in the first weight gain tests greatly favored the growth of the Nellore breed in the following years (Machado, 2015).

Phase 3 - Racial Purity (1945 to 1965)

Period characterized by the appreciation of "Racial Purity", using the standards stablished in 1939 and reaching the extreme of morphological racial detailing (Ears, tail, coat color, dewlap, etc.). Probably perceiving the miscegenation In the Brazilian herd was increasing fast, the purebred selection (i.e. Ongole/Nellore, Gyr and Kakrej/Guzerat) was chosen instead the formation of new breeds, such as Indubrasil. Crossbreeding was indicated only for commercial farms in order to obtain the hybrid vigor (heterosis) in F1s animals (Machado, 2015).

Other imports were carried out in order to increase the zebu population size and, in 1962, the last major import took place when the main Nellore breed Genearchs and individuals of the Sindhi breed arrived, thus completing the four major Zebu breeds of interest in Brazil (i.e. Nellore, Gyr, Guzerat and Sindhi) in Brazil. Toward the end of this period, breeders and technicians began to realize that selection based solely based on racial morphology was not enough and the valorization of characteristics related to production began (Santiago, 1960; Santiago, 1986; EMBRAPA, 1996; Magnabosco *et al*., 1997; Oliveira, Magnabosco, Borges, 2002; Santos, 2013).

Phase 4 - Production-Oriented Breeding (1965 to 1990)

This period was marked by an increasing appreciation of the use of production traits as selection criteria. The first official zootechnical tests controlled weight development "on pasture tests" and weight gain and weight adjusted to standard ages served as selection criteria for beef production.

In 1967, the Triângulo Mineiro Rural Society-SRTM was renamed as the Brazilian Association of Zebu Breeders (Associação Brasileira dos Criadores de Zebu –ABCZ), and delegated to carry out the Genealogical Registry of Zebu Breeds throughout Brazil, by the Ministry of Agriculture. In 1968, the collection of weight development data by private associated farms began. Other efforts aiming at identifying males with high genetic potential for weight gain might be pinpointed, such as the Pasture Performance Test maintained by the Animal Science Institute-IZ of Sertãozinho-SP (Machado, 2015).

During this period, the ideal morphotype of the "modern steer" changed, so that selection favored breeding longer animals with less subcutaneous fa deposition on the carcass. A year earlier, in 1971, the Herdbooks of the Nellore, Gyr, Guzerat and Indubrasil breeds were simultaneously closed, and the Tabapuã breed pedigree record initiated. The first registrations of the Gyr Mocho breed, in the LA (Open Book) category, occurred in 1976, closing in 1986.

The breeding for milk emerged at the end of the 1970s because of the interest in improving the dairy capacity of dairy breed animals; however, it could not be implemented at the time, due to an insufficient number of registered controlled animals. Subsequently, after its creation, the Brazilian Association of Dairy Gyr Breeders (ABCGIL) contacted Embrapa Dairy Cattle- CNPGL in 1980, and, in 1985, with the work of Dr. Mário Luiz Martinez, the Dairy Gyr Progeny Test was implemented, where the characteristics of milk production and milk fat content were evaluated. Subsequently, new traits were incorporated (protein, lactose and total

milk solids contents besides morphological appraisal linear system), expanding it to the National Dairy Gyr Improvement Program (PNMGL) (Machado, 2015).

Phase 5 – Genetic Evaluation (1990´s to present)

During the 1990s, genetic evaluation using genealogical data and all available sources of phenotypic information, whether those provided by breeders, or those obtained in zootechnical tests, produced estimates of genetic value with BLUP (Sire and Animal models) properties. The results of genetic evaluations were made available to all interested parties, through the National Bull Summaries for the different breeds. Among the various research entities and Brazilian researchers who participated in this advance, Embrapa / National Center for Beef Cattle Research (CNPGC) played a fundamental role. During this period, the word "precocity" appears in the technical vocabulary, and the search for animals that are precocious in growth, reproduction and carcass finishing begins.

In 1992, ABCZ reorganized its weight gain tests and launched the "Zebu Genetic Improvement Program" (PMGZ). In 1994, it launched the new visual appraisal method known as EPMURAS (E=body structure, P=precocity, M=muscularity, U=sheath and prepuce, R=race, A=uprightness and S=sexual traits) replacing PHRAS (precocity, harmony, racial characteristics, gait and ligaments and sexual characteristics). The "Weight Gain Test – PGP" was decentralized, allowing breeders, or associations, to carry out the test on their properties. This decade saw the advancement of private genetic improvement programs, independent of ABCZ (de Faria *et al.*, 2007).

Due to the success achieved by the PNMGL, a similar program was launched in 1994 for the Dairy Guzerat breed, in 1998 for the Girolando (Holstein x Gyr) breed, in 2000 started the National Program for the Improvement of the Holstein Breed. In 2010, the implementation of the National Sindhi Breed Improvement Program for milk began (Verneque *et al.*, 2010).

Nowadays, due to the significance of Zebu breeds to Brazilian animal production, various breeding programs are being developed, with a clear predominance of the Nellore and Gyr breeds, but also other zebu and synthetic breeds, which have zebu blood in their formation, such as Girolando, Canchim and Brangus/Ibagé. The general objective of these programs is to increase fertility, growth rate and carcass quality in beef herds, and fertility, milk and solids production and somatic cell count. These programs use classical animal breeding allied with modern biotechnologies, which lead to a significant increase in annual genetic gain and overall productivity (Machado, 2015; Ferraz and Fries 2004).

The Brazilian Association of Zebu Breeders- ABCZ in collaboration with the Embrapa Beef Cattle (Embrapa Gado de Corte) researchers conducts the main Breeding Programs for Zebu Cattle–PMGZ. They generate the possibility of identifying superior animals with Expected Progeny Differences (EPDs), or Predicted Transmitting Ability (PTAs), for weight, weight gain at different ages as well as fertility traits and fertility, among others. This is a national program and includes all zebu breeds selected for meet or milk (Nellore, Gyr, Guzerat, Sindhi, Tabapuã and Brahman) (Rosa *et al.,* 1986; Machado, 2015).

The database contains genealogical information on over 1.5 million animals, 65,000 new animals being included each year from about 3,600 herds distributed throughout the national territory and performance evaluated by the means of its 03 zootechnical tests (i.e. Control of Weight Development-(CDP), Weight Gain Tests-(PGP) and Dairy Control-(CL). The GENEPLUS Program, coordinated by Embrapa Gado de Corte (Embrapa Beef Cattle) that allows the breeder of different Zebu breeds to create a database that will undergo genetic analysis. The produced genetic values aid breeders in the selection of superior animals for fertility, weight gain, sexual precocity, carcass finishing score and muscle development, with EPDs calculated for age at first calving, calving interval, gestation period, service period, scrotal circumference as well as weights and weight gains at different ages (Santos, 2013).

Since Nellore is the major beef breed, there are other breeding programs been conducted in Brazil, such as: (1) Embrapa Cerrados, Polled Nellore-BRGN Delta G, Nellore; (2) CFM, Nellore; (3) Paquetá group, Nellore (4) Paint, Nellore; (5) Qualitas, Nellore and (6) Pastoril Potrilho, Nellore. Besides the Zebu breeds, breeding programs are been conducted for synthetic beef breeds such as: (1) Embrapa, Canchim (Charolais x Nellore cross) and Brangus/Ibagé (Angus x Nellore cross), South American Nature Genetics, Brangus; (2) Delta G,

Braford; (3) CFM, Montana composite; (4-5) Angus Belavista Pecuária, Brangus and Braford and (6) Paquetá group, Brangus (Mariante *et al*., 2003; Buzanskas *et al*, 2015, Embrapa, 2022)

For dairy cattle, as described here, the PMGZ conducts breeding programs for Gyr, Guzerat and Sindhi, which use progeny tests to identify bulls that are genetically superior for 305 days milk production, fat and protein content, fertility, as well as conformation traits. Approximately 15 to 20 young Gyr bulls are progeny tested every year, as well as between eight to ten Guzerat bulls. Among the synthetic milk breeds, only the Girolando has a breeding program, which includes a progeny test. The Holstein breed is the only *Bos taurus* with a breeding program, with few bulls being progeny tested very year. The breeding values results are delivered to the breeders for management decisions such as mating and culling (Mariante *et al*., 2003).

Phase 6 – Genomic Era (2000 to present)

This period was marked by the advancement of Genomics and by an increasing concern regarding carcass quality. In 2004, ABCZ started the Gyr Leiteiro (milk) genetic evaluation at ExpoZebu. In 2009, the sequencing of the cattle genome was announced by an international team including Brazilians (Nogueira, 2009) and, in 2011, the Sao Paulo State University–UNESP announced the mapping of 100% of the genome of the Nellore breed (Yoneya, 2011).

This decade saw the widespread utilization of the Expected Progeny Difference (EPD) in herds selected for meat, and Predicted Transmitting Ability (PTA) in herds selected for milk. The importance and use of Selection Index was better understood by the livestock farmers and became the basis of the germplasm selection. The first works with estimates of Genomic Breeding Values were published (Machado. 2015).

Few examples of breeding programs including genomic information to aid in the identification of superior animals. Clarifide Girolando is the result of a public-private partnership formed by Embrapa, the Brazilian Association of Girolando Cattle Breeders and two private companies (Zoetis and CRV Lagoa). It is the first Brazilian product for genomic assessment of Girolando dairy herds (Gyr and Holstein Friesian cross), which is of great importance for national dairy farming. In 2021, genomic PTA results from 92 bulls were published (Silva *et al*., 2021). Another example is the Embrapa´s Polled Nellore-BRGN, which selection program includes genomic predictions for the traditional EPDs, besides carcass quality and meat tenderness (Magnabosco *et al*., 2016), PMGGL Dairy Gyr (Panetto *et al*., 2023; Fernandes *et al*., 2023), PMGG Guzerat (Carrara, *et al*. 2023) and the DeltaGen and PAINT Nellore breeding programs (Terakado *et al*., 2021).

Phase 7 – Economic Indices (from 2012 to present)

This period, marked by the incorporation of "Economic Indices" into the selection, started in 2012 is still ongoing. Awareness of the importance of administrative management and costs control. "Feed Efficiency" and "Residual Food Consumption" (CAR) of animals becomes one of the main methods for the identification of more productive and efficient animals. In 2013, the first Unified Beef Cattle Summary was launched containing information from the Zebu Genetic Improvement Program -PMGZ in a collaborative work with the National Association of Breeders and Researchers – ANCP (Machado, 2015).

Perspectives for Zebu in Brazil

Over time, cattle farming has faced several challenges, mainly due to public concerns regarding deforestation, environmental degradation, GHG emissions and health aspects. Despite this, livestock farming have been, and is still, an important economic development vector for Brazil as it contributes to guarantee national food security and social stability.

Large investments in technology, i.e. genomics, reproduction and integrated production systems, are being made to improve the productivity and sustainability of livestock farming, resulting in greater profits for Brazilian producers with reduced environmental impacts.

Improvement of genetic improvement programs for different zebu and synthetic breeds, aiming to increase the accuracy of genetic values, especially at young ages. New traits aiming the improvement of feed efficiency, such as RFI- Residual Feed intake, productive longevity, longevity and stayability, carcass quality, meat tenderness and health traits.

Due to its characteristics and importance, Zebu became an integral and indispensable part of Brazilian history and tradition. Zebu changed the history of Brazil. It contributes to the present culture and economy, and will certainly do so in the future as well.

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GENOMIC SELECTION IN INDIA: CURRENT STATUS, OPPORTUNITIES AND CHALLENGES

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Abstract:

Genomic Selection (GS) is widely used in dairy breeding across many countries. With increasing evidences of usefulness of GS methods, farmers have adopted widespread use of genomic bulls and have started use of Genomic Breeding Values (GBVs) for selection of heifers as well. GS has been used for many traits that were difficult to include in a selection program.

From a theoretical concept, GS in our country is now being taken to practical use for selection of bulls for frozen semen production in majority of important dairy breeds of cattle and buffalo. This paper aims to cover the current status of implementation of GS, challenges faced and future opportunities in implementing this technology.

Genomic Selection – Global Scenario:

Genomic Selection has changed landscape of dairy breeding across the globe. This technology has been adopted very fast by the breeding industry. After first use of genomic selection for selection of dairy bulls in 2009, within few years there were evidences that the genetic progress is being accelerated in dairy cattle (Scott et al 2021, Xu et al. 2020, Guinan et. al. 2023) and with increasing evidences of utility of genomic selection, breeding companies and farmers have started heavily relying on genomic selection. This is evident by reports of use of 40-70% genomic young bulls in various countries (Wiggans and Carrillo 2021, https://www.icbf.com/selecting-genomic-bulls-or-daughter-proven-bulls/, https://licnz.com/news/genomics-is-providing-farmers-with-faster-genetic-improvement/#:~:text=How%20many%20 farmers%20are%20using,uptick%20from%2012.6%25%20in%202016).

Apart from selection of bulls for traditional traits, Genomic Selection has provided opportunity to start selection programmes for newer traits like immunity+ bulls, High Fertility Sires, Calving ease sires, built for automation sires etc. by Semex (https://www.semex.com/), feed efficiency by ABS (https://absbullsearch.absglobal.com /BullList?Visibility Country Code=USA&BreedTypeCode=D&BreedCode=HO&ProofCode=USA&comp=&o=NetMerit|true), TB advantage sire +2 by Mastergen (https://mastergen. com/ bulls/all-holstein/tb-advantage-sires-2/), Heat Tolerance ABV in Australia (https://datagene.com.au/sites/default/files/Upload%20Files /Fact %20Sheet%205%20Heat%20Tolerance%20ABV%20Dec%2017_0.pdf)

Farmers have now resorted to genotyping of females for selection of replacement heifers. In 2008 and 2009, more genotypes for bulls were received in CDCB database, than for cows, however, in later years, the number of bull genotypes received has been nearly constant while the number of genotypes of females received increased rapidly (Wiggans and Carrillo 2021). Genomic testing of commercial dairy females benefits genetic evaluations of populations through regular contributions to reference populations (Mc Hugh et al., 2011; Pryce *et al.,* 2012).

Herd-level benefits of genotype include (1) more reliable decisions in selecting herd replacements, (2) fewer errors in parentage assignment, (3) the development of more targeted breeding objectives, and (4) earlier identification of candidates to artificially inseminate (AI) to high-value semen or for use in embryo transfer and in vitro fertilization programs (Newton *et al.,* 2017)

Genomic Selection in India

Genomic Selection in India has progressed well in past few years. In absence of large number of Progeny Tested bulls, genomic selection using female reference population has been implemented for various cattle and buffalo breeds (Gajjar et. al. 2018, Nayee et.al. 2020). At present genomic evaluations of six major cattle

and buffalo breeds viz. Gir, Sahiwal, HFCB, Jersey CB cattle and Murrah and Mehsana buffaloes are available in the country. Below we describe various aspects of genomic selection initiatives in India.

Genotyping chips

Genomic Selection requires set of SNPs that are polymorphic across the breeds that are targeted. Large scale efforts have been done in developing genotyping chips especially for Bos Indicus breeds in the country. National Institute of Animal Biotechnology, Hyderabad has come up with High Density (HD) genotyping chip IndiGau chip for Indigenous cattle (https://pib.gov.in/Press Release Page.aspx?PRID=1745479). Similarly, National Bureau of Animal Genetic Resources, Karnal has come up with HD genotyping chips of various species including Cattle and Buffaloes claiming to capture SNPs specifically selected for Indigenous breeds. (https://nbagr. icar .gov.in/en/salient-achievements/). National Dairy Development Board have developed custom made genotyping chips INDUSCHIP and BUFFCHIP for cattle and buffalo genotyping respectively (Saha et.al. 2020, Thakkar et. al. 2022). BAIF used GeneSeek GGP chips (https://sapac.illumina.com/ products/ ggp-whole-genome-genotyping-arrays.html) for cattle genotyping.

With an aim to get benefit of large volumes and to make data compatible across the organizations, an initiative was taken to combine useful SNPs from various genotyping chips and come up with medium density (around 60K SNPs) unified genotyping chip each one for cattle and buffalo. Now the chips are available to users across the country.

Reference population and GBV estimation methods:

Most important component of GS is availability of set of animals that are having both phenotype data for the traits of importance (e.g. Milk Production, fat %, SNF%, Age at First Calving, Days Open etc.) and genotype data for a predefined set of SNP markers, called reference population. Accuracies of GBVs is dependent upon the size of reference population for the breed of interest (Takeda et. al. 2021, Zhang et.al.2023). This part is very difficult to achieve in our country.

Performance recording systems across various breeds have been established under National Dairy Plan I and was continued under Rashtriya Gokul Mission scheme. The Progeny Testing and Pedigree Selection projects have collected valuable data on performance of individual animals. The information and samples collected from these projects were used as basis to create reference population in the country. Currently, we have reasonable size reference population for various breeds. Number of animals with genotype and phenotype information in the reference population for GBV estimation used at present are provided in table below:

Table1: size of reference population and method used for GBV estimation

Breed	No. of animals with Genotype	No. of animals with records	Availability of pedigree information	GBV estimation method	Used for bull selection
Murrah	8168	13780	Yes	ssGBLUP	Yes
Mehsana	6550	14880	Yes	ssGBLUP	Yes
Gir	7789	4180	No	GBLUP	Yes
Sahiwal	3542	2788	No	GBLUP	Yes
Kankrej	2035	1548	No	GBLUP	No
HFCB	8490	29960	Yes	ssGBLUP	Yes
JCB	5949	29458	Yes	ssGBLUP	Yes

Accuracy of genomic breeding values

Before use of GBVs for selection of bulls or heifers in the field it is important to build confidence that the GBV estimates are better predictor of genetic worth of animal compared to currently used selection criteria. Five-fold cross validation process is generally used for validation of genomic predictions obtained by various models (Schrauf et. al 2021, Runcie and Cheng 2019, Erbe et. al.-https://www.uni-goettingen.de /de/ document/ download/ 11d6ffd2bb779f7b0d2c3ea27bef8512.pdf/erbe.pdf). For each of the breeds under consideration cross validation was done for predictive ability of GBVs compared to Dam's Lactation Yield or traditional EBVs as the case may be. In all the cases GBV had higher correlation with own records/

daughter records of the validation animals compared to other criteria. This indicates higher predictivity of GBV compared to other selection criteria. The correlations between average daughter yield and GBV for bulls ranged from 0.25-0.4 whereas correlation between animal's own performance with GBV ranged from 0.10-0.46 in various validation studies. A table showing validation correlations of GBVs with individual's own performance is given below for 3 major indigenous breeds.

Table 2: Correlation of actual corrected phenotype (305D std. lactation yield) with GBV for validation animals whose record was removed from analysis

Single breed Reference								
Breed	**No. of animals with records**	**No. of animals genotyped (females and bulls)**	**Val set 1**	**Val set 2**	**Val set 3**	**Val set 4**	**Val set 5**	**Across all val sets**
Kankrej	1548	2035	0.122	0.146	0.241	0.233	0.141	0.158
Sahiwal	2788	3542	0.307	0.245	0.254	0.360	0.294	0.289
Gir	4180	7789	0.414	0.429	0.463	0.439	0.416	0.418
Multibreed reference- no PCA as covariates								
Kankrej	NA	NA	0.160	0.153	0.186	0.213	0.106	0.148
Sahiwal	NA	NA	0.245	0.242	0.255	0.348	0.293	0.273
Gir	NA	NA	0.398	0.418	0.458	0.420	0.405	0.404
Multibreed reference - PCA as covariates								
Kankrej	NA	NA	0.163	0.150	0.198	0.243	0.125	0.161
Sahiwal	NA	NA	0.203	0.228	0.254	0.343	0.257	0.254
Gir	NA	NA	0.382	0.404	0.443	0.395	0.386	0.384

The table provides information on how correlations are increasing with increase in reference population. The lowest correlations were seen in Kankrej breed where number of animals with phenotype and genotype information is lowest. There is sampling variation among the validation datasets. However, none of the correlation is less than 0.1. considering heritability of 0.25, it is expected to obtain correlations of around 0.06 when only mother's lactation yield information is used for selection of animals.

As seen in the table, the multibreed approach for creating reference population for 3 important indigenous cattle breeds is not working well. The Kankrej breed that has a smaller number of genotypes, has benefitted slightly when first 3 Principal components were used as covariates in the genomic model. More work is required to apply the method practically in the field.

Genomic breeding value of bulls under semen collection

A total of 6023 bulls of various breeds were genotyped using INDUSCHIP or BUFFCHIP by NDDB and GBV for all the eligible breeds was estimated for bulls under collection during the year 2022-23. Breed wise average GBV for bulls under collection, range of GBVs and % of bulls below average GBV are provided in table below:

Table 3: Genomic Breeding Value of Bulls for 305D std. lactation yield trait

Breed	**No. of bulls evaluated**	**Average GBV**	**Minimum GBV**	**Maximum GBV**	**No. of bulls with negative GBV (% in parenthesis)**
Murrah	1620	15	-339	433	626 (36%)
Mehsana	442	50	-357	442	137 30%
Gir	274	384	-369	949	17(7%)
HFCB	879	213	-676	894	174(19%)
JCB	769	14	-503	407	326(42%)

Many organizations still believe that higher the Bull's Dam's yield, better will be their progenies. Also, there are arguments that GBV estimated for a breed using reference population recorded in one area will not hold good for bulls produced in another area. A similar argument was put forward for Murrah bulls being selected from Haryana, where reference population comprise of majority of buffaloes recorded in Gujarat (more than 50%), Western UP (Around 25%), Punjab (around 15%) and only small portion (10%) buffaloes recorded in Haryana. A comparison was done for bulls having minimum 10 daughter records to evaluate whether GBVs provide better prediction or not. A graph showing correlation of Dam's lactation yield and bull's daughter yield vis a vis GBV and bull's daughter yield is shown in figure below:

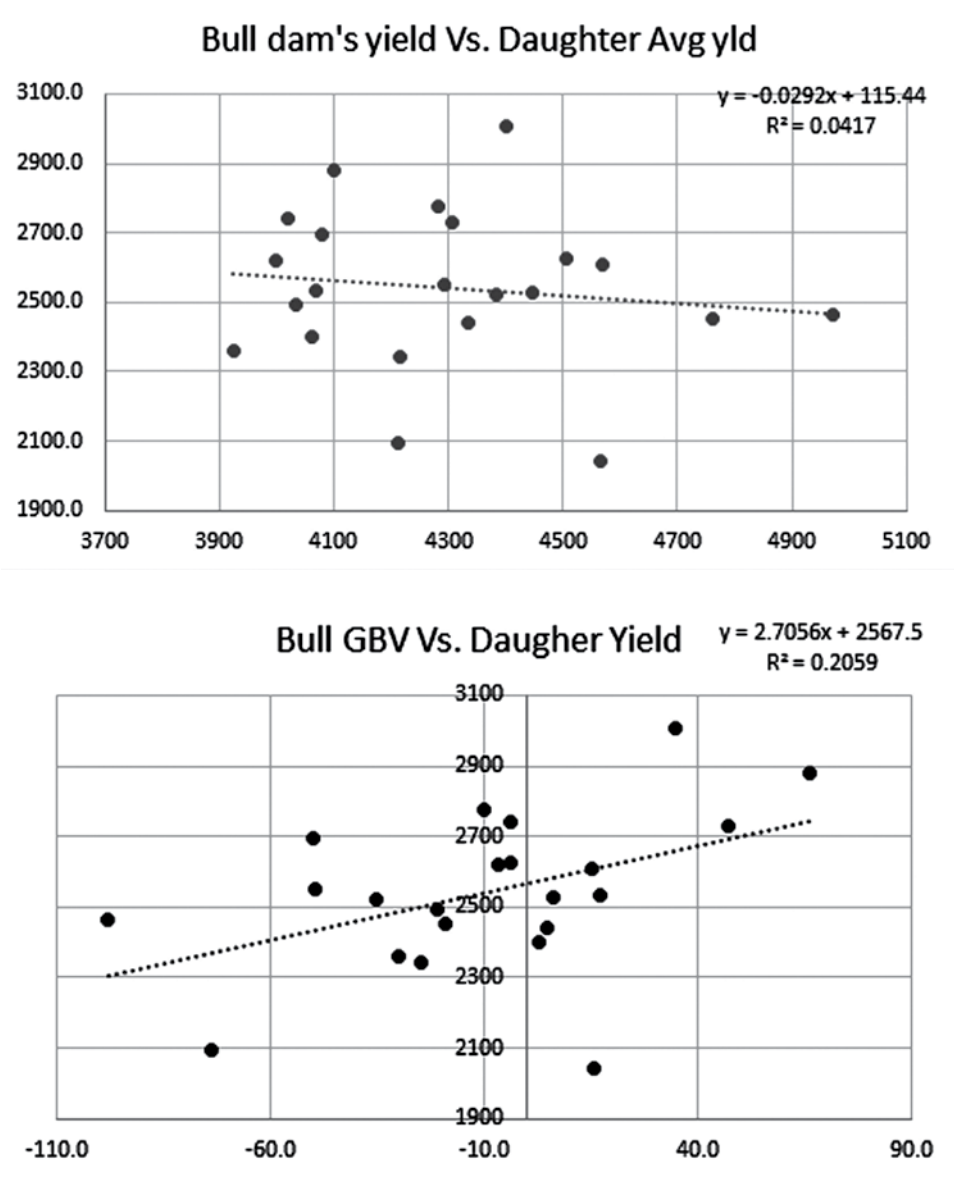

Figure 1: Relationship of Dam's yield and GBV with daughter's yield

Changes in bull selection policy

Considering encouraging results, DAHD have incorporated GBV as bull selection criteria under Minimum Standards for frozen semen production from the year 2022 onwards (https://dahd.nic.in/sites/default/filess/MSP-2022-for-production-of-bovine-frozen-semen.pdf). Also, semen stations are advised to cull bulls with negative or lower GBVs on priority and induct new bulls with higher GBVs. This approach will improve overall genetic composition of bulls at semen stations and will accelerate genetic progress.

Challenges in implementation

Following are some areas posing challenges in adopting GS in the country:

1. Building sizeable reference population is still a bigger challenge for many important breeds like Tharparkar, Red Sindhi, Pure Holstein and Pure Jersey.
2. There is low level of awareness among professionals and farmers regarding scientific process used for GBV estimation. Still there are arguments saying a particular bull or animal is not selected despite showing higher dam's absolute milk yield.
3. With intensified selection, there will be more chances of declining trend in traits that are negatively correlated with milk production like fat & protein content, survival and fertility (Uteral et. al. 2015, Strucken et. al. 2012). These traits need to be included in selectin criteria. However, there is lack of recording of these traits and hence, we are not able to include them in selection programme.
4. There is also lack of trained manpower in quantitative genetics.

Opportunities

With implementation of GS in the country, we have opened up avenues for various interventions for fast tracking genetic progress in the country. Following can be some major initiatives that will help in expanding reference population within breeds, across breeds and across the traits. Also, this expansion will provide platform to improve not only milk production but other traits also could be included in our selection index.

1. We can implement genetic improvement programmes for breeds that do not have extensive progeny testing programme setup.
2. The organizations that are implementing performance recording, may join together to implement same SoP for recording and collect samples for expanding genomic reference for various breeds.
3. With availability of unified genotyping chips, now there will be better data compatibility and organizations will be able to share data across to have larger reference for Genomic Evaluations.
4. Recording more trait will provide scope of selection of bulls and heifers based on index of traits instead of only for milk production
5. Collaborating with various international agencies will increase our knowledge and help in implementing complex models for higher accuracies.
6. Bayesian approach seems to have better utility in breeds where reference population is still limited. However, steps to reduce bias in estimates are to be implemented before actual implementation.
7. Opportunity of producing bulls from young heifers with high GBVs instead of waiting for lactation records, will reduce generation interval and accelerate genetic progress.
8. Farmers now has tool to select replacement heifers early with higher accuracy. This will help in getting more genetic progress at individual farmer level also.

Conclusion

Genomic Selection in the country has opened up various avenues that can really fast track genetic progress of dairy cattle and buffalo breeds. However still there is need to expand reference population covering more breeds and more traits to reap benefits of this technology.

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GENOMIC APPLICATIONS TO UNDERSTAND EVOLUTION AND DOMESTICATION OF ANIMAL GENETIC RESOURCES

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Introduction

The introduction of domesticated animals brought about a profound transformation in human existence, facilitating the transition from a society based on hunting and gathering to one centered around agriculture. This shift was a crucial prerequisite for the emergence of human civilization (Diamond, 2002). It's evident that the major centers where domestication took place often coincide with the epicenters of cultural advancement in diverse advanced civilizations throughout history, such as the Fertile Crescent (Brown et al., 2009), the Inca civilization in South America (Gonalons and Yacobaccio, 2006), and the ancient Egyptian culture (Rossel et al., 2008). Throughout history, humans have endeavored to domesticate wild animals and selectively breed them, shaping their roles as sources of sustenance (such as meat and milk), producers of valuable resources (like wool and silk), or providers of labor (for tasks like transportation and protection (Driscoll et al., 2009).

Domestication ("domus" means "house") can be defined as "*that process by which a population of animals becomes adapted to man and to captive environment by genetic changes occurring over generations and environmentally induced developmental events reoccurring during each generation*" (Price, 1984). It occurs when a species is removed from its natural ecological surroundings and undergoes development through the influence of selective pressure and controlled environments (Herre and Rohrs, 1990). Long-standing animal husbandry and deliberate breeding practices have significantly altered the behavior and physical traits of domesticated animals compared to their wild counterparts, leading to substantial modifications in genetic diversity among various breed populations (Wang et al., 2014).

Major events in animal domestication

Since the time of the Neolithic era, human efforts have beenrevolved around the taming of wild animals for purposes such as food production (milk and meat), generation of valuable resources (like silk and wool), providing protection, and enabling transportation. These domestication processes are commonly categorized into three pathways: the commensal, prey, and direct pathways (Zeder, 2012). In the commensal pathway, wild animals were drawn to human-inhabited areas, primarily attracted by the availability of human food waste or small prey, and later on developed a mutually beneficial association with humans. Species like dogs, cats, and chickens exemplify this pathway. In the prey pathway, humans initially hunted certain animals such as pigs and cattle for their meat when the local stocks of these animals were depleted. With time, these hunting practices evolved into the controlled breeding of these species. In the direct pathway, humans captured wild animals like horses, donkeys, and camelids to harness specific resources by controlling their movement, nutrition, and reproduction, resulting in a significant bottleneck effect (Zeder, 2012).

Dogs (Canis lupus familiaris) were the first species to be domesticated by prehistoric Asian and European foragers, a process that took place during the late glacial period, around 17,000 to 15,000 years before the present (BP) (Pionnier-Capitan et al., 2011; Frantz et al., 2016). Subsequently, the domestication of livestock and crops followed (Mignon-Grasteau et al., 2005). Remarkably, the domestication of certain species like dogs and cattle was actively guided by humans, while in the case of other species like cats, rats, and house sparrows; it happened naturally (Driscoll et al., 2009). During the course of domestication, animals experience genetic expressions of morphological, physiological, and behavioral modifications (Schmitten, 1980). These modifications, when compared to their assumed wild ancestors, are known as domestication traits. Examples of these traits include characteristics such as depigmentation, floppy or smaller ears, decreased brain size, curled tails, brachycephaly, changes in teeth, increased docility, and heightened sexuality (e.g., Darwin, 1875; Winge, 1900; Belyaev, 1979; Herre and Rohrs, 1990).

The collective presence of these traits has been newly labeled as the 'domestication syndrome in mammals,' contributing to a reduction in the animal's responsiveness to external stimuli as these changes progress

(Wilkins et al., 2014). At first, dogs had a role in consuming food scraps (Tchernov and Horwitz, 1991), but over time, they were purposefully bred not only for their physical traits like body thickness, length, fur texture, skull shape, and tail size and shape but also for enhanced behavioral qualities, including improved guarding, herding, speed, agility, and companionship (Ostrander et al., 2000). The need for large horns for combat became obsolete and even undesirable in a farm setting, resulting in the development of cattle with shorter horns or, in some cases, no horns at all as in polled cattle (Felius, 1995).

Genomes of domesticated animals

Numerous technological advancements were created to facilitate the construction of genomes from scratch as part of the human genome project (Lander et al., 2001). The combination of whole-genome sequencing (WGS) and the creation of physical maps proved to be the most efficient method. By 2009, this approach had successfully led to the sequencing and assembly of genomes for four domesticated animals (dog, cat, taurine cattle, and horse) as well as one genome from a wild species (the red junglefowl, a significant ancestor of the chicken) (Wang et al., 2014).

During 2004, the genome of the red junglefowl (Gallus gallus), a significant precursor to the domestic chicken (*Gallus gallus domesticus*), was sequenced. The reference genome was constructed using approximately 6.6-fold coverage of WGS reads produced through Sanger sequencing technology (Wallis et al., 2004). By 2009, the genome assembly of the domesticated cow marked a milestone as it became the initial livestock animal to achieve this feat. A genome of 2.6 gigabases (Gb) in size was constructed using roughly 9.5-fold coverage sequencing data (Zimin et al., 2009). In the same year, a whole-genome sequencing (WGS) technique was employed to sequence the genome of a thoroughbred mare, often regarded as the pinnacle of equine athleticism. This effort produced a high-quality preliminary genome sequence of the horse, with a 6.8-fold coverage, featuring a 112-kilobase (kb) N50 contig size and a 46-megabase (Mb) N50 scaffold size (Wade et al., 2009).

Whole-genome sequencing (WGS), when combined with a physical map, proves to be a valuable method for genome assembly. Nonetheless, the expense of generating WGS reads through Sanger sequencing technology is a limiting factor. Currently, Next-Generation Sequencing (NGS) technologies offer a cost-effective alternative with improved sequencing capacity (Wang et al., 2008). This NGS approach demonstrated its effectiveness in sequencing the giant panda (Li et al., 2010) genome and has since been employed to sequence the genomes of three domesticated animals: turkey (*Meleagris gallopavo*) (Dalloul et al., 2010) and yak (*Bos grunniens*) (Qiu et al., 2012).

Genomic insights into evolution

Compared to nuclear markers, mitochondrial DNA (mtDNA) possesses specific characteristics such as the absence of recombination, a high mutation rate, and multiple copies, which render it valuable for tracing the origins of domesticated animals (Bruford et al., 2003). Since the year 2000, numerous investigations have employed sequences of mtDNA fragments to explore a range of domesticated animals. These include species with global distribution, like pigs (Larson et al., 2005), horses, cattle (Troy et al., 2001), goats (Luikart et al., 2001), and sheep (Hiendleder et al., 2002). Findings from mtDNA research offer insights, particularly from the maternal perspective, into the potential progenitors and candidate lineages involved in domestication. Similar to mtDNA, the Y chromosome (specifically, the male-specific segment of the Y chromosome) serves as an equivalent in revealing the paternal influence on the genetic makeup of domesticated mammals (MacHugh and Bradley, 2001). Combining data from both independently inherited markers, mtDNA and Y chromosome genetic markers, can offer more comprehensive insights into the ancestry of domesticated species.

Both mitochondrial DNA (mtDNA) and Y chromosome markers are valuable tools for tracking the beginnings and subsequent spread of domesticated animals, and this data is frequently beneficial for other genetic investigations. However, the conventional Sanger sequencing method can be impractical and costly for large-scale population-level analyses of mitochondrial genomes and Y chromosomes. By integrating information from a broad range of sources, including mtDNA, the Y chromosome, and autosomes, and

extending the analysis to encompass even ancient DNA, we can achieve a more comprehensive understanding of the origins of domesticated animals (Wang et al., 2014).

Domestication studies on animal genome

Domestication has captivated scientists across diverse disciplines due to its significance as a model for studying evolutionary and demographic shifts (Zeder et al., 2006). To ascertain the origins of domestication for a species, it is essential to uncover their progenitors and pinpoint the approximate geographical sites of domestication (Larson and Fuller, 2014). In initial investigations, DNA was extracted from samples of a particular species, encompassing diverse locations, breeds, and populations. This DNA was then utilized to amplify and sequence control regions of the mitochondrial genome.

Subsequently, researchers generated phylogenetic trees and haplotype networks (Larson, 2011). Mitochondrial DNA (mtDNA) is widely regarded as an ideal marker due to its high mutability within species, making it suitable for studying demographic expansion, genetic diversity, and phylogenetic relationships (Bruford et al., 2003). However, these sequences are inherited exclusively from the maternal lineage and have limited capability in detecting and quantifying hybridization between distinct populations (Larson, 2011). This methodology was extensively applied to various species, including pigs (Giuffra et al., 2000), horses (Jansen et al., 2002), and cattle (Loftus et al., 1994). Moreover, researchers have employed quantitative trait loci (QTL) mapping to pinpoint potential genes linked to traits relevant to domestication. These analyses have led to decipher the advancements in the domestication of various species, such as chickens (Fallahsharoudi et al., 2017), pigs (Rodriguez et al., 2005), and cattle (Khatkar et al., 2004).

GWAS, Microarray and NGS to explore complex traits

In several domesticated species, GWAS have effectively recognized contributing genes both for complex traits and Mendelian traits controlled by loci with big influence size (Hekman et al., 2015). GWAS have identified genomic regions that are associate with diverse traits, such as fattiness and brown coat color in pigs (Duijvesteijn et al., 2010); dwarfism in horses (Orr et al., 2010); abdominal fattiness and body weight in chickens (Abasht et al., 2007); and several diseases in dogs (Awano et al., 2009; Wood et al., 2009)

GWAS offers a rapid and standardized method for investigating complex traits, but it requires specific conditions. Firstly, a relatively large sample size is essential to attain adequate detection sensitivity and minimize the false discovery rate (FDR). Secondly, the SNPs that co-occur with and will be genotyped on the chips should surround the mutations responsible for the observed phenotypic variations (Sutter, 2007). An improved alternative to GWAS involves utilizing Next-Generation Sequencing (NGS) platforms to sequence the entire genome of a population of individuals. This approach offers a distinct advantage by enabling the detection of numerous mutations specific to particular breeds (Allen et al., 2013). Through whole-genome resequencing data, it becomes possible to identify candidate genetic sequences associated with complex traits by detecting selective sweeps. An indicator of a selective sweep is a localized decrease in nucleotide diversity at the selected loci (Tonomura et al., 2015). The advent of Next-generation sequencing (NGS) technologies has transformed the field of gene expression research by eliminating the necessity for preexisting probes for transcripts. Using NGS or RNA-seq data, individuals can be categorized into healthy or diseased groups by identifying the genetic connections related to hereditary diseases or other genetic characteristics (Gautier et al., 2012; Tonomura et al., 2015).

Identification of SNPs for domestication traits

It is imperative to note that the expeditious changes in gene expression associated with domestication likely affect specific tissues during growth and necessitate extensive research to obtain conclusive results. Studies have indicated that domestication is primarily linked to selective sweeps leading to genetic variations in regulatory regions across the animal genome, suggesting micro-evolutionary developments during the early stages of vertebrate domestication (Carneiro et al., 2014). Understanding the significance of individual disease-associated SNP alleles is neither necessary nor sufficient for causing a disease. Instead, it is likely the combined impact of a group of SNP alleles located within key genes, along with environmental factors, that collectively determine whether an individual develops a particular disease (Pruvost et al., 2011). The association study involves assessing the frequency of SNP allele in a group of patients and comparing it to a

control group consisting of individuals of the same race and age. The reliability of this test relies significantly on ensuring that the patients and controls are appropriately matched, taking into account factors like population stratification (Corbett-Detig et al., 2015).

Investigation of the deleterious mutations in animals

Mammalian genomes harbor numerous deleterious mutations, and it's crucial to accurately identify them. In the field of conservation biology, fragmented populations with limited size tend to accumulate harmful variants, making it advantageous to conserve animals with less damaged genomes, thereby optimizing conservation efforts. In the context of livestock breeding, effectively distinguishing deleterious mutations can help strike a better balance between farm performance and overall fitness. This classification is also valuable in addressing issues in veterinary medicine, treatment of infectious diseases, and understanding developmental abnormalities in cattle, pigs, and dogs.

To enhance this classification, transfer learning techniques from machine learning are employed, where a model trained for one task serves as a starting point for a similar but data-limited task. Human datasets, such as Hum Div/HumVar, are leveraged to predict deleterious mutations in animals like dogs, cattle, and partially labeled mouse data. This technique is applied to classify deleterious versus neutral mutations (Plekhanova et al., 2017), identify splice sites (Altun & Ratsch, 2010), and perform image recognition (Xue & Yang, 2011). Notably, this approach delivers more accurate results compared to the SIFT/POLYPHEN-based classification methods.

Investigation of the ancestral alleles

For selection signature studies, it is essential to define the ancestral allele. Ancestral alleles (AAs) are polymorphic sites shared among closely related species, such as Yak, Bison, and Gayal-gaur-banteng (cattle outgroup), found in at least two of these groups. In a study by Naji et al. in 2021, they identified 32.4 million ancestral alleles, which make up approximately 1.2% of the cattle genome. They conducted genomic scans using 10-kilobase windows on taurine and zebu cattle. Regions with a high count of ancestral alleles, representing the top 0.1%, have retained gene functions from ancestral states that remain beneficial in current conditions. In contrast, regions with null counts of ancestral alleles are linked to mutated genes.

In both taurine and zebu cattle, regions with a high count of ancestral alleles are associated with fundamental aspects like basal lipid metabolism, which is essential for coping with various environmental pressures. On the other hand, mutated regions are linked to productive traits in taurine cattle, including higher metabolism, cell development, and behaviors. In zebu cattle, these regions are associated with immune response. The retention and loss of ancestral alleles in various regions of the genome are species-specific, resulting in unique genetic characteristics.

Paleogenomics, also referred to as genome-wide ancient DNA (aDNA) analysis, offers valuable insights and plays a crucial role in investigating the timing, geographic distribution, and speed of the spread of adaptive alleles within populations (Irving-Pease et al., 2018; Brunson and Reich, 2019). DNA extracted from the petrous portion of temporal bone produce highest yield (183x more) as evidenced by Irish Scientist and Archaeologist. Therefore, the era before 2014 was considered as Before Petrous (BP) and after 2014 as after petrous (AP) due to this significant change in sampling aDNA. Moreover, now a days sample collection from non-conducive environment like tropical zones is also feasible. Recent studies employing this approach have yielded fresh perspectives on the evolutionary history of the cave bear (Barlow et al., 2018) and horses (Gaunitz et al., 2018). In the realm of livestock, a combination of biotechnology and conservation tools has significantly contributed to enhancing productivity, preserving genetic diversity, and improving adaptation to various environments (Ko and Takahashi, 2006).

Epigenetics in the Evolution of the Domestic Traits

Various research workers have shown that along with genetic factors, epigenetic influences have the potential to impact behavioural traits and other characteristics within a breed or species (Jensen, 2015; Bélteky et al., 2018). For instance, differences in the behavior of the great tit have been statistically associated with DNA methylation at dopamine receptor genes (Verhulst et al., 2016). Epigenetic factors can impact behavioral traits and other characteristics within a breed or species (Jensen, 2015; Bélteky et al., 2018). These epigenetic

alterations can occur shortly after individuals are exposed to different rearing environments (Pértille et al., 2017). While DNA methylation patterns are typically maintained through cell division, external stimuli can sometimes regulate these patterns (Raynal et al., 2012).

Changes in DNA methylation, influenced by the environment, can be passed down through the germline and persist across generations in somatic tissues (Franklin et al., 2010; Goerlich et al., 2012). These epigenetic changes have the potential to impact phenotypic traits, whether intentionally selected or arising due to environmental factors. As a result, the mechanisms underlying epigenetic changes can play a significant role in the development of rapid phenotypic variations that occur during the process of domestication.

Genes involved in domestication

Selection has played a pivotal role in expediting phenotypic variations during the process of domestication. Among these, skin and coat colour stand out as the earliest traits that humans selectively influenced, making them essential genetic markers. Coat colour can be manifested as patterned (spotted or striped) or non-patterned (solid colours) phenotypes which are determined by the proportions of two pigments: eumelanin (black/brown) and pheomelanin (red/yellow) (Cieslak et al., 2011; Koseniuk et al.,2018). Various genes, such as MC1R, ASIP, TYRP1, CBD103, KIT, and PMEL17, have been linked to different skin and coat colour variations across several species (Schmutz et al., 2002; Andersson, 2003; Fontanesi et al., 2006).

Additionally, genes like Tph1 and Gabra5 have been found to be associated with tameness, leading to the production of tamed animals like foxes (*Vulpes vulpes*) and rats (*Rattus norvegicus*) (Albert et al., 2009). Other genetic factors play a role in determining body size (IGF1), fur growth and texture (FGF5, RSPO2, KRT71), muscle development (MSTN in cattle and goats), stature (HMGA2 in cattle), meat tenderness (CAST2 in pigs and cattle), and even features like wrinkled skin (HAS2) in dogs (Sutter et al., 2007; Cadieu et al., 2009; Parker et al., 2009).

Challenges and prospects

Selecting the right samples for conducting methylomics and transcriptomic analyses is crucial for studying epigenetic factors and gene expression in both wild and domesticated animals, providing valuable insights into variations associated with domestication. It's essential to explore the interplay between environmental factors and the traits of organisms that have undergone adaptations to modern environments, as well as the patterns of selection that have been influenced by these environments during the domestication period.

To address the global challenges posed by domestication, there's a growing need for a more integrated, cross-disciplinary approach in the fields of evolutionary biology and functional genomics. Ensuring the validity and reproducibility of aDNA samples amplified from archaeological sources is vital. Efforts to identify ancestral and deleterious alleles across various livestock species should incorporate advanced machine learning methods, including transfer learning techniques. The ongoing mass extinction event, where species struggle to adapt to rapidly changing environments, underscores the importance of developing evolutionary plans and policies that support sustainable development, better health, and the responsible use of natural resources and biodiversity.

In future endeavors, it is advisable to apply these advanced technologies to obtain genomes from a diverse range of individuals within different species worldwide. This approach, as part of the Digital Sequence Information system, will contribute to a deeper understanding of the genetic, morphological, and behavioral characteristics of various species.

Conclusion

The genomes of domesticated animals have evolved into valuable assets for gaining insights into domestication-related issues. By identifying and categorizing deleterious mutations, it becomes possible to preserve animals with less damaged genomes, thereby optimizing conservation efforts. Utilizing machine learning techniques to identify the most minimal set of markers for distinguishing between different breeds or groups can safeguard the rights to use specific breeds within the international community.

Conducting new genome resequencing at the population or breed level is imperative, particularly for various indigenous breeds that face the risk of displacement by common commercial breeds. This step is

crucial for assessing and conserving genetic resources. These research endeavors will not only enhance our comprehension of the genetic foundations of animal domestication but also support their enhancement through selective breeding.

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MULTIOMICS APPROACHES FOR CHARACTERIZING ADAPTATION AND MILK ATTRIBUTES IN BOVINES FROM HIGH ALTITUDES

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Introduction

Mountains cover about one fifth of earth's surface with number of high-altitude regions above 2400 m and also extreme regions like Tibetan Plateau and the Andean altiplano with average elevation of approximately 4000 m. These regions are inhabited by many native human (12% of the world population) and animal populations across world that are living in highland plateau over centuries such as Tibetans, Andeans, Ethiopians and Ladakhi. These high-altitude regions have characteristic vegetation and harsh climate of high-altitude areas make pasture grazing the predominant farming system and thus ruminants, the main livestock species. These livestock populations have undergone natural evolution to gain adaptation in challenging hypoxia environmental conditions.

The Himalayan Region in India spans thirteen Indian states and union territories. It starts from the foothills of south (Sivaliks) and extend up to the Tibetan plateau on the north (Trans-Himalaya). Ladakh union territory, the highest altitude plateau region in India with much of geographical region being over 3,000 m lies sandwiched between the Kunlun Mountains in the north and the Himadri to the south. Ladakh plateau generally known as "land of high passes" harbours difficult terrain and huge stretches of barren lands. The difficult terrain of Ladakh region with high altitude, cold and arid conditions, has rich heritage of important livestock species viz., yak and their crosses (dzo, dzomo), cattle, goat, sheep, donkeys, horses and double hump camel. These livestock genetic resources have undergone natural evolution to gain adaptation in challenging environmental conditions of cold dry arid region. Evolutionary, each of these species might have developed some effective mechanisms to survive at high altitude and to combat the ill effects of low temperature and low oxygen conditions. This animal wealth plays an important role in the life of the local people as the land resources are meager, the economy of people is majorly based on animal husbandry.

Though these animals are lifeline of local people, much efforts have not been put in either to understand their adaptive mechanism or to increase their production efficiency or disease resistance. Multi-omics approaches *viz*., the high-throughput holistic molecular technologies analysing all genes (genomics), all transcripts (transcriptomics), all proteins (proteomics), all metabolites (metabolomics), accessibility of DNA for transcription (epigenomics), and characterizing microbial groups (metagenomics) in one go can help to gain insights of the biological process and improve these important livestock resources. The overall aim is collective characterization and quantification of biological molecules translating to structure, function, and dynamics of an organism. Many of the international research groups are trying to underpin mechanistic insights in populations adapted to Tibetan, Andeans, Ethiopian highland plateau. Some of the studies have compared highlanders and lowlanders with respect to their distinct phenotypic, physiological, haematological and proteomic responses (Jin-Wei Xin *et al*., 2020; Storz and Bautista, 2021). With the advent of high throughput sequencing platforms, genome and transcriptome scans have also been attempted across many native and non-native highlanders (human and animal species) to identify genes and genomic regions under positive selection (Wang *et al*., 2022).

In the last few years, ICAR-NBAGR has made some tangible efforts to characterize animal genetic resources with special emphasis on high altitude adaptation and bring forth positive attributes. Some of the project on this aspect include; ICAR-National Fellow project, DRDO funded CARS project and DST funded project under ASACODER schemes (Arid semi-arid and cold arid region), Govt. of India. In these projects, a multipronged approach was implemented to characterize the native cattle, yak and Zanskar ponies of Ladakh so that the tribal farming community who are rearing these livestock genetic resources gets benefitted on the longer run. The approach was mainly to bring forth the positive attributes related to their adaptation to almost inhabitable conditions, genetic uniqueness, superior milk quality, and superior endurance trait. A total of 14-15 survey visits were made to difficult terrain of Leh and Ladakh to understand population demography and unique attributes of native cattle, Zanskar ponies and yak population of Ladakh. Morphometric data on more than 300 individuals indicated Ladakhi cattle to be one of the shortest cattle

breeds of India. Haematological, biochemical, antioxidants, cytogenetic and genetic profiling was helped to characterize this unique population. The sequence characterization of candidate genes regulating milk production and genotyping of allelic variants of beta casein gene establishes native cattle of Ladakh to be a natural resource for A2 milk (Sodhi *et al.*,2018; Mukesh *et al.*, 2022). Transcriptomic based approach was implied in PBMCs of Ladakhi cows from high altitude and Sahiwal cows from low altitude to identify genes/ transcripts and pathways associated with high altitude adaptation (Verma *et al.*, 2018). Further, to normalize the gene expression data, panel of stably expressed reference genes were identified in high-lander Ladakhi cows and low-lander Sahiwal cows (Verma *et al.*, 2022). The distinct transcriptome and genome signatures of local native cattle of Ladakh from high altitude compared to native cattle from plain area (at low sea level), resulted in identification of several genes and pathways involved in high altitude adaptation. Based on data generated in our lab, native cattle of Leh and Ladakh region was found to be genetically distinct from native cattle adapted to tropical region of India. Due to our concerted efforts, the native cattle of Ladakh got registered as distinct breed in 2018 named "Ladakhi cattle". Additionally, the superior load carrying capacity and endurance potential of Zanskar ponies, another important species adapted to high altitude was established in field trials in Ladakh at a hight of >10,000 ft (Niranjan et al., 2018). The morphometric and genetic characterization of native yak populations of Ladakh showed its uniqueness from rest of the yak populations found in other part of India. Recently, metabolome data in serum and milk colostrum samples of native cattle and yak was generated to identify unique biomolecules and metabolites associated with high altitude hypoxia.

Physiological adaptation to high altitude

Life at high altitude is physiologically challenging because of multiple risk factors *e.g.* UV exposure, cold desert and hypobaric hypoxia. Body requires constant supply of oxygen to generate energy. Hypoxic stress at high altitude is due to low barometric pressure and lower oxygen content in the inspired air compared to sea level. Hypoxia (0.1–1%), physioxia or physoxia (~1–13%), and normoxia (~20%) of O_2 are terms used to define oxygen concentration in the cellular environment. Till date, many studies have been carried out to gain understanding of high-altitude adaptation across different animal species *e.g.*, Ladakhi cattle (Verma *et al.*, 2018), yak (Qiu *et al.,* 2012), sheep (Wei *et al.,* 2016), domestic goat (Song *et al.,* 2016), snow leopards (Cho *et al.,* 2013), Tibetan antelop (Ge *et al.,* 2013), pigs (Wang *et al.,* 2015), horses (Xu *et al.,* 2007), snub nosed monkeys (Yu *et al.,* 2016), as well as humans (Bigham *et al.,* 2016). To cope with the harsh climate condition, these native animals have acquired morphological adaptations. Most of these animals defend hypoxia by having unique circulatory adaptability with enlarged lungs, heart and and greater heart pumping to supply more O_2 to the cells resulting in higher pulse rate and blood pressure. The yak (*Bos grunniens*), a large ruminant of the bovine family is the best example of high-altitude adaptation through natural selection over millennia. Yak has compact body structure, thick outer long hairs and a fine down undercoat, generating the heat to protect themselves from cold. Because of more accumulated fat in subcutaneous layer and presence of less sweat glands, internal heat does not dissipate from the body and therefore protect the animal from relentless cold. Besides, they have highly efficient energy metabolism (Wang *et al.*, 2011) that enables them to survive easily at high altitude hypoxic condition. Yaks also have higher haemoglobin content and a higher affinity between haemoglobin and oxygen (Wiener *et al.* 2003). Other species like pigs, sheep and goat are also well known for their strong adaptability and robustness at high altitude (Zhu *et al.,* 2009). The Tibetan Cashmere goat (*Capra hircus*), one of the most ancient breeds, is an important source of meat for local farmers. This goat has developed distinct phenotypic traits compared to lowland breeds for adaptation to high altitude (Song *et al.,* 2016). Tibetan goat also exhibits higher adult haemoglobin (Hb) concentrations, large heart and lung as a physiological adaptation to hypoxic condition (Renzheng *et al.,* 1992). Similarly, Tibetan pigs have developed a number of key adaptive physiological features to survive in the harsh environment of high altitude (Chang *et al.,* 2005). They have long hair with black skin and dense bristle that shield them from solar radiation and low temperature. They have well-developed heart and lung for the increase blood flow and oxygen transportation to tissues in response to low O_2 concentration (Ai *et al.,* 2014; Ruan *et al.,* 2004). High-altitude hypoxia results in increase in number of erythrocytes as an adaptive mechanism. Several studies have reported increased red blood cells (RBCs), hematocrit/packed cell volume (HCT/PCV), Hb and decreased mean corpuscle volume (MCV) in animals such as yak (Ding *et al.*, 2014). Increase in RBCs was attributed to the increased secretion of erythropoietin which stimulates RBC

production. Increase in RBC and Hb level was also observed in high altitude cattle (Wuletaw, 2011; Kumar and Pachauri, 2000). Additionally, prolonged exposure to high altitude (5100 m) is associated with decreased oxygen pressure, that could result in oxidative/reductive stress, enhanced generation of reactive oxygen and nitrogen species (RONS), and lipid peroxidation. In order to prevent the body from damage by free radicals; animals at high altitude develop an antioxidant defence system which helps to prevent the formation of free radicals and inhibit the lipid peroxidation, thus reducing the oxidative stress.

Candidate genes, proteins and metabolites in high altitude adaptation

Genomic and transcriptomic studies have revealed several potential candidate genes in high altitude adaptation. Qiu *et al. (*2012) and Zhang *et al. (*2015) have identified disintegrin and metallopeptidase domain17 *(ADAM17),* arginase 2 (*ARG2),* matrix metallopeptidase 3 (*MMP3)* as major genes associated with the high-altitude adaptation in Tibetan yak and Tibetan pigs. *ADAM17* and *ARG2* affect the stability and activity of the hypoxia inducible factor, whereas *MMP3* is involved in several physiological processes such as angiogenesis, wound healing and immunity. *VEGFA*, another important gene that plays major role in angiogenesis have been identified in high altitude adaptation in yak (Wu *et al.,* 2013). Two SNPs g.8430T>C in intron 4 and g.14853G>A in 3' untranslated region of *VEGF-A* genes have been linked to high altitude adaptation in yak. Interestingly, in the study carried out by our group, both these SNPs in *VEGF-A* gene were not associated with high altitude adaptation in native cattle of Leh and Ladakh (Verma *et al.,* 2016).

In addition, 7 important candidate genes; endothelial pas' domain 1 (*EPAS1),* crystalline alpha A *(CRYAA),* lon peptidase 1 *(LONP1),* neurofibromin1 *(NF1),* dipeptidyl petidase4 *(DPP4),* peroxisome proliferator activated receptor gamma *(PPARG),* and suppressor of cytokine signaling 2 *(SOCS2*) were identified in Tibetan sheep (Wei *et al.,* 2016). In another study, *EPAS*-1, methionine sulfoxide reductase B3 (*MSRB3*), beta hemoglobin (*HBB*), cyclin dependent kinase 2 (*CDK2*) and guanine nucleotide binding protein G-Beta polypeptide1 (*GNB1*) genes have been identified as important candidate genes in high altitude adaptation of Tibetan dogs (Wang *et al.,* 2014; Gou *et al.,* 2014). Apart from this, hypoxia related candidate genes *ADORA2A, CCL2, ENG PIK3C2A, NOS3*, and *ALB, ECE1, GNG2* and *PIK3C2G* were also identified in Tibetan antelopes and Tibetan wild boars, respectively (Ge *et al.,* 2013; Li *et al.,* 2013).

In addition, *HIF* and its regulatory genes were found to be associated with high altitude adaptation. *EPAS-1* and *EGLN1* were identified as key regulator of hypoxia pathway (Peng *et al.,* 2011). In Andeans, candidate genes such as *EGLN1* **(**Egl-9 family hypoxia inducible factor1), *PRKAA1* (protein kinase, AMP activated *α* 1 catalytic subunit), and *NOS2A* (nitric oxide synthase 2A) that participate in HIF pathway were found to be associated with the high-altitude adaptation (Labie *et al.,* 2012; Bigham *et al.,* 2010). Earlier, *NOS2A* another important gene that plays significant role in vasodilation, smooth muscle relaxation, and increased uteroplacental blood flow were also found critical for high altitude adaptation (Bigham *et al.,* 2010)

Studies have also been conducted to identify genetic variations in *EPAS1* gene related to high-altitude adaptation in different animals such as yak (Wu *et al.,* 2015), dogs (Wang *et al.,* 2014), sheep (Ai *et al.,* 2014), cattle (Newman *et al.,* 2015). Recently, a missense mutation in *EPAS1* at position Q579L was reported to be associated with higher Hb concentration in Tibetan goats (Song *et al.,* 2016). Similarly, genetic variation at G83065A in *EPAS1* was also found to be associated with increased Hb level in yak (Wu *et al.,* 2015). Wei et al. (2016), showed that mutation at conserved site of 3' UTR of *EPAS1* gene was associated with high concentration of mean corpuscular volume and mean corpuscular hemoglobin concentration in highland sheep. Moreover, several mitochondria genes that are involved in oxidative phosphorylation and electron transport chain were also found to be integral to high-altitude adaptation. For example, SNPs at m.3907 C>T and m.3638A>G within mitochondrial MT-ND1 gene; m.8164 G > A, m.8210 G > A, m.8231 C > T in ATP6 gene and m. 8249 C > T in ATP8 mitochondrial gene were positively associated with high altitude adaptation in Tibetan Yak. (Shi *et. al.,* 2017; Wang *et al.,* 2017). A non-synonymous mutation in NADH dehydrogenase subunit 6 *(NADH6*) was also found to be associated with high altitude adaptation In Tibetan horses (Xu *et al.,* 2007).

Transcriptomic based studies across different species have also helped to identify key genes. Zhang *et al.* (2015) have shown differential expression of hypoxia-related genes (*ADAM17, ARG2, MMP*, and *HIF1A*) in skeletal muscles of Tibetan pigs reared at different altitudes (500 and 3650 m). Similarly, Wang *et al.* (2015)

identified several genes (*EPAS-1*, *CRYAA1, LONP1, NF1, DPP4, PPARG,* and *SOCS)* involved in angiogenesis, erythropoiesis and energy production in high land sheep. Verma *et al.* (2018) generated the transcriptome data in PBMCs of Ladakhi cattle adapted to high altitude hypoxia condition and compared with Sahiwal cows adapted to tropical condition. The genes (*INHBC, ITPRI, HECA, ABI3*, *GPR171*, and *HIF-1*α) that were upregulated in Ladakhi cows were involved in hypoxia and stress response whereas genes (*GRO1, CXCL2, DEFB3* and *BOLA-DQA3*) up-regulated in Sahiwal cows were associated with immune function and inflammatory response. The molecular pathways that were highly impacted included MAPK signaling, ETC, apoptosis, TLR signaling and NF- kB signaling indicating signatures of adaptive evolution in two native cattle adapted to diverse environments.

Recently, proteomic and metabolomic signatures have also been generated in different livestock species to provide insights into molecular mechanisms underlying high-altitude adaptation. Xin *et al.* (2020) have reported large differences in proteome of lung and heart tissues of yak and three cattle strains (Holstein, Sanjiang and Tibetan cattle). The KEGG based analysis shown enrichment of retinol metabolism and toll-like receptor categories in lung tissue which may regulate hypoxia-induced factor and immune function in yaks. On the other hand, in heart tissues, cardiac muscle contraction, Huntington's disease, chemical carcinogenesis and drug metabolism-cytochrome P450 categories were enriched. These may benefit yak by altering cardiac function through regulation of type 2 ryanodine receptor (RyR2) and Ca2+-release channels. Such information was quite useful to understand mechanisms underlying adaptation of animals to high-altitude condition. Xin *et al.* (2020) have compared the proteomic profiles of gluteus between yak and one moderate-altitude cattle strain (Tibetan cattle) and two low-altitude cattle strains (Holstein and Sanjiang cattle) using a label-free quantitative method. Protein-protein interaction analysis indicated that differentially expressed proteins were mainly related to oxidative phosphorylation and electron transport chain. Further analysis revealed that NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 11, NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 4, cytochrome C oxidase subunit 6A2, mitochondrial and cytochrome c oxidase subunit NDUFA4 were all increased in the yak, suggesting that yak might increase mitochondrial capacity to sustain metabolic rates under high altitude conditions. This might be a long-term adaptive mechanism underlying adaptation of yak to high altitude environments. These results provided more information to better understand the molecular mechanisms underlying yak adaption. Wen *et al.* (2019) identified 52 differentially expressed protein in longissimus thoracis muscles in yaks and cattle involved in energy production and provide the molecular mechanism for adaptation at high altitude. Earlier, Zhang *et al.* (2017) have provided insights into the key genes and proteins involved in high-altitude adaptation in the Tibetan pig. The genes and proteins that were enriched in the HIF-1 signaling pathway (NPPA, ERK2, ENO3, and EGLN3), VEGF signalling pathway (ERK2, A2M, FGF1, CTGF, and DPP4), and hypoxia-related processes (CRYAB, EGLN3, TGFB2, DPP4, and ACE) in heat tissues as important candidate genes for high-altitude adaptation in the Tibetan pig. Further, in order to understand the mechanisms underlying the reproductive adaptations, a tandem mass tag (TMT)-labelled quantitative proteomic data was generated in spermatozoa from Tibetan pigs raised in high and low altitudes (Zhao *et al*., 2019). A number of proteins associated with the actin cytoskeleton, the TCA cycle, and ATP metabolism were identified that might play key role in providing tolerance to Tibetan pig sperm in high-altitude environments.

Unlike genomic and transcriptomic related studies, not many data have been published using metabolomics in yak or other high altitude adapted livestock population. One of the interesting studies, common biofluids of yak such as serum, urine and faeces have been utilized to analyse the metabolic signatures using NMR Spectroscopy (Zhu *et al*., 2019). They could identify a total of 108 metabolites linked to diet, protein digestion, and energy generation or gut-microbial co-metabolism. Around 15 metabolites were commonly present across all biofluids. Lactate, acetate, and creatinine were most abundant metabolites in the serum, faeces, and urine of yak, respectively. Earlier, nitric oxide level has shown to be increased with the elevation (Ishizakia *et al*., 2005). In another study, the serum metabolome of yak and the closely related species of low-altitude yellow cattle and dairy cows were compared (Huang *et al*., 2022). The analysis showed a clear separation of 63 differentially expressed metabolites among the three species. These differentially expressed metabolites were functionally annotated to activation of innate immune system, oxidative stress-related metabolism, and energy metabolism in yak. This has given strong clue about their important roles in high-altitude adaptation in yak.

Multi-omics in characterizing milk attributes of high-altitude bovines

The milk from high altitude adapted cows and yak have always been a source of nutrition and various milk products like churpi, butter and curd for the nomads, pastoralists and high-landers from Ladakh and Tibetan plateau. These are important source of proteins, fats, vitamins and minerals especially during lean winter season when there is extreme shortage of vegetables and crops. The milk and milk products from native highlanders' cows and yak are the major ingredients of their daily diet. Due to environmental differences, the milk from high altitude adapted cows, and yak are considered to have distinct composition and constituents in comparison to milk from bovines from mean sea level. Since these animals' graze on local grasses, mountain pastures and alpine meadows their milk is considered to be rich in several nutrients and growth factors. Further, milk from these animals is highly concentrated due to high fat, and protein content. In last few years, there has been lot of excitement amongst the researchers to understand the nutritional value of milk from high altitude animals as their milk and milk products may offer several health benefits especially for GI functioning, immunity, antioxidant properties and overcoming stress and inflammation. In recent years number of studies have been conducted to characterize the milk constituents and milk traits of bovines especially in yak from high altitude using various omics tools such as genomics, transcriptomics, proteomics and metabolomics. Though there is still a long way to go but with the advent of high throughput omics tools, now there is better understanding of molecular and genetic aspects of key differences in milk of high and low altitude adapted bovines.

Similar to the understanding of adaptive mechanism, the omics approaches (genomics, transcriptomics, proteomics and metabolomics) individually or in combination have unparallel applications in understanding and improvement of milk traits. These approaches along with computational techniques allow the simultaneous quantification of a large number of transcripts, proteins or metabolites. The milk composition, structure and physicochemical properties differ across dairy species and breeds due to difference in genetic makeup. Therefore, since the genomes of yak and high land native cows have lots of differences in comparison to animals of low altitude, it is expected that milk traits between high and low altitude bovines will vary to a large extent.

Yak milk has a high content of TS (16.9–17.9%), protein (4.9–5.9%), and fat (5.5–7.5% (Wiener *et al*., 2003). Though these values were similar to milk of buffaloes (*Bubalus bubalis*) but were significantly higher from dairy cows (*Bos taurus*) and goat (*Capra hircus*). Therefore, it is very much pertinent to understand the specific molecular signatures associated with milk synthesis in yaks. This kind of information is crucial to understand the milk biology of such a unique species adapted to high altitude and pastoral ecosystem. Several research groups in world are trying to uncover which genes control the production and composition of milk of yak, and how these genes are regulated.

The approach of transcriptomics has been well implemented to understand the lactation biology of yak (*Bos grunniens*) surviving under harsh conditions of low temperature, humidity, and oxygen levels; strong winds, high ultraviolet radiation at high altitude (Zhang *et al*., 2020; Xia *et al*., 2023). Recently, Wie *et al*. (2023) delineated the transcriptome of the mammary tissue of yak during the whole lactation cycle and identified >6000 differentially expressed genes (DEGs), with a large number of DEGs at the onset (1 d vs. −15 d) and at the end of lactation (240 d vs. 180 d). The study has helped to annotate several lactation-associated genes present on BTA3, BTA4, BTA6, BTA9, BTA14, and BTA28. Further, they concluded that unlike dairy cattle, yaks have to adapt to an environment with a low oxygen concentration, and induction of the 'VEGF signaling pathway' in their study could indicate the potential adaptive mechanism of yak mammary gland. In one of the study, Maiwa yak's mammary gland gene expression changes was captured during transition from colostrogenesis to lactogenesis using Bovine Genome Array (Wang *et al*., 2017). The data from 1-day and 30-day post parturition revealed 517 differentially expressed genes. GO terms "fatty acid transport" and "monocarboxylic acid transport" were induced significantly while "hormone binding", "positive regulation of tissue remodelling", and "synaptic vesicle" were significantly inhibited during the colostrum period. The strongly impacted KEGG pathways were chondroitin sulfate biosynthesis, glycosphingolipid biosynthesis, and glycerolipid metabolism. Role of non-coding RNA in during lactation and the dry period of the yak has also been explored (Wu *et al*., 2020). In India, a comparative transcriptomic study has been attempted wherein, mammary epithelial cells isolated from Jersey (high milk producer) and native Kashmiri (low

producer) cows were included. The study has revealed a total of 1103, 1356 and 1397 genes in samples collected at day 15, day 90 and day 250 post calving, respectively (Bhat *et al*., 2019). Interestingly, the top 13 expressed genes at each stage of lactation were similar for both breeds except RPS12 and CCL14, which were highly expressed in Kashmiri cows and Jersey cows only, respectively. The study has shown that expression of candidate genes for milk synthesis and yield traits were more in mammary epithelial cells derived from Jersey cows as compared to high-land Kashmiri cattle. Such kind of studies will go a long way in understanding the mammary gland biology of high altitude adapted native bovines.

Similar to transcriptome, studies have been conducted to characterize the proteome of milk in high altitude bovines using LC-MS/MS approach. In one of the recent studies by Gao *et al.* (2023), a total of 632 proteins were identified in milk whey samples of Chineese yak. The study has included three breeds of Chinese yak such as Gannan, Maiwa and Huanhu yak milk samples. The proteins that were most abundant in the milk of all the three yak breeds were immune-related proteins. The study has provided information on proteomics constituents of yak milk from different breeds in China, and improved our understanding of the biological functions of yak milk proteins. Earlier, Yang et al. (2015), used quantitative proteomics to compare the protein profiles of whey from yak colostrum and milk (1 day vs 28 day) using isobaric tag for relative and absolute quantification (iTRAQ)-labelled proteomics. They could identify a total of 183 proteins in milk whey and expression of majority of proteins (86) differed significantly between the whey from colostrum and milk. The haemoglobin abundance was significantly higher in colostrum than in mature milk. Some recent studies also characterized the whey proteome and glycoproteome in bovine milk and a total 240 whey proteins and 315 N-glycosylation sites on 214 glycoproteins were quantified (Zhang *et al*., 2023). Chopra *et al.* (2020) has identified the global proteome signature of Indian zebu (Sahiwal) cows using LC–MS/MS. Apart from bovine, a large number of research examined and compared the whey protein of different mammalian species. For example, Weixuan *et. al.* (2020) has conducted quantitative proteomics study to compare whey proteins of colostrum and mature milk in donkey. Li *et al.* (2023) also characterized the proteome signature of donkey and bovine colostrum. Their study has provided insight into the biological functions of donkey milk proteins. Similarly, Anagnostopoulos *et al.* (2016) compared the whey proteomes of three indigenous pure-bred Greek sheep and goats.

Besides transcriptomics and proteomics, in recent years researchers have also tried to understand the milk architecture of bovines at metabolome level. Metabolomics is one of the most widely used OMICS technologies, measures small molecules in biological samples at a particular time. Comprehensive characterization of milk metabolome has been undertaken in recent past for various species such as cows, sheep, goat, camel and donkey using various metabolomic tools such as 1H-NMR spectroscopy, GC-MS and LC-MS/MS spectroscopy (Sundekilde *et al*., 2013; Klein *et al*., 2010; Sen *et al*., 2020). However, such an effort are relatively missing and milk metabolites present in high altitude adapted yak and native cows are mainly unexplored. Recently Li *et al.* (2023) have characterized the metabolites of yak colostrum and yak mature milk using gas chromatography–mass spectrometry (GC–MS). A total of 354 metabolites were identified and out of which 109 were differentially expressed between colostrum and mature milk of yak. Comparative metabolomic analysis identified distinct metabolites present in colostrum and mature milk indicating compositional differences. The study has also compared the yak and human breast milk and identified key differences in metabolomic composition of two species. Interestingly, the hypoxic stress-tolerant pathways were enriched in yak milk-specific metabolites. Such kind of scientific information indicating role of yak milk metabolites in providing tolerance to hypoxic stress is so important for the value addition of yak milk in future.

The metabolic profiles of yak biofluid (serum, feces, and urine) was characterized by using 1H-NMR (Zhu et al., 2019). A total of 108 metabolites could be identified and out of which only 15 metabolites were commonly present across all the three biofluids. Lactate, acetate, and creatinine were reported be be most abundant metabolites in serum, feces, and urine, respectively. Four metabolic pathways namely valine, leucine, and isoleucine biosynthesis; phenylalanine, tyrosine, and tryptophan biosynthesis; glutamine and glutamate metabolism; and taurine and hypotaurine metabolism were identified. Such kind of study has helped to understand the mechanism of its high-altitude adaptation. Not many metabolomic based study has not been

reported for the milk of high-altitude bovines. In contrary, several efforts have been made to characterize the metabolome signature of milk and colostrum of major dairy livestock species.

Very recently, Yuan *et al*. (2023) performed comparative milk metabolic profiles of Italian Mediterranean buffaloes and Chinese Holstein cows using liquid LC-MS/MS platform. Majority of differential metabolites were enriched in glycerophospholipid metabolism, nicotinate and nicotinamide metabolism, glycine, serine and threonine metabolism, as well as purine metabolism. Similarly, O' Callaghan *et al*. (2021) generated colostrum and milk metabolome data using 1H NMR. Interestingly, the colostrum metabolome was distinctly different from milk samples collected during early lactation stages. Li *et al*. (2020) provided comprehensive insight into the metabolome signature of colostrum and mature milk in donkey. Similarly, Picone *et al*. (2018) have characterized the colostrum metabolome of three pig breeds (Large White, Landrace and Duroc). The major focus of their study was to evaluate the compositional differences of colostrum from swine three breeds and identify the colostrum metabolites that influences newborn survival and litter growth rates. Yu *et al*. (2023) performed systematic analysis of differential metabolites in colostrum and mature milk of Guanzhong dairy goats using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. In addition, O'Callaghan *et al*. (2018) used 1H-NMR to examine the impact of diet on the milk metabolome and identified several metabolites such as valine, leucine, isoleucine, choline and orotic acid associated with pasture-based diets. Zhao *et al*. (2018) showed higher concentrations of leucine, valine, and glutamate in the calves that were fed with colostrum.

Conclusion

There exist lot of challenges for both livestock and livestock keepers at high altitudes, especially the presence of low atmospheric pressure, lower oxygen concentration, low temperature and extreme winter period. In the high-altitude environment, as animals are constantly subjected to multiple stressors due to a persistent hypoxic-hypobaric environment, this condition may lead to oxidative stress in animals. However, the nature provides several kinds of modifications at physiological, molecular and cellular levels to help animals adapt to the conditions of high-altitude high altitude. In future, it would be interesting to explore insights about their remarkable high altitude specific adaptive trait. In recent years, cross breeding with non-native population has become a major threat to precious bovine germplasm of high altitude developed through adaptation after natural selection. Hence, strategies should be designed to improve management practices and production traits for sustainable utilization of yak and native cattle from high altitude region. Under the changing climate scenario, identification of climate-resilient populations will help to protect the high-altitude ecosystems of the world. The valuation of such unique populations on the basis of diversity index, adaptation, and availability of health-related biomolecules in milk is key for their sustainable utilization.

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Technical Session - I

Genetics and Genomics in Livestock and Poultry

ABSTRACTS

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UNVEILING POSITIVE SELECTION IN THARPARKAR CATTLE THROUGH DEEP NEURAL NETWORKS

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Identifying genomic regions influenced by natural selection provides fundamental insights into the genetic basis of local adaptation. However, it remains challenging to detect loci under complex spatially varying selection. In the current investigation, we used a deep learning-based framework, called DeepGenomeScan, which can detect positive selection signatures of spatially varying selection. Although, now researchers have started exploring genomic footprints of selection in indigenous cattle breeds all these investigations relied on traditional summary statistics methods. These methods have a condition of working under assumptions and thus produce bias. A deep neural network, DGS could rectify these shortcomings and enhance the accuracy of such analyses. In this study, we constructed a Multilayer Perceptron (MLP) network with two hidden layers. We used geographic coordinates (longitude and latitude) as response variables to detect the spatially varying selection. Hence, we applied DeepGenomeScan to Tharparkar cattle chip SNP data. Genome scan identified 70 loci with top 1% normalized values. Further, annotation was carried out using the latest bovine reference genome, i.e., ARS UCD 1.2. A total of 25 genes were acknowledged to be overlapped with the regions under positive selection. We identified some well-known genes under selection and a substantial number of production, disease, and behavior-related important genes (*TLR3, CPT1A, PALLD, BMP6*, and *AMN*) that were not identified by traditional summary statistics when applied to the similar dataset earlier in other studies.

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DIVERSITY ANALYSIS OF SOUTH INDIAN CATTLE BREEDS USING MICROSATELLITE MARKERS

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The study aimed to characterise the genetic diversity of Kangayam, Umblachery, Alambadi, Bargur, Pulikulam, Deoni and Ongole cattle breeds present in the South India. A total of 96 animals were genotyped for 27 FAO recommended microsatellite loci. The genotypes were analysed using various bioinformatics tools. A total of 1340 alleles were observed over the 27 microsatellite markers and 7 breeds with ranges from 6 to 13 alleles. The average number of observed alleles obtained in Alambadi, Bargur, Deoni, Kangayam, Ongole, Pulikulam and Umbalacherry cattle were 6.59 ±2.15, 6.96 ± 2.05, 7.52 ±2.42, 6.19 ±2.32, 6.67 ± 2.11, 8.07 ± 2.13 and 7.63 ± 2.34 respectively. The highest number (Na=14) of alleles was observed in Deoni, whereas the lowest number (Na=3) of alleles was noticed in Ongole, Kangayam and Alambadi breeds of cattle. The mean F_{IS} value observed over different microsatellite loci was 0.0678 ± 0.028 and it was ranged from -0.0883 to 0.6067. The F_{IS} value was positive for all the breeds analyzed indicating significant heterozygosity deficiency. Most of the microsatellite markers considered informative (PIC > 0.50) and the PIC values showed significant positive correlation with the number of alleles (r = .517, p = .006), regardless of differences in allele size revealed. The dendrogram among the seven breeds revealed that Alambadi and Barugur breed of cattle formed one cluster and the Kangayam, Umblacherry and Ongole cattle were distinctly different from all other breed of cattle.

ISAGB-2023/Abst/TS-I-003

POLYMORPHISM OF DWARF (ACAN) GENE IN SMALL SOUTH INDIAN CATTLE BREEDS

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The present study was conducted on 200 Punganur, 50 Nattukuttai, 50 Malnad Gidda and 10 Kangayam cattle to identify genetic polymorphisms in dwarf gene i.e ACAN gene. A total of 521 bp of exon 11 of ACAN gene was studied in South Indian cattle breeds. One set of HRM primer and one set of sequencing primer for exon 11 of ACAN gene on the basis of Bos Taurus sequence (ACCESSION. No - NC_037348.1) were designed using Primer3 software and PCR products of 98 and 521 base pairs were obtained. A 521 bp amplicon was Sanger sequenced and subjected to Clustal W analysis which showed nucleotide changes in coding and non-coding region in cattle breeds as compared to Bos taurus. Analysis of novel SNP 44312 G>A was performed, using restriction fragment length polymorphism (PCR-RFLP) to detect nucleotide changes in the sequence in dwarf gene of small cattle as well as Kangayam breeds. Polymorphism at 44312 G>A was present in the sampled population of Punganur, Nattukuttai, Malnad Gidda and Kangayam cattle.

ISAGB-2023/Abst/TS-I-004

INVESTIGATING SELECTIVE PATTERNS ON THE X CHROMOSOME ACROSS DIVERSE CATTLE BREEDS

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During the divergence of mammal-like synapsid reptiles from other reptiles (310 million years ago), the X and Y chromosomes independently emerged from different autosomal counterparts in the course of mammalian evolution. Considering that its effective population size is only three-quarters that of an autosome, the X chromosome undergoes more drift than an autosome. Due to its high gene density, the X chromosome presents a hotspot for the identification of selection signatures. To discover selection signals on the X chromosome across various cattle breeds, we have conducted a detailed genome-wide survey in order to fully understand the relationship between genomic makeup and the emergence of phenotypic variation during the evolutionary process. In this study, we have prepared the datasets of 184 individuals of different cattle breeds and explored the complete X chromosome by utilizing different summary statistics such as Tajima's D, CLR, iHS, ROHXP-EHH, and F_{ST}. There were 23, 25, 30, and 17 outlier regions identified in Tajima's D, CLR, iHS, and ROH respectively and 17 and 12 regions identified in F_{ST} and XP-EHH. Bioinformatics analysis showed that these regions harbor important candidate genes like *AMOT, AKAP4,and CDK-16*were relevant to reproduction in Brown-Swiss, Gir, and Jersey. We also identified some other genes like *MAGEA11,* and *CACNA1F* for production traits in Jersey and Holstein, *CXCR3* and *SH2D1A* for immune response in Gir and Guernsey, and *CITED1* and *EDA* for pigmentation pathway in Nelore and Gir. Our results have the potential to offer novel perspectives on X-linked selection within diverse cattle breeds.

ISAGB-2023/Abst/TS-I-005

A COMPREHENSIVE STUDY OF SELECTION SIGNATURES IN DAIRY CATTLE

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The current study's major goal was to find, annotate, and highlight certain regions of the bovine genome that are under intense positive selection. We aimed to find selection signatures in crossbred (*Bos taurus* X *Bos indicus*), taurine (*Bos taurus*), and indicine (*Bos indicus*) cattle. Indicine cow breeds are known throughout India for their strong heat tolerance and disease resistance. More breeds and approaches can help us better grasp the selection signature. So, we worked on nine different cattle breeds with seven different summary statistics, which is a holistic approach. Using bovine 50K SNP data, we conducted a comprehensive genome-wide assessment of selection signatures. For a total of 320 animals, we included genotyped data from two taurine, two crossbred, and five indicine cattle breeds. We used seven summary statistics for this work, including intrapopulation data like Tajima's D, CLR, iHS, and ROH, as well as interpopulation statistics like F_{ST}, XP-EHH, and Rsb. After identifying the essential locations for selection, the NCBI database, PANTHER 17.0, and CattleQTL database were utilised for annotation. *EPHA6, CTNNA2, NPFFR2, HS6ST3, NPR3, KCNIP4, LIPK, SDCBP, CYP7A1, NSMAF, UBXN2B, UGDH, UBE2K*, and *DAB1* were identified to be shared by three or more techniques. As a result, it demonstrates the most intense selection in these areas. These genes are largely associated with milk production and adaptation. This research also uncovered selection zones including genes involved in a variety of biological activities, such as milk production, coat colour, glucose metabolism, oxidative stress response, immunology, and circadian rhythms.

ISAGB-2023/Abst/TS-I-006

PROFILING OF EVOLUTIONARY SIGNATURES REVEALS UNIQUE SELECTIVE HOTSPOTS IN INDIAN SAHIWAL CATTLE

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Sahiwal, known as the "Champion dairy breed of India," is highly regarded for its superior milk production compared to other indigenous dairy breeds. To effectively implement conservation and breed improvement plans, it is crucial to conduct genome-wide scanning to identify potential genomic regions under selection. The majority of studies leveraging the ddRAD sequencing approaches have been conducted so far in taurine breeds, and very few studies have been done on indigenous cattle breeds such as Sahiwal. Therefore, the objective of this study is to identify the genome-wide selective sweeps operating across the Sahiwal cattle genome using statistical techniques.Advances in sequencing technologies have addressed the issue of ascertainment bias and improved the detection of rare variants, leading to more efficient and comprehensive genotyping of multiple individuals. One cost-effective and efficient approach for this purpose is reduced representation sequencing, often referred to as ddRADseq. A total of 82 Sahiwal samples were included in the study and after initial QC and variant calling,we utilized five different intra-population statistics: Tajima's D, CLR, |iHS|, |iHH12|, and ROH. After applying filtering criteria, 78,193 high-quality SNPs were

retained for analysis. The study identified 146 regions undergoing selective sweeps using five statistical tests. These regions encompassed genes associated with improved immune systems *(IFNL3, IRF8, BLK)*, disease resistance *(NRXN1, PLCE1, GHR)*, tick resistance (*IL2* on Chr17), and heat stress adaptation *(HSPA8, UBASH3B, ADAMTS18, CRTAM)*.These findings demonstrate the evolutionary impact of natural selection on Sahiwal cattle's environmental adaptation. The identified genomic regions can guide targeted breeding and conservation efforts for future research and breed improvement initiatives.

ISAGB-2023/Abst/TS-I-007

UNIQUE SIGNATURES OF BALANCING SELECTION IN BOVINE GENOMIC LANDSCAPE

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The role of balancing selection in evolution has been debated for a long time. The method of selection that preserves different alleles within a population is known as balancing selection. It is critical to investigate the areas undergoing balancing selection because it protects genetic diversity in a population. The purpose of this study is to find genes that show signals of balancing selection. Balancing selection is an important concept in evolutionary biology, as it describes a mechanism by which genetic diversity is maintained within a population over time. This type of selection occurs when multiple alleles (different versions of a gene) are actively maintained in a population, rather than one allele becoming fixed or the other alleles being lost. We studied the cattle genome to identify places where variability has persisted in the cattle herd for millions of years. We used bovine 50k SNP data to conduct an extensive genome-wide examination of selection signatures for balancing selection in this study. The genotyped data from 427 animals, including five taurines, two crossbreds, and eight Indian cattle breeds, were included. The breeds included in our study are Ayrshire, Brown-Swiss, Frieswal, Gir, Guernsey, Hariana, Holstein Friesian, Jersey, Kankrej, Nelore, Ongole, Red Sindhi, Sahiwal, Tharparkar, and Vrindavani. We used Tajima's D method to identify regions undergoing balancing selection for this investigation. After locating the necessary areas under balancing selection, the annotation was conducted out using the NCBI database, PANTHER 17.0, and CattleQTL database. *KIT, NFATC2, GBP4, LRRC32, SYT7, RAG1, RAG2, LOC513659*, and *ZBTB17* are among the immune system-related genes got identified in our study that may be subject to balancing selection.

ISAGB-2023/Abst/TS-I-008

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISM (SNPS) IN *HSP90AB1* GENE BY ALLELE SPECIFIC PCR (AS-PCR) IN KANKREJ CATTLE

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Heat shock proteins play an important role in the development of thermo-tolerance and protection from cellular damage associated with heat stress. In the present work, Allele-Specific PCR was performed to identify single nucleotide polymorphism (SNP) within the *HSP90AB1* gene in the Kankrej breed of Indian cattle. AS-PCR has a simple strategy that introduces mismatch at the 3' end of forwarding primer in the identification of SNPs. Although there are many techniques for the identification of SNPs, AS-PCR is a cost-effective and time-saving technique to identify previously reported SNP. The study was done on 70

Kankrej cattle maintained at LRS, Kodamdesar, RAJUVAS, Bikaner. About 5 ml of the blood sample was collected aseptically and genomic DNA was isolated from whole blood through spin column method as per standard protocols described by Sambrook *et al* (1989) with slight modification. A final reaction volume of 25 µl containing 5X PCR buffer, dNTP's, MgCl2, primers and Taq DNA polymerase was used. Two forward primers and a common reverse primer were used in the technique. Three genotypic patterns (CC, CT and TT) were observed in this study. The genotypic frequencies of CC, CT and TT genotypes were observed as 0.16 (n = 11), 0.48 (n = 34) and 0.36 (n = 25), respectively. The allelic frequency was 0.40 and 0.60 for C and T alleles, respectively. The calculated allele frequency indicates that the T allele was predominant in the Kankrej breed. In the identification of polymorphism in DNA of previously known allele and unknown genotype, AS-PCR is a very useful technique.

ISAGB-2023/Abst/TS-I-009

POPULATION GENETIC CHARACTERIZATION OF WILD *ANABAS TESTUDINEUS* STOCKS USING MTDNA D-LOOP MARKER

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The cornerstone of every fish farming lies in the genetic information which provides a variety of data required for an efficient conservation and production strategy. *Anabas testudineus* is a hardy fish with many nutritional benefits and is considered as one of the important food fish native to India. In the present study, genetic variation among four populations of *A. testudineus* was studied using the mitochondrial control region partial DNA sequences. In total sequences of 479 bp length for 74 wild individuals collected from Assam, Manipur, Kerala and Uttar Pradesh were obtained. The sequence composition of anabas D-loop sequences is C: 16.37%, T: 30.62%, A: 24.92 % and G: 28.09 %. The anabas D-loop is A+T rich as observed in other teleosts. In total 61 variable sites were observed comprising 3 singletones and 58 parsimony informative sites. The total number of haplotypes observed was 9 with a haplotype diversity value of 0.709 and a nucleotide diversity value of 0.05693. The number of haplotypes ranged from 2 to 5. The haplotype diversity and nucleotide diversity ranged from 0.00 to 0.484 and 0.00 to 0.00174, respectively. Minimum spanning tree analysis revealed that haplotype 5 is the ancestral haplotype. The AMOVA analysis for anabas mitochondrial D-loop revealed that 99.06% variation was due to among population and 0.94% was due to within-population variation. Pairwise F_{ST} ranged from 0.050 to 0.997. In the present study, significant genetic differentiation was observed among different anabas populations. The information generated in the present study would help in efficient strategy planning on the conservation, selective breeding of this species along with the maintenance of population structure and variability.

ISAGB-2023/Abst/TS-I-010

UNRAVELING GENETIC DIVERSITY AND POPULATION STRUCTURE IN SHEEP BREEDS: INSIGHTS FROM ADMIXTURE ANALYSIS

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The genetic diversity and population structure of livestock species are of critical importance for both conservation and selective breeding programs. In this context, admixture analysis serves as a powerful tool to uncover the intricate genetic relationships among different populations and to detect and eliminate outlier individuals that may distort the interpretation of genetic data. This study focuses on the admixture analysis of multiple datasets containing sheep breeds, aiming to unravel the complex genetic makeup of these animals. The barplots revealed genetic relationships, such as shared ancestry between Changthangi and Tibetan sheep and a historical connection between Bangladeshi Garole and Garole. Stringent filtering-maintained cluster purity. The significance of this study lies in its ability to provide insights into the genetic diversity and population structure of sheep breeds, which have substantial implications for breeding strategies and conservation efforts.

ISAGB-2023/Abst/TS-I-011

UNRAVELLING THE GENOMIC SIGNATURES OF SELECTION IN WORLDWIDE CATTLE POPULATIONS

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The natural selection followed by domestication and artificial selection had led to an increased genetic differentiation among the cattle breeds all over the globe. The selection process thrust some influence on certain genomic areas that set distinct breed attributes. These remarkable genetic footprints that persist in an individual's genome are described as 'Selection Signature'. Detection of selection signatures helps in understanding domestication, evolution, and identification of genomic regions associated with adaptation and production traits in cattle. In this study, we sought to identify signatures of selection in cattle populations. BovineSNP50 chip data from 3438 animals belonging to 135 populations (including 61 taurine, 23 zebu and 51 admixed cattle breeds) from different parts of the world were analysed in the present study. We attempted to detect footprints of selection by utilizing pcadapt method. In total, we identified 66 genomic regions under selection mapping to 67 genes across 22 chromosomes. Maximum number of selection signatures were observed on chromosome 14 (n=12) followed by chromosome 6 (n=9). Some of the important candidate genes were found to be associated with QTLs for disease resistance, milk production, reproduction, growth and carcass traits. Our findings provide insight into mechanisms of artificial selection and are a valuable resource for future cattle breeding research.

ISAGB-2023/Abst/TS-I-012

GENOMIC DISSECTION OF DIVERGENT BREEDS/STRAINS OF CHICKEN: A SNP-BASED STUDY

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Single nucleotide polymorphisms (SNPs) play a critical role in the genomic dissection of any population, breed, or strain. In the current study, we investigated the population features amongst divergent poultry germplasm by exploring genome-wide spanned SNP information retrieved through double digest restriction-site associated DNA (ddRAD) analysis (using Illumina Novaseq 6000) in 70 chicken samples from six divergent breeds/strains (Rhode Island Red (RIR), Assel (A), Commercial Broiler (CB), Punjab Brown (PB) including similar indigenous strain (Ind), and Kadaknath (K)). The overall SNP data indicated the total number of Sites: 205356, Proportion Not Missing: 0.98192, Average minor allele frequency 0.222. The study on the annotation of high-confidence SNPs revealed that 99.044% of SNPs had modifier impact. About 30.9% of the SNPs in the poultry breeds were found in the coding region and 36.7% in the intronic region. The data were subjected to analysis using TASSEL to explore linkage disequilibrium (LD), kinship analysis principal component analysis (PCA), etc population parameters. The result indicated that the Kadaknath breed is closely related to the reference genome (https://www.ncbi.nlm.nih.gov/genome/111?genome_assembly_id=2065830, GenBank assembly accession: GCA_027408225.1). The data obtained have been submitted to NCBI SRA (BioProject Id: PRNJA1033377). In the study, it was found that the 'GAA' codon was replaced by the 'TAA' (2,822 times) and by the 'GAC' codon (2,600 times), and the 'AAG' by the 'AAT' codon (1,874 times) in the dataset. The SNPs on sex chromosomes, linkage groups, and uncertain marker placements were eliminated from the analysis. The study provides important information for future allele/gene identification using genome-wide association studies (GWAS) and marker-assisted selection (MAS), which will aid in formulating relevant genetic improvement programs for divergent poultry breeds.

ISAGB-2023/Abst/TS-I-013

MOLECULAR CHARACTERIZATION, POLYMORPHISM AND ASSOCIATION OF TLR4 GENE IN INDIAN DROMEDARY CAMELS

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The present study was aimed to characterize, access the genetic polymorphism and study the association of Toll-like receptor 4 (TLR4) gene in Indian Camels. Blood samples were collected from 118 Indian camels (Bikaneri-29, Kachchhi-34, Jaisalmeri-28 and Mewari-27). The complete coding sequence of the camelid TLR4 gene was amplified using 3 pairs of primers and sequence was analysed using polymerase chain reaction-sequence based typing (PCR-SBT) technique. Sequence analysis revealed no SNPs in the exonic region and only one SNP was found at nt 475C>T in the intron-1 region, resulting in 3 genotypes, CC, CT and TT in the Jaisalmeri, Kachchhi and Mewari breed. CC genotype was absent in Bikaneri breed. The genotype frequency for CC, CT and TT genotypes were 0.102, 0.381 and 0.517 and the allele frequency of C allele was (0.292) and T allele was (0.708) in the population. The heterozygote genotype CT was predominant in the Mewari breed, whereas TT genotype was predominant in Bikaneri and Kachchhi. The frequency of T allele was higher than C allele in all the breeds. The population was in Hardy-Weinberg Equilibrium indicating population

meet the HWE assumptions and genetic variations are conserved. Sequences of camelid TLR4 gene obtained were manually edited and submitted at NCBI with accession no- MT492152, MT492153, MT365024 and MW507720. Association of this SNP with somatic cell score and milk production traits was assessed in 40 lactating she camels. Association study inferred that the effect of genotype on SCS, lactation yield (LY) and peak yield (PY) was non-significant however heterozygote (CT) genotypes recorded lower SCS and higher LY and PY. The association study also indicated that heterozygote animals possess better udder health status and production performance, the statistical significance of which need to established using large data set.

ISAGB-2023/Abst/TS-I-014

IN VITRO CONSERVATION OF INDIGENOUS LIVESTOCK 'AT RISK': EMBRACING THE HALFWAY MARK

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India is home to one of the world's largest and most diverse livestock populations. Diversity is crucial for facing future challenges like climate change, diseases, drought, and land degradation. However, a multitude of processes including socio-economic factors are contributing to the continuous loss of indigenous livestock genetic diversity across the globe including India. The erosion of indigenous livestock diversity in the country is a matter of major concern for conservation biologists, policymakers, and the state governments. 38 native breeds of India are presently 'At risk' according to the National Breed Watchlist-2022 (NBW) for indigenous livestock (ICAR-NBAGR, Karnal). Thus, an initiative was undertaken to preserve the livestock breed's 'at risk' under the CRP on agrobiodiversity project. Somatic cell conservation being one of the viable tools for *in vitro* conservation was utilized and so far 19 breeds belonging to 6 livestock species have been conserved for posterity. These included 4/5 breeds categorized as critical (Teressa goat, Malvi, Mewati & Mewari camel), 9/19 categorized as endangered (Belahi cattle, Karnah sheep, Agonda goan pig, Kachchhi-Sindhi, Manipuri & Zanskari horse, Jalori, Kharai & Marwari camel), and 6/14 (Mewati & Siri cattle, Gurez, Kedrapada & Poonchi sheep, Konkan Kanyal goat) under the vulnerable category. Ear skin tissue was collected from representative animals of each breed from their respective breeding tract. Tissue explants were cultured in the DMEM + 10% FBS in a humidified incubator (37°C, 5% CO_2). Fibroblast cells were harvested and passaged and 4th to 6th passage cells were preserved in liquid nitrogen stocking ≥ 30 vials (1×10^6 cells/ml) for each animal. Cells cultured after thawing were morphologically indistinguishable from the cell stocks before freezing. Conserving livestock biodiversity by somatic cell banking will have far-reaching implications in dealing with future challenges. This endeavor also contributes to India's commitment to the United Nations Sustainable Development Goals (SDG) target 2.5 (Indicator 2.5.1.a).

ISAGB-2023/Abst/TS-I-015

GENOME-WIDE RUNS OF HOMOZYGOSITY SIGNATURES IN DIVERSE INDIAN GOAT BREEDS

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Runs of Homozygosity (ROH) play a potential role in studying demographic patterns, estimating inbreeding levels of various populations, mapping recessive alleles, and identification of selection signatures. Present study involved analysis of ROH and consensus ROH regions with elevated frequencies in 102 animals of

eleven diverse Indian goat (Capra hircus) breeds using whole genome sequencing. A total of 51705 ROH and 21271 consensus regions were identified. The mean number of ROH per animal was highest in meat breed, Jharkhand Black (2693) and lowest in pashmina breed, Changthangi (60). The average length of ROH (ALROH) was maximum in Kanniadu (974.11 Kb) and minimum in Tellicherry (T) (146.98 Kb). Long ROH is typically associated with more recent inbreeding, whereas short ROH is connected to more ancient inbreeding. The overall ROH- based genomic inbreeding (FROH) was highest for Jharkhand Black (JB) (0.602) followed by Kanniadu (KAN) (0.120) and Sangamneri (SAN) (0.108) among all breeds. FROH of JB was higher than KAN till the 5Mb ROH length category but in >20Mb ROH length category KAN (0.98) had significantly higher FROH than JB (0.46) suggesting that KAN had higher levels of recent inbreeding than JB, but due to the abundance of both recent and ancient inbreeding, JB had higher overall FROH than KAN. ROH patterns revealed dairy and pashmina breeds as less consanguineous while recent inbreeding was apparent in meat breeds. Analysis of ROH consensus regions identified selection sweeps in key genes governing intramuscular fat deposition, meat tenderisation, lean meat production and carcass weight (CDK4, ALOX15, CASP9, PRDM16, DVL1) in meat breeds; milk fat percentage and mammary gland development (POLD1, NOTCH2, ARHGAP35) in meat & dairy breeds; while cold adaptation and hair follicle development (APOBEC1, DNAJC3, F2RL1, FGF9) in pashmina breed. MAPK, RAS, BMP and Wntsignaling pathway associated with hair follicle morphogenesis in pashmina were also identified.

ISAGB-2023/Abst/TS-I-016

DETECTION OF MULTIPLE ALLELISM AND LINKAGE DISEQUILIBRIUM ANALYSIS REVEALS KASHMIR MERINO SHEEP IS MORE SUSCEPTIBLE TO FOOT-ROT

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Foot rot is one of the most economically important contagious hoof diseases of sheep caused by mixed action of gram negative anerobes *Dichelobacter nodusus* and *Fusobacterium necrophorum.* Globally the disease results in production losses, welfare issues and negative economic implications with significant prevalence in Kashmir valley. Gene *Ovine Leucocyte Antigen-DQA2* (*OLA-DQA2)* is one of the important genetic marker that has been found to be associated with foot-rot. The present study was carried out to genotype Kashmir Merino and Corriedale sheep maintained at Govt. Sheep Breeding Farm, Goubal and Mountain Research Centre for Sheep & Goat, Kashmir India for DQA2 gene polymorphism. Genomic DNA was isolated from blood samples by phenol-chloroform method and DNA quality and quantity was evaluated by agarose gel electrophoresis and UV spectrophotometer respectively. Exon 2 of *DQA2* was amplified using nested polymerase chain reaction (PCR). Fragment of size 828 bp (comprising entire exon 2 flanked by 209 bp of intron 1 and 370 bp of intron 2) and then product of size 242 bp fragment of *DQA2* gene was successfully amplified using internal primers . PCR-SSCP technique for 242 bp fragment of exon 2 of *DQA2* gene was carried out by 1-2% Polyacrylamide Gel Electrophoresis (PAGE). Five different band patterns were observed in Kashmir Merino (n=30) with genotype frequency of 0.10, 0.24, 0.13, 0.20 and 0.33 while, only three polymorphic variants were observed in corriedale sheep (n=30) with genotype frequency of 0.33, 0.47 and 0.20. PCR products showing distinct banding pattern in PAGE gels were subjected to DNA sequencing. Comparision of allelic sequences and deduced amino acid translations suggested highly polymorphic nature of *DQA2* gene due to various Single Nucleotide Polymorphism (SNP). The results of the present study showed that exon 2 of *DQA2* gene has rich genetic diversity and may be considered as an important marker for marker assisted selection in breeding of animals for disease resistance. In-silico analysis revealed an interesting insights for between breed comparison having significant difference in susceptibility to foot-rot due to linkage disequilibrium between foot rot gene marker and the genes controlling finess of fibre, thus supporting that Kashmir Merino are more prone to foot-rot. The existence of polymorphism and non

random association between foot rot genetic marker and genes related to fibre finess may be utilized in future with more numbers to test the association with economic traits and sustainable selection program.

ISAGB-2023/Abst/TS-I-017

IDENTIFICATION OF GENOMIC VARIANTS IN KANNIADU GOAT USING WHOLE GENOME RESEQUENCING

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Natural and artificial selections are known to fix certain genomic regions with reduced heterozygosity, which is a stepping stone in the process of breed development. Comprehensive information on genetic variants viz genome-wide single nucleotide polymorphisms (SNPs) and INDELs, offers valuable genomic insights linked to key Kanniadu goat traits, has not yet been investigated. Exploring the genomic signals under positive selection and the overlapping genes in the Kanniadu goat breed is the major goal of the current investigation. High throughput genomic sequence data of Kanniadu (n=10) were generated from whole genome re-sequencing (~10X) using 150 bp paired-end chemistry on the Illumina NOVASEQ 6000 platform. FastQC (version 0.11.9) was utilized to assess the read quality parameters. Fastp (http://opengene.org/fastp/fastp) was employed for adapter trimming and quality filtering. The samples were then aligned to the goat (Capra hircus) genome assembly ARS1 (GCF 001704415.1 ARS1 genomic.fna.gz) from USDA ARS using Burrows Wheeler Aligner (BWA). The Picard tool was used to remove duplicate reads and the GATK tool to call variants (SNPs and INDELs). We generated around 202.7 million clean reads which covers an average of 99.5 percent of the reference genome (ARS1), suggesting that high-quality sequences are generated. We identified 22.5 million SNPs and 3.7 million INDELs in the Kanniadu goat. SNPs found in intronic, intergenic, and exonic regions were annotated. Overall , the present study highlights the genes under selection in Kanniadu goats, which will be useful in determining the genetic potential of this breed.

ISAGB-2023/Abst/TS-I-018

GENOMIC LANDSCAPE REVEALS EVOLUTIONARY FOOTPRINTS OF ADAPTABILITY IN INDIAN HILL CATTLE

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The present study aims to assess the population structure of hill cattle and analyze the genomic footprints of selection and adaptation. The population structure and signatures of selection were analyzed in hill cattle as compared to Indian milch, non-milch, and exotic cattle populations, using Illumina high-density genotyping data. The present study used high-density SNP genotyping data generated on Illumina BovineHD SNP BeadChip on a total of 299 individuals representing 14 bovine populations across three groups i.e., Hill cattle (Ladakhi and Siri), Indian milch cattle (Sahiwal, Gir, Tharparkar), Indian non-milch cattle (Hariana, Hallikar, Kankrej, Ongole, and Vecheur) and exotic group (Holstein-Friesian, Jersey, Brown Swiss and Guernsey). The grouping of populations was based on utility, inheritance, and characteristics related to the terrain of the breeding tract. After quality pruning using different thresholds, the final merged dataset covered 4,68,671 common autosomal SNP markers. The population structure was assessed using principal component analysis

and maximum likelihood-based TreeMix analysis. In contrast, twin methods (iHS and XP-EHH) were used to scan the whole genome for haplotype homozygosity statistics. Multiple chromosomes were observed to harbour SNPs under selective sweeps in different comparisons. TRIM44, SLC5A12, MYO1D, and NAPB were important genes that showed overlap with SNP markers whose frequency was significantly different from the overall hill cattle genome under selection. Various genes were found as part of selection footprints along multiple comparisons, including U6, ADGRL3, RELN, DAP3, STO1, and DNAJA2. A functional analysis of the genes harbouring SNPs under evolutionary pressure (as part of selection sweeps) was undertaken to gain deeper insights into the biological relevance of selection footprints. As part of selection sweeps, the genes were mainly involved in important adaptation traits, including cell-cell signaling, neuronal development, mitochondrial functioning, hyaluronidase functioning, ion-channel transport of solute and nutrients, and response to reactive oxygen species and other stressors. The present study provides detailed insights into the genomic landscape changes resulting from the adaptation of hill cattle to agro-climatic conditions under which they are reared.

ISAGB-2023/Abst/TS-I-019

UNRAVELING X CHROMOSOME WIDE RUNS OF HOMOZYGOSITY IN INDIAN *ZEBU* CATTLE

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Research on Runs of Homozygosity (ROH) and admixture patterns of cattle primarily autosome centric, excluding X chromosome. In the present study, X chromosome-wide SNPs based admixture patterns as well as the size, frequency, distribution, and associated inbreeding (F_{ROH}) were explored in seven Indian native cattle breeds (n =92), classified into dairy, draft, and dual-purpose categories. In order to investigate recent and ancient inbreeding/selection events in each breed, two ROH length classes >1Mb (25 generations ago) and >8Mb (5 generations ago) were examined. The minimum and maximum mean number of ROH on the X chromosome per animal were recorded in Tharparkar (8.31±1.57) and Kangayam (12.15±0.61), respectively. The longest ROH segment was observed in Ongole cattle (143.82 Mb), and the mean genome length covered by ROH on the X chromosome was found to be highest in Tharparkar (79.38 Mb; 57.10%) and lowest in Kangayam (29.05 Mb; 19.50%). Kangayam had the highest F_{ROH}> 1Mb based inbreeding (0.16 ± 0.001), while Sahiwal (0.12 ± 0.001), Gir (0.15 ± 0.01), Hariana (0.15 ± 0.001), and Ongole (0.12 ± 0.01) had intermediate levels. This suggests that Kangayam underwent ancient inbreeding. Inbreeding in Tharparkar cattle, however, is recent, as evidenced by the highest level of inbreeding based on F_{ROH}> 8 Mb (0.44±0.13) and lowest level in Kangayam (0.03±0.02). Based on inbreeding similar to the X chromosome, a study on autosomal ROH of the same Indian cattle breeds revealed F_{ROH}> 1Mb, with the highest levels in Kangayam (0.113 ± 0.059) and intermediate levels in Hariana (0.042 ± 0.031) and Sahiwal (0.043 ± 0.048). Furthermore, autosomal inbreeding based on F_{ROH}>8 Mb produced different results with regard to the X chromosome, where the highest levels found in Kangayam (0.052±0.038) and intermediate levels in Hariana (0.022±0.028) and Sahiwal (0.023±0.040). The levels of admixture on the X chromosome and the chance factor of inheritance through male/female path in successive generations may be the primary causes of the difference in F_{ROH}>8 Mb between the autosome and X chromosome. Therefore, researchers should focus on the F_{ROH}> 1Mb on the X chromosome in order to rule out this chance factor of inheritance. These results are similar to those of the autosome, with the highest levels in Kangayam and intermediate levels in Sahiwal. However, F_{ROH}> 8Mb values based on autosome and X chromosome may differ due to chance factor of inheritance, admixture as well as differential recombination frequency. Genes and QTLs related to immunity, milk production, and reproduction were discovered in high frequency ROH islands. The preponderance of ROH islands with genes linked to reproduction traits was found, particularly in the draught breed Kangayam. This suggests that this breed is more efficient at reproducing than dairy breeds. For X chromosome wide association studies in

the future, the genomic regions enriched with agronomically significant traits identified in this study will be a valuable resource.

ISAGB-2023/Abst/TS-I-020

COMPARATIVE GENOME WIDE SELECTION SIGNATURES USING DIFFERENT DENSITY ARRAY IN SAHIWAL CATTLE

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The Sahiwal breed of cattle is renowned for its exceptional milk production capabilities in the tropics and subtropics environment. The detection of genomic regions as selection signatures can explain the distinctive genetic variations observed in this breed. In the past decades many study has been conducted for detection of selection signature in cattle and few studies also reported for sahiwal cattle from Asian counteries. But the studies reported varies in terms of density of genotyping array and also different density platforms are available. This study was conducted with the aim to compare the selection signature identified by different platform in selecting genotyping array and making genomic studies cost effective in sahiwal cattle. In this study, a total of 245 animal genotyped with medium density (n=193) and high density (n=52) were were considered. The Linkage disquilibrium (LD) based integrated haplotype score (iHS) method were used for detection of selection signature. Both the methods revealed significant selection regions mapping to 491 on medium density and 428 protein coding genes on high density array. The study identified 54 common genes between medium and high density array. The key genes found in both medium and high density array were related with traits like coat color (*KIT*), facial pigmentation (*SLC44A2 and LEF*), lactation persistency (*NNT*), milk fat % (*MAP3K21*), sperm membrane integrity (*OSTC*) and bovine tuberculosis susceptibity (*RNF144A*). The genes identified were further analysed and candidate gene prioritization revealed 20 genes in medium density and 17 hub genes on high density array. The present study identified genes associated with various economic traits using both the arrays.The study indicated not much significant difference in selection signature region and hub genes in both the array. Thus it can be concluded that both the density array can be equally effective in genomic studies and imputation of medium-density array to the high density array can be implemented for cost-effective genomic research.

ISAGB-2023/Abst/TS-I-021

COMPARATIVE ANALYSIS OF SELECTION SIGNATURES IN TRANSBOUNDARY SAHIWAL CATTLE

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Sahiwal cattle is considered as one of the best germplasm in the Indian sub-continent as far as dairy merits are concerned. The original breeding tract lies in the Montgomery in Pakistan, Punjab region of Indo-Pakistan border and Haryana, Rajasthan and adjoining areas of India. There is a limited exchange of Sahiwal germplasm across the border and animals were propagated with differential selection criteria. This lead to unique traits in two populations. The study thus aimed to compare the genomic selection signatures in these two Sahiwal population comprising of n= 193 from ICAR-NDRI, Karnal, India and n=17 from Pakistan genotyped with Illumina Bovine SNP 50K. The quality control of the samples was done using Plink v1.9 software and after quality filtration, further analysis was done. To identify the selection signatures, five statistics were combined known as de-correlated composite of multiple signals (DCMS) viz., F_{ST}, Haplotype Homozygosity (H1), Modified Haplotype Homozygosity (H12), Tajima's D index, and Nucleotide diversity (pi). A total of 115

and 52 protein coding genes from NDRI herd and Pakistan herd were identified which affect a number of important economic traits. The annotation of genes was done through PANTHER 18.0 and the result revealed that traits like milk composition traits (*NEK11*), lactation persistency (*HMGCS1*) was found in Sahiwal cattle of NDRI herd whereas *MCOLN2* gene was associated with immune defence mechanism. *DEK* and *RNF144B* genes were associated with resistance to Bovine Tuberculosis. In case of Pakistan Sahiwal, *ASAP1, RALGAPA2* and *TCEA3* genes were associated with production traits in beef cattle, subcutaneous fat thickness, bovine MDSCs (muscle-derived satellite cells) and *ADAMTS9* was associated with milk protein processing. These findings revealed that selection of Sahiwal cattle in Indian herd is more towards milk production traits whereas selection of Sahiwal cattle in Pakistan herd is more of meat production traits.

ISAGB-2023/Abst/TS-I-022

GENOMIC CLUES TO MITHUN'S UNIQUE QUALITIES: MEAT, RESILIENCE, AND IMMUNITY

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Domestication and artificial selection are the major driving forces responsible for the determinative and huge genetic variability in modern day bovids. One such massive bovine, Mithun (*Bos frontalis*) also known as Gayal, found at varying altitudes (300 – 3000 msl) is very promising meat animal. Its distinct genetic makeup and immune responses reflect its exceptional capacity to withstand local environmental challenges and pathogen pressures, setting it apart as a truly unique bovine species. These distinctive attributes have emerged from the unique genetic imprints etched within its genome, shaped by years of selective pressures and adaptation. For such candidate gene discovery important to its adaptation and evolution, Illumina Bovine 50k bead chip data was obtained WIDDE (**W**eb-**I**nterfaced next generation **D**atabase dedicated to genetic **D**iversity **E**xploration) (http://widde.toulouse.inra.fr/widde/). Data from 21 Mithuns were analyzed in the present study using intra-population summary statistics. Three complementary approaches, including Tajima's D, Runs of Homozygosity (ROH), and Nucleotide Diversity, were utilized to uncover selection signatures. In total, 248 (200), 397 (352), and 146 (138) genetic regions unveiled candidate genes as identified by the respective methods of Tajima's D, ROH, and Nucleotide Diversity. We found several candidate genes related to meat production traits (*MEF2C, MYOD1,* and *RYR3*) and the adaptation of Mithun to suit its ecological habitat in forest (*EPHA5, GPR39,* and *SLC24A3*) and environmental constraints such as high altitude and cold temperatures (*NOS3, ADRB2,* and *PDPK1*). Investigation of immune-related gene selection in the Mithun genome reveals potential adaptations to local environmental challenges and pathogen pressures especially insects and ticks (*CCR1, IL6, IL22,* and *CD25*), shedding light on the genetic basis of immune resilience in this unique bovine species. Furthermore, selection signals found to be part of different quantitative trait loci (QTLs) associated with important traits. However, further studies are warranted to refine the findings using a larger sample size, whole-genome sequencing, and/or high-density genotyping.

ISAGB-2023/Abst/TS-I-023

MOLECULAR CHARACTERIZATION OF *HSP60 G*ENE IN POONCHI CHICKEN

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Heat shock protein 60 (HSP60) is a multifunctional protein, playing a crucial role in protecting organisms from environmental stress. The present study Molecular characterization of *HSP60* gene in Poonchi chicken was undertaken with the objectives of amplifying and sequencing of *HSP60* gene and studying the genetic similarity and distance between different species to local chicken of Poonch. 2 ml blood was collected from wing vein of chicken. Total RNA was extracted from freshly collected chicken blood using All Blood RNA Purification Kit (HiPura) and cDNA was synthesized by reverse transcription from RNA using Revert aid first strand cDNA synthesis kit. *HSP60* gene was amplified from cDNA using specific primers designed by Primer 3 software. The amplified product was purified by GenElute Gel extraction kit and thereafter cloned and sequenced. A partial cDNA sequence of chicken *HSP60* gene of 1002 bp encoding 327 amino acids was obtained. The pair wise distance between sequences aligned with ClustalW method was estimated by MEGA X software. On its comparison, chicken partial cDNA sequence demonstrated 91.30%, 90.61%, 83.63%, 97.46%, 96.85%, 95.83%, 82.67%, 92.35% and 93.37 % homology with pigeon, ostrich, rabbit, guinea fowl, turkey, quail, guinea pig, geese and duck, respectively which indicates close evolutionary relationship and high sequence homology among the species. Deduced amino acid sequence of 327 residues of chicken *HSP60* gene was 98.47%, 96.94%, 96.64%, 99.30%, 98.17%, 99.00%, 96.33%, 98.47% and 99.08% similar to pigeon, ostrich, rabbit, guinea fowl, turkey, quail, guinea pig, geese and duck, respectively. The phylogenetic tree drawn by MEGA X software at nucleotide level showed conserved nature of *HSP60* gene. Maximum divergence from chicken partial cDNA was observed with guinea pig CDS with value of 0.20695875 and minimum divergence was observed with guinea fowl with value of 0.02806869. Z test was conducted in order to test whether positive selection is operating on a gene. It was found selection is of purifying type. From the present study it can be concluded that chicken *HSP60*, is highly conserved among different species. The *HSP60* gene might have evolved by purifying selection (dS > dN) and chicken CDS sequence is the most close to quail, ostrich, turkey, pigeon duck and the most divergent to guinea pig.

ISAGB-2023/Abst/TS-I-024

GENOME-WIDE SNP MINING AND ESTIMATION OF POLYMORPHIC SNPS IN BACHAUR CATTLE (*BOS INDICUS*)

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Bachaur is the first registered draft cattle breed of Bihar. A total of 21 samples of Bachaur cattle breed were analysed in the present study. DNA samples were genotyped with GGP Bovine 50K chip. The results were obtained as intensity data and files were loaded into the Illumina Genome Studio v1.9.0 software, where fluorescent intensities were converted into SNP geotypes. A final custom report from the Genome Studio using Plink Input Report v2.1.1 was used to create ped (Pedigree) file and map (SNP mapped file). A total 74,151 SNPs were obtained. Unmapped and SNPs present on X, Y chromosome and mitochondrial DNA were filtered out from the dataset. Only 69,967 autosomal SNPs were considered for the final analysis. Minor Allele Frequency (MAF) is the frequency of the least common allele of a SNP in a population. It provides the information about the degree of polymorphism and genetic variability in the population, which is helpful

for genomic selection, genetic diversity analysis and genome-wide association studies. Therefore, MAF was calculated by using PLINK v1.9 software. After removing 3,447 missing values, MAF values of the remaining 66,520 SNPs were classified into four groups *i.e,* monomorphic/fixed (MAF = 0), rare (>0 MAF <0.05), intermediate (≥0.05 MAF <0.1) and common (≥0.1 MAF <0.5). The proportion of SNPs under each category was estimated. In Bachaur cattle, the proportion of fixed, rare and intermediate SNPs were, 28.5%, 5.69% and 4.48%; respectively. The proportion of the common SNPs was highest (61.33%) among all the MAF catagories *ie* 40,794 SNPs. The overall mean MAF was found to be 0.2184±0.1838. Further, chromosome-wise MAF was calculated and the value ranged from 0.1863 to 0.2405. The highest and lowest MAF was observed on chromosome 9 and 3; respectively. The proportion of polymorphic or informative SNPs (P_N) at 5% level was found to be 65.81%, which is relatively higher. This indicates the appropriateness of the SNP panel used for genomic study as well as substantial genetic diversity in this breed.

ISAGB-2023/Abst/TS-I-025

GENETIC DISTINGUISHING OF SPPV FROM LSDV BY GENE-SPECIFIC RFLP ANALYSIS

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Sheep pox (SPPV) and Lumpy Skin Disease Virus (LSDV) are significant viral pathogens affecting small ruminants, including sheep and cattle, with the potential for substantial economic losses and cross-species transmission. Both viruses belong to the Poxviridae family and share nearly identical genomic features. They are large, enveloped, double-stranded DNA viruses with genome sizes of around 150 kilo base pairs (kbp), exhibiting approximately 96% nucleotide identity throughout their genomes. To differentiate between SPPV and LSDV, four specific genes have been identified: EEV Maturation Protein Gene, Virion Protein Gene, RNA Polymerase Subunit Gene, and EEV Glycoprotein Gene. Restriction Fragment Length Polymorphism (RFLP) analysis is employed with restriction enzymes that cleave DNA at single recognition sites, generating two distinct fragments for one virus while leaving the other intact. For example, the EEV maturation protein gene (649 bp) is cleaved by AccI and BsawI, yielding two fragments of 233 bp and 235 bp for LSDV, while SPPV remains unaltered. Similarly, the virion protein gene (660 bp) is cleaved by HpyCH4III, resulting in two fragments of 300 bp and 360 bp for LSDV, with no corresponding site in SPPV. The RNA polymerase subunit gene (341 bp) is selectively cleaved by BcivI and EarI, affecting LSDV but not SPPV. The EEV glycoprotein gene (432 bp) is specifically cleaved by BtscI and SfcI for LSDV, with no cutting site for SPPV. Utilizing these enzymes with single cutting sites in partial gene fragments allows for the differentiation of SPPV and LSDV through gene-specific RFLP analysis, enabling accurate diagnosis and disease management in livestock.

ISAGB-2023/Abst/TS-I-026

ANALYSIS OF GENETIC DIVERSITY OF A NEW OVINE GENETIC GROUP "GANG-FATEHPURI" OF UTTAR PRADESH

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The present study was aimed to assess the genetic diversity of a new ovine population "Gang Fatehpuri" in their breeding tracts in Uttar Pradesh based on 15 highly polymorphic, co-dominantly inherited and ubiquitous microsatellite markers. Total 186 alleles were detected across 15 microsatellite loci. The actual number of observable alleles at each locus ranged from 6 (OarCP20) to 23 (MAF214) with an average of 12.

The effective number of alleles ranged between 2.246 (CSSM47) to 13.740 (OarHH41). The average observed heterozygosity was less than the expected heterozygosity. The observed heterozygosity ranged from 0.525 (CSSM47) to 0.977 (OarJMP29) with mean as 0.751, while the expected heterozygosity per locus was varied from 0.555 (CSSM47) to 0.927 (OarHH41) with mean as 0.823. The values of mean observed heterozygosity and mean expected heterozygosity were relatively similar to those of other domestic sheep breeds investigated earlier. The PIC values varied from 0.535 (CSSM47) to 0.923 (OarHH41). Fairly high mean informative estimates of PIC (0.805) supported the utility of used set of microsatellites in biodiversity evaluation of native Indian sheep. Heterozygote deficiency analysis revealed significant deviation from HWE ($p < 0.001$) 11 loci out of 15. The average within population inbreeding estimate (F_{IS}) was observed to be 0.083 ranging from -0.242 (OarCP20) to 0.437 (OarHH41). The low rate of inbreeding may perhaps be due to absence of any directional selection. The findings of Bottleneck analysis suggested the absence of any recent reduction in the effective population size and non-bottlenecked Gang Fatehpuri sheep population. The results show that Gang Fatehpuri sheep possessed a high level of genetic diversity.

ISAGB-2023/Abst/TS-I-027

EXPLORING GENOMIC FOOTPRINTS OF SELECTION IN WORLDWIDE SHEEP POPULATIONS USING MEDIUM-DENSITY SNP DATA

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Domestication followed by natural and artificial selection resulted in the development of various sheep breeds with distinct phenotypic attributes, including morphological, physiological, and adaptability characteristics. These selection processes are expected to impose pressure on certain genomic regions that are detected as selection signatures. Analysis of these signature regions provides insights into the evolution and identification of beneficial mutations associated with economically important traits. The advent of Next Generation Sequencing and SNP genotyping had expanded our ability to detect these signatures of selection. Medium-density SNP data of 3066 animals from 90 different sheep populations worldwide were obtained from ISGC (International Sheep Genomics Consortium). We used single marker based pcadapt approach to detect the genomic footprints of selection. After applying False Discovery Rate (FDR) adjustment at 1% across the genome, we identified 132 genomic regions under selection. Annotation revealed 161 candidate genes overlapping with those genomic regions under selection. Among these, several candidate genes were related to adaptation, growth and carcass traits. Our findings offer valuable insights into the selective sweeps within the Sheep genome, potentially indicating adaptive and productive significance. This may further assist in genome-wide association studies, genomic selection, developing breed-specific SNP panels, implementation of breed improvement, and conservation programs in Sheep.

ISAGB-2023/Abst/TS-I-028

EXPLORATION OF DELETERIOUS MUTATIONS IN MURRAH BUFFALOES ANALYSING GENOME SEQUENCE DATA

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Murrah, known as the "black gold of India," holds a distinguished reputation as one of the finest milk-producing buffalo breeds in the world. It has been extensively utilized in various breed improvement programs within the country as well as globally. Extensive use has also raised the concerns about the potential spreading of harmful DNA mutations, and consequently, requires to study for their occurrence in the population. The present study was intended to identify genome-wide deleterious non-synonymous SNPs in Murrah buffalo (*Bubalus bubalis*) using *in silico* approach. The ddRAD sequence data from 96 Murrah buffalo was aligned with the buffalo reference genome (UOA_WB_1) and a total of 814,919 single-nucleotide polymorphisms (SNPs) were identified at read depth 10. These SNPs were filtered down to 4,742 non-synonymous SNPs (nsSNPs) after annotation using SnpEff tool and further analyzed for functional consequences by employing the Variant Effect Predictor, PANTHER, and Consensus Classifier PredictSNP tools. A total of 47 nsSNPs mapped to 41 genes were commonly predicted as deleterious by these tools. The functional annotation of the mapped genes with deleterious mutations revealed a predominant association with catalytic activity. Six genes (OR10AG83, FAM135B, SQLE, RB1CC1, SDR16C5 and ARFGEF10) at chromosome 14 had high concentration of deleterious mutations. As a lead, these identified mutations may be further analysed for functional impact by using supporting analyses and experiments.

ISAGB-2023/Abst/TS-I-029

GENOMIC FOOTPRINTS FOR HIGH-ALTITUDE ADAPTION IN NATIVE CATTLE, YAK AND THEIR HYBRIDS OF TRANS-HIMALAYAN REGION OF LADAKH (UT) OF INDIA

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Ladakh (UT) is in high-altitude Trans Himalayan region (3000 meter and above from msl) of Northern India, characterized by extreme low temperature, hypoxia, high UV radiation and low precipitation. Native animal populations are well adapted to such extreme climatic conditions; possibly due to intense selection-based evolution. Ladakhi cattle (*Bos indicus*) and Ladakhi yak (*Bos grunniens*) are native bovine populations of Ladakh. Yaks are generally adapted to extremely high altitude, distributed mainly at above 5000 meters of msl, whereas, the cattle are more distributed between 3000 to 4000 meters of msl. Cross-hybrids (*Bos indicus* x *Bos grunniens*) of these species are well adapted to transitional regions of these two species. Owing to different morphological, physiological and biochemical adaptive changes, some unique kinds of genomic evolutions are presumed in these species. To dissect the differences of molecular evolution, DNA samples of 12 each of cattle and yak and six of cross-hybrids were sequenced using double digestion restriction-site associated DNA (ddRAD) technique. Further using a pipeline of online bioinformatic tools, species-specific selection sweeps as well and commonly selected genes were also identified in these native stocks. A total of 4985 and 1168 and 1945 selection sweeps were identified in Ladakhi cattle, Ladakhi yak and their hybrids, respectively using iHS method. However, selection sweeps to tackle the high-altitude stressors responded to different sets of genes in cattle and yak. Major co-selected genes pertaining to adaptive processes (hypoxia, cardiac energy metabolism regulation) in three bovines were also identified using the XP-EHH based pairwise comparison.

The study dissects the genomic regions of three native bovines under selection to high altitude and identify common as well as diverging genomic regions, evolved in view of high-altitude adaptation in native cattle, yak and their hybrids in Ladakh .

ISAGB-2023/Abst/TS-I-030

GENOMIC SELECTION SIGNATURES IN SAHIWAL CATTLE REVEAL GENES ASSOCIATED WITH ECONOMICALLY IMPORTANT TRAITS

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Selection signatures are genomic regions that have experienced, or are currently subject to selective pressures of natural or artificial origin. These signatures in the genome serve as markers of sequence variations that are functionally significant, shedding light on the impact of evolutionary forces, especially recent selection on the genetic makeup of a domesticated animal populations. High-density genotyping offers a valuable tool for precisely pinpointing genomic areas that have experienced positive selection. Through the application of double digest restriction site associated DNA (ddRAD) sequencing, the present study aimed to discern signatures of positive selection within the Sahiwal cattle maintained at the Livestock Research Complex of NDRI, Karnal. Dataset comprising 67823 autosomal single nucleotide polymorphisms (SNPs) in 36 female Sahiwal cattle was used to detect selection signatures by applying three statistical methods, namely composite likelihood ratio (CLR), integrated haplotype score (iHS) and Tajima's D. Genes associated with the olfactory response (OR4C46), heat tolerance (BHMT2, ZFP91), immune response (IL18RAP, SH3BP2), productive life length, pregnancy rate and calving ease (TMEM61) reproductive performance (ADIPOR2, OVOS2, and RBBP8), lactation persistency (RABGGTA), fertility (SCP2D1) and initiation of puberty (SPIN1) were detected as being under selection by at least two methods. The PANTHERdb protein classification analysis unveiled a significant proportion of genes predominantly involved in the catalysis of metabolite interconversion reactions, indicating their pivotal role in cellular metabolism. The enrichment analysis highlighted the prominence of the WNT signalling pathway, which plays a significant role in reproductive tissues and in early pregnancy events. Although, this study could not distinguish the signatures due to artificial and natural selection, the prominence of signatures in genomic regions associated with economically important traits are underlined.

ISAGB-2023/Abst/TS-I-031

GENOME WIDE RUNS OF HOMOZYGOSITY REVEALS SELECTION SIGNATURES AND INBREEDING IN INDIAN MILCH CATTLE BREEDS

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Runs of homozygosity (ROH) can be used to study population history, estimate genomic inbreeding, understand the genetic basis of complex traits and diseases, and identify genes linked to economically important traits in livestock. In the present investigation, ROH were characterized in 198 animals of four native cattle breeds (Gir-50, Rathi-49, Sahiwal-50, and Tharparkar-49) using BovineLD Chip. During the analysis, minor allele frequency with 0.05, hwe with 0.001 were used, SNPs with more than 10% missing genotypes were also removed. Further, following parameters: --homozyg-window-snp 50 ,--homozyg-snp 40, --homozyg-kb 1000, --homozyg-gap 1000, --homozyg-density 1000, --homozyg-window-threshold

0.05, --homozyg-window-missing 5, --homozyg-window-het were used for ROH detection in Plink v1.9. The results revealed highest number of ROH in Tharparkar cattle (1062) while the lowest in Sahiwal cattle (366). The average Froh in Gir, Rathi, Sahiwal, and Tharparkar were found to be 3.11873E-07, 2.22184E-07, 1.47199E-07, and 4.3971E-07, respectively. Further, Tharparkar was the only cattle containing common ROH regions in more than 20% of the individuals. These regions were detected on chromosome 2,3,7,10,12,23. The common ROH regions were annotated and genes were identified. Further, identified genes were used for pathways analysis. This study has provided the valuable information for understanding the genetic basis of relevant traits.

Technical Session - II

ISAGB Young Scientist Award

ABSTRACTS

National Conference

Advances in Genetics and Genomics
for Sustainable Livestock Transformation

&

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ISAGB-2023/Abst/TS-II-001

DEEP LEARNING FOR DISCERNING NATURAL SELECTION IN THE BOVINE GENOME

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High-throughput genomic sequencing coupled with machine learning allows us to disentangle the evolutionary forces acting in populations. The discovery of natural selection signatures in the cattle genome has the potential to reveal the genetic processes underlying important candidate genes. Popular techniques for finding these signals rely on compressing genomic data into summary statistics, which results in information loss. However, this issue can be addressed by detecting loci under spatially varied selection. We utilized a deep learning-based framework, DeepGenomeScan, which can detect signatures of spatially varying selection and was initially developed for the human genomic dataset. We constructed a deep neural network with two hidden layers using DeepGenomeScan. We used geographic coordinates (longitude and latitude) as response variables to detect the spatially varying selection. The generalized model, Y=G (WX+b), in which Y is the geographic coordinates and X is the genotypes (54,610 SNPs), was used to build the multilayer perceptron (MLP). We applied DeepGenomeScan to an indicine and taurine cattle genetic dataset and identified 50 well-known genes under selection harbored under 112 loci. These include *ADCY8, KCNQ1, SP110,* and *DACH1* having a crucial role in milk production, mastitis, paratuberculosis, and feed efficiency respectively. These important genes were not identified by traditional summary statistic methods when applied to a similar dataset earlier in other studies. Our approach also identified several additional outlier SNPs that are not located in known genes. However, they might be related to regulatory genes, making them interesting for research on the genetics of cattle.

ISAGB-2023/Abst/TS-II-002

TRANSCRIPTOMIC PROFILING OF HEART TISSUES IN CHANGTHANGI AND MUZZAFARNAGRI SHEEP: INSIGHTS INTO ADAPTIVE GENE EXPRESSION PATTERNS

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Sheep are invaluable genetic resources integral to Indian agriculture and economies. Among 44 registered Indian sheep breeds, Changthangi sheep demonstrate remarkable resilience in the challenging Himalayan environment, showcasing their adaptability. In the current study, the transcriptome profile of Changthangi sheep was compared with Muzzafarnagri sheep (low-land), to understand the genes and pathways enabling them to thrive in their respective habitats. cDNA libraries were prepared from heart tissue samples of four animals each of Changthangi and Muzzafarnagri sheep, all of the similar age, sex and raised under similar conditions. RNA sequencing was conducted using the Illumina HiSeq 2000 platform, producing 90-140 million reads per library. Quality assessment was performed with FastXToolKit, and reads were mapped

to known *Ovis aris* reference genome (GCA_000298735.1_Oar_v4.1_genomic.fna). A total of 173 known differentially expressed genes with p value ≤ 0.05 and a fold change of ≥2 were identified. Out of these, 51 genes including *ADRB3, DRD1, ACE, AGT* were upregulated and 122 genes including *WNT2, FOS, FOSB, HSPA6* were downregulated in Changthangi. Gene ontology of these DEGs revealed their association with regulation of blood pressure, positive regulation of heart rate, adenylate cyclase-activating G-protein coupled receptor signalling pathway, diet induced thermogenesis, regulation of systemic arterial blood pressure by renin-angiotensin. These DEGs were also involved in cellular components like extracellular matrix, plasma membrane and smooth muscle contractile fiber. The predicted pathways enriched for these genes were Fluid shear stress and atherosclerosis, Neuroactive ligand-receptor interaction, IL-17 signalling pathway. At high-altitude, these processes facilitate adaptation by increasing heart rate and blood pressure to enhance oxygen delivery, activating thermogenesis to generate heat in cold conditions, ensuring the body's efficient response to reduced oxygen levels and cold temperatures. The present study therefore, contributes to a better understanding of gene expression in heart muscles of sheep breeds adapted to diverse environments.

ISAGB-2023/Abst/TS-II-003

APPLICATION OF STATISTICAL AND MACHINE LEARNING FOR CLASSIFICATION OF CATTLE BREEDS AND LINEAGES USING MINIMUM INFORMATIVE MARKERS

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Assigning individual animals to their respective breed lineages has great importance in the analyses of evolutionary history of global cattle populations besides detecting the genetic variation that likely facilitated the adaptation of these breeds to environmental conditions and discovering the geographic patterning of genetic variation in cattle. The present study was undertaken to select the minimum number of SNP markers, originally generated using medium density Bovine BeadChip, that will be helpful to assign individual animals to its corresponding lineage and respective populations within that lineage using two well-known supervised machine learning (ML) algorithms, Random Forest (RF) and the XGBoost. Each of the two ML models was trained with the most informative SNPs panels (of 48, 96 and 192 size) that were selected using two statistical methods (PCA and F_{ST}), and two ML methods (RF with Gini, and RF with MDA). Using these four SNPs selection approaches, three panels of 192, 96 and 48 SNPs consisting of the topmost discriminant SNPs were created for each SNPs preselection approach. We evaluated these panels based on their performance vis-à-vis assignment of each animal to one of the lineages/ population groups (either African or European or Indicine lineage or admixed) as well as assigning the animal to its breed/population within its prospective lineage/ group. The results showed that XGBoost achieved the best accuracy of 95% with 192-SNP panel selected by RF with MDA followed by RF that achieved an accuracy of 93% with 192-SNP panel selected by RF with either GINI or MDA for animal to lineage assignment. Similarly, for animal to breed/population assignment, RF trained with 48-SNP panel selected by RF with Gini algorithm achieved the best accuracy of 97% for assigning animal to African breeds, while it achieved the best accuracy of 89% for assigning animal to admixed populations using 96-SNP panel selected by PCA. On the other hand, XGBoost achieved the best accuracy of 88% for assigning animal to European breeds using 192-SNP panel selected by F_{ST}. On the other hand, the results with both RF and XGBoost achieved a poor performance of assigning animals to Indicine breeds as the best accuracy for such assignment was 66% and was achieved using RF with 192-SNP panel selected by F_{ST}. In conclusion, the study reports the applicability of statistical and machine learning approaches for identification of discriminatory SNPs for assignment of individuals to corresponding lineages and to respective populations within lineages. The machine learning approach was more useful for assignment of individual to specific lineages while it faired comparable for assignment of individuals to populations within specific lineages or population groups.

ISAGB-2023/Abst/TS-II-004

DISCOVERING THE GENETIC UNIQUENESS OF NON- DESCRIPT CATTLE POPULATIONS OF MAHARASHTRA

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India has registered 53 cattle breeds adapted to different agro-climatic conditions; thus, breeding and management practices differ for breeds catering to a variety of traits and utilities, *viz.*, dairy, draft and dual use. In the present study, genomic compositions of the targeted non-descript populations *viz.*, Kamgaon, Vidarbha White, and crossbred, were analyzed. A total of 51,600 autosomal SNPs shared between the GGP indicus 50K and Illumina HD chip genotyping platforms were retained for analysis in order to maintain methodological consistency. The top 1000 Ancestry Informative Markers (AIMs) were identified based on delta values, F_{ST} and I_n, out of which 207 Breed Informative Markers (BIMs) and 17 Utility Informative Markers (UIMs) were extracted using the ensemble model to classify individuals as per breed and utility, respectively. Upon application of quality control, 50,033 SNPs and 314 individuals were retained. Admixture analysis revealed 67.84% and 30.75% uniqueness in Kamgaon and Vidarbha White, respectively, while crossbreds were found to be highly admixed. Recent admixture was traced 11.35, 10.5 and 1 generations ago, in Kamgaon, Vidarbha White and crossbred, respectively while ancient admixture was traced 37.5 generations ago in crossbred. Local ancestry analysis in crossbred revealed post admixture positive selection (PAPS) signatures addressed within BTA- 4, 11, 17 and 26 attributed to *Bos indicus* and BTA- 5, 12 and 15 attributed to *Bos taurus.* The *f3* statistics revealed crossbred and Vidarbha White were admixed but not detected any significant admixture in Kamgaon cattle. Based on evidence, Kamgaon can be considered for breed registration, while grading-up can be suggested for Vidarbha White. These insights offer valuable contributions to cattle genetic research and inform strategies for effective breeding programs.

ISAGB-2023/Abst/TS-II-005

GENOMIC REVELATIONS OF ZEBU AND TAURINE INTROGRESSION IN KARAN FRIES CROSSBRED CATTLE

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Crossbreeding was pivotal in India's self-sufficiency in milk production. In alignment with this approach, ICAR-NDRI developed Karan Fries (KF) cattle by crossbreeding zebu Tharparkar cows with *taurine* Holstein Friesian, Brown Swiss, and Jersey bulls, intending to increase milk yield while retaining heat tolerance and disease resistance characters of indigenous cattle. The present research aimed to ascertain genomic divergence and inheritance stability across seven overlapping generations spanning four decades of KF development. Additionally, we investigated the local ancestry of KF cattle to explore the genomic regions exhibiting higher recombination from parental breeds. The study was conducted utilizing 777k Bovine HD SNP array genotyping data of KF cattle (n=44) and its parental breeds: Tharparkar (TP=48), Holstein Friesian (HF=60), Brows Swiss (BS= 22), and Jersey (JS=31).The results revealed that KF cattle exhibited closest genetic relationship to taurine HF cattle in terms of genetic distance (F_{ST}=6.5%) and PCA based clustering.Population structure revealed KF cattle as an admixture with the greatest contribution from HF (50.6%), followed by TP (38.3%), JS (5.12%), and BS (5.98%). There was no sub-structuring present within the KF population.KF can be classified as a two-breed cross, primarily derived from HF and TP, as Brown Swiss (BS) and Jersey (JS) genetic contributions are notably lower, and the breeding strategy involves only HF and TP. The local ancestry inference revealed average TP and HF ancestry for the autosomes ranged from

0.28 (TP) and 0.72 (HF) to 0.44 (TP) and 0.56 (HF). Average genomic ancestry was 0.37 (TP) and 0.63 (HF) which was in concordance with the global ancestry of KF cattle. The selective sweep regions of KF cattle that were rich in TP ancestry harbored tropical adaptation genes (ADAM19, MAT26, PLCH2, PEX10, RER1, MORN1, SKI, FAAP20, PRKCZ) and HF rich regions comprised genes associated with reproduction traits (RIPPLY2, TASP1, ADAP2, VEGFA, PSMB8, ADIRF, GLUD1, GDF2, GDF10, ARGHAP22), udder health (MAPK8), and milk production traits (CRYB5R4, RHBDL3, KCNMA1, SNCG). The study shows Karan Fries cattle achieved the desired level of exotic and indigenous inheritance, forming a distinct crossbred with introgressed genes related to milk, fertility, and adaptation from parental breeds.

ISAGB-2023/Abst/TS-II-006

ANALYZING THE COMPLETE GENOME OF INDIAN NATIVE GOATS ALONGSIDE GLOBAL BREEDS UNCOVERS RICH WITHIN BREED GENETIC DIVERSITY AND DISTINCT POPULATION STRUCTURE

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The genomic diversity of Indian goats displays varying degrees of adaptability, productivity, and resistance to diseases, while thriving in extreme conditions that offers valuable insights for establishing a sustainable goat production system. We studied diversity of 11 Indian breeds (n=102, Assam Hill-ASL, Changthangi-CHA, Jhakrana-JAK, Jharkhand Black-JB, Kanni Adu-KAN, Kahmi-KAH, Marwari-MAR, Sangamneri-SAN, Surti-SUR, Tellicherry-T, Uttarakhand Local-UK) and compared them to worldwide goats from 30 breeds and 5 outgroups (n=101, public domain). Indian goat samples were sequenced using Illumina NOVASEQ6000 platform. Sequences were combined to generate a VCF file; annotation was done using the *Capra hircus* reference. Nucleotide diversity(π), inbreeding coefficient(F), observed (H_o) and expected heterozygosity (H_e), proportion of polymorphic SNP (P_N), average pairwise genetic distance (D_{ST}), effective population size (N_e), linkage disequilibrium (LD, r^2) and LD decay were obtained. NJ tree, PCA and Admixture analysis were carried out to assess population structure. 21.44 billion reads remained after quality control and alignment with ARS1 reference genome. Average coverage was 99.2% and17.2%duplication rate; depth of sequencing was ~9X. Kanni Adu (KAN) had 1.95M SNP while Jharkhand Black (JB) had 30.77K; average r^2 was highest in JB (0.859) and lowest in Jakhrana (JAK) (0.496). LD showed rapid decay(r^2<0.2) within 5kb in all the breeds except JB, Sangamneri (SAN) and KAN. Changthangi (CHA) (0.379) and JAK (0.363) had the highest π, while KAN had the highest P_N (0.340). Average Ho was lowest in JB (0.119) and highest in CHA (0.505); He was lowest in KAN (0.249) and highest in CHA (0.378). KAN, Tellicherry (T), SAN and JB were distinct in PCA. Admixture graph at k=2-12 showed that k=8 had minimum error (0.423). PCA revealed that other Capra species were distinct from *C. hircus*. Indian goats were widely distinct from exotic; 3 breeds from Pakistan clustered with Indian goats. Goats spread over the Indian subcontinent have high within breed diversity (>94%), harbouring substantial genetic variations to respond to future demands and climate change. Within breed diversity, gene flow among indigenous animals and shared genomic regions are contributors for admixed nature of animals. The distinct breeds based on the population structuring were JB, KAN, SAN and T. Selection sweeps identified in JB, KAN, SAN and T, compared to all the other Indian breeds, using Decorrelated Composite of Multiple Signals (DCMS) demonstrated that they shared huge similarities in the protein coding genes found in the selected regions compared to other Indian goats. Selected regions that coded for miRNAs that were differentially expressed in many reported studies. The historical evolutionary patterns and varied genetic bottlenecks may have resulted in shared genomic regions being selected.

ISAGB-2023/Abst/TS-II-007

EFFECTS OF ALLELIC VARIANTS OF STAT GENES ON MILK FAT AND PROTEIN YIELD IN HOLSTEIN FRIESIAN CROSSBRED CATTLE

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The present study was conducted to find polymorphisms in STAT1 and STAT5A genes and their associations with milk fat and protein yields in Holstein Friesian (HF) crossbred cattle. Milk composition data of first lactation of 222 adult HF crossbred cows for a period of 12 years i.e., 2009 – 2020 was collected from the records maintained at Livestock Farm, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. Data was corrected for various non-genetic factors viz. season of calving (SoC), period of calving (PoC), interaction effect of season and period of calving (SoC*PoC); and age at first calving (AFC). After data correction, these animals were divided into two groups based on milk fat and protein yields. Out of 222 cows, 40 animals (20 from each group) were selected with highest and lowest milk fat and protein yields, respectively for blood sample collection and DNA extraction. Specific regions of STAT1 and STAT5A genes were amplified and further subjected to PCR-RFLP technique using *BspHI and AvaI* restriction enzymes, respectively. Sequence alignment revealed 2 SNPs at position 201 (C>T) and 260 (C>T) in 314 bp long fragment of STAT1 gene. The genotype frequencies for AA and Aa were observed to be 0.80% and 0.20% at 201 nucleotide position; and for BB and Bb were observed to be 0.25% and 0.75% at 260 nucleotide position, respectively. Due to close proximity of SNPs, only 3 genotypes were found, thus indicating presence of linked alleles A & B. A significant association of all genotypes for STAT1 with milk fat and protein yields was observed. For STAT5A gene, a monomorphic pattern at 181bp and 34 bp were found in all the individuals. This indicates that the locus for this gene is fixed with only the presence of homozygotes in the studied population. These SNPs can prove to be very useful in various marker-assisted selection and breeding programs in HF crossbred cattle.

Technical Session - III

Animal Breeding Strategies in the Era of
Genomics and Phenomics

ABSTRACTS

National Conference

Advances in Genetics and Genomics
for Sustainable Livestock Transformation

&

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ISAGB-2023/Abst/TS-III-001

ASSESSMENT OF THE EFFECT OF VARIOUS FACTORS ON THE CONCEPTION RATE IN SEX-SORTED SEMEN-INSEMINATED BUFFALOES OF RAJASTHAN

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A study was performed for a period of approx. 3.5 years (Feb. 2020 to August 2023) at various BAIF cattle development centres (CDC) in Pali district of Rajasthan to evaluate the performance of sex-sorted semen in Murrah Buffaloes. A total of 4955 Buffaloes were inseminated with sex semen to observe the effects of genetic and environmental factors on the conception rate. Several variables were considered for the study, including the dam's breed, body condition, lactation number and the season of insemination. It was observed from statistical analysis of data that the overall conception rate was 34.99±0.68% in buffaloes. The conception rate was significantly affected by body condition, lactation number and the season of insemination ($P<0.001$) while there was no significant difference ($P>0.05$) found due to the dam's breed. The highest conception rate was noticed in the winter season (37.04%) as compared to the summer (33.71%) and rainy (29.71%) seasons in sex semen-inseminated buffaloes. The lowest conception rate percent was recorded in buffalo heifers with 28.51% which went on to increase till the third lactation (43.67%) being the highest whereas the highest CR (44.16%) was noted in three ribs exposed Murrah buffaloes. while the sex ratio of female to male was found to be 88: 12.

In conclusion, considering these genetic and non-genetic variables influencing the conception rate in sex sorted semen-inseminated buffaloes may improve the outcomes of sorted semen technology, resulting in increased milk productivity.

ISAGB-2023/Abst/TS-III-002

DELIVERING IMPROVED DAIRY GENETICS THROUGH NEW TECHNOLOGY INTERVENTION OF SORTED SEMEN TECHNOLOGY IN RAEBARELI DISTRICT UTTAR PRADESH

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The current research aimed to assess the conception rate (CR) and sex ratio after artificial insemination using sex-sorted semen in Gir, Sahiwal & crossbreed cattle of farmers from Raebareli district of Uttar Pradesh. Main objective of this study was -To cover more number of farmers under AI with Sorted Semen. To popularize Sorted Semen technology to produce more number of female calve. Overall increase milk production and income of farmers towards contributing to doubling of farmer income. Sexed sorted semen was supplied from BAIF semen production, Pune using the technology process of sexing technologies, USA. The study was conducted at different cattle development centres operated by the BAIF Institute for Sustainable Livelihoods and Development (BISLD) in Uttar Pradesh, from April 2021 to July'2022. Total of 1200 insemination were performed to animals belonging to 811 farmers.

The findings revealed an overall CR of 53.13% and a female-to-male sex ratio of 91.36%. In terms of the sex ratio, cattle had 91.36% females over males. Conception rate recording in Gir, Sahiwal, Holstein-Friesian cross and Jersey Crossbreed cattle was 57.3±9.04%, 50.5±6.07%, 53.4±5.45%, 49.8±6.56% respectively. In season with study highest conception rate was observed in Winter 55.7±5.94%, followed by Summer 54.8±5.8% & Rainy season 48.9±5.95%. The promising results obtained with sex-sorted semen have instilled confidence among artificial insemination technicians and farmers, encouraging the adoption of this technology.

Additionally, this technology presents an opportunity for the accelerated multiplication of offspring with desired genders, benefiting livestock breeders.

ISAGB-2023/Abst/TS-III-003

PHENOTYPIC CHARACTERIZATION OF A DISTINCT GOAT POPULATION FROM VINDHYAN REGION OF EASTERN UTTAR PRADESH

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The study was undertaken to characterize the goats of Vindhyan region, especially Sonebhadra districts of Eastern Uttar Pradesh under a project funded by UP Council of Agricultural Research (UPCAR). The morphological and morphometric data were recorded from 80 male and 302 female goats spanning over 9 blocks and 45 villages. The study revealed that these goats were small in size with black or brown coat colour. Both sexes had small to medium sized flat and spiral/twisted horns, of which, most of them had curved shaped with upward-backward-outward orientation. Ears were medium sized, flat and leafy in shape with pendulous orientation. Forehead was straight to slightly convex; eyelids were light to dark black; muzzle and hooves were mostly black. Tail was short to medium in size, bunchy and curved upward. Beard was present on 36.91% of goat. The average (Mean ± SE) adult body weight, body length, height at withers and chest girth were 30.78±0.40 kg, 65.92±0.42 cm, 72.76±0.46 cm and 77.14±0.67 cm, respectively in males and 28.85±0.13 kg, 64.77±0.14 cm, 69.41±0.21 cm and 74.45±0.19 cm, respectively in females. The horn, ear and tail length were 13.72±0.33, 17.82±0.20 and 18.26±0.45 cm, respectively in male and 12.77±0.10, 17.70±0.09 and 15.86±0.14 cm, respectively in females. These goats were found to seasonal breeder which exhibit signs of estrus mostly during summer (64.7%) followed by rainy season (27.45%). The age of first estrus was recorded as 11.4±0.34 months whereas duration of estrus cycle and estrus were observed as 19.90±0.26 days and 27.65±1.28 hours, respectively. Natural service was practiced for breeding. These goats are reared by the farmers and tribes for meat purpose under low input extensive management system. The majority of flock size rages from 4-45 per farmers. This goat was found to be phenotypically distinct from the other goats of the adjoining area and has potential to be recognized as a new breed. This goat may be named as Vindhi or Sonpari after its distribution in Vindhyan region/Sonbhadra district.

ISAGB-2023/Abst/TS-III-004

ECONOMICS OF GOAT FARMING IN TRIBAL AREAS OF RAJASTHAN

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The aim of present study was to assess the feed intake and feeding management practices of tribal farmers in Rajasthan. A total of 120 tribal goat farmers were selected from 12 villages from 6 blocks in 3 tribal dominated districts viz., Banswara, Dungarpur and Udaipur. Ten farmers from each village were selected purposively based on the number of goats. The selected goat farmers were grouped into three categories based on flock size as small (<25 goats, N= 60), medium (26-50 goats, N = 36) and large (>50 goats, N = 24). The data on feed intake and feeding management practices were recorded on-field. A half of goat farmers (51.57 %) were adopting partial grazing followed by complete grazing (48.33%) and none of the selected farmers was

practicing complete stall feeding. About two-thirds of farmer (75%) sent their goats for grazing for more than 5 hours daily and the remaining 25 per cent farmer sent their animals for grazing for less than 5 hours. The proportion of goat farmers who sent their animals for more than 5 hours was 76.67, 66.67 and 83.33 per cent among small, medium and large farmers respectively. Most of goat farmers (77.5%) were feeding colostrum after the birth of the kids whereas some of the farmers (22.5%) were not feeding colostrums at all to kids at all due to the myth of spread of diseases. About a half of the farmers (49.17 %) offered grasses, fodders (berseem, lucerne, bajra, jowar and oats) followed by 34.16 per cent goat farmers feeding tree leaves (ber, neem, babool, khejri) and 16.67 per cent farmers were feeding weeds and grass (stylo, cenchrus spp., crop weeds) and about the same number of farmers (16.66 %) in small, medium and large flock size practiced feeding weeds and grass (stylo, cenchrus spp., crop weeds). It was observed that overall average amount of green fodder offered to milking goats, dry goats, goatlings, kids and breeding buck was 1.33 ± 0.07, 0.85 ± 0.07, 0.45±0.03, 0.37±0.02 and 1.71±0.10 kg/day respectively. The average amount of green fodder offered daily was significantly (p<0.05) higher in case of small farmers as compared to medium and large goat farmers in case of milking as well as dry goats and significantly higher in case of breeding bucks in case of large farmers as compared to other categories of farmers. The overall available dry fodders fed to milking goats, dry goats, goatlings, kids and breeding buck was 0.95±0.67, 0.93±0.07, 0.87±0.06, 0.37±0.02 and 1.72±0.11 kg/day respectively. Being significantly (p<0.05) higher in small farmers followed by medium and large goat farmers. Overall average amount of concentrate mixture offered to milking/pregnant goats, dry goats, goatlings, kids and breeding buck was 210.09±14.26, 85.37±6.84, 86.76±5.83, 85.65±5.86 and 246.11±16.89 g/day respectively. Being significantly (p<0.05) higher in small farmers followed by medium and large farmers among milking goats and breeding bucks. A sizable majority of farmers (56 %) were offering fattening ration to their male kids for their higher body weight gain so that they attain early market weight and on an overall average 255.79±7923.12 g of concentrate mixture per buck/day was fed as fattening ration. The overall total DM intake through stall feeding in case of milking goats, dry, goatlings, kids and breeding bucks was 1.16, 0.90, 1.01, 0.52 and 1.38 kg respectively. The total DM intake in different categories of goats was similar among the three flock size categories. It was concluded that feeding management practices were mostly traditional without much regard to scientific recommendations. However, these management practices in general were better in case of small farmers as compared to medium and large farmers.

ISAGB-2023/Abst/TS-III-005

EFFECT OF SELECTED MANAGEMENT INTERVENTIONS ON GROWTH AND PRODUCTION PERFORMANCE OF GOATS IN TRIBAL AREAS OF RAJASTHAN

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among small, medium and large farmers respectively. Most of goat farmers (77.5%) were feeding colostrum after the birth of the kids whereas some of the farmers (22.5%) were not feeding colostrums at all to kids at all due to the myth of spread of diseases. About a half of the farmers (49.17 %) offered grasses, fodders (berseem, lucerne, bajra, jowar and oats) followed by 34.16 per cent goat farmers feeding tree leaves (ber, neem, babool, khejri) and 16.67 per cent farmers were feeding weeds and grass (stylo, cenchrus spp., crop weeds) and about the same number of farmers (16.66 %) in small, medium and large flock size practiced feeding weeds and grass (stylo, cenchrus spp., crop weeds). It was observed that overall average amount of green fodder offered to milking goats, dry goats, goatlings, kids and breeding buck was 1.33 ± 0.07, 0.85 ± 0.07, 0.45±0.03, 0.37±0.02 and 1.71±0.10 kg/day respectively. The average amount of green fodder offered daily was significantly (p<0.05) higher in case of small farmers as compared to medium and large goat farmers in case of milking as well as dry goats and significantly higher in case of breeding bucks in case of large farmers as compared to other categories of farmers. The overall available dry fodders fed to milking goats, dry goats, goatlings, kids and breeding buck was 0.95±0.67, 0.93±0.07, 0.87±0.06, 0.37±0.02 and 1.72±0.11 kg/day respectively. Being significantly (p<0.05) higher in small farmers followed by medium and large goat farmers. Overall average amount of concentrate mixture offered to milking/pregnant goats, dry goats, goatlings, kids and breeding buck was 210.09±14.26, 85.37±6.84, 86.76±5.83, 85.65±5.86 and 246.11±16.89 g/day respectively. Being significantly (p<0.05) higher in small farmers followed by medium and large farmers among milking goats and breeding bucks. A sizable majority of farmers (56 %) were offering fattening ration to their male kids for their higher body weight gain so that they attain early market weight and on an overall average 255.79±7923.12 g of concentrate mixture per buck/day was fed as fattening ration. The overall total DM intake through stall feeding in case of milking goats, dry, goatlings, kids and breeding bucks was 1.16, 0.90, 1.01, 0.52 and 1.38 kg respectively. The total DM intake in different categories of goats was similar among the three flock size categories. It was concluded that feeding management practices were mostly traditional without much regard to scientific recommendations. However, these management practices in general were better in case of small farmers as compared to medium and large farmers.

ISAGB-2023/Abst/TS-III-006

LEVERAGING MACHINE LEARNING ALGORITHMS FOR INDIAN CATTLE BREED TRACEABILITY WITH SNP MARKERS

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Allocation or assignment tests use genetic information to determine an individual's population membership, providing the most direct methods for determining the population of origin of unknown individuals. SNP chip data has been used for the identification of several breeds/species in recent years, thanks to the rapid development of single nucleotide polymorphisms (SNPs) Bead Chips and the availability of public databases. In the current investigation, we used Bovine SNP 50K chip data to tailor breed-specific SNPs for the target population, i.e., Tharparkar cattle breed via machine learning models for the first time in an Indian animal genomics scenario. We used a total of eight populations in the study, involving several exotic cattle breeds as well. This was done to increase the robustness of the final SNP panel. Machine learning algorithms, a genome-wide association study (GWAS), linkage disequilibrium (LD) analysis, and principal component analysis (PCA) were used to distinguish a target (case) group for comparison with control chicken groups. A total of 216 individuals were available, including 72 of Tharparkar cattle breed after quality control comprising 8,765 SNPs in total. After applying more filter parameters, we had a total of 500 SNPs to work with machine learning models. Moreover, 23, and 48 SNPs were selected as the minimum numbers of markers by the AdaBoost, Bagging Tree, Gradient Boosting Machines, and Random Forest machine learning classification models, which had accuracy rates of 95.2%, 95.2%, 98.4%, and 98.4%, respectively for 23 SNPs panel. For

48 SNPs panel accuracies were as follow, 98.4%, 95.2%, 96.8%, and 98.4%, respectively in same order. The GWAS, PCA, and machine learning algorithms used in this study are efficient in determining the optimal marker combination with the fewest markers that can distinguish the target population from a large number of SNP markers.

ISAGB-2023/Abst/TS-III-007

GENETIC PARAMETERS AND CORRELATIONS OF BODY WEIGHT TRAITS IN MITHUN (*BOS FRONTALIS*) USING ANIMAL MODEL

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This study focuses on the analysis of body weight traits in Mithun (*Bos frontalis*) using an animal model. Eight body weight traits were investigated at various time points, ranging from body weight at week 1 to body weight at month 45. The study considered fixed effects, including the season of birth (categorized into summer, rainy, and winter), the period of birth spanning from 2011 to 2022, and the sex of the calves born(female and male). A fixed-effect model employing Least Squares Analysis was applied to the data. Genetic parameters were estimated using a Bayesian approach through Markov Chain Monte Carlo (MCMC) and Gibbs sampling, with a multitrait animal model. The BLUPF90 suite of programs, including RENF90, GIBBSF90, and POSTGIBBSF90, was used for this purpose. The MCMC iterations were conducted with 500,000 runs, a burn-in length of 50,000, and a thinning interval of 50, resulting in 8,000 saved samples. POSTGIBBS analysis provided the heritability estimates for each body weight trait using the multi-trait model. The heritability estimates ranged from 0.48519 to 0.64121, suggesting that a considerable portion of the phenotypic variation is influenced by genetic factors. Additionally, genetic and phenotypic correlations were assessed among body weight traits. These correlations highlight the relationships between the traits, indicating that some traits are more strongly correlated than others. Understanding these correlations can be valuable in breeding and selection programs for Mithun. In summary, this study provides valuable insights into the genetic parameters of body weight traits in Mithun, offering a foundation for selective breeding strategies and management practices to enhance the growth and productivity of this unique bovine species.

ISAGB-2023/Abst/TS-III-008

HARNESSING GENOMIC DATA TO OPTIMIZE BUFFALO PRODUCTION TRAIT UTILISING SELECTION SIGNATURES

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Domestication, driven by natural and artificial selection led to diverse buffalo breeds with unique traits. Riverine buffaloes *(B. bubalis)* originated from wild Asian water buffaloes *(B. arnee)* in the Indian subcontinent approximately 6000 years ago. Over generations, buffaloes have served as sources of milk, meat, and draught in tropical and subtropical regions. The selection processes leave distinct genomic footprints that serve as indicators of selection signatures. Analyzing these signatures offers valuable insights into how both natural and artificial selective forces have shaped the genome. In our study, we strived to explore the genomic footprints related to production traits in five buffalo populations. The dataset consists of Brazilian Murrah

(n = 15), Bulgarian Murrah (n = 11), Indian Murrah (n = 6), Kundi (n = 10), and Nili-Ravi (n = 15). SNP filtering and quality control (QC) were performed using PLINK. After quality control, the dataset contained 48,080 SNPs with a genotyping rate of 99.19%. We used differentmethods such as Tajima's D, ROH, FLK, and hapFLK.After identifying the candidate regions under selection, structural, and functional annotation has been performed using NCBI, PANTHER, and QTLdb. After utilizing these various summary statistics, we found distinct regions undergoing selective sweeps. Among them, six regions with seven annotated genes *(LOC100849046, LOC107132121, IRF5, ANKFN1, LOC104971319, CTSO,* and *SLC10A7)* were detected in more than one method. We have identified genes associated with production traits *viz. NPFFR2, TMTC1* using Tajima's D, and *SHROOM3* using ROH in Kundi, *ANKRD17,* and *RASSF6* usingTajima's Din Nili-Ravi. In Brazilian Murrah, The *LEP* gene using Tajima's D (chr. 4) was reported to be associated with 93 QTLs including milk yield and milk composition. Few candidate genes (*CHST, MYO16, SORCS1* using Tajima's D, and *POU1F1* using FLK) detected in Indian Murrah have been associated with milk protein and milk fat traits.These findings demonstrate the evolutionary impact of natural selection on the production traits of different buffalo breeds. The identified genomic regions can guide targeted breedingand conservation efforts for future research and breed improvement initiatives.

ISAGB-2023/Abst/TS-III-009

ELUCIDATING THE EFFECT OF HEAT STRESS ON MILK PRODUCTION AND COMPOSITION IN JERSEY CROSSBRED COWS USING TEST DAY RECORDS INTEGRATED WITH NASA POWER SATELLITE DATA

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The present study was undertaken to determine the effect of heat stress on milk production (test day milk yield) and compositional traits (fat%, protein%, fat yield, protein yield) as well as to observe the pattern of response to increasing heat load on these traits in Jersey crossbred cows, maintained at ICAR-National Dairy Research Institute, Eastern Regional Station, Kalyani, West Bengal, India. The weather information, obtained from the NASA POWER database based on the location of the farm latitude and longitude, was used to calculate the Temperature Humidity Index (THI). Two statistical approaches were used. First, a linear model was fitted to the production records, which were adjusted for additive genetic effect of animal, permanent environmental effect of animal and known environmental sources of variations. In the second analysis, a segmented linear regression model was fitted, and the least squares estimates of production and composition traits in different classes of THI were used as the dependent variable. Two THI break-points (BP) for milk yield, fat yield, protein %, and one THI BP for protein yield were found in this study. The first and second BP for milk yield was at THI 59 and 73, respectively. The rate of decline in milk yield occurs at BP-2 at THI 73 with decline of -0.01 kg/unit of THI at population level. The BPs for fat yield at THI were 76 and 82, with rate of decline of -1.25 g/ unit to -4.25 g/unit of THI. The BPs for protein % were 71 and 75 with rate of decline of -0.04 and -0.02g / unit of THI respectively, whereas for protein yield, BP was 74 with rate of decline of -0.62 g/ unit of THI. Continuous climatic changes showed significant adverse effects on milk production and composition traits in Jersey crossbred cattle in this study.

ISAGB-2023/Abst/TS-III-010

NON-GENETIC FACTORS AFFECTING WOOL QUALITY TRAITS OF CHOKLA SHEEP

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The objective of the study was to evaluate the effect non-genetic factors on wool quality traits in Chokla sheep maintained at ICAR-Central Sheep and Wool Research Institute,Avikanagar, Rajasthan from 1998 to 2012 and at Arid Region Campus (ICAR-Central Sheep and Wool Research Institute), Bikaner, Rajasthan from 2013 to 2020. The overall least-squares means for staple length, fibre diameter, and proportion of pure, hetero, hairy and medullated fibres were 5.64±.07 cm, 30.35±0.23 μ, 77.17±0.95, 9.72±0.38, 13.12±0.79 and 28.30±1.13, respectively at ICAR-CSWRI, Avikanagar and 5.80±0.06 cm, 31.99±0.17 μ, 81.54±0.66, 13.92±0.47, 3.08±0.18 and 28.71±1.22, respectively at Arid Region Campus, Bikaner. Staple length improved from period to period at both locations. Fiber diameter remained within limit as per industry need at Bikaner. The significant factors should be given due importance in general management in order to obtain better wool quality. Moreover, these significant factors will be helpful to adjust the data for the calculation of accurate genetic parameters of the present flock.

ISAGB-2023/Abst/TS-III-011

ESTIMATION OF FIRST LACTATION PERFORMANCE PARAMETERS IN KANKREJ CATTLE

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The present study was conducted on 274 first lactating Kankrej cattle maintained at Livestock Research Station, Kodamdesar, Rajasthan University of Veterinary and Animal Sciences, Bikaner, calved during the period 2012 to 2022. The aim of present investigation was to access the effect of season and period (non-genetic factors) on first lactation production and reproduction traits in Kankrej cattle. The production and reproduction traits evaluated as first lactation length (FLL), first dry period (FDP), first lactation total milk yield (FLTMY) and first calving interval (FCI). To estimate the effect of non-genetic factors, computer package programme, IBM SPSS version 26.0 was used for least-squares analysis. The least-squares means estimated for FLL, FDP, FLTMY, FCI were 236.07±8.79 days, 133.76±6.7days, 1416.04±66.45kg, and 427.72±6.79 days, respectively. Season and period of calving had significant effect on first lactation total milk yield (FLTMY) and non-significant effect on other traits considered for the study. Findings of present study will help in further research in a large population of indigenous cattle.

ISAGB-2023/Abst/TS-III-012

FARM ANIMAL GENETIC RESOURCES IN AGRO ECOSYSTEM OF NORTH EAST INDIA

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North East Region (NER) of India is the homeland of diverse animal genetic resources and represents a unique agro-ecosystem with integrated subsistence low input tribal production system. Farm animals play an important role in improving the socio-economic status of the people. The total livestock and poultry population of the region is about 70.13 million (6.85% of India) of which 92.76% is indigenous population and remaining is crossbred population. Among the 183 registered breeds of livestock and poultry in India, the region has 21 registered breeds consisting of three cattle, one buffalo, three goat, two sheep, four pig, two horse and pony, one yak, four chicken and one duck breed. Besides, many uncharacterized farm animal breeds/populations are reared by tribal farmers in the region described by their local names. The review enumerates the farm animal genetic resources of NER, their current status, descriptions, unique features, utility, economic valuation, cultural importance and future conservation strategies. Precise and reliable evaluation and estimation of important economic and climate resilient traits of indigenous germplasm, genetic characterization, documentation and registration are highly warranted. It has proposed a model to central and state agencies for strict policy implementation to facilitate *in situ* conservation with active community participation and *ex situ* conservation through application of modern biotechnological tools which is warranted to maintain the diversity of farm animals in NER of India

ISAGB-2023/Abst/TS-III-013

MULTIVARIATE PRINCIPAL COMPONENT ANALYSIS OF MORPHOMETRIC TRAITS IN NATIVE SHEEP BREEDS OF INDIAN HIMALAYAN REGION.

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This study was performed to analyze the morphometric traits and indices in 3000 animals of 5 registered sheep breeds viz., Karnah, Gurez, Poonchi, Bakerwal and Changthangi in the Himalayan region under a multivariate approach. Data was recorded under field conditions on body length (BL), height at withers (HW), chest girth (CG), ear length (EL), and tail length (TL). Furthermore, four derived traits (indices) were studied, which included an index of body frame (IBF), index of thorax development (ITD), Baron-Crevat index (BCI), and an index of body weight (IBW) and PCA was undertaken on nine morphometric traits. Kaiser's criterion was used to reduce the number of principal components for further analysis and interpretation. The adequacy of sampling was evaluated using Kaiser-Meyer-Olkin (KMO) and Bartlett's test of sphericity. The mean BL ranged from 52.15 (Changthangi) to 71.13 (Gurez). The estimates of HW, CG, EL, TL (63.49) and derived IBF (112.22) were highest in Gurez breed. Upon PCA the first four principal components were able to explain 92.117% of the total variance. The Kaiser-Meyer-Olkin test, Bartlett's test of sphericity and estimated communalities showed the appropriateness of PCA on the evaluated traits. Four eigenvalues were greater than one and were extracted for further analysis. Morphometric traits were highly correlated, except for ear length and tail length which showed lower correlation estimates with other traits. The present study ascertained important morphometric traits/indices that can help in developing selection criteria and formulating sustainable breeding and conservation plans vis-à-vis the unique sheep breeds of the temperate Himalayas.

ISAGB-2023/Abst/TS-III-014

DEMOGRAPHIC VARIATION AND ENVIRONMENTAL EFFECTS ON BIOMETRIC TRAITS OF KASHMIR MERINO SHEEP

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Kashmir Merino sheep is a major synthetic breed developed in Jammu and Kashmir by crossing Gaddi, Bhakarwal and Poonchi with Tasmanian and Delain Merino at Reasi Jammu during 1942-1964 for apparel wool, better growth, and adaptability. Measurements of body conformation in sheep are vital in judging the quantitative characteristics of meat and also helpful in developing suitable selection criterion. Data on 564 Kashmir Merino sheep for body length (BL), chest girth (CG), height at weather (WH), ear length (EL), tail length (TL), face length (FL), body weight (BW) and horn length (HL) were analyzed with sex, age, and locations as fixed factors. Stepwise regression equations were generated from regression analysis of body measurements as independent variables and body weight (BW) as dependent variable in adult sheep above 1.5 years of age. The overall estimates of 58.16±0.51cm, 74.72±0.71cm, 62.07±0.68cm, 10.69±0.19, 32cm, .25±0.48cm, 32.25±0.48cm, 20.05±0.28cm, 31.90±0.54kg and 27.41±2.2cm for BL, CG, WH, EL, TL, FL, BW and HL, respectively. The effect of age, sex and location was highly significant ($P<0.01$) on all traits under study with sexual dimorphism of 1.04, 1.06, 1.03, 1.05, 1.07, 1.10, 1.08 and 1.23 for BL, CG, WH, EL, TL, FL, BW and HL, respectively was observed in favour of males. All the biometric traits depicted increasing trend up to four years. Phenotypic correlation among all the traits was positive, significant and ranging from low to very high. Body weight had moderate phenotypic association with ear length, and horn length and high association with face length and tail length. When biometric traits were considered separately in regression analysis the highest R^2 was obtained for height at withers followed tail length in females whereas highest R^2 in males was obtained for CG followed by body height. The best prediction equation from single trait regression equation in females was height at withers and in males was with chest girth. It is concluded that Kashmir Merino is a variable sheep genetic resource and environments factors significant influence on the biometric traits. Further morphometric measurements can be used to determine live body weight under field conditions in the absence of weighing balance.

ISAGB-2023/Abst/TS-III-015

NEW PROSPECTIVE FOR IMPROVING DISEASE RESISTANCE IN POULTRY

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Breeding in poultry for disease resistance is a central focus of poultry breeding programs.The major genes considered are ;Tv-A and Tv-B, gene loci are responsible for the resistance from avian leukosis virus (ALV); Major histocompatibility complex (MHC) in the domestic fowl genetically influence the reaction and resistance to Marek's disease (MD); endogenous viral (ev) genes influence the reactions to leukosis infection. Nowadays, with the development of molecular and quantitative genetics, some poultry diseases can be addressed by breeding for disease resistance. With the need to accelerate the development of improved varieties, genomics-assisted breeding is becoming an important tool in breeding programs. As the genetic architecture of resistance shifts from single major R genes to a diffused architecture of many minor genes,

the best approach for molecular breeding will shift from marker-assisted selection to genomic selection. In general, whole-genome models for disease resistance can produce prediction accuracy suitable for application in breeding. With the implementation of genomic selection for yield and other agronomic traits, whole-genome marker profiles are available for the entire set of breeding lines, enabling genomic selection for disease at no additional direct cost. In this context, the scope of implementing genomics selection for disease resistance is a powerful approach in breeding programs.

ISAGB-2023/Abst/TS-III-016

MORPHOMETRIC CHARACTERS OF BANGALORE LONG EAR GOATS

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Bangalore Long Ear Goats have been maintained by farmers for more than sixty years, and the same germplasm has been distributed to neighboring states. The typical characteristics of this breed include long ears, and they are always reared under rooftops in a completely intensive stall-feeding system. The present study was designed to characterize the Bangalore Long Ear breed of goats in its breeding tract, mainly consisting of Bengaluru Urban and Bengaluru Rural, Kolar, Chikkaballapura, Ramanagara and Mysore districts of Karnataka state. This enables us to bring about breed development through selective breeding. Data was collected on the morphological traits and body measurements of 476 Bangalore Long Ear Goats (Adult males and females). The overall body weight of adult males and females, at more than 12 months of age, was 86.09 ± 1.19 kg and 61.26 ± 1.73 kg, respectively. In adult male goats, the averages for chest girth, body length, and height at withers were 84.19 ± 1.43 cm, 118.19 ± 2.09 cm, and 85.29 ± 1.35 cm, respectively. In females, the average measurements were 80.71 ± 1.51 cm, 114.29 ± 2.11 cm, and 83.16 ± 1.41 cm, respectively. The main characteristic feature of this breed is ear length and ear width, which are 53.48 ± 0.41 cm and 17.76 ± 0.24 cm, respectively, while in females, the average measurements were 44.73 ± 0.28 cm and 19.61 ± 0.21 cm, respectively. Most of the goats are brown in color with white patches; however, a few are black with white patches, and rarely, a few goats with a mixture of brown, black, and white are also seen. Rarely, white goats are also observed. These results indicate that phenotypic characterization, body weight, and other linear body measurements can help us to characterize the Bangalore Long Ear breed of goats and develop the breed descriptor.

ISAGB-2023/Abst/TS-III-017

PHYSICAL CHARACTERISTICS AND MORPHOMETRIC TRAITS OF BUFFALOES IN KASHMIR VALLEY

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Physical characteristics and morphometric traits of buffaloes in Kashmir Valley from two geographically isolated districts with highest buffalo population viz, Anantnag in South Kashmir and Baramulla in North Kashmir was recorded. Black coat colour (66.68%), black colour of muzzle (77.77%), black colour of eyelid (72.96%) black colour of hooves (71.12%) curved horns (84.07%) oriented backward and upward in 35.56

per cent and backward, upward and forward in 28.16% was recorded. 52.22% had straight forehead followed by convex (in 46.67%. Horizontal orientation of ear and absence of dewlap was universal. The navel flap was absent in 82.22% animals. 72.89 per cent females had bowl shaped udder, of small size with teats of cylindrical shape in 85.04% and pointed tip of teat in 65.88%. The overall mean body length, chest girth, height at withers, ear length, and horn size was 51.70±0.23, 76.00±0.33, 52.22±0.36, 9.16±0.08 and 13.19±0.23 inches respectively. It was therefore inferred that buffaloes in Kashmir Valley had predominantly black coat colour, curved horns, horizontal orientation of ears, straight forehead and absence of dewlap. Most of the animals had bowl shaped, small sized udder and cylindrical teats with pointed tip.

ISAGB-2023/Abst/TS-III-018

ESTIMATION OF (CO) VARIANCE COMPONENTS AND GENETIC PARAMETERS OF PRE-WEANING GROWTH AND EFFICIENCY-RELATED TRAITS IN INTER-CROSS SHEEP

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Quantitative traits such as pre-weaning growth are expressed as a result of combined genetic and environmental effects. The phenotypic expression of these traits in offspring is affected by direct genetic effects and sometimes also by maternal genetic effect which is the ability of the dam to provide optimum nurturing conditions**.** The objective of the current study was to estimate the (co)variance components and genetic parameters of pre-weaning growth and efficiency related traits in inter-cross sheep maintained at NTRS, ICAR-CSWRI, Garsa, India. Data records of 1891 lambs for birth weight (BW) and 1763 lambs for weaning weight (WW) descending from 183 rams and 667 dams between 2012 and 2023 were utilized in the study. Pre-weaning daily weight gain (PDWG), pre-weaning Kleiber ratio (PKR), pre-weaning growth efficiency (PGE) and pre-weaning relative growth rate (PRGR) were calculated. Estimation of covariance components was carried out by fitting a series of animal models using restricted maximum likelihood (REML) algorithm in WOMBAT software. The best-fitting model was determined after testing for improvement in log-likelihood values. The overall least squares means ± standard error for BW, WW, PADG, PKR, PGE and PRGR were 3.29±0.04kg, 14.83±0.22kg, 127.87±2.46g, 16.78±0.13, 357.35±7.13 and 1.66±0.02, respectively. Analysis revealed that pre-weaning traits were significantly (P<0.05) affected by year of lambing, parity of dam, birth type and sex of the lamb. Direct heritability estimates from best-fitted models were 0.11±0.04, 0.16±0.04, 0.16±0.04, 0.15±0.06, 0.14±0.05 and 0.12±0.05 for BW, WW, PADG, PKR, PGE and PRGR, respectively. Maternal effects were important for BW, PKR, PGE and PRGR and corresponding maternal heritability estimates for these traits were 0.19±0.03, 0.11±0.04, 0.09±0.03 and 0.12±0.04, respectively. For WW and ADWG, only direct effects were significant. However, estimate of maternal permanent environmental effects were not significant for all the traits. The genetic and phenotypic correlations between BW and WW were moderate and positive. However, both genetic and phenotypic correlations of BW with all remaining traits were negative except phenotypic correlations of PDWG which were low and positive (0.10 ± 0.02). The correlations of WW with efficiency-related traits and among the other traits were quite high and positive. The moderate estimates of heritability with high and positive genetic correlations among the traits in the study suggest that moderate genetic improvement of the flock can be achieved through selection for these traits.

ISAGB-2023/Abst/TS-III-019

INDIAN CAMEL (*CAMELUS DROMEDARIUS* AND *CAMELUS BACTRIANUS*) BIODIVERSITY CONSERVED *IN VITRO* AS SOMATIC CELLS

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Camel invokes an intriguing chapter of Indian desert history being an integral component of its ecosystem. Camel population has reached a crisis point after three decades of decline causing major concern to the policy makers. It is imperative to conserve the country's camel germplasm for posterity. Thus, Somatic cell banking was selected for their long-term cryopreservation. To propagate somatic cells, ear tissue samples of male and female animals of all the registered dromedary camel breeds of India (Jaisalmeri, Bikaneri, Jalori, Kharai, Kachchhi, Mewari, Mewati, Marwari, and Malvi) as well as double humped camel of Leh & Ladakh were collected. Explants were cultured in DMEM+20% FBS in a humidified incubator at 37°C and 5% CO_2. Fibroblast cells with typical fusiform morphology and centrally located oval nuclei were harvested and passaged in the DMEM+10% FBS. Fibroblast cells (1x10^{6}cells/ml) of the 4th passage that were following normal sigmoid growth curve and free from any contamination were selected for cryo-conservation in the LN_2 container at -196°C at the National Gene Bank of ICAR- NBAGR, Karnal, India. The freezing media was made up of Culture media with 10% DMSO. The cells had more than 90% viability after freeze-thaw cycle. This small step is an important milestone in conserving India's unique livestock genetic resources. Cryopreservation of indigenous camelid biodiversity as somatic cells also contributes towards the fulfillment of the national obligation towards the Conservation of animal genetic resources embedded in the United Nation's Sustainable Developmental Goal 2.5.1.

ISAGB-2023/Abst/TS-III-020

GENOME WIDE ASSOCIATION STUDY OF E2 ANTIBODY RESPONSE REVEALED NOVEL SNPS AND GENES AFFECTING IMMUNE RESPONSE AGAINST CLASSICAL SWINE FEVER VACCINE

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Vaccination against classical swine fever (CSF) is one of the standard procedures to control this devastating and highly contagious disease of pigs. The host genetic makeup plays a significant role in causing the within-breed variation among individuals after vaccination. The present study was undertaken to elucidate the genetic basis of differential immune response (in terms of genome-wide informative SNPs) between high and low responder Landlly (Landrace X Ghurrah) piglets vis-à-vis CSF vaccination. E2 antibody response against CSF vaccination was estimated in sampled animals on the day of vaccination and 21-day post-vaccination as an analogy to humoral immune response. Reduced representation sequencing (Double-digestion restriction associated DNA (ddRAD) was undertaken on 96 randomly chosen Landlly piglets using the Illumina HiSeq platform. SNP markers were called using standard methodology. Genome-wide association study (GWAS) was undertaken in the PLINK program to identify the informative SNP markers (genotype) significantly associated with differential immune response (E2 antibody phenotype). The results revealed significant SNPs to be associated with E2 antibody response against CSF vaccination. The genome-wide informative SNPs for the humoral immune response against CSF vaccination were located on SSC10, SSC17, SSC9, SSC2, SSC3, and SSC6. The overlapping and flanking genes (500Kb upstream and downstream) of significant SNPs were

CYB5R1, PCMTD2, WT1, IL9R, CD101, TMEM64, TLR6, PIGG, ADIPOR1, PRSS37, EIF3M, and *DNAJC24*. Functional enrichment and annotation were undertaken for these genes to gain maximum insights into the association of these genes with immune system functionality in pigs. In conclusion, genetic makeup was associated with the differential immune response against CSF vaccination in Landlly piglets while the identified informative SNPs may be used as suitable markers for determining variation in host immune response against CSF vaccination in pigs.

ISAGB-2023/Abst/TS-III-021

POLYMORPHISM AND ASSOCIATION OF *MAP3K1* GENE WITH SCROTAL CIRCUMFERENCE OF AI BULLS

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Scrotal circumference measurements are now a requirement for assessing bull fertility due to their significant influence on semen quality, including sperm concentration and the percentage of normal sperm in a bull's ejaculate. These measurements play a crucial role in identifying undesirable bulls and are indicative of a bull's total reproductive potential and utility in breeding programs. This study explores the genetic and non-genetic factors influencing bull fertility, with a specific focus on scrotal circumference as a key parameter. We used Chilibot and Connected paper, web-based bioinformatic tools, to identify a gene linked to scrotal circumference in bulls. The study was conducted with 239 bulls of various breeds in the FSBS of Andhra Pradesh. We utilized the *MAP3K1/CviQ*1 PCR-RFLP assay to reveal allele frequencies in different cattle and Murrah populations. The T allele frequency was found to be higher in the Ongole population, while the C allele was predominant in other cattle breeds. Notably, Jersey and Ongole populations exhibited the highest and lowest homozygosity values, respectively. Negative F_{IS} values indicated heterozygous excess in the studied populations due to outcrossing. In the Murrah population, the T allele frequency was higher, and heterozygosity excess was observed at this locus. We found no significant influence of *MAP3K1/CviQ*1 on scrotal circumference. Age groups had a significant impact on scrotal circumference in HF crossbred and Ongole cattle. Scrotal circumference significantly increased with age until 11-13 years and then decreased in Murrah bulls aged over 13 years. Additionally, the season at the time of measurement significantly affected scrotal circumference in the Murrah population. To obtain more reliable estimates and identify additional SNPs responsible for scrotal circumference variations, future studies should consider larger sample sizes and screen for causal mutations in the vicinity of the studied SNP. This research contributes valuable insights into the factors affecting scrotal circumference and provides a foundation for further investigation and improvement of cattle breeding practices.

ISAGB-2023/Abst/TS-III-022

ASSOCIATION OF SHORT TANDEM REPEAT POLYMORPHS WITH BODY WEIGHT TRAITS IN VRINDAVANI COWS

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Selection among the dairy animals has been primarily practiced for production traits with little attention towards the other non-lactational traits. However, with increasing emphasis on sustainable livestock

transformation selection for several non-production traits along with the traditional dairy traits is now necessary. Birth weight (BW), yearling weight (YW) and body weight at 18 months (BW-18m) of age can be considered as important indicators of growth among dairy heifers which may have a bearing on their future milk yield. The objective of this study was to detect polymorphism in short tandem repeats (STRs) flanking QTLs governing body weight in cattle and comprehend the association of identified polymorphs with BW, YW and BW-18m in Vrindavani cows. Three pairs of STR loci viz., CSSM34 and ETH10; BMS1248 and BM315; BM6026 and RM500 flanking three QTLs for body weight on chromosome 5 were investigated in 95 Vrindavani cows. All the loci were found to be polymorphic. On analysis of phenotypic data collected for the three traits least square mean values of 22.9 ± 0.4 kg, 156 ± 2.0 kg and 233 ± 3 kg were obtained for BW, YW and BW-18m, respectively. Association analysis revealed a significant effect of genotypes at BM6026, BMS2503 and BMS2389 on YW. Also, genotypes at CSSM34 and BM6026 had a significant effect on BW-18m. Identification of favourable genetic variations for improved body weight and growth will facilitate the development of appropriate selection strategies for overall genetic improvement of dairy population.

ISAGB-2023/Abst/TS-III-023

SNP MINING AND ASSOCIATION OF VARIANTS WITH MILK FATTY ACID TRAITS IN VRINDAVANI COWS

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Association of single nucleotide polymorphisms (SNPs) is a sophisticated technique for predicting genetic quality by correlating enormous volumes of genomic and phenotypic data. In various regions of the world, SNPs have been linked to milk component properties in crossbred cattle. Our study was an attempt towards finding association of the SNPs with fatty acids. Milk fat comprises mono- and polyunsaturated fatty acids, some of them are recognized to have a beneficial impact on human health. A total 58 lactating Vrindavani cattle which were maintained at LPM Section of ICAR-IVRI, Izatnagar, were included in the study. DNA of selected animals were isolated using conventional phenol-chloroform extraction method. The SNPs for the study were chosen from the Cattle QTL database. Then it was fed to ensembl genome browser to retrieve the nucleotide sequence. With the help of NEB cutter, the suitable enzymes were selected. In-silico primer designing for target region of selected SNPs were carried out using PRIMER 3 software and. SNPs governing different milk fatty acids were shortlisted, based on the availability of the unique restriction enzyme at the site of nucleotide variation. PCR-RFLP technique was employed in order to genotype these animals. Optimum conditions were followed for PCR-RFLP. The PCR-RFLP analysis of 150 bp fragment containing SNP rs41944626 with BseNI RE (corresponding to Caprylic acid) revealed identical genotype i.e., AA in all the animals. Hence no polymorphism was found in this fragment. The PCR-RFLP analysis of 154 bp fragment containing SNP rs207783265 with ApaI RE (corresponding to Lauric acid) revealed that 8 animals had AA genotype, 43 had AB genotype and 7 had BB genotype. Least squares analysis of variance showed significant genotype effect for rs207783265 on lauric acid ($P<0.05$) whose least square mean was 0.04 ± 0.004 gm lauric acid/100 gm of milk.

ISAGB-2023/Abst/TS-III-024

GENETIC CHARACTERIZATION OF F_4 INBRED SWISS ALBINO MICE USING MICROSATELLITE MARKERS AND THEIR ASSOCIATION WITH FITNESS TRAITS

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Inbred mice are a reliable animal genetic model for biological research. However, their genetic monitoring at regular intervals is essential to avoid genetic contamination and to estimate population genetic parameters. Thus, the present study was aimed to characterize the F_4 inbred female mice (n=102) populations using 14 microsatellite markers. The F_3 inbred Swiss albino mice maintained at LAR section of Animal Genetics Division, ICAR-IVRI were used as base population to produce F_4 generation. Fitness i.e., litter size at birth (LSB), litter weight at birth (LWB), litter size at weaning (LSW) and litter weight at weaning (LWW) traits recorded on 102 F_4 inbred mice populations. The mean estimates of LSB, LWB, LSW and LWW were 6.00±0.20, 9.22±0.31, 5.21±0.17 and 83.22±2.76 in F4. All microsatellite loci were found to be polymorphic in present investigation. The number of alleles at all microsatellite loci ranged from 3 (D2Mit61, D3Mit55, D8Mit14, D9Mit27, D10Mit180, D11Mit167, D15Mit16, DXMit172 and DXMit187) to 4 (D1Mit15, D2Mit51, D5Mit18, D7Mit323 and D16Mit4) alleles. The PIC estimates ranged from 0.47 (DXMit187) to 0.68 (D16Mit4). The estimates for heterozygosity ranged from 0.11(DXMit187) to 0.31(D7Mit323). The allelic diversity was maximum for D16Mit4 (0.73) and minimum for DXMit187 (0.54) locus. Chi square test showed that all the loci significantly ($p<0.05$) deviate from HWE. The number of observed alleles (N_a) per locus varied from three to four, while the number of effective alleles (N_e) varied from 2.192 (DXMit187) to 3.699. (D16Mit4). The observed heterozygosity (Ho) was maximum for D7Mit323 (0.314) and minimum for DXMit187 (0.108), with mean ± SD (0.196 ±0.061). The expected heterozygosity (H_e) was highest for D16Mit4 (0.730) and lowest for DXMit187 (0.544), with mean ± SD (0.631±0.057). The F_{IS} estimate was lowest (0.526) for D5Mit18 and highest (0.802) for DXMit187 locus. The average "F" based on 14 microsatellite markers was estimated as 0.690 in the F_4 population. D1Mit15, D7Mit323, D8Mit14, DXMit172 and D9Mit27 had significant ($P<0.05$) genotypic associations with fitness traits.

ISAGB-2023/Abst/TS-III-025

OPTIMISING SELECTION STRATEGY FOR ENHANCING REPRODUCTION EFFICIENCY IN INDIAN CROSSBRED GOATS USING MILK PRODUCTIVITY AS A SELECTION CRITERION

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The present study was aimed at optimising the selection strategy for enhancing reproductive efficiency and milk productivity of Alpine x Beetal crossbred goats. The data set included 2949 milk trait records across parities and 1389 milk records from first parity and corresponding reproductive traits. The traits included for analysis were 150-day milk yield (150DMY), days in milk (DIM), peak yield (PY), and total milk yield (TMY). The litter size (LS) and litter weight (LW) were used for specifically formulating selection plan using indirect selection. The least squares mean for lactation traits during the first parity were 150DMY:

195.32±2.09 kg, DIM: 236.42±3.04 days, PY:1.82±0.02 kg, TMY: 269.62±4.52 kg. Notably, Alpine x Beetal goats demonstrated genetic superiority pan India for milk productivity as compared to other native goat breeds. The least squares mean for 150DMY across all parities was 236±3.13 kg. An animal model employing average information restricted maximum likelihood (AIREML) was used for (co)variance component estimation to get the genetic parameters. The analysis revealed total heritability estimates for 150DMY, DIM, PY and TMY as 0.18±0.06, 0.04±0.04, 0.12±0.06 and 0.08±0.05, respectively. Repeatability estimates for 150DMY, DIM, and TMY were 0.28±0.04, 0.21±0.03, and 0.37±0.03, respectively. Bivariate analysis of 150DMY with reproductive traits revealed heritability for litter size (LS) and litter weight (LW) as 0.05±0.01 and 0.10±0.01, respectively using Gibbs sampling. Strong and positive genetic correlations of 150DMY with other production and reproduction traits was observed, such as DIM (0.72), PY (0.98), TMY (0.88), LS (0.57) and LW (0.33). Moderate heritability and repeatability estimate of 150DMY, along with its positive correlation with production and reproductive traits suggested it as a suitable selection criterion for early selection and overall genetic progress of lactation traits. The genetic trend analysis showed an overall improvement in all these traits, with observed gain of 98.4g per year for 150DMY, 0.04 days per year for DIM, 0.5g per year for PY, and 220.5g per year for TMY. We observed that selecting based on 150DMY would lead to a favourable indirect improvement for litter weight as 79g and litter size 0.04 units per generation. We, therefore, recommend employing 150DMY as the single trait selection criteria to enhance both milk productivity and reproductive potential of Alpine x Beetal goats.

ISAGB-2023/Abst/TS-III-026

INFLUENCE OF DIFFERENT NON-GENETIC FACTORS ON YOUNG CROSSBRED CATTLE'S DISPOSAL PATTERN (MORTALITY AND CULLING).

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Data of 2128 female crossbred calves, born between 1988 and 2019 from 1000 crossbred cattle at IDF, Nagla, Uttrakhand, were analyzed to study the influence of different non-genetic factors on disposal pattern (mortality and culling) of young crossbred cattle up to age at first calving (AFC). The analysis of influence of various non-genetic factors on disposal pattern was carried out by Chi-square method using the SPSS software. It turned out that before reaching the milking herd, 14.80% of the total number of female calves born died and 17.53% were culled. Only the effect of period was significant on total mortality. Age specific mortality among female calves from 0-1, 1-3, 3-6, 6-12 and 12 months to age at first calving was noted as 5.87, 3.06, 2.13, 1.68, and 3.13 percent, respectively. While, Age specific culling among female calves from 0-1, 1-3, 3-6, 6-12 and 12 months to age at first calving was recorded as 0.47, 0.70, 1.82, 4.72 and 13.17 percent, respectively. The period of birth had highly significant effect (p<0.01) from birth to 1 month as well as significant effect (p<0.05) by period and season of birth was recorded on 1-3 months age mortality. It was concluded that the early phase of life is more crucial for calves' survival. To decrease involuntary disposal, careful oversight of feeding and healthcare is necessary.

ISAGB-2023/Abst/TS-III-027

ESTIMATION OF DEMOGRAPHIC PARAMETERS IN CROSSBREED CATTLE

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Data for the present investigation were collected from history sheet of crossbred cattle maintained at Instructional Dairy Farm of G. B. Pant University of Agriculture and Technology, Pantnagar. The data pertained to 1000 crossbred cattle with a total of 3583 calving records from 87 sires were distributed over a period of 32 years from 1988 to 2019. Cows with abnormal and incomplete records were excluded from the study. Demographic parameters specific to lactation were calculated. Survival rate and loss rate in the first lactation were found to be 82.24% and 17.61%, respectively. The probability of survival was found to be highest during the first lactation, which gradually decreased with in subsequent lactations. In the first lactation the loss rate calculated was 17.61%, which was one-sixth of the total cows present in the first lactation. The overall values for survivorship were observed as 0.8239, 0.5090, 0.2732, 0.1198, and 0.0244 in the first, third, fifth, seventh, and ninth lactation, respectively. A total number of 24.65 percent cows were present in first lactation, while 20.31, 09.52, 02.95 and 00.60 percent in second, fifth, eighth and tenth lactations, respectively. It was found that the proportion of female cows being lost from the herd due to death and culling were 0.1761, 0.1659, 0.1488, 0.0733 and 0.0204 at first, second, third, seventh and tenth lactations. The expected herd life in crossbred herd was obtained as 3.043, 2.695, 2.374, 1.760, and 0.163 years in first, second, third, fifth and tenth lactations, respectively. This decline in expected herd life is a common pattern observed in dairy herds. Understanding the distribution of cows across lactation numbers is important for monitoring herd demographics and implementing appropriate management strategies to optimize productivity and longevity in the herd.

ISAGB-2023/Abst/TS-III-028

GENETIC EVALUATION OF BODY WEIGHTS AND BIOMETRIC TRAITS USING MULTI-TRAIT ANIMAL MODEL IN INDIGENOUS ASEEL AND KADAKNATH CHICKEN

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Knowledge of genetic parameters like heritability, genetic and phenotypic correlation is essential to evaluate the population under selection in every generation for various economic traits and it is essential that bird should have optimum weight during growing and laying period. The data used in this study included 529 birds of Aseel and 530 birds of Kadaknath to evaluate body weight traits (BW_{0D} BW_{4}, BW8, BW_{16}, BW_{20}, BW_{24}, BW_{28}, BW_{32}, BW_{36}, BW_{40}), and biometric traits such as body length, tarsus length , back length, comb length, comb height, wattle length, beak length, head length, neck length, wing length, wing span and breast girth at 40 weeks of age. Multi-trait animal model with the average information-restricted maximum likelihood (AI-REML) was used to estimate the variance components, genetic parameters, and breeding values. The findings indicated that males outperformed females in the above-mentioned traits for both breeds. The least squares mean for body weights were found to be higher in Aseel than Kadaknath at all ages in both male and females. Effect of sex was highly significant ($p \leq 0.01$) on all body weight traits *viz*., body weight at 8 weeks of age to

body weight at 40 weeks of age for Aseel except for BW_4. Similarly, the effect of hatch was significant ($p \leq 0.05$) for BW_{12} , BW_{16} , BW_{20} , BW_{24} and highly significant ($p \leq 0.01$) for BW_4 , BW_8. Effect of sex was significant ($p \leq 0.05$) for BW_{12} , BW_{32} and highly significant ($p \leq 0.01$) for BW_{36} and BW_{40} in Kadaknath. Heritability estimate of body weights (BW_4 to BW_{40}) ranged from 0.19±0.08 (BW_{16}) to 0.50±0.16 (BW_{40}) and 0.15±0.06 (BW_{24}) to 0.45±0.14 (BW_8) in Aseel and Kadaknath, respectively. The lowest heritability estimate was observed for traits, like head length (0.14±0.11) and breast angle (0.12±0.11) while highest heritability was observed for comb length (0.67±0.23) and back length (0.67±0.09) for Aseel and Kadaknath, respectively. The genetic and phenotypic correlations among the growth traits (BW0 to BW40) were low to highly positive. Positive phenotypic correlation was observed amongst the various morpho-metric traits and the body weight in both Aseel and Kadaknath. Therefore, indirect selection of body weight using highest genetic and phenotypic correlated linear traits will result in rapid genetic improvement of the breeds.

ISAGB-2023/Abst/TS-III-029

QUANTIFYING THE GENETIC PARAMETERS GOVERNING THE PERFORMANCE TRAITS IN INDIGENOUS ASEEL CHICKEN

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Aseel breed, originating in Andhra Pradesh, is well known for its endurance, fighting spirit and majestic gait. Knowledge of genetic parameters is crucial for formulating effective breeding strategies. The data pertaining of 529 Aseel birds over 3 generations (2018-19 to 2020-21) maintained at Poultry Breeding Farm of Department of Animal Genetics and Breeding, LUVAS, Hisar have been utilized for this study to estimate genetic parameters of performance traits. Mixed linear model was used including sire as random and generation and hatch were fixed effect. The performance traits including age at first egg (AFE), body weight at 20 weeks of age (BW_{20}), body weight at 40 weeks of age (BW_{40}), egg number upto 40 weeks (EN_{40}), egg weight at 40 weeks (EW_{40}) egg mass at 40 weeks of age (EM_{40}). Highly significant ($p \leq 0.01$) effect of generation was observed on all performance traits except $EM_{40.}$ The least square means for performance traits AFE, BW_{20}, BW_{40}, EN_{40}, EW_{40}, EM_{40} were 175.37±0.71 days, 1198.03±7.91 g, 1674.62±7.01 g, 67.78±0.77 eggs, 46.71±0.14 g, and 2893.82± 54.06 g, respectively. The heritability estimates were moderate to high for all performance traits. Heritability estimate ranged from 0.14±0.11 to 0.67±0.23. The age at first egg (AFE) was negatively correlated with egg weight at 40 weeks (EW_{40}) of age and egg mass at 40 weeks of age (EM_{40}). Body weight at 20 weeks of age (BW_{20}) had strong association with $EW_{40.}$ The results were indicative of the fact that egg number can further be improved using an appropriate combined selection. Moderate to high estimates of heritability for performance traits indicated that these traits can be improved through family selection.

ISAGB-2023/Abst/TS-III-030

PEDIGREE-BASED ANALYSIS OF POPULATION STRUCTURE AND GENETIC DIVERSITY IN HIGH-MILCH VRINDAVANI CROSSBRED CATTLE OF INDIA

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The present study was aimed to elucidate the population and genetic diversity along with estimate genealogical parameters in Vrindavani cattle using pedigree data. The study included pedigree data on 12718 animals, spread across multiple generations during a 52-year period between 1971 to 2023. The pedigree data was

used to estimate population genealogical including the generation interval, pedigree completeness, rate and level of inbreeding, effective population size, and parameters characterizing the probabilities of gene origin. Endog program was used for estimation of different parameters while using population before after 2012 as reference population. The maximum number of generations (MG) was 13, while the number of completed (CG) and equivalent generations (EqG) were 3.23 and 1.95, respectively. The mean generation interval was 6.9 years. The average inbreeding coefficient of animals in the whole and reference population was 1.11 and 3.44 %, respectively; with rate of inbreeding as 0.68 % per generation. The average additive relationship among all the animals and those in the reference population was 1.16 and 5.49 %, respectively. The average effective population size for the maximum, equivalent, and complete generation/s was 115.56, 56.42, and 46.02, respectively. The effective population size on the basis of regression and log regression on birth date was 77.40 and 71.24, respectively. The probabilities of gene origin were estimated by the effective number of founders (f_e) and ancestors (f_a), which was 115 and 78, respectively. The analysis revealed a loss of 5.3% of total heterozygosity as compared to base population, though significant variability exists in the latest generations. The results revealed that considerable genetic variability remained in the population that may be exploited through appropriate animal breeding program and breed improvement be made with repeated various economic traits.

ISAGB-2023/Abst/TS-III-031

COMPARISON OF EXTERNAL AND INTERNAL EGG QUALITY PARAMETERS IN DIFFERENT STRAINS/BREEDS OF CHICKENS

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The present work was undertaken on five different strains of chickens (synthetic White Leghorn, CARI-Dhanraja, dual purpose Sire Line, Aseel and Kadaknath) maintained at the Poultry Farm, Department of Animal Genetics and Breeding, LUVAS, Hisar. A total of 500 eggs i.e., 100 each were analysed for assessing the external and internal egg quality parameters. External egg quality parameters included were egg weight, egg length, egg width, shape index, shell weight, shell thickness and shell ratio whereas internal egg quality traits were albumin length, albumin width, albumin height, albumin index, yolk length, yolk width, yolk height, yolk index and Haugh unit. Egg weights were found to be breed dependent and varied from 43.01 to 59.03 g. There was no significant difference in the shape index of eggs from Synthetic White Leghorn, CARI-Dhanraja and Sire-Line. Minimum shell ratio was observed for the Kadaknath eggs. There was no significant difference in the albumin length and width in Assel and Kadaknath, however, albumin height was significantly different for the two breeds. Minimum yolk length as well as width was observed in synthetic White Leghorn eggs. Minimum value of Haugh unit was observed for layers' eggs and was significantly different from the eggs of broiler and dual-purpose breed. Among external egg quality parameters, maximum phenotypic correlations were observed among shell weight and shell ratio in Harlay, CARI-Dhanraja, Sire Line, Aseel and Kadaknath. Among internal egg quality parameters, maximum phenotypic correlations were observed among albumin height and Haugh Unit in all the considered breeds/strains. The results of this study will be valuable to the both poultry breeders and egg consumers in selecting high-quality eggs for propagation of next generation and consumption, respectively.

ISAGB-2023/Abst/TS-III-032

GENETIC ANALYSIS OF REPRODUCTIVE AND LONGEVITY TRAITS IN LANDLLY CROSSBRED SOWS.

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There is considerable interest in global swine breeding programs to genetically select better sows for reproduction and lifetime productivity as they have an increasing impact on sow productivity, welfare, and profitability. For the genetic improvement programme to be successful for these traits, accurate heritability estimates for variables related to reproduction and longevity as well as genetic correlations between the pair of traits are essential. Therefore, the present study was conducted with the objectives to examine the reproductive and longevity traits and estimating the genetic and phenotypic parameters for these traits in Landlly sows kept at a swine production farm, Izatnagar, Uttar Pradesh, India. The effect of non-genetic factors on different reproductive traits was estimated using SPSS software (version 16.0) while the Bayesian approach was utilized to estimate the genetic parameters for reproductive and longevity traits. The current study's result that the year and season of birth have a significant effect on reproductive characteristics generally suggests that management practices may be optimised to increase production performance. The heritability estimates for the reproductive and longevity traits were low which indicates that there is a very little additive genetic variance in these traits, and individual selection will not be helpful for improving them. Some traits, such as LSB_FF (litter size at birth during first farrowing) & LSW_FF (litter size at weaning during first farrowing) and LSB_SF (litter size at birth during second farrowing) & LSW_SF (litter size at weaning during second farrowing) had a moderate genetic correlation, suggest that indirect selection can be used to improve these pairs of traits.

ISAGB-2023/Abst/TS-III-033

ESTIMATE OF (CO) VARIANCE COMPONENTS AND GENETIC PARAMETERS FOR KLEIBER RATIO IN SALEM BLACK GOAT UNDER FARM CONDITIONS

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Salem Black goat is one among the 37 registered breeds of goats in India having considerable production potential under semi-arid, tropical conditions of north-western agro-climatic zone of Tamil Nadu. Periodic genetic evaluation is essential for monitoring progress in a breeding program. Kleiber ratio (KR) is the proportion of average daily gain to the three-fourth metabolic body weight and provides a more accurate measure of growth since it considers metabolic body weight. The present study was carried out to genetically evaluate pre weaning (0-3) and post weaning KR (3-6, 6-9, 9-12, 3-12). Data accrued over 19 years at Mecheri Sheep Research Station, Pottaneri, Salem district, Tamil Nadu was utilized for the study. (Co)variance components were estimated by Restricted Maximum Likelihood (REML) using the WOMBAT programme of Meyer (2007), by fitting an animal model. Single trait linear models as described by Meyer (1992), which accounts for the direct, maternal genetic and permanent environmental effects were used. Least squares mean for pre weaning KR (0-3) was 14.34 ± 0.15 and post weaning KR at 3-6, 6-9, 9-12, 3-12 were 5.72 ± 0.28, 3.09 ± 0.32, 5.43 ± 0.36 and 4.12 ± 0.13 respectively. The direct heritability estimates of pre and post weaning (3-6, 6-9, and 3-12 months) KR were 0.164 ± 0.066, 0.184 ± 0.081, 0.051 ± 0.070 and 0.226 ±

0.116 respectively. Post-weaning KR at 9-12 months had negligible heritability estimate. Pre-weaning KR was affected by maternal genetic and maternal permanent environment effects. Maternal influence was not seen in post-weaning KR at 3-6 and 3-12 months, whereas KR at 6-9 and 9-12 months were affected by maternal genetic and maternal permanent environment respectively.

ISAGB-2023/Abst/TS-III-034

IMPACT OF NON-GENETIC FACTORS ON PRODUCTION AND REPRODUCTION TRAITS IN HARDHENU (BOS TAURUS × BOS INDICUS) DAIRY CATTLE

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The objective of the current study was to quantify the non-genetic factors influencing traits related to milk production and reproduction in Hardhenu crossbred cattle. The Least square method of General Linear Model using parity, season of calving and period of calving as the fixed effects were utilized for the analysis and Pearson correlation was used to calculate phenotypic correlation between targeted traits. Parity had a highly significant ($P<0.01$) association with TMY, 305d MY, PY and lactation length in the targeted population whereas season of calving had a significant ($p<0.05$) association with TMY in first parity and with PY in the third parity but not with lactation length and 305d MY ($p>0.05$). Period of calving had a highly significant impact ($p<0.01$) on TMY, PY, and 305d MY in the first parity and lactation length in second and third parity and a significant effect on 305d MY ($p<0.05$) in the third parity. In contrast, non-genetic parameters did not reveal significant association with AFC, SP, CI, or DP whereas season of calving showed significant ($P<0.05$) effect on the A.I/conception in the second parity. Pearson's correlation analysis revealed that AFC showed a significant positive correlation with AI/Conception ($p < 0.01$) and significant negative correlation with LL ($p < 0.01$). Total Milk Yield (TMY) displayed strongly positive correlations ($p<0.01$) with TMY 305d, Peak Yield, and Calving Interval. Furthermore, MY 305 was significantly positively correlated with PY ($p < 0.01$) and Calving Interval (CI) ($p < 0.05$). The findings show that there is a significant amount of variance in milk production and reproduction traits in resource population. In order to improve selection criteria by taking into account non-genetic factors, the aforementioned factors must be taken into account throughout the genetic analysis phase of the cattle evaluation process.

ISAGB-2023/Abst/TS-III-035

EVALUATION OF TYPE TRAITS IN RELATION TO PRODUCTION AND THEIR IMPORTANCE IN EARLY SELECTION FOR MILK PERFORMANCE IN KANKREJ CATTLE

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Type traits (TTs) can significantly contribute to economic traits such as production, longevity, fertility, and thereby profitability of breeding animals. Indigenous cattle are the second largest source of milk supply in the India, and their TTs can be taken into consideration for future dairy cattle breeding programmes. However, the relationship between TTs and milk production traits in Indian cattle remains largely unknown. The relationship between TTs and milk production traits were analysed using records from 257 Kankrej cows maintained at organized and unorganized farming systems in Banaskantha district of Gujarat in present study. We examined the correlations between 20 TTs (10 body structural, 10 udder and teat morphological traits) and five milk production traits *viz.*, standardized (305 days) milk yield (SMY), total milk yield (TMY), peak yield (PY) as well as fat percent (%) and lactation length (LL). The results showed that the highest phenotypic correlation was found between TMY and foot angle (FA) (r = 0.345), body depth (BD) (r = 0.290), rump angle (RA) (r = 0.209), body stature (BS) (r = 0.196), body length (BL) (r = 0.169), rear teat placement (RTP) (r = 0.138), fore udder attachment (FUA) (r = - 0.105), central ligament (CL) (r = -0.112), rear udder width (RUW) (r = -0.128) and rear udder height (RUH) (-0.179). Significant correlation between SMY and BL (r = 0.240), FA (r = 0.230), RA (r = 0.201), BS (r = 0.167), RTP (r = 0.147), FUA (r = -0.140), RUH (r = -0.164) and udder depth (UD) (r = -0.261). The phenotypic correlations between the type traits and other production traits (PY, Fat% and LL) were low to medium. FA, RA, BL, BD and BS were the only trait that had a significant positive correlation with milk production traits, suggesting that milk performance could be comprehensively improved by including mentioned body structural and udder type traits in the selection procedure. Most of the type traits in Kankrej cows were of intermediate nature and of desirable type but have good amount of variation. The phenotypic correlations amongst the most of type traits were low to moderate but significant ($p<0.05$) and positive. Our findings provide insights of theoretical basis as well as practical aspects of type traits for the early selection of Kankrej cows with desirable milk performance.

ISAGB-2023/Abst/TS-III-036

FIXATION OF G ALLELE IN A>G POLYMORPHISM IN INTRON-2 AND EXON-3 OF FOLLICLE STIMULATING HORMONE BETA GENE IN INDIAN GOAT BREEDS

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Follicle-stimulating hormone (FSH) is member of gonadotropin hormones family. They not only control steroid hormone synthesis and gamete production but also play important role for the genetic improvement of reproductive traits. FSH consists two subunits: alpha (FSHα) and beta (FSHβ), which are coded by two distinct genes. However, the beta subunit shows variations and provides biologic specificity. The study of genetic polymorphism of FSHβ gene and its effects on semen quality traits explore the possibilities of FSHβ gene being used as candidate marker gene for these traits. In view of the above, this study was aimed to investigate genetic polymorphism of follicle stimulating hormone beta (FSHβ) gene by PCR-RFLP assay and association of different polymorphic variants of FSHβ with body weights, scrotal measurements and semen quality traits in Sirohi, Barbari and Black Bengal bucks. Genomic DNA was extracted from venous blood of bucks of Barbari (n=35), Black Bengal (n=26) and Sirohi (n=31) breeds, respectively. The amplified PCR products of size 313 bp was obtained for FSHβ gene, indicating the primer specific amplification. PCR-RFLP analysis of FSHβ was found to be monomorphic in all the three breeds therefore association study could not be done. It revealed only one type of uncut banding pattern of 313 bp and a single fragment of 157 bp by using *PstI* and *HinfI* restriction enzymes, respectively. Absence of recognition site for *PstI* in FSHβ gene was also confirmed by sequence analysis, which revealed A>G substitution at nucleotide position 4531. Frequency of AA genotype and A allele was found to be maximum. Least square analysis of variance revealed significant effect ($p<0.05$) of breed for body weights, scrotal measurements and seminal attributes except mass motility. Based on the findings, it was concluded that targeted region of FSHβ gene was fixed in present population of goats.

ISAGB-2023/Abst/TS-III-037

BAYESIAN MULTI-TRAIT AND PRINCIPAL COMPONENT ANALYSIS PROVIDED PRECISE ESTIMATES OF GENETIC PARAMETERS FOR BEHAVIOURAL, FUNCTIONAL AND LINEAR TYPE TRAITS IN SAHIWAL CATTLE

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A balanced approach in selection and breeding strategies must be adopted to bring about genetic improvement in the indigenous breeds of dairy cattle. Selection of dairy cattle on the basis of multi-trait strategy is scanty so far in India. For this purpose, precise estimates of genetic parameters are essential. Genetic improvement programme is going on for a considerable period of time for Sahiwal, one of the most producing indigenous dairy cattle. Keeping this in view, a multi-trait Animal model with Gibbs sampling using Bayesian approach

was utilized to estimate genetic parameters of linear type, behavioural (milking temperament) and functional traits (milking speed, days open) and to find their association with production traits in Sahiwal cattle. The results indicated that majority of linear type traits, milking behaviour and milking speed exhibited low to high heritability, ranging from 0.14±0.01 to 0.69±0.01. High heritability estimates of 0.51±0.11, 0.54±0.02, 0.68±0.01, 0.51± 0.01, and 0.54± 0.01 were obtained for stature, body length, rump width, teat length and rear udder height respectively. The moderate heritability estimates (0.26±0.02 to 0.34±0.03) were found for chest girth, foot angle, rear leg rear view, milking temperament, milking speed. Few of the linear type traits viz. stature, body length and chest girth was having high positive genetic correlation, ranging 0.24±0.06 – 0.31±0.09 with 300 DMY and TMY. This can be utilized for selection and genetic improvement of Sahiwal cattle in future. Milking temperament was having negative phenotypic correlation with 305 days milk yield and total milk yield, indicating docile cows are producing higher milk. Our result showed that the Bayesian multi-trait analysis could generate precise estimates of genetic parameters, suggesting the reliability and robust nature of this technique for animal breeding data analysis with limited sample size.

ISAGB-2023/Abst/TS-III-038

GROWTH CURVE OF KADAKNATH CHICKEN AS PREDICTED BY DIFFERENT NONLINEAR FUNCTIONS REARED UNDER INTENSIVE SYSTEM

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The growth curve shows the relationship between age and body weight for any given age corresponding body weight can be estimated from the growth curve formula. These parameters are used to describe growth pattern over time and to estimate the expected weight of animals at specific ages. The accuracy of this estimation depends on the R^2 value and the root mean square error. This study was carried out to estimate the parameters of polynomial, exponential, logarithmic, Inverse polynomial, logistic and Gompertz growth curve models in Kadaknath chicken from hatching to 20 weeks of age. The model comparison based on the coefficient of determination (R2) showed that Gompertz model led to an improved fit of data compared to other models. In the present study the highest value for RMSE was obtained for logarithmic model indicating lack of fit; RMSE values calculated for Gompertz is 5.08 and adjusted R2 is also 0.99. The RMSE values for Polynomial, Exponential, Logarithmic, Inverse polynomial and Logistic are 5.23, 8.25, 173.09, 10.64 and 8.15, respectively, the corresponding adjusted R2 value are 0.98, 0.94, 0.57, 0.78 and 0.94, respectively. In conclusion, when evaluated on the basis of both the parameters, i.e. R2 and RMSE the Gompertz model was found to be the best.

ISAGB-2023/Abst/TS-III-039

POLYMORPHISMS WITHIN INTRON-9, 3'UTR AND EXON-24 REGIONS OF PPARGC1A GENE AND THEIR RELATION WITH MILK PRODUCTION TRAITS IN GAOLAO CATTLE

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The current study was planned to detect DNA level variations within widely reported PPARGC1A candidate gene in Gaolao cattle population. The study included around 258 unrelated animals of true to the type Gaolao cattle population. The genomic DNA was isolated and the test day traits were recorded. The genetic variation

was revealed using PCR-RFLP, PCR-SSCP & Direct DNA sequencing tools. The PCR-RFLP screening in intron-9 region of PPARGC1A gene at PPARGCA1G2-*HaeIII* locus revealed two genotypes with genotype frequency for AA genotype as 0.0232 & for AB as 0.9768. The allele frequency for the A alleles was 0.5116, and for the allele B was 0.4884 at the PPARGC1AG2-*HaeIII* locus. The significant (P<0.05) Chi-square value for all the genotypes showed Hardy-Weinberg law was not in equilibrium. The association analysis using logistic regression model reveled non-significant differences between PPARGC1AG2-*HaeIII* genotypes on milk yield and components. PPARGC1AG3-SSCP screening of 191 bp fragment of 3'UTR region revealed monomorphism; however monomorphic amplicon followed by direct sequencing reveled T-G SNPs at 98th and 101st position of sequence. PPARGC1AG5-SSCP within 414 bp fragment of exon-24 region revealed polymorphism with frequency of pattern A=0.25 and Pattern B=0.75 in 56 samples of Gaolao cattle. Direct sequencing of pattern A and B confirmed presence of SNP G-A at 194th position in 325 bp sequence within the region. Results of the current study indicate exitance of genetic variation within the intron-9, 3'UTR and exon-24 regions of PPARGC1A gene in Gaolao population. The identified polymorphisms can be useful in future gene aided selection along with traditional methods for genetic improvement in Gaolao cattle.

ISAGB-2023/Abst/TS-III-040

ESTIMATION OF GENETIC AND PHENOTYPIC PARAMETERS OF LINEAR TYPE, BEHAVIORAL AND PERFORMANCE TRAITS IN CROSSBREED CATTLE (KARAN FRIES)

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Linear type traits are considered as crucial in dairy cattle selection procedure because of the high correlation with production performance which not only focuses on production performance but also takes into account for longevity and overall health of animal. Multi-trait selection procedure was used to estimate the genetic parameters of the behavioural, functional, and linear type traits and their relationship with production performance, were Age at first calving (AFC) was considered as coverable and parity, season, calving period, and stage of lactation were considered as fixed effects, while data on adult Karan fries cattle (N=134) were analyzed by using both Bayesian and Least Square Maximum Likelihood (LSMLMW) approaches. The majority of linear type traits, milking temperament, and milking speed were found to be low to moderately heritable. (Ranging from 0.40±0.03 to 0.25±0 04), with low heritability (0.20±0.25) observed for days open. Among the linear type traits, a strong positive genetic correlation were recorded (ranging from 0.74±0.03 to 0.63±0.017) between stature, body length, and chest girth and total milk yield. The study suggested negative phenotypic correlation (-0.018± 0.081 and -0.050±0.099) between the rear leg rear view and the total milk yield. Central ligament had a negative phenotypic correlation with total milk yield (-0.018± 0.081 and -0.050±0.099) respectively. The study showed that Karan Fries cows may be selected based on few important linear type traits (stature, body length, chest girth, body depth, and angularity) having a positive genetic correlation with production performance. The present study may be continued to include a larger data set for validation and better accuracy of results for the selection of Karen Fries cattle.

ISAGB-2023/Abst/TS-III-041

ESTIMATION OF GENETIC PARAMETERS FOR MILK YIELD IN CROSSBRED CATTLE OF KERALA USING PEDIGREE AND GENOMIC-BASED MODELS

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Estimates of genetic parameters are required for implementing any selection program. Accurate estimation of parameters is essential to predict unbiased breeding values and also to predict accurate response to selection. The present study aimed to estimate genetic parameters for 305-day milk yield in crossbred cattle of Kerala. Genotypic data, phenotypic data, and pedigree information were collected from the Kerala Livestock Development Board (KLDB), Gokulam, Kerala, India. The data set included 17,980 records of the first parity of crossbred cattle of Kerala descended from 534 sires and 2579 dams. The statistical significance of fixed effects such as geography, period of calving, season of calving, and age at first calving (AFC), fitted in the model were performed by R software. As per the results, period of calving ($P < 0.001$), geography ($p < 0.001$), season of calving ($p < 0.001$), and AFC ($p < 0.001$) were significant sources of variation for milk yield. The overall least squares mean of lactation yield was 3099.19±9.72 kg. Animal model employing average information restricted maximum likelihood (AIREML) was used for (co)variance component estimation. The heritability estimate of 305-day milk yield from Pedigree based-AIREML was 0.23±0.02, which was moderate and augured favorable scope of selection. The genomic data was also used to estimate genetic parameters for milk yield. The genomic data comprised 918 animals genotyped by Affymetrix Axiom 50K SNP chip, 687 animals by Mini HD chip, and 878 animals genotyped by Affymetrix Axiom HD chip. Phasing and imputation of 50k to HD data and Mini HD to HD data set was done using Beagle 5.4 (Browning and Browning, 2009). The average accuracy (DR^2) of imputing 50k data set to HD data was found to be 80% and the Mini HD to HD data set was around 86.34%. The final imputed genomic data set included 2273 animals having 6,29,171 SNPs. Quality control of genotypic data was performed using PLINK 1.9 and 4,80,227 SNPs were finally used for further analysis. Genomic AIREML estimates for milk yield were obtained and were 0.34±0.13. The moderate heritability estimates indicated existence of sizable additive genetic variance for 305-DMY and hence further scope to select for enhancing per-animal productivity using genomic selection.

ISAGB-2023/Abst/TS-III-042

CHARACTERIZATION OF RAMPUR HOUND DOG AN IMPORTANT DOG BREED OF UTTAR PRADESH

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In India, exotic dog breeds like German shepherd, Labrador, Doberman, etc., are kept as pets but little emphasis has been given to the native dogs. As per Livestock census 2019, indigenous dog population is 15.31 million, which are of diverse phenotype and utility. Indian native dog breeds/populations reported are Caravan Hound, Combai, Chippiparai, Rajapalayam, Rampur Hound, Mudhol Hound, Himalayan sheep dog, Bhutia, etc. Rajapalayam, Chippiparai and Mudhol hound are the three dog breeds characterized, documented and registered for the first time in India. The remaining indigenous dog populations of the country need to be studied, characterized and documented systematically and registered as new breeds. Keeping this in view the study on Rampur Hound dog was undertaken with the objective to study the geographical distribution, management practices & utility and the physical, morphometric and reproduction characteristics. The survey

on Rampur Hound was undertaken in the villages (Rampur & Suar area) of Rampur district, Lucknow of Uttar Pradesh, Parbhani (Maharashtra) Coimbatore and Thoothukudi (Tamil Nadu). During the survey it was observed that only very few breeders maintain the Rampur Hound dog in the Rampur area, but as per the interaction with the breeders good true to type breed animals found in Lucknow also. Few breeders from Tamil Nadu also maintain these dogs. The dog is believed to be developed by Nawab of Rampur by crossing the English grey hound and Thazi hound. The dogs are maintained as a guard dog by the breeders and the dogs are excellent trainable animals. The dogs are locally called as Shikari. Four coat colours were observed during the field visit i.e Black coat colour, Brindle, Light brown and Fawn colour. Among these four coat colours fawn and light brown colours are predominant coat colours and black and brindle coat colours are found in about 3-4% of the animals. About 63% of animals are having straight head, normal fore head and straight nasal bridge. The top line is straight and with tucked-up abdomen. Animals are about 69.02±1.03 cm in height at withers. Age at first oestrus in bitch is about 15-18 months with October and November months are main breeding season and June-July months are minor breeding season. Age at first mating in dogs is about one year. Litter size ranges from 6-10 puppies and weaning is done at the age between 40-60 days. There is a huge demand for puppies among the dog lovers and the selling price ranges from Rs 10,000 to 15,000 per puppy depending on the pedigree of the parents and coat colour. Parvo is the major disease prevalent in this dog breed and breeders used to vaccinate the dogs with seven in one combined vaccine. Most of the breeders maintain these dogs under non-veg food feeding twice a day. As per the preliminary survey conducted the number of Rampur Hound is in alarming stage. Hence, there is an urgent need to document this important canine genetic resource of India and register as new breed and further steps to be initiated to conserve this canine germplasm.

ISAGB-2023/Abst/TS-III-043

DEVELOPMENT OF GENOMIC SELECTION MODELS TO ADDRESS THE LOOSE STRUCTURED DAIRY CATTLE BREEDING IN INDIAN CONDITIONS

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This study aimed to develop and test genomic selection models for India's loose-structured dairy breeding system. Simulated data replicated two scenarios: one with incomplete pedigree data and another with joint genomic prediction for four related, small dairy breeds where pedigree data was absent. Shallow pedigrees in such systems often lead to biased breeding value predictions using BLUP. In complete pedigrees, PBLUP yielded unbiased EBV with accuracies of 0.35±0.02 and 0.26±0.01 for heritabilities of 0.3 and 0.1, respectively. Shallow pedigrees led to biased predictions with decreasing accuracy as more distant pedigree information was removed. For the removal of four distant pedigree generations, accuracy and bias were 0.30±0.02 and 0.55±0.03 in moderate heritability. We observed that ssGBLUP with selective genotyping all female reference (missing 4 distant generations) resulted in high accuracy ($r = 0.50 \pm 0.02$) and least bias ($\rho = 0.96 \pm 0.02$). The study also explored joint genomic prediction and selective genotyping for sex-limited traits across four breeds. Combining breeds improved prediction accuracy and reduced bias. Ancestry-specific factors had minimal impact due to genetic similarity. The multi-breed two-tailed selective genotyping model consistently outperformed other models. The study recommends ssGBLUPF with a two-tailed selectively genotyped all-female reference in shallow pedigrees for unbiased and accurate GEBV for sex-limited traits with limited resources. For small breeds without historical data, adopting a multi-breed joint evaluation with two-tailed selective genotyping is advised. This strategy integrates crucial breeds into genomic selection while conserving resources in resource-poor regions.

ISAGB-2023/Abst/TS-III-044

GENETIC CHARACTERIZATION OF 17 TYPE TRAITS IN AN ORGANIZED HERD OF SAHIWAL CATTLE IN NORTHERN INDIA

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The Present investigation was undertaken to profile and classify Sahiwal cattle based on their linear type traits, addressing a critical research gap, *i.e.*, the non-incorporation of linear type traits for dairy cattle selection in India to date. Data was systematically collected from adult Sahiwal cows, spanning up to their 10th calving, from the Institute herd of ICAR- National Dairy Research Institute Karnal, Haryana. The analysis employed the Least Squares Maximum Likelihood approach, adopting a sire model as the basis for this investigation. Fixed effects incorporated considerations of parity, calving season, and calving period, while age at first calving was included as a covariate. Moreover, the sire or animal factor was treated as a random variable. The study revealed the least squares means for objective linear type traits in Sahiwal cattle: stature (125.97±0.68 cm), body length (136.58±1.01 cm), chest girth (173.04±1.14 cm), body depth (203.40±1.39 cm), rump angle (14.50±0.39 cm), rump width (20.62±0.28 cm), udder depth (20.87±0.41 cm), rear udder height (18.38±0.33 cm), and teat length (6.16±0.24 cm). Conversely, least square mean scores on a 1-9 scale were obtained for subjective linear type traits, including fore teat placement (6.32±0.46), rear teat placement (7.27±0.35), rear leg side view (6.26±0.26), rear leg rear view (6.28±0.27), foot angle (6.86±0.27), angularity (34.97±0.50 degrees), central ligament (6.55±0.39), fore udder attachment (5.91±0.32), and fore teat placement (6.32±0.46), and rear teat placement (7.27±0.35) for Sahiwal cattle. Parity showed a significant effect ($p<0.05$) on udder depth, and the season of calving also exhibited a significant effect ($p<0.05$) on udder depth in Sahiwal cattle. Furthermore, the period of calving displayed a significant effect ($p<0.05$) on rump angle, udder depth, teat length, angularity, and central ligament within the Sahiwal cattle population. These findings emphasize the importance of a balanced scoring pattern for linear type traits in Sahiwal cattle, promoting their sustainability and long-term production performance.

ISAGB-2023/Abst/TS-III-045

GROWTH TRAITS IN MARWARI GOAT UNDER FIELD CONDITION

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The data for the present investigation were collected from farmers' flocks of Marwari goat maintained under sponsorship of All India Co-ordinated Research Project (AICRP) on goat improvement. The information of the 8381 Marwari goats were collected during the period 2018 to 2023 from the different cluster villages of the Bikaner district. The data on body weight at birth, 3, 6, 9 and 12 months of age were collected for the study. The data were assigned to linear mixed model equation (LMME) analysis using IBM SPSS version 25.0. The overall least-squares means were estimated as 2.64±0.003, 8.35±0.010, 14.46±0.020, 18.57±0.055 and 23.47±0.10 kg at birth, 3, 6, 9 and 12 months of age, respectively. The effect of sex was significant ($P\leq0.05$) on birth, 6, 9 and 12 months body weights and non-significant for 3 months body weight. The effect of cluster was non-significant on birth, 3, 6, 9 and 12 months body weights.

ISAGB-2023/Abst/TS-III-046

GENETIC ASSOCIATION OF STAT1 GENE SNPS WITH MILK CONSTITUENTS IN HF CROSSBRED CATTLE

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Present study has been conducted to identify genetic effect of STAT1 gene polymorphism on milk composition in Holstein Friesian crossbred cows. Milk composition data for first lactation on 222 adult cows were collected from Livestock Farm, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (India) for a period of 12 years (2009 – 2020). Further, the data was corrected for non-genetic factors viz. season of calving (SoC), period of calving (PoC), interaction effect of season and period of calving (SoC*PoC), and age at first calving (AFC). After data correction, these animals were divided into high and low milk fat and protein yield groups, and 20 cows of each group were finally selected for genetic analysis. DNA was isolated from the blood samples of these cows and further amplified for 314 bp long region of STAT1 gene using PCR. Amplified PCR products were subjected to RFLP analysis using *BspHI* restriction enzyme to identify the gene polymorphism. Two SNPs at position 201 and 260, with C-T substitution at both positions were identified in the whole 314 bp fragment of STAT1 gene. Genotype frequencies for AA and Aa were observed to be 0.80% and 0.20% at 201 nucleotide position; and for BB and Bb were observed to be 0.25% and 0.75% at 260 nucleotide position, respectively. Least square analysis showed a significant association of AA and Aa genotypes at 201 and BB and Bb genotypes at 260 nucleotide position of STAT1 with milk fat yield as well as milk protein yields in these cows. The SNPs identified under this study may be possible candidate for marker-assisted selection programs in HF crossbred cattle for milk trait improvement.

ISAGB-2023/Abst/TS-III-047

PREDICTION OF 40 WEEK EGG PRODUCTION FROM EARLY PERFORMANCE TRAITS IN SYNTHETIC WHITE LEGHORN STRAIN OF CHICKENS USING MULTIVARIATE ANALYSIS

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The current study was carried out on synthetic White Leghorn chicken strain, which was maintained at the Poultry Farm, Department of Animal Genetics and Breeding, LUVAS, Hisar. The primary objective of this study was to explore the potential for predicting 40-week egg production based on early performance traits using multiple regression (MRA) and principal component analysis (PCA). The performance traits considered were body weight at 20 week of age (BW_{20}) and age at first egg (AFE), part egg production traits viz. egg production from age at first egg (AFE) to 24 week of age (EPP_1), 25 to 28 week of age (EPP_2), 29 to 32 week of age (EPP_3), 33 to 36 week of age (EPP_4) and 36 to 40 week of age (EPP_5) and cumulative part egg production traits were egg production up to 24 weeks of age (EP_{24}), egg production up to 28 weeks of age (EP_{28}), egg production up to 32 weeks of age (EP_{32}), egg production up to 36 weeks of age (EP_{36}). In MRA, the best prediction equation for 40-week egg production had a R^2 (90%) when using only EP36. When combining EP36 with EPP4, it achieved the highest R^2 at 92%. When combined with EPP2 or EPP3, it explained 90% of the variation in 40-week egg production (EP_{40}). Furthermore, PCA was applied to extract essential components from the dataset. Through PCA, four principal components were derived, collectively accounting for an impressive 99% of the total variance explained in the original traits. The findings of this

study are significant for predicting egg production in the synthetic White Leghorn chicken strain. Moreover, these results hold importance for optimizing genetic selection programs within this poultry population.

ISAGB-2023/Abst/TS-III-048

USE OF MULTIVARIATE ANALYSIS FOR PREDICTION OF 40 WEEK EGG PRODUCTION FROM MORPHOMETRIC MEASUREMENTS IN SYNTHETIC WHITE LEGHORN STRAIN OF CHICKENS

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The present study was undertaken on a population of synthetic White Leghorn strain of chickens, maintained at the Poultry Farm, Department of Animal Genetics and Breeding, LUVAS, Hisar. The primary objective of this study was to investigate the possibility of prediction of the 40 week egg production from morpho-biometric traits using multiple regression and principal component analysis. A total of 727 individual birds were included in this study. The selection of morpho-biometric traits for prediction encompassed specific measurements taken at 20 weeks of age, namely, back length (BaL), beak length (BeL), body length (BoL), comb length (CL), radius-ulna length (RUL), breast girth (BG), breast angle (BA), keel length (KL), tail length (TL), shank length (SL), and shank circumference (SC). In the context of multiple regression analysis, a prediction model was developed, incorporating all morpho-biometric measurements. This comprehensive model exhibited a noteworthy capacity to explain 92% of the variance observed in the 40-week egg production. Concurrently, Principal Component Analysis (PCA) was employed in conjunction with orthogonal rotation techniques to extract essential components from the dataset. Through PCA, three principal components were derived, collectively elucidating a remarkable 96% of the total variance found in the original morpho-biometric variables. The outcomes of this study are valuable for the prediction of egg production in the synthetic White Leghorn strain of chickens. Furthermore, these findings hold significance for the optimization of genetic selection programs within this poultry population. Notably, the regression models generated, utilizing the PCA methodology was found more accurate in the prediction of egg production compared to conventional multiple regression analysis techniques.

ISAGB-2023/Abst/TS-III-049

IMPACT OF VARIOUS SOURCES OF INFORMATION ON THE BLUP BREEDING VALUES AND THEIR RANKING OF MAHA-MAGUR (CLARIAS MAGUR (HAMILTON, 1822))

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Clarias magur, an Indian catfish species, has the potential to be a candidate species for commercial aquaculture. ICAR-CIFE has been implementing a genetic selection program to improve body weight at one year of pond age in Magur since 2013. The present study aimed to evaluate the impact of various information sources on the animals' BLUP BV rankings. Body weight at the one-year pond age of the Maha magur was the trait of interest. The data were analysed for both genetic and non-genetic factors employing LMM equations. The non- genetic factors (generation, sex and pond) affected selected growth traits significantly. The heritability estimates for body weight in the base, first, second and third generation by the animal

model were 0.67±0.08, 0.48±0.10, 0.30±0.08 and 0.25±0.08, respectively. The genetic correlations between various growth traits were positive and high (0.91 to 0.97), and the phenotypic correlations were positive and moderate (0.54 to 0.87). The BLUP BV for the animals was predicted by adding only full-sibs, relatives of progeny generation, grand progeny generation, etc. In the earlier generations, adding information from progeny or grand-progeny information to predict the BVs substantially altered the animals' ranks. However, adding immediate successive generations to predict the BV did not alter the rank significantly. Also, adding information from more than two generations did not significantly alter the BLUP BV rankings, indicating that information beyond three generations to estimate the BLUP BV may not be helpful. The rank correlation between PBVs by various combinations of BLUP was high (0.8 to 1). The accuracy of BLUP PBVs was high in each generation.

ISAGB-2023/Abst/TS-III-050

FCR ESTIMATION FOR BROILER

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The present experiment was conducted in the department of Livestock Production and Management, Veterinary College Bidar, KVAFSU Karnataka, to evaluate the feed conversion ratio of 100-day-old Cobb 425 broiler strain broilers. Birds were maintained with standard management practices. The experiment was carried up to 35 days. The brooding temperature was maintained at 34 degrees Celsius, and dropped to 26°C by the twenty-first day. Later broiler chicks were maintained at room temperature consistently. All the chicks were vaccinated on Day-old, 7, 14, 21, and 28 days by MD (s/c), RDVF1 (V/O), IBD (I/O), RDV-Lasota (drinking water), and IBD-booster (drinking water). Chicks were housed on newspaper or other bedding material up to seven days; after that, deep litter made of 2.5–3 cm rice husks is used. In accordance with the BIS-2007 standard, the broilers were fed pre-starter (0–4 days), starter (5–28 days), and finisher (28–37 days) feed. The chicks were on ad-libitum feed and water throughout the experimental period. Calculated the Body Weight, Feed intake and Feed Conversion Ratios, were as feed intake per unit body weight gain at intervals. Throughout the experiment the mortality rate was 2%. The average body weight of day-old, 17th and 35th days was 0.0423 kg, 0.889 kg and 1878.56 kg, respectively and feed intake for pre-starter, starter and finisher was 39.5 kg, 73.5 kg and 197 kg, respectively. The FCR calculated on the 35th day of experiment and it was 1.659.

ISAGB-2023/Abst/TS-III-051

COMPREHENSIVE GENOME-WIDE ASSOCIATION STUDY OF PRODUCTION TRAITS IN VRINDAVANI CATTLE USING MULTIPLE MODELS

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Genetic variants, especially single nucleotide polymorphisms (SNPs), are commonly investigated through genome-wide association studies (GWAS). Traditionally, Vrindavani cattle have been primarily chosen for their milk production potential, thereby prompting a need for the exploration of their other genetic traits in the context of enhancing their genetic quality. This study aimed to identify SNPs associated with production traits in Vrindavani cattle by genotyping 96 animals using the Bovine50K BeadChip. The study examined three traits, including fat-corrected milk (FCM), energy-corrected milk (ECM), and the adjusted number of days in milk (ANDIM). The association study was conducted using four linear models {GLM (General

Linear Model), MLM (Mixed Linear Model), CMLM (Compressed Mixed Linear Model), and ECMLM (Enriched Compressed Mixed Linear Model)} integrated in the GAPITv.3 (Genome Association and Prediction Integrated Tool) package in R software. The GLM model controlled for population stratification, while the MLM added kinship information to the model. In contrast, the CMLM and ECMLM utilized clustering algorithms to better account for the relationships among individuals and optimize the results. In this study, a Bonferroni-corrected genome-wide threshold of -logP = 5.92 and a suggestive threshold of -logP = 4.62 revealed no significant SNPs associated with the studied traits. To validate the GWAS results, QQ plots and lambda values (λ_{gc}) were employed, which suggested that these models controlled population stratification (λ_{gc}~1.00) while studying the traits. Furthermore, the study suggested the use of multiple models for elucidating the significant SNPs as suggested by the literature. This research provided valuable insights into the genetic basis of production traits in Vrindavani cattle, offering potential applications in selective breeding.

ISAGB-2023/Abst/TS-III-052

COMPARATIVE ANALYSIS OF MILK FATTY ACID PROFILING OF KARAN FRIES, THARPARKAR, BELAHI AND HF CROSSBRED CATTLE

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The present study was undertaken to comparatively analyse the milk fatty acid profile of various breeds of cattle, namely Karan Fries (34), Tharparkar (10), Belahi (10) and HF crossbred (46), using a total of 100 samples. Samples were collected from organised herd of NDRI for Karan Fries (KF) and Tharparkar (TP), whereas for HF crossbred (HFCB) samples were taken from organised herds in field. Belahi samples were obtained from the migratory herds. Out of 4 breeds, Belahi were typically on pasture feeding, whereas other breeds received feed on farm. In this study, only the most important fatty acids (N=18) were taken into analysis, out of which 56-69 % were saturated. For the saturated FA, HFCB, KF, TP and BL milk comprised 69.41%, 59.43%, 60.3% and 56.06%, respectively. It was observed that 8.36% saturated FA in HFCB were short-chain fatty acids, majority of which is butyric acid (C4:0). Their proportion in KF, Belahi and TP were 7.48%, 7.62 and 7.74%, respectively. Among long-chain saturated fatty acids, majority comprised of palmitic acid (C16:0) which accounted for 25-35% of saturated fatty acids. Breed-wise proportions of palmitic acid were 35.07%, 24.17%, 29.98% and 26.88% in HFCB, KF, Belahi and TP, respectively. Approximately 25-32% of the fatty acids in milk are mono-unsaturated fatty acids (MUFA).The MUFA were 31.27% in KF, 24.38% in HFCB, 30.53% in TP and 28.83% in Belahi cattle. For polyunsaturated fatty acid (PUFA), the proportions were 2.28% in KF, 1.0% in HFCB, 2.05% in TP and 1.43% in Belahi cattle. PUFA are essential fats which the body cannot synthesise and they need to be supplemented in diet. The MUFA and PUFA are considered as good fats for heart health. The proportion of SFA were higher in HFCB as compared to other three breeds. The proportionate volume of unsaturated fats such as MUFA and PUFA were lowest in HFCB as compared to other three breeds. With existing nutritional dietary knowledge, we understand that unsaturated fats are better as compared to saturated fats for better health prospects, as traditionally saturated fat intake has been linked with increased heart disease risks. However, this idea has been called into question more recently. This study could reveal increased MUFA and PUFA composition as well as less Saturated FA in indigenous as well as KF (62.5% exotic inheritance) cows as compared to higher-grade HFCB. We could not see impact of migratory grazing pattern of FA profiling.

ISAGB-2023/Abst/TS-III-053

EXPLORING GENETIC DETERMINANTS OF PROLIFICACY IN SHEEP THROUGH A META-ANALYSIS OF GWAS

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Meta-analysis has emerged as a powerful approach to identify real genetic associations with traits for animal-based breeding methods. This study performed a meta-analysis of Genome-wide association studies (GWAS) summary statistics from 2125 sheep across 19 breeds, investigating prolificacy using 286784 SNPs. Twelve significant associations with prolificacy were identified at Bonferroni corrected threshold of P<1E-06, with the most significant association observed at rs418841713, followed by rs418228681 and rs412553064, which mapped to the BMPR1B, MTX2 and CNTNAP2 genes, respectively. The presence of breed-specific top SNPs suggests that certain genetic variations may have a more pronounced effect on litter size in specific breeds, thus indicating that the genetic architecture of prolificacy is diverse, and multiple genes and SNPs likely contribute to the observed variation in litter size among different sheep breeds. Furthermore, the meta-analysis uncovered previously unidentified SNPs (such as rs402117622 on LRIG3 and rs409990001 on SAMD12), as significant in the initial GWAS. The lack of recognition in the original GWAS may be attributed to the limitations of the sample size used in those studies. Gene set analysis revealed pathways related to cellular response to endogenous stimulus and peptide cell signalling. Among the twenty-four KEGG pathways exhibiting significant enrichment (FDR<0.05) for the associated SNPs, the GnRH secretion pathway demonstrated the highest fold enrichment compared to other pathways. Network analysis of protein-protein interactions identified TYRP1, PLCB4, GHR, PIK3C2G, SLC45A2, and PIK3CD as hub genes. The present study contributes towards a broader understanding of the genetic makeup of prolificacy in sheep and also underscores the significance of sharing GWAS summary statistics to uncover previously overlooked SNPs.

ISAGB-2023/Abst/TS-III-054

STUDY ON GENETIC EVALUATION OF PRODUCTION TRAITS OF MURRAH BUFFALO THROUGH REPEATABILITY MODEL

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The present study was conducted to genetically evaluate Murrah buffaloes for economically important production traits recorded across parity. The study utilised lactation records of Murrah buffalo, maintained at ICAR-National Dairy Research Institute, Karnal from the period 1971 to 2020. Through Bayesian analysis utilising the BLUPF90 family of programme, genetic analysis was carried out using animal and repeatability model with a combination of random factors, such as the direct additive genetic effect of the animal, the direct maternal genetic effect, and the maternal permanent environmental effect. Using the most appropriate models (based on DIC value) that account for direct and maternal influences fitted to the traits, (co)variance components and genetic parameters were estimated. The heritability estimates of 305MY, TMY, LL, and PY in the most appropriate models were 0.19 ± 0.007, 0.20 ± 0.011, 0.04 ± 0.001, 0.10 ± 0.001, respectively with repeatability estimates of 305MY, TMY, LL, PY, as 0.39,0.40, 0.20, and 0.21. In comparison to the animal model with only additive genetic effects of the animal in each trait, lower error variance and higher additive variance were found for production traits through various models based on partition of variance, indicating that it is crucial to use the best statistical model for elucidating genetic parameters that incorporate suitable random and fixed effects.

Technical Session - IV

Functional Genomics and Epigenetics
in Livestock and Poultry

ABSTRACTS

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XVII Annual Convention of
Indian Society of Animal Genetics & Breeding

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ISAGB-2023/Abst/TS-IV-001

COMPREHENDING THE CORRELATION BETWEEN THE SEASONAL VARIATIONS ASSOCIATED GENE EXPRESSION WITH CELLULAR CHANGES IN MURRAH BUFFALO SPERM CELLS

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The degree of oxidative stress has a significant role in regulating male fertility. Testicular tissue is vulnerable to oxidative stress due to its rapid cell division and mitochondrial oxygen consumption. Antioxidants protect against harm by either neutralizing free radicals or inhibiting their production in testicular cells. To correlate the mitochondrial oxidative stress and apoptosis-related functions with the semen quality, the expression of genes responsible for both the characteristics, *ATF4*, *ATF5*, *BAK1*, and *MCL2*, was analyzed across seasons (hot summer and winter) and across groups of Murrah bulls (bulls with poor semen quality only during hot summer, or SA; bulls with consistent good semen quality across all the seasons, or SNA). Additionally, for the same group of animals, flow cytometry derived data was generated: (high mitochondrial membrane potential (HMMP), low mitochondrial membrane potential (LMMP), dead/live acrosomal reaction (DI, LI) and dead/live acrosomal reaction (DR, LR). The RNA was isolated from the purified sperm pellets obtained from Frozen Murrah semen using the hot TRIzol method; the cDNA was synthesized from 1 µg of RNA and checked for gDNA, epithelial cells, somatic cells, and leucocyte contamination. The winter samples were considered controls, and *EEF2* and *B2M* were chosen as internal control genes for real-time data normalization. *BAK1* and *ATP5* were found to be down-regulated, whereas *ATP4* and *MCL2* were up-regulated in the hot summer in comparison to the winter season. No significant fold-change expression was recorded for any of the genes in the summer stress-affected and non-affected buffalo bulls. The Pearson correlation matrix for flow cytometry-derived data and expression fold change suggested that only during the winter season, the expression of apoptotic genes was positively correlated with the oxidative stress genes. Overall, the results show that during either season, the acrosomal integrity, dead and live spermatozoa and mitochondrial membrane potential were negatively correlated.

ISAGB-2023/Abst/TS-IV-002

UNRAVELING THE EVOLUTIONARY HISTORY OF LIVESTOCK: INSIGHTS FROM MITOCHONDRIAL DNA

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The domestication of animals represents a pivotal moment in human history, profoundly influencing our demographic and cultural progress. It has resulted in significant genetic, behavioral, and physical changes in livestock species when compared to their wild ancestors. Therefore, comprehending the evolutionary history and genetic diversity of Indian livestock and categorizing them based on their evolutionary significance is of paramount importance. Mitochondrial DNA (mtDNA) has emerged as the preferred marker for investigating molecular diversity in animals in view of its highly conserved gene contentacross animal

species,minimal duplications, absence of introns, and short intergenic regions. While mtDNA is traditionally believed to exhibit strict maternal inheritance, recent studies have unveiled instances of paternal leakage and heteroplasmy. Comparative analysis of mitochondrial and nuclear genes frequently reveals significant variations in their phylogenetic information, with mitochondrial DNAoffering superior resolution for closely related species and taxa.Investigating divergence and phylogenetic relationships within the framework of mitochondrial DNA is crucial for formulating effective policies for conservation and breeding programs. Adopting a holistic approach incorporating mtDNA will further illuminate our understanding of evolution. This will enable us to gain deeper insights into the intricate processes that have shaped the genetic landscape of domesticated animals, ultimately facilitating more informed and effective strategies for their management and preservation.

ISAGB-2023/Abst/TS-IV-003

TRANSCRIPTOME ANALYSIS OF BOVINE SPERMATOZOA REVEALS SEX SELECTION SIGNATURES

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Sex-sorted bovine semen is a disruptive change for the dairy sector. We find gross deficiencies in omics knowledge bases concerning X- and Y-chromosome bearing spermatozoa (X- and Y-sperm) across mammalian species notably native cattle (Bos indicus). Previously, we reported proteome profile of sperm including differential plasma membrane-associated proteins between X- and Y-sperms of native cattle (Laxmivandana et al., 2021). Here, we report the transcriptomes of X- and Y-sperms of native cattle along with validation of differential gene expression (DGE) to provide original insights into the multifaceted sex determination. Mapped reads were considered for transcript assembly and quantification of transcript abundance. Further, differential gene expression (DGE) analysis was performed. The DGE analysis revealed upregulation of 737 genes in bovine Y-sperm and downregulation of 1,016 genes vis-à-vis X-sperm. The GO enrichment analysis of genes upregulated in Y-sperm revealed a significant association with molecular functions such as oxidative phosphorylation, glycolysis and motility of sperm. The major biological processes were related to mitochondrial translation and regulation of MAPK cascade. The pathway and network analysis also pointed out that genes unique to Y-sperm are involved in sperm motility like the cAMP signalling pathway, cGMP-PKG signalling pathway and Calcium signalling pathway. The top 22 DGE were validated using qPCR which showed significant up/down-regulation of the genes in Y-sperm ($P<0.01$) in sync with the transcriptome data. Thus, we deciphered the transcriptomes of X- and Y-sperms of Bos indicus cattle along with identification and validation of the differential transcripts that pave the way for following a proteogenomics approach for sex selection signatures.

ISAGB-2023/Abst/TS-IV-004

COMPARATIVE TRANSCRIPTOMICS: INDIGENOUS VS. IBL-80 BROILER CHICKENS

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Indigenous chicken breeds have traditionally played a crucial role in ensuring nutritional security and supporting the rural economy due to their adaptability to local environments and disease resistance. However, they typically lag in performance, especially in growth traits, making them less popular for commercial broiler farming. In this study, we conducted a comparative transcriptomic analysis of lung and trachea tissues in three chicken groups: Aseel, Punjab Brown, and IBL-80 commercial broilers. The RNA-seq analysis (raw data available at Bioproject: PRJNA972351, Biosample: SAMN35074231) identified 5,282 differentially expressed genes (DEGs) across the three chicken groups. Surprisingly, a higher number of DEGs were downregulated in all three groups. Gene Ontology (GO) enrichment analysis revealed similar terms enriched in the DEGs among the chicken groups. Notably, we focused on immune-related genes using the PANTHER tool. We found that Aseel had a greater number of innate-immunity-related genes compared to Punjab Brown and IBL-80 commercial broilers. These genes primarily included chemokines and chemokine receptors. Aseel displayed a higher abundance of genes associated with chemokines and chemokine receptors when compared to Punjab Brown and IBL-80 commercial broilers. This research sheds light on the transcriptomic differences in immune-related genes among these chicken groups, providing valuable insights into their immune responses and potentially contributing to the future development of poultry farming strategies.Top of Form

ISAGB-2023/Abst/TS-IV-005

MAY OUR ENVITONMENT EDITING MODEL, EEM SAY GENE & GENOMICS ARE CONTROLLED DIGITALLY IN FISHERIES, LIVE-STOCK, MANKINDS' AND EVERY OTHER SPP

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Every gene, genomics are inherited from parental sources found every plant and animal are well known. In digital biochemistry every genetic materials are synthesised from the inputs of bio-molecules of environmental as for example we get extra bases of genetic materials in Tea and Coffee etc and Ayurveda products. Similar to plants, we find all the dwarf or small cattle in highest digital cation exchange capacity i.e., CEC environments, whereas all large cattle in low CEC environments. Since every metal, heavy metal in digital CEC has very high anabolic inhibitions with every livestock resulting highest CEC yields dwarf live-stocks. A long ago once authors used to maintain a bushy environment with highest digital CEC environments and naturally managed that yielded only smallest ever bird, locally named 'Tuntuni" existence. In similar highest CEC Environment authors find only smallest intra and inter Fish species any fish species and Puntius respectively in highest digital CEC environment. There are relations with intra or inter species tallness and dwarf-ness with environmental digital CEC. This science is communicated since years. This environment supported

genetic traits can be altered with DISPER EEM or EEM. Environmental Editing Models. In DISPER EEM we find acquiring a zero DISease PERcentage may become possible when we keep an environmental having digital Cation Exchange Capacity bellow 20 meq per 100 grams of environmental sample to every live-stock including Fisheries and cattle management In recent days super high-tech Western CRISPR Model may also be applicable in Livestock management with very high investments may supported by DISPER EEM. Authors find that EEM that if live-stocks are fed with volatile Isoprene yielding plants then every live-stocks may enjoy a zero diseased life-spans since the most hygiene bio-molecule, Isoprene at a conc. above 1 pbb either in air, water or in soils, can completely destroy diseases causing Virus by means of inhibiting genetic replication through a chain biochemical cellular reaction, recently communicated for livestock including fisheries and mankind. In other EEM we find below a digital TDS with 165 ppm, there may be no-diseases amongst the fish species.

ISAGB-2023/Abst/TS-IV-006

IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH FITNESS TRAIT USING RNA-SEQ ANALYSIS OF OVARIAN TISSUE OF INBRED SWISS ALBINO MICE

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Litter size in mice is an important fitness and economic feature that is influenced by several genes as well as non-genetic factors. High- and low-prolific lines of inbred Swiss albino mice showed substantial variability in the litter size at birth (LSB) in each generation, despite uniform experimental conditions. To explore the difference in gene expression between high litter sized (HLS; LSB ≥12; n=3) and low litter sized (LLS; LSB ≤3; n=3) lines of F_4 inbred Swiss albino mice, expression analysis of ovarian tissue was done using RNA-Seq. A total of 870 differentially expressed genes (DEGs) were identified on transcriptome analysis; among these, 287 genes were significantly up-regulated while 583 genes were down-regulated in HLS as compared to the LLS inbred Swiss albino mice group. DEGs were assigned to 166 Gene Ontology (GO) terms and KEGG pathways. In HLS, the significantly up-regulated DEGs were involved in ovarian cell-cell signaling, regulation of biological activity and ovarian metabolic associated pathways. The majority of down-regulated DEGs were expressed in immune-related pathways, indicating that immunological dampening is associated with a high ovulation rate and higher level of progesterone concentration leading to physiological changes responsible for higher fecundity. A total of 24 DEGs (ACOD1, CLEC4E, ADAMDEC1, ALDH3A1, PROM2, SPTSSB, ALDH1A1, SLC5A11, COX8C, FAT2, KRT15, COLQ, S100A8, CLSTN3, S100A9, TP63, SLC5A7, WNT11, CNR1, UCP3, SLC1A3, PRKAR2B, HADH and IRS2) were identified as candidate genes for LSB traits in HLS F_4 inbred Swiss albino mice. Further, expression profile of these genes needs to be investigated for finding expression Quantitative Trait Loci (e-QTL) associated with fitness traits in comparatively larger population.

ISAGB-2023/Abst/TS-IV-007

WHOLE MITOGENOME BASED GENETIC DIVERSITY ANALYSIS REVEALS DISTINCT MATERNAL ORIGINS FOR THE DROMEDARY AND BACTRIAN CAMELS

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There are two major tribes called Lamini (New World camels) and Camelini (Old World camels) in the Camelidae family. The Camelini tribe encompasses the larger camelids, specifically the wild double-humped camels (*Camelus ferus*), domestic double-humped camels (*Camelus bactrianus*), and domestic single-humped camels (*Camelus dromedarius*). In India, there are nine recognized breeds of dromedary camels, namely Bikaneri, Jaisalmeri, Kutchi, Mewari, Jalori, Malvi, Marwari, Kharai, and Mewati. These breeds primarily inhabit the nation's hot deserts. Additionally, the Nubra valley of Ladakh hosts a small population of two-humped Bactrian camels. This research represents an exploration into the whole mitogenome-based genetic diversity of Indian camel populations. It employed whole genome re-sequencing data from 44 dromedary and 7 Bactrian camels. For all the animals studied, a consensus mitogenome sequence was generated through alignment with the reference mitochondrial genome. Among the dromedary camels, there was a notably high haplotype diversity (Hd: 0.9979), with 42 haplotypes found among the 44 samples studied. Their nucleotide diversity was measured at 0.00215. In contrast, the Bactrian camels displayed lower genetic diversity, with haplotype and nucleotide diversity values of 0.524 and 0.00005, respectively. The Analysis of Molecular Variance (AMOVA) revealed a significant 97.15% genetic variance between the two species, with a mere 2.15% genetic variance noted within each species. To further analyze the genetic relationships of Indian camels within the broader global genetic diversity of the Camelidae family, a Neighbor-Joining tree was constructed using representative mitogenome sequences retrieved from the NCBI. The phylogenetic analysis clearly segregated the Camelini and Lamini tribes into distinct clusters. Moreover, within the Camelini tribe, a clear differentiation was observed among the three major camelids, suggesting unique maternal origins for each species.

ISAGB-2023/Abst/TS-IV-008

MOLECULAR INSIGHTS INTO PASHMINA FIBER PRODUCTION: COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF CHANGTHANGI GOATS AND SHEEP

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Situated in one of the most inhospitable environments on Earth, Ladakh is home to the distinctive Changthangi goat, famed for its production of Pashmina, the most luxurious natural fiber known. In contrast, the wool from the region's Changthangi sheep ranks below Pashmina in terms of quality. Our study aimed to examine and contrast the skin transcriptomic patterns of both Changthangi goats and sheep to uncover the genetic determinants that contribute to recognition of Changthangi goats as premier Pashmina producers. Based on data from prior research, we selected 225 genes linked to fiber traits from the differentially expressed gene pool observed between the two species, using stringent criteria (adjusted p-value of ≤ 0.05 and a Log2 fold change of ≥ 1.5). These genes underwent further analysis through DAVID software for biological

function interpretation and pathway enrichment in KEGG and Reactome databases. The protein-protein interaction networks were constructed via Cytoscape, cytoHubba, and STRING tools to pinpoint pivotal genes and ascertain their roles. The comparative transcriptomic analysis showed a marked upregulation of genes in pathways including Wnt, MAPK, PI3K-Akt, and Hedgehog in Changthangi goats, all critical to fiber formation and its quality attributes. Such pathways are integral to hair follicle development, the preservation of epidermal stem cells, and defining fiber properties. Additional insights were gained into cell adhesion molecules and ECM-receptor interactions, which are crucial for hair follicle structure, growth, and signaling. Our research provides a detailed molecular portrait of Pashmina fiber genesis in Changthangi goats, shedding light on the genetic mechanisms that contribute to the unique attributes of Pashmina fibers.

ISAGB-2023/Abst/TS-IV-009

IDENTIFICATION OF STABLE REFERENCE GENES FOR QUANTITATIVE REAL-TIME PCR ACROSS DIFFERENT OVINE TISSUES

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Quantitative Real-Time PCR (qPCR) is a popular method for measuring gene expression levels in a variety of tissues and under various disease and healthy states. However, this technique faces the challenge of selecting appropriate reference genes (RGs) that exhibit stable expression across a range of experimental conditions to guarantee valid biological conclusions. In our research, we evaluated 18 potential RGs (ACTB, BACH1, B2M, GAPDH, HMBS, HPRT1, PGK1, PPIA, PPIB, RPLP0, RPL19, RPS9, RPS15, RPS28, SDHA, TBP, UXT, and YWHAZ) in 10 different sheep tissues (muscle, skin, kidney, liver, intestine, rumen, lung, testis, heart, and spleen), collected from five sheep, to identify consistently expressed genes. Through an extensive review of the literature, we selected our list of potential RGs, which included representatives from various functional categories across different species of livestock. To determine the stability of these genes, we utilized a combination of four analytical approaches: geNorm, NormFinder, BestKeeper, and the Delta Ct method. The stability rankings from these algorithms showed a coherent pattern. For an aggregate stability ranking, we applied RefFinder, which amalgamated data from the different approaches. Our results indicated that ACTB, PPIB, BACH1, and B2M were the most stable RGs, whereas RPS9, RPS15, and PGK1 had inconsistent expression patterns. We confirmed our results by analyzing the expression of four selected genes (ACTN2, CRYAB, DLK1, and TRIM54) via qPCR in skin samples from two sheep breeds (Changthangi and Muzaffarnagari). From these observations, we suggest ACTB, PPIB, BACH1, and B2M as dependable internal control genes for qPCR studies in sheep tissues.

ISAGB-2023/Abst/TS-IV-010

DIVERSE ROLES OF CIRCULAR RNAS IN BUFFALO COTYLEDONARY TISSUES: INSIGHTS FROM DIFFERENTIAL EXPRESSION ANALYSIS ACROSS DEVELOPMENTAL STAGES

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Circular RNAs (circRNAs) represent a unique class of non-coding RNA molecules with a distinct circular structure and conserved binding sites for microRNAs (miRNAs), distinguishing them as key players in the regulation of gene expression. In this study, we conducted a comprehensive exploration of circRNAs in buffalo cotyledonary tissues across three critical developmental stages: early (0-40 days of pregnancy), mid (41-135 days), and post-parturition. Using the seekCRIT STAR splice-aware aligner-based pipeline, we analyzed nine tissue samples (three from each developmental stage) to identify circRNAs. Our analysis revealed a vast catalog of circRNAs, including 35,260 known and 504 novel circRNAs, enhancing our understanding of the dynamic circRNA landscape in buffalo pregnancy. We further investigated the potential functional roles of circRNAs through a differential gene expression study using edgeR methodology. Comparing the early and mid-stages of pregnancy, we identified 175 circRNAs with significant differential expression, including two novel circRNAs. Interestingly, these differentially expressed circRNAs were distributed across various genomic regions, encompassing exonic, intronic, intergenic, and antisense locations. Expanding our analysis to include the early vs. post-parturition and mid vs. post-parturition stages, we discovered 836 and 876 significantly differentially expressed circRNAs, respectively. These comparisons also led to the identification of novel circRNAs, with five in the early vs. post-parturition and four in the mid vs. post-parturition comparisons. Notably, these circRNAs were distributed across various genomic regions, reflecting the diversity of circRNA regulation. This study highlights the pivotal roles of circRNAs in regulating gene expression during buffalo pregnancy across different developmental stages. The identification of novel circRNAs enriches our understanding of the intricate regulatory networks involved in buffalo pregnancy. Ongoing research will continue to elucidate the specific functions and mechanisms through which circRNAs contribute to the multifaceted processes of buffalo pregnancy. The findings from this study provide valuable insights into the dynamic landscape of circRNAs in the context of buffalo cotyledonary tissues and their potential impact on developmental processes.

ISAGB-2023/Abst/TS-IV-011

EXPLORING THE LANDSCAPE OF LONG NON-CODING RNAS (LNCRNAS) IN BOVINE COTYLEDONARY TISSUES DURING PREGNANCY AND POST-PARTURITION

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Long non-coding RNAs (lncRNAs) are pivotal regulators of gene expression in diverse biological processes. In the realm of bovine reproductive biology, lncRNAs have gained prominence for their roles in feto-

maternal communication and embryonic development. This study aimed to elucidate the lncRNA landscape within bovine cotyledonary tissues during early pregnancy (0-40 days), mid-pregnancy (41-135 days), and post-parturition. Rigorous measures were taken to maintain sample purity and prevent contamination. High-throughput RNA sequencing was conducted using the KAPA RNA HyperPrep Kit with RiboErase (HMR) on the Illumina Platform, generating paired-end 100 bp reads on the NOVA 6000 NGS platform. Raw sequencing data underwent stringent quality control and adapter trimming via FASTQC and Trim Galore on the Galaxy platform. Clean reads were aligned to the reference genome using Tophat (v2.1.1), followed by transcript assembly with Cufflinks (v2.2.1) to identify both known and novel transcripts. To identify potential lncRNAs, a systematic series of five stringent filters were applied. The first step involved merging transcripts from all nine samples using the Cuffcompare program. Transcripts shorter than 200 base pairs were excluded in the second step. In the third step, transcripts with insufficient expression levels (FPKM < 0) were filtered based on cufflinks calculations. Cuffcompare was then used in the fourth step to search for known lncRNAs and non-mRNA sequences, including rRNA, tRNA, snRNA, snoRNA, pre-miRNA, and pseudogenes. Any transcripts closely resembling these sequences were removed. In cases with no known lncRNAs in existing databases, transcripts were compared directly with non-coding mRNAs of the species. Transcripts surviving these stringent filters were classified as potential lncRNAs and further screened using CPC2, distinguishing coding from non-coding transcripts based on CPC2 predictions. This yielded 22,017 lncRNAs, which were analyzed for differential expression across the three stages. The study unveiled 28 novel lncRNAs in bovine cotyledonary tissues. Differential expression analysis was conducted with edgeR, setting a significance threshold at an adjusted p-value of < 0.05 and a fold change ≥ 2. The results of three group comparisons were as follows: (i) Early vs. Mid pregnancy group exhibited 114 differentially expressed genes (DEGs), (ii) Early vs. Post-parturition group displayed 1,346 significant DEGs, and (iii) the Mid vs. Post-parturition group comparison identified 1,587 significant DEGs and two novel lncRNAs. Functional annotation and enrichment analysis underscored the significance of these DEGs in cellular components, molecular functions, biological processes, and KEGG pathways. Notably, processes linked to embryo development, organogenesis, and other critical functions during pregnancy were enriched.

ISAGB-2023/Abst/TS-IV-012

METABOLOMIC PROFILE OF MILK-DERIVED EXOSOMES: APPROACH FOR VALUE ADDITION OF NATIVE INDIAN CATTLE

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Bovine milk is highly composite nutrient system providing important biomolecules with nutritional benefits. Amongst these bioactive compounds, milk derived exosomes (MDE) and the cellular components therein, involved in various signaling pathways hold special importance. These MDE have been shown to have several potential health benefits. The composition of MDE cargo varies with source of origin that includes the bovines species/breed as well. In the present study metabolome profile of exosomes derived from milk of Indicine, Taurine, and crossbred cows was studied to understand the distribution of important metabolites. Exosomes were isolated and characterized as per the guidelines of MISEV. One-dimensional NMR spectra of exosomes from different milk sources was analyses and a total of 41 metabolites were identified for three groups of MDE. Comparison across groups indicated that there were 19 metabolites significantly different ($p < 0.01$, VIP >1, FC >2) between Sahiwal (SW) and Holstein Friesian (HF) MDE, between Sahiwal and Karan Fries (KF) MDE, 12 metabolites showed significant difference. However, between HF and KF MDE, only 6 metabolites were differentially expressed. Among the three clusters, Sahiwal MDE showed clear distinction indicating the significant differences in metabolite concentration compared to HF and KF MDE. Discriminatory potential of the metabolites was further identified based on the VIP score plot analysis,

which indicated that 16 metabolites have the most discriminating power (VIP≥1.2; $p < 0.05$) to differentiate the 3 groups of MDE and six most discriminating metabolites with fold change >5 and VIP≥1.2 were betaine, leucine, valine, tyrosine, methyl histidine and O-Acetyl carnitine. These discriminating metabolites were involved in many physiological processes and pathways with impact factor >1 phenylalanine, tyrosine and tryptophan biosynthesis etc. Better understanding of the interactions between the metabolites and their physiological effects may help in future research on the nutritive value of exosomes derived from Indicine, crossbred or exotic cows and also their suitability for drug delivery.

ISAGB-2023/Abst/TS-IV-013

COMPREHENSIVE MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF COAT COLOUR ASSOCIATED AGOUTI SIGNALING PROTEIN (*ASIP*) GENE IN INDIAN DROMEDARY CAMEL

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The coat colour is a qualitative trait controlled by only a few genes responsible for phenotypic variation. The trait is of immense importance for breed characterization, domestication pattern, aesthetic value, behavioural adaptation, and the survival of animals. The coat colour schemes of mammalian skin and hair play a vital role in the development, differentiation, proliferation, and migration of melanocytes for melanogenesis. The *ASIP* gene is one of the major candidate genes that produce the agouti signaling protein. The agouti signaling protein (*ASIP*) gene has a critical role in melanin production and pigment distribution. There is a void of studies available for regulatory genes and proteins involved in the coat colour variation in Indian dromedary camel (*Camelus dromedarius*) breeds. In this first report, the molecular and functional characterization of the *ASIP* gene in three Indian camel breeds, Bikaneri, Jaisalmeri, and Mewari is described based on a comprehensive analysis of the nucleotide and amino acid sequences. The representative animals of extreme coat colors (dark: black; light: white) were amplified, sequenced, and analyzed using in silico platforms. We found a 1-bp deletion (23 delT/T) and a SNP (25 G/A) in exon 2 of *ASIP*, gene in black-coat-colored Bikaneri camels which stop *ASIP* protein formation due to an immature stop codon in the sequence. However, the complete 399 bp long coding sequence (CDS) of the *ASIP* gene gives rise to 132 amino acids (AA) long ASIP protein in white-coat-colored Mewari and Jaisalmeri camels. A mean percent divergence of 0.00 and 0.75 was observed with the *ASIP* gene sequence of the dromedary camel and other camelidae (*Camelus dromedarius* and *Camelus ferus*) species, respectively. The amino acid sequence-based analysis revealed high hydrophilicity, a high proportion of specific amino acids, the presence of one signal peptide site, and important post-translational modifications (PTMs) sites, viz., 32 phosphorylation sites, 1 pro-peptide cleavage site, 35 internal acetylation sites, and 2 O-glycosylation sites in the ASIP protein. The protein-protein association network analysis of the *ASIP* protein by the STRING server predicted direct and indirect interactions with other regulatory genes and proteins critical for skin pigmentation and the melanin biosynthesis process. This study provides first-hand information about CDS of the *ASIP* gene and functional prediction of translated protein in an Indian dromedary camel. Further, the deletion in the exon-2 region indicated its role in determining the black colour coat in dromedary camels. The epistatic interaction with other genes and mutational modifications of the *ASIP* gene need to be explored in intermediate-coat color animals for a better understanding of the color pattern of the Indian dromedary camel. the survival of animals.

ISAGB-2023/Abst/TS-IV-014

DIFFERENTIAL EXPRESSION OF MIRNAS ELUCIDATE THE ASYMMETRIC OVARIAN DEVELOPMENT OF EMBRYONIC CHICKEN

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In mammals both left and ovaries are functional, however, in birds only the left ovary is functional while the right ovary gets regressed. In the present study, the left and right ovaries of chicken at embryonic day 18.5 were used to identify the differentially expressed miRNAs to understand about the right ovary regression. A total of 106 miRNAs were differentially expressed, among them 42 miRNAs were found to be upregulated and 64 miRNAs were found to be downregulated in the right ovary compared to the left ovary. The upregulated miRNAs were found to target a total of 3,749 genes in the chicken. Target effects of the differentially expressed miRNAs were determined using TargetScan. The upregulated miRNAs targeted 3,749 genes, while the downregulated miRNAs targeted approximately 4,220 genes in chicken. Enrichment analysis of the upregulated miRNAs revealed their involvement in the various pathways related to the process of right ovary degeneration, primarily by regulating steroidogenesis, oocyte maturation, and various signalling pathways. The gga-miR-6553-5p and gga-miR-1769-5p revealed their involvement in the notch signalling pathway thereby regulating the cell-to-cell contact mechanism, follicle assembly, growth, steroidogenesis and ovarian vascular development. The gga-miR-6663-5p was found to be involved in the Mitogen-activated protein kinase (MAPK) pathway thereby inhibits the cell proliferation, differentiation and migration in right ovary. The miRNAs, gga-miR-6663 and gga-miR-1329-5p regulates the genes that prevent the germ cells of right female gonads from entering into oocyte meiosis. The gga-miR-6553-5p and gga-miR-1724 regulate the genes involved in the mitophagy resulting in meiotic defects and accumulation of damaged mitochondria in oocytes leading to poor oocyte quality. The involvement of gga-miR-6553-5p and gga-miR-1769-5p in the transforming growth factor-β (TGF- β) signalling pathway regulates apoptosis, proliferation, senescence, inflammation, cell fate, and tissue repair during the developmental and adult life resulting in the right ovary degeneration. The gga-miR-6553-5p, gga-miR-1724 and gga-miR-1782 affect the maturation of oocyte. The down regulated miRNAs, gga-miR-302c-5p, gga-miR-302a, gga-miR-302d and gga-miR-302b-5p (gga-miR-302 cluster) have a dominant role in the regulation of avian primordial germ cell proliferation by increasing the doubling time of the PGCs. This study revealed about the miRNAs that play a significant role in regulating the development of the right ovary in chicken.

ISAGB-2023/Abst/TS-IV-015

TRANSCRIPTOMIC PROFILE OF MILK-DERIVED EXOSOME FROM INDICUS, TAURINE, AND CROSSBRED COWS

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Milk exosomes are nano-sized vesicles that are secreted by mammary epithelial cells into milk. Exosome cargo contains a variety of biological molecules, including proteins, lipids, and nucleic acids, the composition of which varies according to genetic background. This study aimed to compare the transcriptomic profiles of milk-derived exosomes (MDE) from Indicus, taurine, and crossbred cows. Exosomes were isolated from

milk samples using ultracentrifugation and small RNA was extracted. The samples were found to be enriched in different small RNAs like miRNA, lncRNA, piRNA, tRNA, rRNA, mRNA, snRNA, snoRNA, scRNA, etc. The percentage of miRNA was relatively higher in all the samples across different groups of Indicus, taurine, and crossbred cows. RNA sequencing analyses performed to identify miRNA with differential expression revealed a high level of diversity in the miRNA repertoire of milk-derived exosomes across the different cow types. A total of 606 miRNAs were identified of which 349 were novel and 257 were known miRNAs. Comparative analysis revealed upregulation of 78, 54, and 38 miRNAs in indigenous, exotic, and crossbred cow MDE. Functional enrichment analysis of the differentially expressed miRNAs revealed that upregulated miRNAs in indigenous cow MDE were involved in immunity, inflammation, and cell signaling while miRNAs involved in metabolism and transport were significantly enriched in taurine cow MDE. Crossbred MDE showed enrichment of miRNAs involved in cell communication and adhesion. These findings suggest that the transcriptomic profile of milk-derived exosomes varies with the source of milk and needs to be studied further for the benefit of local communities as well as the stakeholders.

ISAGB-2023/Abst/TS-IV-016

EXPLORING THE DISTRIBUTION AND METHYLATION DYNAMICS OF NON-CPG ISLANDS IN BUFFALO SPERMATOZOA UNDER HEAT STRESS

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The CpG island concept helps to define the distance between methylated Cs in 5'-C-phosphate-G-3 stretches and the promoter region. As it is anticipated that CpG aggregations in the regions surrounding the promoters are otherwise in a non-methylated state and a methylation event marked in this region, modulates the gene-expression. Conversely, the length, CpG fraction, and GC content thresholds are used to define CpG in an arbitrary manner. We would not be able to explore the CpG islands, which might include methylated CpG portions linked to gene regulation, in such a circumstance. The CpG shore (2 kb up and downstream), the CpG shelf (4 kb to 4 kb up and downstream), and the CpG open sea (non-CpG) are thus worth investigating even though their CpG density is lower. The study involved developing a unique pipeline to extract the CpG island, CpG shore, CpG shelf, and CpG open sea by intersecting the bed files obtained from the Bismark pipeline with the UCSC genome browser CpG annotation track database. Further differences in the groups of samples (bulls with bad semen quality and bulls with good semen quality) were examined, and the DMCs specific to CpG islands, CpG shore, CpG shelf, and CpG open sea were identified. The average percentage of methylation at the CpG islands differed between the two buffalo bulls' categories by 16.61 and 18.21, the CpG shore by 55.54 and 54.50, the CpG shelf by 59.18 and 57.90, and the open sea by 72.92 and 75.14, percent. The percentage of methylation increased with increasing distance from the CpG islands. This is the first time that buffalo sperm genome has been investigated for distribution of CpG across these regions. This work further establishes the basis for such ad-hoc determined CpG fractions by offering a new approach to carry out the CpG component analysis for species for which annotation databases are not available in traditional DMC estimation tools like MethylKit. The study will help in understanding the epigenetic regulation of methylome patterns in buffalo spermatozoa, having impact on bull semen quality and fertility.

ISAGB-2023/Abst/TS-IV-017

TRANSCRIPTOME MAPPING OF MILK SOMATIC CELLS DERIVED FROM COLOSTRUM OF NATIVE LADAKHI COWS ADAPTED TO HIGH ALTITUDE REGION OF LEH-LADAKH

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Ladakhi cattle is a genetically unique population of Leh and Ladakh region of India which has been constantly exposed to hypobaric hypoxia environment prevalent at high-altitude. These animals are integral component of livelihood for the native population of Ladakh especially as a major milk and protein source. They provided 2-5 kg of milk per day with high fat and protein content. Their milk is generally preferred for making Churpi and butter and fetch good price in local market. In last few years, there has been extensive intermixing of Ladakhi cattle with exotic Jersey animals especially in and around Leh city and peri-urban areas. This is diluting the important and unique gene pool developed after years of natural selection to survive under high altitude conditions. In the present study, an effort was made to characterize the transcriptome signature of 12 milk somatic cells derived from colostrum (0-day) and transition period (2-day, 4-day). The data was compared with Sahiwal cows adapted to hot arid or semi-arid region. The RNA-Seq data revealed a total of 236 and 312 million reads for Ladakhi and Sahiwal cows, respectively. The filtered reads were mapped to the Btau4 (Bos taurus) reference genome assembly with an average of more than 90% mapping rates. The transcriptome data could be annotated to a total of 27271 genes. The most abundant transcripts based on RPKM values (> 3500 RPKM) revealed that major milk protein genes such as kappa casein (CSN3=34157.27), beta casein (CSN2=22414.76), alpha S1-casein (CSN1S1=16941.81), alpha S2-casin (CSN1S2=4535.55), whey protein (PAEP=79027.31), alpha-lactalbumin (LALBA=3542.95), glycosylation-dependent cell adhesion molecule (GLYCAM1=8042.13), translationally controlled 1 (TPT1=4591.42) expressed at higher level in somatic cells of Ladakhi cows *vis* a *vis* Sahiwal cows during colostral stage of the lactation. Similarly, regulatory genes such as (JAK2=36.35 and STAT5A=33.08) were found to be more abundant in somatic cells of Ladakhi cows. Additionally, genes/transcripts involved in metabolic pathways related to translation, energy, and oxidative phosphorylation (with RPKM > 6000) were highly abundant in Ladakhi cows. The fold change criterion of ≤ or ≥ 2 (FC = 2) revealed a total of 4915 differentially expressed genes between milk somatic cells of Ladakhi and Sahiwal cows. The most differentially expressed genes were found to be Metazoans-RNA (334.12), Vault_RNA (290.80), CSN3 (266.83), TFF3 (196.56), PAEP (180.41), SPDTC (174.62), EBF2 (124.70), ANXA13 (124.52), and ZFPM2 (120.13). The majority of the DEGs were linked to pathways such as TNF-alpha signalling, T-cell receptor and co-stimulatory signalling, MAPK cascade, angiogenesis, apoptosis, cytokines and inflammatory response, TCR signalling, and Toll-like receptor signalling. Furthermore, qPCR analysis was performed for several genes such as milk synthesis (CSN3, CSN2, CSN1S1, RPLP4, RPS15, CSN1S2, LF, LGB, and LALBA), milk fat synthesis (FASN, ACACA, SCD FABP3, GPAM, and BTN1A1), and regulatory (JAK2, STAT5, and EIF4BP41) mainly to validate the transcriptome data. The expression difference of these genes that play important roles in regulating milk attributes, growth, immunity, and development of newly born calves, indicated distinct adaptive evolution of Ladakhi and Sahiwal cattle in response to diverse environments.

ISAGB-2023/Abst/TS-IV-018

DELINEATING PROTEOMIC SIGNATURES OF COLOSTRUM AND MATURE MILK OF NATIVE COWS ADAPTED TO HIGH ALTITUDE ENVIRONMENT OF LEH-LADAKH

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Ladakhi cows are naturally adapted to the high-altitude hypobaric hypoxic environment of Leh and Ladakh. These animals are reared by native community of Ladakh for milk, churpi and butter as source of protein and nutritional security especially during extreme winter periods. Due to the fact that Ladakhi cattle is naturally adapted to high altitude and geographically separated from rest of the Indian cattle breeds, they are considered to be genetically distinct gene pool. Cow milk has always been regarded as nature's perfect food due to its presence of vital nutrients. Along with the presence of a higher concentration of major vital (casein) proteins, bovine milk whey (20%) contains an enormous class of minor proteins, not all of which have been comprehensively reported especially for Indian native cattle breeds. This study has allowed to characterize the proteomics signatures of colostrum and mature milk of Ladakhi cows in a comprehensive manner. In the present study, we performed the LC-MS/MS-based ultra-deep characterization of 14 whey protein samples of colostrum (0-day: N = 7) and mature milk (>60 days: N = 7). Each sample was subjected for casein depletion using series of ultracentrifugation in order to increase the chances of identifying minor whey proteins in the data set. Three independent search engines, Comet, Tandem, and Mascot-based analysis, resulted in the discovery of over 6,000 non-redundant proteins. The filtering of spectra at 0.05 probability error and at 1% FDR, resulted in identification of 815 proteins with high confidence. Further, the identified proteins were subjected for quantification using MS2-based Normalised Spectral Index by implementing StPeter algorithm of TPP software. A total of 268 proteins were present in high abundance in colostrum as well as mature milk of Ladakhi cows. The principal component analysis grouped the colostrum and mature milk into separate clusters. Some of the most abundant proteins in colostrum *vis* a *vis* mature milk of Ladakhi cows (with a fold change > 2) was: Clusterin (4.14); Alpha-1-acid glycoprotein (3.89); Alpha-1B-glycoprotein (3.57); 40S ribosomal protein SA (3.50); Tyrosine-protein kinase (3.44); DNA polymerase delta catalytic subunit (2.98); Serotransferrin (2.90); Alpha-2-macroglobulin (2.68); Alpha-2-HS-glycoprotein (2.68); Complement component C9 (2.65); and Vitamin D-binding protein (2.32). However, alpha-lactalbumin (1.55), albumin (1.11), glycosylation-dependent cell adhesion molecule 1 (1.03), and beta-lactoglobulin (0.744) were the proteins that were more abundant in mature milk. The functional annotation of these proteins on the basis of GO terms grouped them into categories such as: B cell activation, angiogenesis, PDGF signalling pathway, VEGF signalling pathway, Toll receptor signalling, EGF receptor signalling, apoptosis signalling, oxidative stress response, and hypoxia response via HIF activation. These results demonstrate that the milk proteome profile of Ladakhi cows is quite unique and warrant further research.

ISAGB-2023/Abst/TS-IV-019

ESTIMATION OF GENETIC AND PHENOTYPIC PARAMETERS OF LINEAR TYPE, BEHAVIORAL AND PERFORMANCE TRAITS IN CROSSBREED CATTLE (KARAN FRIES)

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Linear type traits are considered as crucial in dairy cattle selection procedure because of the high correlation with production performance which not only focuses on production performance but also takes into account for longevity and overall health of animal. Multi-trait selection procedure was used to estimate the genetic parameters of the behavioural, functional, and linear type traits and their relationship with production performance, were Age at first calving (AFC) was considered as coverable and parity, season, calving period, and stage of lactation were considered as fixed effects, while data on adult Karan fries cattle (N=134) were analyzed by using both Bayesian and Least Square Maximum Likelihood (LSMLMW) approaches. The majority of linear type traits, milking temperament, and milking speed were found to be low to moderately heritable. (Ranging from 0.40±0.03 to 0.25±0 04), with low heritability (0.20±0.25) observed for days open. Among the linear type traits, a strong positive genetic correlation were recorded (ranging from 0.74±0.03 to 0.63±0.017) between stature, body length, and chest girth and total milk yield. The study suggested negative phenotypic correlation (-0.018± 0.081 and -0.050±0.099) between the rear leg rear view and the total milk yield. Central ligament had a negative phenotypic correlation with total milk yield (-0.018± 0.081 and -0.050±0.099) respectively.The study showed that Karan Fries cows may be selected based on few important linear type traits (stature, body length, chest girth, body depth, and angularity) having a positive genetic correlation with production performance. The present study may be continued to include a larger data set for validation and better accuracy of results for the selection of Karen Fries cattle.

ISAGB-2023/Abst/TS-IV-020

INSILCO GENOME WIDE IDENTIFICATION AND VALIDATION OF A FUNCTIONAL MIRSNP ASSOCIATED WITH HEAT STRESS RESPONSE IN KARAN FRIES AND THARPARKAR CATTLE

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Genetic variations especially SNPs that occur at miRNA binding sites on the target genes that, if functional, can alter the miRNA-mediated regulation of these variant genes. So in the present study, an in-silico genome wide scan for mapping of SNPs onto miRNAs and within the thermal stress-related QTLs using modifications in the customized SNPtool-miRNAQTLsnp software, was carried out. The QTLs considered under the study include heat tolerance, Respiration Rate, Body Temperature, coat colour, Dry matter intake, Blood glucose and cold tolerance. Among the SNPs, 13 SNPs which are related with the gene CUX1 and miRNAs bta-mir-2388 were found associated with QTLs for heat tolerance, respiratory rate and body temperature. A total of 90 blood samples were collected from Tharparkar and Karan Fries animals from LRC, ICAR-NDRI, Karnal and Genomic DNA was isolated from 3 ml of whole blood and quantitative and qualitative analysis of extracted DNA was carried out. Physiological (Respiration Rate, Skin Temperature and Heart Rate) and hematological parameters of all the 90 animals were collected. Tetra-primer ARMS-PCR protocols were developed for genotyping rs43722771 (ENSBTAT00000014098.6:c.1050+4197C>T) in CUX1 gene and seed region of bta-miR-2388 and all the three genotypes identified in Karan Fries. But Tharparkar animals showed

monomorphic pattern with CC genotype. Association analysis including breed and genotype as fixed factor indicated significant association between the mutation ENSBTAT00000014098.6:c.1050+4197C>T in CUX1 gene and physiological parameters such as heart rate and respiration rate. Further, the expression of bta-miR-2388 varied significantly between KF and Tharparkar heifers which are exposed to heat stress in climate chamber So the identified SNP can be used as a genetic marker for genetic program for selecting the animals with thermotolerance.

ISAGB-2023/Abst/TS-IV-021

CHARACTERIZATION OF LIPIDS USING UHPLC-QTOF-MS LIPIDOMICS IN THE COLOSTRUM OF LADAKHI CATTLE ADAPTED TO HIGH ALTITUDE LEH-LADAKH

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Ladakhi cattle are a unique cattle population adapted to the high altitude of Leh-Ladakh and are well known for their adaptability in hypoxia and hypobaric stress environments. Milk from Ladakhi cows is an important protein source for the local people, especially in lean winter seasons. It contains various constituents like protein, carbohydrates, vitamins, and thousands of lipid species. Lipids are generally considered the most complex material in nature. These are involved in several biological processes, like cell membrane formation, energy storage, and signalling processes. Till date, no study has been conducted to characterize the lipidome profile of milk from Indian native cows especially those adapted to high altitudes. In the present study, we performed an LC-MS/MS-based lipidome profile of colostrum fat sample (zero day) of Ladakhi cows. A total of 541 lipid molecules were identified in the colostrum of Ladakhi cows. We utilized web-based tools like Lipid Sig and Lipid Suite for the characterization of lipidome datasets and classified lipid biomolecules using LIPIDMAPS, Chemspider, Lipidpedia, and the HMDB database. The lipidome was divided into eight main lipid categories: sphingolipids [SP], glycerophospholipids [GP], fatty acyls [FA], glycerolipids [GL], sterol lipids [ST], prenol lipids [PR], polyketides [PK], and saccharolipids [SL]. In Ladakhi cow colostrum fat samples, the most abundant lipids were identified as GP (59) followed by FA (119), SP (80), and GL (59). Furthermore, under the category of glycerophospholipids, the most abundance subclasses were glycerophosphoethanolamines, glycerophosphoserines, and glycerophosphocholines.

ISAGB-2023/Abst/TS-IV-022

COMPREHENSIVE TRANSCRIPTOMIC ANALYSIS TO DECIPHER NOVEL CANDIDATE GENS REGULATING HEAT STRESS ADAPTABILITY IN PIGS

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Selection for stress adaptability needs immediate attention in pigs, which is highly susceptible to thermal stress due to absence of sweat glands, thick subcutaneous fat and limited respiratory cooling mechanisms. Hence, the present study aimed to explore the transcriptomic signature of heat stressed granulosa cells and signalling pathways regulating their adaptability to thermal challenge. For the purpose, the primary cell culture system of porcine granulosa cells from Indian Ghoongroo pigs was established, which was subsequently subjected to in vitro heat stress challenge at 42 ^{O}C for 6 hours. RNA sequencing was conducted for heat stressed (treated; n=3) and non-heat stressed (control; n=3) groups using Illumina NextSeq2000 sequencing platform. The significant differentially expressed genes (DEGs) were selected using NOISeq R package with cut-offs,

probability value ≥ 0.95 and absolute $\log_2$(fold change) ≥1. Bioinformatics analysis of DEGs were conducted to explore the transcription factors, gene interaction network and hub genes regulating the cellular homeostasis and survivability during heat stress challenge. The differential expression of randomly selected DEGs were validated by RT-qPCR with technical replicates. The analysis pipeline yielded a total of 12156 protein coding transcripts, which were expressed during heat stress challenge in granulosa cells, out of which 4904 were differentially (prob. ≥ 0.95) expressed; 2936 were upregulated and 1968 were downregulated. The large number of DEGs and gene ontologies in the study specifies the concerted mechanisms involving multiple signalling pathways like MAPK, Hippo, Wnt, PI3-Akt, NFKB, Notch and many more operating in the cell to maintain cellular homeostasis. The hub genes *HSP90, HSPA8, HSPA5, TGFB1, PPARG, RADS1, CDK1* are key elements in stress, regulating multiple pathways and expression of transcription factors. The study observed high fold change and significance level in genes ENSSSCG00000061267 and ENSSSCG00000029160 which were upregulated and gene ENSSSCG00000009221 which was downregulated, and these can be regarded as novel candidate genes for stress adaptation in pigs.

ISAGB-2023/Abst/TS-IV-023

INSTABILITY OF THE PLASMID WITHIN BACTERIAL HOST: A FOREMOST CHALLENGE OF *PESTIVIRUS* PLASMID-BASED REVERSE GENETICS SYSTEM

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Molecular cloning involves assembling recombinant DNA molecules and introducing them into a suitable host system to replicate successfully. The efficient generation of identical copies of recombinant plasmids depends on the host system that can maintain its stability during large-scale industrial cultivation. Plasmid stability is critical for maintaining the integrity and fidelity of a recombinant plasmid during propagation. The aim of the present study was to investigate the effect of a full-length plasmid-based cDNA of classical swine fever virus (CSFV) within the bacterial host system used for plasmid propagation. In the study, we used a novel RNA polymerase II-driven pestiviral pCSFV rescue plasmid of approximately 18 kb, which was transformed into the Stbl-3 *E. coli* bacterial cells by the heat shock method. Two phenotypically different colonies appeared in kanamycin-containing Super Optimal Broth (SOB) agar plates at different hours after transformation. The larger colonies appeared at 16-18 hours, while the smaller colonies appeared 72-96 hours post-transformation. All transformed colonies were positive for the presence of the vector and inserted sequence and showed expected 2549 bp amplicons in colony PCR by forward primer (RFF) and reverse primer (SF474R). Although colony PCR was positive, some colonies produced cleaved plasmids when cultured in Super Broth (SB) media. However, intact full-length pCSFV rescue plasmid was also isolated from the transformed colonies, which was confirmed by 0.8% agarose gel electrophoresis. This study highlights the major hurdle of plasmid instability issues with pestiviral-based rescue plasmids in bacterial host systems used for plasmid propagation.

ISAGB-2023/Abst/TS-IV-024

DECIPHERING ROLE OF SHUTDOWN CHICKEN PULMONARY MIRNA DURING HIGHLY PATHOGENIC AVIAN INFLUENZA (H5N1) INFECTION

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Avian influenza (AI) is a highly contagious viral infection mainly affecting the avian species. AI results in huge economic losses and resulted in the breakdown of the poultry industry worldwide. In the present study, we deep sequenced the miRNAs expressed in highly pathogenic avian influenza virus (HPAIV) H5N1 infected and phosphate-buffered saline (PBS) mock-infected specific pathogen free (SPF) chicken lungs. Other than differentially expressed miRNAs, 97 were found be expressed only in SPF chickens. miRDB database was used to identify predicted target genes. A total of 10771 genes were found to be targeted by shutdown miRNAs. Gene ontology (GO) analysis of the bioinformatically predicted target genes of the shutdown miRNAs revealed significant pathways such as protein phosphorylation, positive regulation of transcription from RNA polymerase II promoter, protein dephosphorylation, regulation of transcription from RNA polymerase II promoter, regulation of alternative mRNA splicing, via spliceosome, potassium ion transmembrane transport, intracellular protein transport, protein binding, metal ion binding, identical protein binding, guanyl-nucleotide exchange factor activity, protein kinase binding, ubiquitin protein ligase binding etc. These significant pathways might be having a significant role during viral pathogenesis during HPAIV infection.

ISAGB-2023/Abst/TS-IV-025

MICROARRAY ANALYSIS OF GENES EXPRESSED IN THE SKIN OF VECHUR AND *BOS TAURUS* CROSSBRED CATTLE FOLLOWING *RHIPICEPHALUS ANNULATUS* INFESTATION

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This study was conducted to identify the differentially expressed genes in the skin of Vechur (*Bos indicus*) and Holstein Friesian crossbred (CBHF) cattle in response to *Rhipicephalus annulatus* (*R. annulatus*) infestation using microarray analysis on Agilent platform. Vechur and CBHF crossbred calves (two each) of comparable age were artificially infested repeatedly with about 20,000 larvae to ensure stable resistance against tick infestation. Then these calves were infested with about 10,000 larvae. Skin biopsies were taken before tick larval infestation (T_0) and 24 hours after infestation (T_{24}) from the neck area and used for gene expression profiling by microarray. The comparison of microarray data of crossbred to Vechur, pre-infestation revealed 18 upregulated and 19 downregulated while post-infestation profile revealed 20 upregulated and 55 downregulated genes. After infestation, *LOC781146* and *HS3ST1* genes were upregulated in both genetic groups while there were no common genes which were downregulated. *Galectin 9* was the only gene upregulated in CBHF which was downregulated in Vechur after infestation while the majority of the DEGs were unique to the genetic group and produced no change in expression in the other.

The observations made in the present study indicated the difference in immune response mechanisms to *R. annulatus* infestation between cattle of *Bos indicus* and *Bos taurus* lineage. Further investigations on the DEGs within the genetic group will yield more insight into the genetic control of these mechanisms.

ISAGB-2023/Abst/TS-IV-026

INTERACTOME (CERNA AND PROTEOME) THAT DETERMINES DISEASE PHENOTYPE OF PPRV INFECTED GOAT

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Peste des petits ruminants virus (PPRV), a member of the genus Morbillivirus within the family Paramyxoviridae, causes a devastating disease that affects the lives of sheep and goat. The host immune response to PPRV involves secretion of cell mediators by natural killer cells, macrophages, dendritic cells, B cells,T cells, etc.. However,the virus can evade or modulate the immune response by altering the expression of several immune-related genes. Long non-coding RNAs (lncRNAs), messenger RNAs (mRNAs), and microRNAs (miRNAs) are all part of the competitive endogenous RNA (ceRNA) network, which govern the host response. The connectivity or interaction between these molecules defines the role of these molecules in influencing the disease phenotype. Transcriptome analysis identified 7724 mRNAs, 1023 lncRNAs, and 383 miRNAs to be differentially expressed (log2FC, $p < 0.05$). Interactome analysis on the long non-coding RNA, messenger RNA, microRNA, and proteome data generated from PPRV-infected goat peripheral blood mononuclear cells revealed downregulation of CALY, CPEB4, DAGLA, Mx1, Mx2 protein and upregulation of AQP9,BCL6,CHI3L1, CIITA, DTX3L, HAVCR2, HMOX1,IL1R2, NFKBIZ and ZFPM1 genes involved in immune process. Transcriptome analysis of spleen and lung revealed lesser enrichment of immune response processess than in PBMCs. This indicated an initial balance between viral and immune response in PBMCs which further tilted in favour of viral load, which must have resulted in the death of animals. This is the first interactome study among the animal disease viruses.

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REGRESSION MODELING FOR PREDICTING LIFE-TIME MILK PRODUCTION IN HARIANA CATTLE

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The present investigation was undertaken to prepare the regression models to predict actual lifetime milk yield based on early expressed traits of Hariana cattle in organized farms. The data of 702 Hariana cattle with date of birth from 1962 to 2018 and 1995 to 2018 were collected from DUVASU farm, Mathura and Babugarh farm, Hapur, respectively. Variance inflation factor (VIF) was used to know correlations existing among the independent traits in the study. Earlier on use of various combinations of age at first calving (AFC), first dry period (FDP), first calving interval (FCI) and first lactation total milk yield (FLTMY) as independent variables to predict the actual lifetime milk yield (ALT) where we observed high correlations between FDP and FCI. For estimating unbiased co-efficient of determination, the FDP was excluded from VIF analysis. Step wise regression procedure was used to predict the actual lifetime milk yield from first lactation traits viz. AFC, FCI and FLTMY. The models with lower Akaike information criterion (AIC), Bayesian Information Criterion (BIC) and root mean square error (RMSE) values with higher co-efficient of determination (R2) was selected as optimum. Out of 07 models developed a model with all three traits (R2 - 32.95%) was selected as optimum model to predict the actual lifetime milk yield in Hariana cattle.

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ESTIMATION OF GENETIC PARAMETERS OF TEST DAY MILK YIELD AND FIRST MILK YIELD IN MURRAH BUFFALOES

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The research aimed to assess the impact of non-genetic factors and estimate the genetic parameters for the milk yields of Murrah buffaloes on the first ten test days (TD1 to TD10), first lactation 305-days milk yield (FL305DMY), and the total lactation milk yield (TLMY). Data was gathered from 646 buffaloes at the Buffalo Research Centre, Department of Livestock Production Management (LPM), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar over a 28-year period spanning from 1993 to 2020. The results showed that the period of calving had a statistically significant impact ($p<0.01$) on all test day records (TD1 to TD10), FL305DMY, and TLMY. The season of calving was statistically significant for TD2, TD3, and TD4. Additionally, the regression effect on AFC had a statistically significant impact on TD1 to TD5, FL305DMY, and TLMY. The overall least squares means for these parameters were 3.94±0.06, 7.79±0.07, 8.58±0.07, 8.61±0.08, 8.18±0.08, 6.65±0.13, 6.26±0.12, 5.61±0.12, 5.03±0.12, and 4.42±0.11 in kg/day, 2135.72±18.69 kg, and 2273.68±23.41 for TD1, TD2, TD3, TD4, TD5, TD6, TD7, TD8, TD9, TD10, FL305DMY, and TLMY, respectively. The heritability estimates for the first lactation ten test days, FL305DMY, and TLMY were 0.34±0.09, 0.39±0.09, 0.41±0.09, 0.31±0.08, 0.43±0.09, 0.40±0.09, 0.24±0.08, 0.52±0.10, 0.11±0.06, 0.33±0.08, 0.35±0.09, and 0.34±0.10, respectively. The genetic and phenotypic correlations between FL305DMY and the first ten test days ranged from 0.20±0.18 (TD1) to 0.67±0.02 (TD5) and 0.17±0.04 (TD1) to 0.64±0.02 (TD6).

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NON - GENETIC FACTORS AFFECTING KLEIBER RATIO IN SALEM BLACK GOATS UNDER FARM CONDITIONS

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India is a rich repository of goat genetic resources in the form of 37 well defined breeds. Tamil Nadu has three recognised breeds of goat namely Kanniadu, Kodi Adu and Salem Black. Salem Black goat are tall animals, completely black in color and reared mainly for meat. The native tract of Salem Black goat is Salem, Dharmapuri, Krishnagiri, Erode, Karur and Namakkal districts of Tamil Nadu. Evaluation of the growth performance of Salem Black goats at Mecheri Sheep Research Station, Pottaneri, Salem district, Tamil Nadu with data (n=1011) accrued over 19 years was carried out. The Kleiber ratio (KR) is an essential selection criterion for growth efficiency and a good measure of feed conversion. Single trait analyses were done by fitting a general linear model (GLM) to study the influence of various non-genetic factors viz., period of kidding, season of kidding, parity of dam, type of birth, sex of kid, dam weight at kidding, inbreeding coefficient on KR using SPSS (v.26.0) software. Pair-wise comparison for sub-class means within each fixed effect was done by Duncan's Multiple Range Test. Overall least squares mean for pre weaning KR (0-3) was 14.34 ± 0.15 and post weaning KR at 3-6, 6-9, 9-12, 3-12 were 5.72 ± 0.28, 3.09 ± 0.32, 5.43 ± 0.36 and 4.12 ± 0.13 respectively. Period of birth showed a significant ($P<0.01$) influence on pre- and post-weaning KR (3-6, 6-9, 9-12, 3-12). Season of birth had significant effect on pre- and post-weaning KR at 3-6, 6-9 and 3-12 months ($P<0.01$) and 9-12 months ($P<0.05$). Type of birth was found significant in post weaning KR at 3-6

and 9-12 months (P<0.05). Sex of the kid had significant effect (P<0.01) on pre- and post-weaning KR except for KR at 3-6 months. Season of birth had significant effect on pre- and post-weaning KR at 3-6, 6-9 and 3-12 months. Weight of dam at kidding had significant effect on pre-weaning KR (P<0.05) as well as post-weaning KR at 6-9 months (P<0.05). Parity of dam and inbreeding was found non-significant in pre- and post-weaning KR (3-6, 6-9, 9-12, 3-12).

Key words: Salem Black goat, Kleiber ratio, Non-genetic factors, General linear model

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UNRAVELLING THE POPULATION STRUCTURE OF CATTLE POPULATIONS OF BIHAR

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Bihar is the 3rd largest state for cattle population in India according to 20th livestock census and is divided into four agro-climatic zones. There are three registered native breeds viz., Bachaur, Gangatiri and Purnea in Bihar along with other non-descript populations. However, these local cattle populations are bred with other indigenous and exotic breeds to improve their productivity. Therefore, evaluation of population structure is a fundamental assessment of the breeding strategies and development of conservation and management plans. The aim of the present study was to estimate the population structure of Bihar cattle populations, for which Bachaur (n=21), Gangatiri (n=21), Punea (n=21) and Shahbadi (n=22) were genotyped GGP Bovine50K array. Genotyping data (Illumina BovineHD BeadChip) of Sahiwal (n=19), Hariana (n=18), Tharparkar (n=17), Gir (n=43), Kangayam (n=18), Ongole (n=24), Vechur (n=20), Jersey (n=32) and HF (n=60) were taken from the lab and WIDDE database. The datasets were merged for 336 samples keeping only 51525 common SNPs. Genotyping quality control was performed using PLINK v.1.9 with the following exclusion criteria: sample call rate <90%, genotype call rate <90%, minor allele frequency <0.003. The screened SNP dataset included 308 samples of 13 breeds/population with 50631 variants. Pruning was applied for linkage disequilibrium >0.5 and the final pruned SNP dataset with 40596 SNPs was used in the analysis. Population structure was performed using ADMIXTURE v.1.3 assuming ancestral population (K) ranging from 2 to 13. Principal Component Analysis (PCA) was performed using PLINK v.1.9 by estimating eigenvalues and eigenvectors and plotted in R environment. The lowest cross validation (CV) error was found at K = 10 and derermined as optimum K. Bihar cattle populations and Sahiwal share their ancestry and group as one cluster and rest other breeds grouped into separate clusters. Shahbadi and Gangatiri showed nearly 16 and 18% of Haryana inheritance; respectively. From the PCA of 13 breeds/population, PC1 (55.29%) separated Bos indicus from Bos taurus and then PC2 (11.78%) separated Jersey from Holstein-Friesian. Considering all Indian populations with PC1 (10.07%) and PC2 (8.73%), Bihar populations along with Sahiwal and Hariana created one group and other breeds separated as different groups. Bachaur and Purnea breed grouped together with more homogeneity and similarity, while Shahbadi has more within population diversity.

ISAGB-2023/Abst/TS-IV-031

COMPREHENSIVE TRANSCRIPTOMIC ANALYSIS TO DECIPHER NOVEL CANDIDATE GENS REGULATING HEAT STRESS ADAPTABILITY IN PIGS

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Selection for stress adaptability needs immediate attention in pigs, which is highly susceptible to thermal stress due to absence of sweat glands, thick subcutaneous fat and limited respiratory cooling mechanisms. Hence, the present study aimed to explore the transcriptomic signature of heat stressed granulosa cells and signalling pathways regulating their adaptability to thermal challenge. For the purpose, the primary cell culture system of porcine granulosa cells from Indian Ghoongroo pigs was established, which was subsequently subjected to in vitro heat stress challenge at 42 OC for 6 hours. RNA sequencing was conducted for heat stressed (treated; n=3) and non-heat stressed (control; n=3) groups using Illumina NextSeq2000 sequencing platform. The significant differentially expressed genes (DEGs) were selected using NOISeq R package with cut-offs, probability value ≥ 0.95 and absolute log2(fold change) ≥1. Bioinformatics analysis of DEGs were conducted to explore the transcription factors, gene interaction network and hub genes regulating the cellular homeostasis and survivability during heat stress challenge. The differential expression of randomly selected DEGs were validated by RT-qPCR with technical replicates. The analysis pipeline yielded a total of 12156 protein coding transcripts, which were expressed during heat stress challenge in granulosa cells, out of which 4904 were differentially (prob. ≥ 0.95) expressed; 2936 were upregulated and 1968 were downregulated. The large number of DEGs and gene ontologies in the study specifies the concerted mechanisms involving multiple signalling pathways like MAPK, Hippo, Wnt, PI3-Akt, NFKB, Notch and many more operating in the cell to maintain cellular homeostasis. The hub genes HSP90, HSPA8, HSPA5, TGFB1, PPARG, RADS1, CDK1 are key elements in stress, regulating multiple pathways and expression of transcription factors. The study observed high fold change and significance level in genes ENSSSCG00000061267 and ENSSSCG00000029160 which were upregulated and gene ENSSSCG00000009221 which was downregulated, and these can be regarded as novel candidate genes for stress adaptation in pigs.

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EVIDENCE OF ADAPTIVE SELECTION IN MITOGENOME OF INDIAN CATTLE BREEDS

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Origin of domestic cattle have puzzled the archaeologists for more than a century. It is widely believed that cattle domestication independently took place in the Near East and the Indian subcontinent around 10,000 to 8,000 years ago, leading to the emergence of two primary domestic types: *Bos taurus* (taurine) and *Bos indicus* (zebu), respectively. Over the past three decades, mitochondrial DNA has been widely utilized as a valuable tool for studying the evolutionary and demographic history within and among different livestock species. The importance of *Indicine* cattle breeds in the livelihood of small and marginal farmers cannot be overstated, as they serve as valuable sources of milk, draught power, and manure. The Bos genus provides a compelling framework for investigating adaptive evolution within the mitochondrial genome, given that the two existing cattle species occupy both hot, low-altitude environments and cold, high-altitude regions. In the current study, signatures of natural selection on the complete mitogenome of 140 cattle individuals from various populations, including Europe (49), Africa (36), Aurochs (9), and Indian (46) were analyzed. Through the Global MK test on the 13-protien coding genes, it was observed that there is evidence of purifying selection in the Indian population, considering all possible combinations. The MK test conducted on individual genes revealed that in the comparison between the Indian population and others, purifying selection was evident in 3 out of the 13 genes (*ND1, COX1, CYTB*). The CODEML package of PAML software was used for analysis of adaptive selection in the mitogenome using codon-based methods which showed 9 sites distributed across 5 genes, specifically selected in the native population compared to both the exotic and ancestral populations. Three genes (*ND2, CYTB, CO3*) within the mitogenome was identified as potential sites under positive selection associated with high altitude adaptation in Ladhaki cattle and *ND6* gene exhibited signals of positive selection in Kangayam cattle. Divergence time estimation displayed that *Bos indicus* diverged from *Bos taurus* approximately 1.43 million years ago with further diverging into different haplotypes around 0.1 million years ago. The study provided evidence of purifying and adaptive selection across the mitogenome of cattle. These mt-DNA variants under positive selection in the cattle population might be associated with their adaptation to their contrasting environments The divergence pattern observed in zebu cattle indicates the likelihood of a distinct ancestor and supports the notion of independent divergence for *Bos indicus*.

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