RESEARCH

GARIMA Delivers 2nd Calf "Karishma"

(M. S. Chauhan, S. K. Singla, R. S. Manik, P. Palta, Shiv Parsad, S. S. Lathwal, Anuj Raja, Amol Sahare and Basanti Jyotsana)

Garima, a cloned buffalo, earlier born at NDRI produced second female calf named "**Karishma**" on 27th December, 2014 through normal parturition. The weight of the calf at the time of birth was 35 kg and the newborn calf is keeping good health.

The Garima was born on 22nd August, 2010 using embryonic stem cells as donor cell through hand guided cloning technique. GARIMA was inseminated with frozen-thawed semen of a progeny tested bull of NDRI No. 5258 on 27th February 2014, which resulted in conception. She was maintained under standard scientific management system during her gestation. Earlier, a female buffalo calf MAHIMA was born to 'Garima' a cloned buffalo mother at NDRI, Karnal on 25th January, 2013, which was the first calf born from cloned buffaloes, produced through hand guided cloning technique.

ICAR - NDRI Produced Clone of Endangered Wild-buffalo of Chhattisgarh

(M. S. Chauhan, S. K. Singla, R. S. Manik, P. Palta, S. S Lathwal, Anuj Raja and Amol Sahare)

A clone of the only wild-buffalo in Chhattisgarh in semi-captivity has been produced through the 'Hand-guided Cloning Technique' at ICAR- National Dairy Research Institute, Karnal. The female calf was named **"Deepasha"** and was born on December, 12, 2014. The calf was born by normal parturition, and its weight at the time of birth was 32 kg.

Udanti in Chhattisgarh is left with a lone female wild buffalo in the State's Udanti Wildlife Sanctuary and popularly named as ASHA and is a darling of the whole department. The lone female bred with males during several natural-matings has delivered male calves only and is a cause of concern for Chhattisgarh state, as nobody wants that this buffalo gets eliminated from the system due to aging or other risks. Through their technical partners Wildlife Trust of India, they approached NDRI for assistance by initiating research to copy their wild animals. NDRI accepted the challenge of exploring the totally unknown path of research with their partial funding from the state and support in sampling the tissue from the animals.

An *in-situ* breeding programme to save wild buffaloes from becoming extinct is going on for the past many years in Udanti. Chhattisgarh's state animal, known locally as 'Ban Bhainsa', is similar to the bison in appearance but is a different species and it is their pride animal. The species is in the Red List of International Union for Conservation of Nature (IUCN). It is also a Schedule I animal under the Wildlife Protection Act 1972.

Scientists are of the opinion that besides multiplication of superior germplasm through cloning, conservation of endangered species through cloning has great potential. As our domestic buffalo has evolved from wild-buffalo, there is a need to extract few traits of biological and/or economic importance from these wild animals in future.

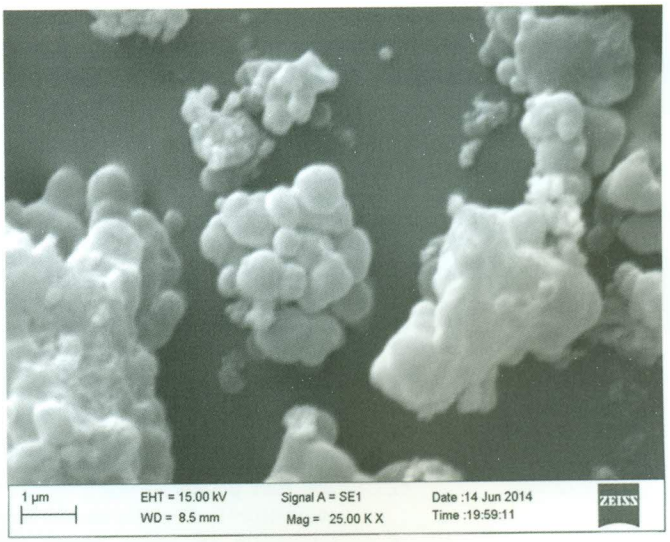
This novel achievement of producing cloned calf from endangered species has opened up new windows of applications of cloning technology.

A cloned female calf named Deepasha

Magnet can Attract Antibiotic from Food Matrix

(Y. S Rajput, Rajan Sharma, Sneha Aggarwal and Gulab Singh)

Imprinted polymer against oxytetracycline, cephalexin and cefquinome were prepared over the surface of iron magnetite and evaluated for extraction of antibiotics from food matrix. The imprinted polymers against these antibiotics were prepared by polymerization of methacrylic acid and ethylene glycol dimethacrylate in presence of respective antibiotics. Iron magnetite was prepared from $FeCl_2$ & $FeCl_3$ and exhibited superparamagnetic property which behaved like magnet in magnetic field. The selectivity of imprinted polymers over non-imprinted polymer was determined under different experimental conditions for selecting appropriate binding



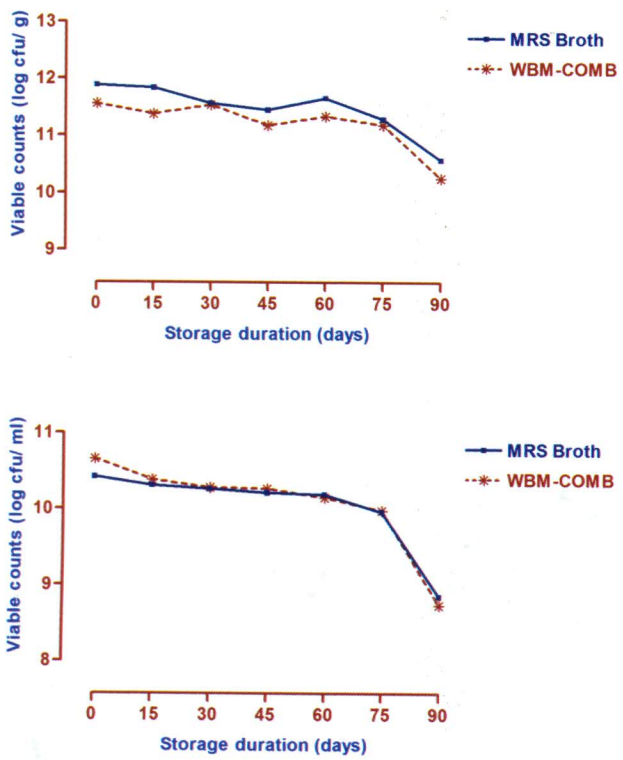
SEM Image of Oxytetracycline imprinted magnetic polymer

and elution conditions. These polymers extracted 62% to 94% antibiotics from water, milk, honey and egg white.

Direct Product Probiotic (DPP) *Lactobacillus casei* NCDC 298 Culture

(S. Mandal, Pritee, P. V. Behare, K. Khamrui and S. K. Tomar)

The cell biomass of a probiotic potential strain of *Lactobacillus casei* NCDC 298 was produced under batch fermentation in MRS broth and Whey Based Medium at pH 6.0 (inoculation rate 6.0-7.0 log cfu/ml). The early stationary phase culture (9.0-10.0 log cfu/ml) was obtained after 10 h at 37°C. Cell biomass was concentrated from the culture media by centrifugation (10000 rpm for 10 min at 4°C) and preserved as frozen concentrate as well as freeze dried powder forms. Viable counts were in the range of 11-12 log cfu/g of freeze dried powder, which was stable during storage at -20°C till 75 days. On the other hand, viable counts were in the range of 10 - 11 log cfu/ml of frozen concentrate, which were stable during storage at -20°C till 60 days. Preserved *L. casei* NCDC 298 biomass was incorporated in fermented milk beverages @ 8 log cfu/ml of final product. The viable counts were also found stable during storage under refrigeration condition till 7 days. The concentrate *Lactobacillus* culture can be used for value addition to fermented and non-fermented dairy products.



Survival of preserved L. casei NCDC 298 during storage (-20°C): (a) freeze dried & (b) frozen concentrate

Production of α-glucosidase Inhibitory Milk Bioactive Peptides by using *Lactobacillus* spp.

(P. Patil and S. Mandal)

Diabetes is a metabolic disorder characterized by high blood glucose level (Hyperglycemia). One therapeutic approach to decrease postprandial hyperglycemia is to retard absorption of glucose through inhibition of carbohydrate-hydrolyzing enzymes, e.g., α- amylase and α-glucosidase. Bioactive peptide fragments are formed during degradation of the milk proteins by digestive enzymes in the gastrointestinal tract or by proteolytic lactic acid bacteria (LAB) during fermentation of milk. Therefore, inhibition of α-glucosidase by using milk derived bioactive peptide is an effective strategy for controlling/managing of Type 2 diabetes. Hence, the present study was carried out to exploit the proteolytic activity of *Lactobacillus* spp. for the production of milk peptides having α-glucosidase inhibitory activity. Hydrolysates from all fermented milk samples of *Lactobacillus* spp. were evaluated for their proteolytic activity and potential to inhibit α-glucosidase. Among 21 strains of Lactobacilli, the proteolytic activity measured using OPA method ranged between 1.77 to 3.13 mg of leucine/ml whereas unfermented milk sample had very low peptide content (0.046 mg/ ml). It is evident that during fermentation milk protein undergoes proteolysis to varying extent by extracellular proteinases of Lactobacilli. Among the different species of *Lactobacillus*, the proteolytic activity was maximum for *L. rhamnosus* NCDC 24 (3.13 ± 0.02 mg/ml) followed by *L. helveticus* NCDC 288 (3.10 ± 0.02 mg/ml). The α-glucosidase inhibitory activity was highest for the hydrolysate from *L. helveticus* NCDC 288 and *L. helveticus* NCDC 292 i.e. 46.62 ± 0.88% and 43.83 ± 0.53%, respectively, followed by *L. rhamnosus* NCDC 24 (40.12 ± 1.38%). The α-glucosidase activity of all strains assessed increased with most of the cases during fermentation with increase proteolysis. High correlation coefficient between development of α-glucosidase inhibitory activity and proteolytic activity was found with eight *Lactobacillus* strains i.e. (r >0.90). Five *Lactobacillus* cultures were selected for further study. The peptide fractions of 10 and 3KDa were prepared by ultrafiltration process and tested for α-glucosidase inhibitory activity. The results from this study showed that peptides with α-glucosidase inhibitory activity can be generated by using *Lactobacillus* spp. from milk proteins. Therefore, these peptides can be produced in fermented dairy product by selected proteolytic strains of Lactic Acid Bacteria or peptides rich formulation can be incorporated into functional foods or administered via nutraceuticals.

Effect of Humectants on Water Activity Modification and Sensory Properties of *Khoa* Using Model *Khoa* System

(R. Badola, R. R. B. Singh and A. K. Singh)

Khoa, produced by heat desiccation of different types of milk, resulting different total solids and textural attributes, constitute the base and filler for the production of variety of popular traditional sweets. During the preparation of *khoa,* desiccation destructs most of the microorganisms, but the high water activity (0.96) of khoa limits its shelf-life not to be more than 3 days at room temperature and 14 days at refrigeration temperature. Further desiccation may reduce the water activity but could lead to dryness and discolouration in final product. Therefore, attempts were made using certain humectants viz., polyols (sorbitol and mannitol), polydextrose, maltodextrin and corn-syrup in model *khoa* system on water activity modification of *khoa*. Effect of addition of humectants on

in their career development. Technical consultants from the industry as well as HR consultants mentored the students at SRS on various aspects of placement.

Hindi Week Celebration

Hindi Week was celebrated at SRS, Bangalore from 22nd – 30th September, 2014. During the celebrations, hindi competitions were conducted for the staff members as well as for their children and students of the Station. Hindi Day was celebrated at the Station on 30th October, 2014. The occasion was graced by Sri. D. N. Shyam Prasad, Director, Airport Authority of India, Bangalore. Dr. Satish Kulkarni, Head, NDRI, Bangalore presided over the function. Prizes to the winners/runners of the competitions were distributed by the Chief Guest.

Visit of Deputy Director General (AS), ICAR

Dr. K. M. L. Pathak, Deputy Director General (AS), ICAR alongwith Dr. A. K. Srivastava, Director, NDRI Karnal visited SRS of NDRI on 18th of December, 2014. A meeting of Scientists, Technical Staff & Ph.D. Scholars of the station was convened. Dr. B. Surendra Nath and Dr. P. Heartwin highlighted the salient achievements of the section and activities of the recent period. On the occasion, DDG released the Course Manual of the Engg. Section & Publication by Dairy Production section. DDG also inaugurated the Facebook page of the SRS of NDRI. Later, Dr. A. K. Srivastava had detailed meetings with Scientists, Staff and Students, where he gave a presentation on the Vision 2050 of NDRI.

EASTERN CAMPUS, KALYANI

RESEARCH

Effect of Supplementation of Area-specific Mineral Mixture (KALMIN) on Productive and Reproductive Performance in Black Bengal Goats

(M. K. Ghosh, A. Chatterjee, M. Mondal, M. Karunakaran, C. Bhakat, T. K. Dutta)

The present study was conducted to observe the effect of area specific mineral mixture (ASMM) on growth performance, body condition score, puberty, blood metabolites (Glucose, NEFA and AAN) and enzymes(SGOT and SGPT) in Black Bengal goats. For the purpose, goats were divided into four groups viz. Group I, II, III and IV supplemented with 0, 1, 2 and 3 g/day/animal ASMM, respectively. It was inferred that area specific mineral mixture @2.0g/day/animal in growing Black Bengal goats may be of great use for enhancement of growth and age at puberty.

Rumen Fermentation Pattern and Ciliate Protozoal Population in Growing Crossbred Cattle Fed Animal Feed Grade Wheat alongwith Paddy Straw

(D. Chandrashekhar Keshav, A. Santra, A. Mandal, S. K. Das and T. K. Dutta)

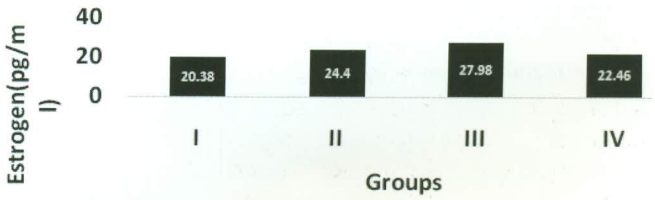
Quality of a good sizable proportion of wheat grain deteriorated during storage at FCI due to lack of proper storage facility and declared unfit for human consumption which is designated as animal feed grade wheat. Therefore, experiments were conducted to study the rumen fermentation pattern and ciliate protozoal population in growing crossbred cattle fed graded level of animal feed grade wheat. Twelve growing Jersey crossbred male calves about 7-8 months of age, were randomly divided into 3 groups (G1, G2 and G3) of 4 animals each, so that average body weight of each group was similar. These animals were maintained under individual feeding on roughage (paddy straw) and concentrate based ration under stall feeding to meet out maintenance and growth (600 g average daily gain) requirement (NRC, 2001). Roughage and concentrate mixture were offered separately and their ratio was tried to maintain at 40:60 throughout the experimental period. 3 types of iso-nitrogenous concentrate mixtures (C1, C2 and C3) were prepared in which, maize grain was serially replaced by animal feed grade wheat at 0, 30 and 50% level in concentrate mixture C1, C2 and C3, respectively. Animal feed grade wheat (AFW) which was used in the present experiment, contained 55 to 70 % sound grains.

It was concluded that animal feed grade wheat may be used in the ration of growing cross-bred calves by replacing maize grain to formulate economize ration.

Effect of Area-specific Mineral Mixture (KALMIN) Supplementation on Endocrine Parameters in Prepubertal Goats

(M. Mondal, M.K. Ghosh, A. Chatterjee, M. Karunakaran, C. Bhakat, T. K. Dutta)

The present study was undertaken to find out the effect of area specific mineral mixture (ASMM) on endocrine parameters during pubertal process in growing Black Bengal goats. It was revealed that progesterone level at starting of experiment differed significantly ($P< 0.05$) among different groups, except at day 30 and 60. From starting to end of experiment, value of progesterone showed inclined trend. In conclusion, progesterone level varied with different level of area specific mineral supplementation. Supplemented groups showed significantly higher level of progesterone hormone in comparison with non-supplemented group. Plasma progesterone concentrations were found to be highly correlated with onset of puberty.



Plasma estradiol-17β concentrations (mean ± SEM; pg/ml) in different groups at various time points

EVENTS

Scientists-Dairy Industry Partners Interface Meet

ERS-NDRI-Dairy Industry Meet was organized on 27th October, 2014 at ERS-NDRI, Kalyani, Nadia, West Bengal under the Chairmanship of Prof. (Dr.) A. K. Srivastava, Director, NDRI to discuss the various issues and challenges of dairy industry in the eastern part of India. Scientists' from NDRI, Karnal and ERS-IVRI

and other researchers. State Livestock officers were present in the meet. Representative from Kishan Milk Union, Krishnagar, Ichamati Milk union, Red Cow Dairy Ltd., Kolkata, Natures Dairy, Hooghly, NEST Dairy, Kolkata, Metro Dairy, Kolkata, and other Dairy industry partners, NABARD, Kolkata, NGOs', and progressive farmers from West Bengal also participated in this Industry meet. The keynote address pertaining to issues and challenges of dairy industry at this region was delivered by Prof. (Dr.) A. K. Srivastava, Director, NDRI, He informed about various technologies available at NDRI, Karnal and these technologies were presented afterwards by the scientists of NDRI, Karnal to participant from Industries with a focus on commercial transfer of technologies.

Brain Storming Session on Breeding Policy of ERS, NDRI

A Brain Storming Session on "Breeding policy of ERS, NDRI herd" was organized on 28th October, 2014 at ERS-NDRI, Kalyani, Nadia, West Bengal under the chairmanship of Dr. R. K. Malik, Joint Director (Research), NDRI, Karnal. Experts from NDRI, Karnal, ERS-IVRI, Kolkata, West Bengal University of Animal and Fishery Sciences, State Livestock sectors actively participated to frame out the new breeding policy of ICAR-NDRI-ERS herd.

Midterm IRC Meeting

The midterm IRC meeting was held at ERS-NDRI, Kalyani on 20th Oct, 2014 under the chairmanship of Dr. G.R.Patil, Joint Director (A). Dr. R. K. Malik, Joint Director (Research), NDRI co-chaired the meeting. The progress of the ongoing projects was presented by the respective principal investigators.

Dairy Mela - 2014

Dairy Mela was organized as a part of Golden Jubilee Celebration on 11th December 2014, in the adopted village Muratipur (near Kalyani, West Bengal). Dr. G. R. Patil, Joint Director (Academic), NDRI chaired the celebration. Approximately 200 people including Livestock farmers, Students, Trainees, Scientists, Technicians and Local officials attended the celebrations. Various programmes were organized throughout the day viz. Cattle Health- cum- Fertility Camp, Cattle Show and Judging, Distribution of mineral mixture to the livestock owners, Display of Transferable Technologies through Posters, Quiz on Dairy Cattle Management, Distribution of bilingual (English and Bengali) Technical Folders to the dairy farmers, distribution of prizes to the best cattle owners /dairy farmers etc.

Golden Jubilee Meet Celebrated

Eastern Regional Station of National Dairy Research Institute (ERS-NDRI), Kalyani, Nadia celebrated the Golden Jubilee Meet on 13th December, 2014, under the chairmanship of Prof. (Dr.) A. K. Srivastava, Director, NDRI. The theme was Dairying in Eastern India: Opportunities and Challenges. Representative from Milk Union, ERS-IVRI, Kolkata, CEO and Director, PBGS and ARD, WB Govt., Director, ZPD-II, Kolkata, Dean, F/O Dairy Technology, Ex-Dean, Vet. faculty, WBUAFS, and other dignitaries from ICAR institutions and universities and farmers from different districts also participated in this celebration. The chairman released the golden jubilee 'SOUVENIR' and 'ERS-NDRI at a Glance' on this occasion. The achievements of ERS-NDRI for the last 50 years were presented by Dr. T. K. Dutta, Head, ERS. In his key note address, the Director, NDRI stressed on efforts to be made by the ERS team for the development of technologies for higher milk production for nutritional and economic security of small and marginal dairy farmers of this region. He has also emphasized on the practices to be followed by the farm women and children for their nutritional security.

Dr. A. K. Srivastava, Director & Vice-Chancellor, NDRI releasing a Golden Jubilee Souvenir and ERS-NDRI at a Glance

FEATURE ARTICLE

Current Status of Embryonic Stem Cells Technology in Buffalo

(M.S. Chauhan, R.S. Manik, P. Palta, S.K. Singla and M.K. Singh)

Embryonic stem cell (ES) cell lines were first established in mice by Evans and Kauffman in 1981 by culturing ICM in the presence of murine embryonic fibroblast (MEF) feeder layer and leukemia inhibitory factor (LIF), and since then they have been used extensively for studying the mechanism of pluripotency and cell differentiation. Upon removal of LIF from the culture medium, they cease to express pluripotency markers such as Oct 4, Nanog, Sox, rapidly losing the capacity for self-renewal, differentiating into a variety of cell types and forming embryoid bodies. Well-characterized ES cell lines have been derived only from mice. An important point in the production of ES cells is the criteria used to define them. These include morphological similarities to ES cells of other species, indefinite undifferentiated proliferation *in vitro*, potential to differentiate into three embryonic germ (EG) layers and specific characteristics detected through immunohistochemical and molecular markers. Attempts to establish stem cell lines have been made in a number of mammalian species (Toyoka *et al.*, 2003., Sharma *et al.*, 2011).

Isolation and Characterization of Embryonic Stem Cell

The ability of buffalo ES cells produced to form random and specific cell types through spontaneous and directed differentiation, respectively, confirmed their pluripotency. Study showed the presence of surface markers SSEA-1, SSEA-3, SSEA-4 and intracellular markers OCT-4, SOX-2 and NANOG on buffalo ES cells both by immunofluorescence and RT PCR (Sharma *et al.*, 2013, Zandi *et al,* 2013). At NDRI, Karnal, buffalo ES cells have been isolated from *in vitro* produced blastocysts. The ability of buffalo ES cells to form embryoid bodies (EBs) and to spontaneously differentiate to neuron cells, muscular cells and epithelial cells was demonstrated by our group (Verma *et al.*, 2007, Anand *et. al.*, 2009) at NDRI, Karnal. Developed buffalo ES cells express *NF-68* and *NESTIN*, specific for ectodermal lineage; *BMP-4* and α-skeletal actin, specific for mesodermal lineage and α-fetoprotein, *GATA-4* and *HNF-4* specific for endodermal lineage confirming the ability of ES cells to differentiate to all the three germ layers.

Many other transcription factors, besides OCT4, NANOG and SOX2 appear to maintain ES-cell pluripotentiality in other species (Saito *et al.*, 2003). Some of these include c-Myc, Rex1, B-Myb, Foxd3, Gbx2, UTF1, Fgf4, Pem, Sall4, and Zfx. However, many of these factors are not exclusively expressed by pluripotent ES cells and can be found in other cell types. Oct4, Sox2, and Nanog has been recognized as the regulatory core activating genes critical for self-renewal and to repress genes initiating differentiation, thus, controlling ES-cell pluripotency. The exogenous supplementation of growth factors is very species specific e.g. for human ESCs, FGF-2 is essential but for murine ES Cells, LIF is able to support their undifferentiated state (Nicholas *et al.*, 2009).

Application of ES Cells in Farm Animals

ES cells have varied applications for farm animals as well as humans, like enabling studies on the fundamental events in embryonic development, production of therapeutic delivery systems, gene targeting, and regenerative medicine. Production of pluripotent ES cells from farm animal species might have a big influence on the genetic modification of these animal species. Availability of ES cells is expected to be especially useful in cloning technology, gamete (oocyte, sperm) formation. Also, in the context of gene targeting, use of ES cells could overcome current limitation on efficient gene transfer by providing an abundance of stem cells to be genetically manipulated by using conventional recombinant DNA techniques.

Somatic cell cloning through nuclear transfer, to produce healthy cloned animals, remains remarkable but highly inefficient and prone to epigenetic errors. The high rates of mortality throughout development create serious animal welfare issues, which limit the acceptability of somatic cloning (David *et al.*, 2003). In animal breeding, improved genetic markers, correlated to specific livestock production traits, will provide confidence in cloning selected embryos and their derivatives, especially undifferentiated embryonic stem cells. This will enable rapid dissemination of the most recent elite genotypes to avoid the genetic lag associated with cloning adults. Furthermore, for the production of transgenic animals, embryonic stem cells might also be beneficial, because they are more amenable to precise genetic modifications and result in higher cloning efficiencies than somatic cells in the mouse. It can be said that for agricultural applications, embryonic stem cell cloning will ultimately prove more useful than somatic cell cloning.

Normal Physiology of Cloned Buffalo 'Garima-II' Produced from Embryonic Stem Cell

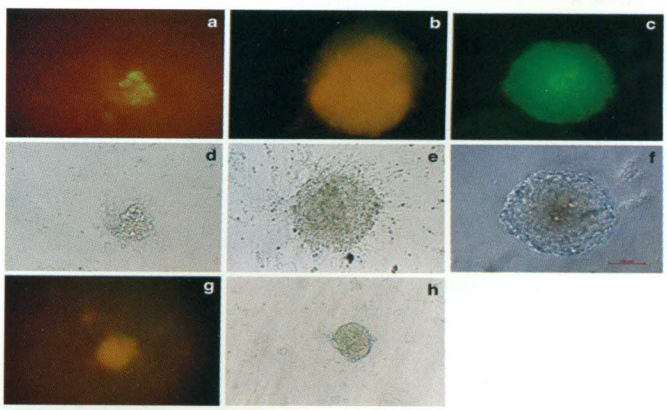
It is now established that transgenic animals having foreign gene, have played and anticipated to continue to play an important role in pursuit of knowledge for understanding the genetic basis of human disease. There is an ever increasing need for animal models instead because of the complexity of biological processes that form the basis of most diseases. Moreover, the pluripotency of ES cell can also be judged by developing ES cell into whole animal. Having realized the potential of embryonic stem cells, NDRI achieved successful cloning using Embryonic Stem Cell as a donor cell and produced a female buffalo calf through 'Hand-guided Cloning Technique'. The calf named 'Garima- II' was born at NDRI, Karnal on August 22, 2010. The ES cell used for producing Garima-II was from an ES cell colony derived from buffalo blastocysts (stage of embryo when it gets preimplanted in the uterus) generated using *in vitro* fertilization (IVF) technique.

Garima II produced through cloning has absolutely normal physiological processes as has been proved by the successful delivery of offspring female calf named as "Mahima" on January 25, 2013 and another female calf "Karishma" in December, 2014 after Garima-II was inseminated with frozen-thawed semen of a progeny tested bull.

'Karishma' a second normal calf born from cloned buffalo Garima-II

Buffalo Spermatogonial Stem Cells

Spermatogonial Stem Cells (SSCs) have the unique ability to self-renew and to produce progeny that undergo differentiation to spermatozoa. The SSCs were isolated from testes of 3–7 months old buffalo calves and disaggregated by double enzymatic digestion. Mixed population of isolated cells were then plated on Datura stramonium agglutinin (DSA) lectin coated dishes for attachment of Sertoli cells. Spermatogonial cells isolated have spherical outline and two or three eccentrically placed



Buffalo type-A spermatogonial colonies: (a, d) OCT 4 was detected within the colony cells, (b, e) Positive expression of CD-9, (c, f) colonies showing SSC specific marker i.e. Dolichos biflorus agglutinin, (g, h) SSEA-1 was also seen in the colonies.

nucleoli, created a colony after proliferation during first week or immediately after passage. After 7–10 days of culture, the resulted developed colonies of spermatogonial cells expressed the spermatogonial specific genes like Plzf and VASA and other pluripotency related markers viz. alkaline phosphtase, DBA, CD9, CD90, SSEA-1, OCT-4, NANOG and REX-1. (Kala *et al.*, 2012)

Major Outcome by Buffalo Embryonic Stem Cell Research

- Buffaloes 4 embryonic stem cell (ES) lines have been developed and characterized.
- Embryonic stem cell research translated into cloned buffalo calf *'Garima-II'*
- GFP expressed transgenic embryos using ES cell as donor cell were produced.
- Buffalo Oct-4 and *NANOG* gene was cloned and characterized.
- Transfection of NANOG, LIF and FGF2 gene construct into buffalo fibroblast cell and ES cells and maintained their pluripotency for longer time.
- Spermatogonial stem cells are maintained under *in vitro* culture system.

Conclusion

The generated ES cell lines will have great potential/use in buffaloes. These ES cells have very varied applications like enabling studies on the fundamental events in embryonic developmental, production of therapeutic delivery systems, regenerative medicine, etc. The use of ES cell technology in farm animals may overcome current limitation on efficient gene transfer by providing an abundance of stem cells to be genetically manipulated by using conventional recombinant DNA techniques. Besides these uses, ES cells provide a powerful tool for the studies of early embryonic development, gene targeting, cloning, chimera formation and transgenic animal production. Because of their potential use for targeted gene manipulation, ES cells could have enormous agricultural, biomedical and pharmaceutical applications through cloning and transgenesis.

Spermatogonial Stem cells have great potential for self proliferation and then differentiation ultimately leading to formation of spermatozoa. Spermatogonial stem cells are the only adult stem cells that contribute genes from one generation to the next. High genetic merit semen of exotic bulls could be produced from indigenous bulls in harsh tropical climate. Also, transgenic animals could be produced by transfer of transgenic donor stem cells. Furthermore, these cells will also provide an opportunity to preserve the genetic material of valuable males.

Acknowledgements

The financial support received from the DBT, New Delhi and NAIP (ICAR) is duly acknowledged.

References

Anand T, Kumar D, Singh M K, Shah R A, Chauhan M S, Manik RS, Singla S K and Palta P. (2009). Buffalo (*Bubalus bubalis*) embryonic stem cell-like cells and preimplantation embryos exhibit comparable expression of pluripotency-related antigens. *Reprod. Dom. Anim. doi: 10.1111/j.1439-0531.2009.01564*

David N. Wells, Bjo rn Oback and Go tz Laible (2003) Cloning livestock:a return to embryonic cells *Trends in Biotechnology* , 21, 428-432.

George A, Sharma R, Singh K P, Panda S K, Singla S K, Palta P, Manik R, Chauhan M S. (2011). Production of Cloned and Transgenic Embryos Using Buffalo *(Bubalus bubalis)* Embryonic Stem Cell-Like Cells Isolated from *In Vitro* Fertilized and Cloned Blastocysts.) *Cellular Reprogramming*, 13(3):263-72.

Kala S, Kaushik R, Singh K P, Kadam P H, Singh M K, Manik R S, Singla S K, Palta P, Chauhan M S. 2012. *In vitro* culture and morphological characterization of prepubertal buffalo putative spermatogonial stem cells. *J. Assist. Reprod. Genet.* 29(12):1335–1342.

Nicholas C R, Haston K M, Grewall A K, Longacre T A, Reijo Pera R A. (2009).Transplantation directs oocyte maturation from embryonic stem cells and provides a therapeutic strategy for female infertility. *Hum Mol Genet.*;18(22):4376-89.

Saito S, Sawai K, Ugai H, Moriyasu S, Minamihashi A ,Yamamoto Y et al. (2003). Generation of cloned calves and transgenic chimeric embryosfrom bovine embryonic stem-like cells. *Biochemical and Biophysical Research Communications* 309 (2003) 104–113

Sharma R, George A, Kamble N M, Singh K P, Chauhan M S, Singla S K, Manik R S, Palta P. (2011) Optimization of Culture Conditions to Support Long-Term Self-Renewal of Buffalo *(Bubalus bubalis)* Embryonic Stem Cell-Like Cells.*Cell Reprogram.*13(6):539-49.

Sharma R, Kamble N M, George A, Chauhan M S, Singla S, Manik R S, Palta P. (2013) Effect of TGF-β1 superfamily members on survival of buffalo (Bubalus bubalis) embryonic stem-like cells. *Reprod Domest Anim.*;48(4):569-76. doi: 10.1111/rda.12126.

Toyoka Y, Tsunekawa N, Akasu R, Noce T. (2003) Embryonic stem cells can form germ cells *in vitro. Proc Natl Acad Sci USA* 100:11457–11462.

Verma V, Gautam S K, Singh B, Manik R S, Palta P, Singla S K, Goswami S L, Chauhan M S.(2007) Isolation and characterization of embryonic stem cell-like cells from in vitro-produced buffalo *(Bubalus bubalis)* embryos. *Mol Reprod Dev.*;74(4):520-9.

Yadav P S, Kues W A, Herrmann D, Carnwath J W, Niemann H. (2005) Bovine ICM derived cells express the Oct4 ortholog. *Mol Reprod Dev.*;72(2):182-90.

Zandi M, Muzaffar M, Shah S M, Kaushik R, Singh M K, Palta P, Singla S K, Manik R S, Chauhan M S. (2013). *WNT3A* signalling pathway in buffalo *(Bubalus bubalis)* embryonic stem cells. *Reprod Fertil Dev.* doi: 10.1071/RD13084.

Published by: **Director,** NDRI Karnal
Tel.: 0184-2252800 | Fax: 0184-2250042 | E-mail : dir@ndri.res.in | Gram : DAIRYRESEARCH