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Conservation and Multiplication of Indigenous Animals (*Bos indicus*) Through *in vitro* Embryo Production

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ABSTRACT

The objectives of the study were conservation and multiplication of superior germplasm in cattle by ovum pick up-*In vitro* fertilization technology, standardization of OPU process and standardization of IVF techniques/process. for this purpose, indigenous breeds *viz*. dangi, deoni, gaolao, gir, red kandhari and sahiwal reared in BAIF's central cattle breeding farm were selected on the basis of minimum standard protocol set by Govt. of India. OPU was performed to aspirate the oocytes followed by IVM and the matured oocytes were processed for IVF which then kept for *In vitro* Culture (IVC). A total of 891 OPU Sessions were carried out. Totally 6416 oocytes were processed for *In vitro* culture and 2016 embryos were produced. The effect of breed, age of the animal and interval between two OPU sessions were evaluated for both number of oocyte and embryos produced per session. The average breed wise oocyte recovery was 6.58±0.45 and the average breed wise embryo production was 2.11±0.33 per session. Breed of cow and age at collection of oocyte were found to be significantly affecting the oocyte and embryo production. It was found that Gir breed has better oocyte recovery and embryo production efficiency in our study. Gir, Dangi and Sahiwal presented higher oocyte recovery and embryo production followed by Deoni, Gaolao and Red Kandhari. In conclusion, the process is standardized for Ovum Pick Up, *In vitro* Maturation, *In vitro* Fertilization, and *In vitro* Culture.

Key words: *In vitro* culture, *In vitro* fertilization, *In vitro* maturation, Ovum pick up.

The scientific and technological advances achieved during the past decades in animal reproduction have resulted in the development of a variety of tools commonly referred to as assisted reproductive technologies (ART). The primary focus of these tools is to maximize the number of offspring from genetically superior animals and disseminate germplasm. Furthermore, ART allows for the effective utilization of donors with anatomical disabilities and sub-fertile conditions, for safeguarding germplasm of threatened species and domestic breeds and for reducing disease exposure and transmission. Ferré *et al.* (2020). The major advances in *In vitro* Embryo Production (IVEP) today seeks to improve overall performance at all procedural stages *viz*. ovarian stimulation, oocyte recovery, maturation, fertilization, embryo development, embryo freezing and embryo transfer and pregnancy establishment.

The native breeds need to be conserved for genetic assurance in future, scientific study, as a part of our ecosystem, cultural and ethical requirements and for energy sources in future. The conservation includes the preservation along with up-gradation of the genetic potential and management of a breed for use in future. The effective management of indigenous cattle resources includes identification, characterization, evaluation, documentation and conservation. The future strategy should be to combine genetic improvement and conservation. Role of establishment of regional gene banks and people's participation by involving breeders, communities, gaushalas, NGOs and other relevant stakeholders is important in conservation programs srivastava *et al.,* 2019.

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The objectives were conservation and multiplication of superior germplasm in cattle by OPU-IVF technology, standardization of OPU process and standardization of IVF techniques/process.

Location of study and period

BAIF, Central Cattle Breeding Farm, Urulikanchan, Pune is located at India with the GPS coordinates of latitude 18° 29′27.6180″N and longitude 74° 8′4.3584″E and at an altitude of 559 m above sea level. The study was conducted from April 2019 to March 2022.

Animal health, breed wise number of donors and bulls, criteria of choosing the donors and bulls

All the donors were screened though disease testing and vaccinated as per Minimum Standard Protocol (MSP) set by Govt. of India.

Breed wise donors available

The number of donors of Sahiwal, Gir, Gaolao, Red Kandhari, Dangi, and Deoni were 10, 16, 5, 5, 3 and 2 respectively. The donors were selected by screening of all MSP guidelines given by Govt. of India.

Bull semen used

The semen doses used for fertilization were procured by BAIF, Frozen Semen Laboratory; which qualifying all the MSP guidelines given by Govt. of India.

In vitro embryo production (IVEP) was done by using commercially available media; IVF Bioscience, UK and Vitrogen, Brazil both. Use of media was random for IVEP. The steps were followed by ovum pick up, *in vitro* maturation, *in vitro* fertilization and i*n vitro* culture.

Ovum pick up (OPU)

In all the OPU sessions during the experimental period, 20 gauge OPU needle was used, and the vacuum pump pressure was maintained in between 70 to 90 mm of Hg and temperature range maintained of vacuum pump was in between 37 to 38°C. OPU was performed without using pre-stimulation protocols for the non-lactating donors. Kumar *et al.* (2020). All OPU donors have lactation range from 1 to 4 and their average age ranged from 4 to 10 years. Throughout the study period, these donors were not inseminated to make them pregnant.

In vitro maturation (IVM)

The recovered oocytes were kept in incubator (Make - Memmert) for 22-24 hours for maturation at 38.5°C temperature by maintaining 5% of CO_2 with 90% relative humidity Aiman *et al.* (2022).

In vitro fertilization (IVF)

After completion of IVM, the sperm interact with oocyte in the fertilization dish in a microenvironment of 80 to 100 µl. Usually the spermatozoa were washed and selected using swim-up or density gradient centrifugation procedures to remove freezing media, seminal plasma, debris and dead spermatozoa and to select the more motile fraction. The concentration of the sperm determine by Neubauer's chamber and the amount of sperm suspension is used in each fertilization drop of 100 µl (final sperm concentration was 2×10^6/ml). The oocytes along with sperms were kept in CO_2 incubator for 16-18 hours at 38.5°C temperature by maintaining 5% of CO_2 with 90% relative humidity (Aiman *et al.,* 2022).

In vitro culture (IVC)

After 16-18 hours the oocytes along with sperms were taken out from incubator and processed for denudation *i.e.* slowly denuded (removing loose cumulus cells and sperm) the presumptive zygotes by using denuding pipette (Singh *et al.,* 2016). All the presumptive zygotes were denuded, washed with Wash media and IVC media. After washing of zygotes; all the zygotes were transferred in pre-equilibrated IVC drops. It was in Mixed Gas bench top incubator (Make-K-Systems G185) at 38.5°C temperature and 5% of CO_2, 5.5% O_2 was maintained. It was kept for 7 days from the date of IVF.

Grading of embryos

Standardized grading of the quality of the embryo has been prescribed by International Embryo Technology Society (IETS) depending upon the cell mass, developmental stage compared to age of the embryo, percentage of cell extrusion from the embryo and overall appearance of the embryo. (Adopted from manual of the IETS, 4th edition). The evaluation is subjective and vary between embryologists. But if the IETS guidelines are followed, there should not be very high variation between grading. The code for embryo quality is also numerical and is based on morphological integrity of embryos.

Statistical analysis

The data was categorised in breed, age at collection of oocyte and interval between two oocyte recovery sessions. The age at collection was categorised in age group of above 10 years, between 9-10 years, between 8-9 years, between 7-8 years, between 6-7 years, below 6 years.

The interval between two oocyte recovery sessions was categorised in above 28 days, between 22-28 days, between 16-21 days, between 8-15days. A general linear model was applied to estimate the effects like in breed, age at collection of oocyte and interval between two oocyte recovery sessions. The significance level was adjusted at 0.05 level.

Oocyte recovery and embryo production

There were 891 OPU sessions of *Bos indicus* covering different breeds viz. Dangi, Deoni, Gaolao, Gir, Red Kandhari and Sahiwal. A total of 6416 oocytes were processed for *In vitro* culture and 2016 embryos were produced. (Breed wise oocytes recovered and embryo produced are presented in Table 1). The average breed wise oocyte recovery was 6.58 ±0.45 and the average breed wise embryo production was 2.11 ±0.33 per session.

Breed wise oocytes recovered and embryo produced are presented in Table 2 and age group wise oocytes recovered and embryo ovum pick up day's interval wise oocytes recovered and embryo produced are presented in Table 2 Collection interval wise: Age group wise oocytes recovered and embryo produced are presented in Table 2.

The data compiled on embryo production, embryo conversion rate and embryo produced per session from 891 ovum pick up sessions in a large-scale program for IVEP from dairy (*Bos indicus*) cattle. The results would facilitate the expansion of OPU-IVEP programs in cattle because they demonstrated the process for conservation and multiplication of indigenous animals (*Bos indicus*) through *In vitro* embryo production (Srivastava *et al.,* 2019).

The study showed that production of higher number of embryos from a wider range of potential female donors, including open cyclic females and ones up to 3 months pregnant. It required a reduced number of sperm to produce embryos and increased chances of obtaining the offspring.

Gir, Dangi and Sahiwal presented higher oocyte recovery and embryo production followed by Deoni, Gaolao and Red Kandhari. The variability in the results for different breeds might be associated with the donor nutritional status. Kouamo *et al.* (2014), estrous cycle phase (Reis *et al*., 2006), reproductive stage (Landry *et al*., 2016), age, number of deliveries (Su *et al*., 2012) and even gene sequence of each animal (Biase *et al*., 2008), which were not evaluated in the present study. A variation was observed with means of 15.7 to 24.9 oocytes per OPU for the Gir breed (Watanabe 2017), similar results were found in the present study. Age group of 6-8 years animals presented higher oocyte recovery and embryo production. The effect of age on follicular population and oocyte recovery was observed (Kouamo *et al.,* 2014) which showed that there was follicular population and fertility decline with age in all species. The cows less than 10 years old were remain the best choice to increase the population of good embryos. OPU between 22-28 days, the oocytes recovery and embryo production rate presented higher than rest all. It was found that no difference in number of viable oocytes when performing OPU in the same donor at intervals shorter than 15 days (Pontes *et al*., 2011). Moreover, a study with Holstein, Nellore and buffalo heifers, and OPU performed every 14 days found that the number of OPU only affected the performance of zebu cows, with a decrease in the number of viable oocytes and cleavage rate (Gimenes *et al*., 2015), as observed in the Brahman, Gir and Nellore donors evaluated in the present study. The number of OPU sessions in the donors and the interval between them can affect the number of aspirated follicles and, consequently, the number of recovered oocytes (Gimenes *et al*., 2015). The number of OPU and other factors such as interval between OPU could have affected the results found. Several factors such as nutrition T. (Kruip *et al.,* 1996) and harvesting techniques may explain this divergence.

Table 1: Breed wise embryo production.

Breed	Sessions	Oocytes recovered	Total embryos produced
Dangi	73	574	148
Deoni	39	198	43
Gaolao	116	691	216
Gir	295	2605	850
Red kandhari	93	399	102
Sahiwal	275	1949	657
Grand total	891	6416	2016

Table 2: Breed wise, age group wise and ovum pick up day's wise interval-oocyte recovery and embryo production.

Levels of factors	Sessions	Oocyte recovery	Sessions	Embryo produced
		Mean±SE		Mean±SE
Overall mean		6.58 ±0.45		2.11 ±0.33
Breed		**		**
Dangi	73	8.11^{ab} ±0.77	72	2.47^{b} ±0.37
Deoni	39	4.77^{d} ±1.01	39	1.42^{c} ±0.48
Gaolao	116	5.79^{c} ±0.70	114	2.23^{ab} ±0.33
Gir	295	9.24^{a} ±0.44	291	3.25^{a} ±0.21
Red kandhari	93	4.1^{d} ±0.78	91	0.86^{d} ±0.37
Sahiwal	275	7.5^{b} ±0.48	270	2.41^{ab}±0.23
Age-group		*		*
above 10 years	113	7.47^{a} ±0.78	112	2.81^{a} ±0.37
below 6 years	145	7.13^{ab} ±0.58	142	1.83^{ab} ±0.28
between 6-7 years	205	7.26^{ab} ±0.52	203	2.11^{ab} ±0.25
between 7-8 years	130	4.97^{b} ±0.59	127	1.46^{b} ±0.28
between 8-9 years	130	6.25^{ab} ±0.64	127	2.17^{ab} ±0.30
Between 9-10 years	168	6.43^{ab} ±0.66	166	2.25^{ab} ±0.31
Interval		NS		NS
above 28 days	285	6.29^{a} ±0.38	185	1.98^{a} ±0.18
Between 16-21 days	154	6.25^{a} ±0.48	100	2.02^{a} ±0.23
between 22-28 days	100	7.50^{a} ±0.58	68	2.24^{a} ±0.28
Between 8-15 days	338	6.00^{a} ±0.35	222	1.75^{a} ±0.17

Significance level: **, P<0.01; *: P<0.05, NS: Not Significant. Means with at least one common superscript within classes do not differ significantly

However, it is known that follicular recruitment can also be influenced by other factors besides genetics. The main factors are nutritional and metabolic factors (Sartori *et al*., 2016) and environmental factors, mainly induced by thermal stress (Baruselli *et al*., 2017).

CONCLUSION

The standardized protocol has shown a promising result in terms of embryo production. The OPU-IVF-ET program can be designed with respect to breed specific conversation program to achieve the desired outcome in very short duration. Such an efforts should be encouraged to boost the effective *ex-situ* conservation and recommended on broad scale.

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Conflict of interest: None.

REFERENCES

Aiman, A. Ammari, Ramzi, A., Amran, Muath, G.A. Ghadi, Ahmad, R.A. (2022). Morphometric assessment of the bovine ovary for *in vitro* matured oocyte quality to determine developmental competence. Indian Journal of Animal Research. 56(5): 557-562. (10.18805/IJAR.BF-1471/.

Baruselli, P.S., Batista, E.O.S., Vieira, L.M., Sales, J.N.S., Gimenes, L.U., Ferreira, R.M. (2017). Intrinsic and extrinsic factors that influence ovarian environment and efficiency of reproduction in cattle. Animal Reproduction Science. 14(1): 48-60.

Biase, F.H., Fonseca, M.G.K., Santos, B.W.K., Martelli, L., Meirelles, F.V. (2008). Global poly(A) mRNA expression profile measured in individual bovine oocytes and cleavage embryos. Zygote. 16(1): 29-38.

Ferré, L.B., Kjelland, M.E., Strøbech, L.B., Hyttel, P., Mermillod, P. and Ross, P.J. (2020). Review: Recent advances in bovine *In vitro* embryo production: reproductive biotechnology history and methods - Animal, © The Animal Consortium. 14(5): 991-1004 doi: 10.1017/S1751731119002775).

Gimenes, L.U., Ferraz, M.L., Fantinato-Neto, P., *et al*. (2015). The interval between the emergence of pharmacologically synchronized ovarian follicular waves and ovum pickup does not significantly affect *In vitro* embryo production in Bos indicus, Bos taurus and Bubalus bubalis. Theriogenology. 83(3): 385-393.

Ka¸tska, L. and Smora¸g Z. (1984). Number and quality of oocytes in relation to age of cattle, Animal Reproduction Science. 7(5): 451-460.

Kouamo, J., Dawaye, S., Zoli, A.P. and Bah, G.S. (2014). Evaluation of bovine (*Bos indicus*) ovarian potential for *In vitro* embryo production in the Adamawa plateau (Cameroon), Open Veterinary Journal. 4(2): 128-136.

Kruip, T. and Postma, A.V. (1996). Influence of food intake antepartum on the quality of oocyte pospartum in dairy cows, Journal of Reproduction Science. 47: 189-195,

Kumar, S., Otávio, M. Ohashi, William, G. Vale, Luciana, M.M., Vicente, J.F.F. (2020). State-of-the-art and emerging technologies for *in vitro* embryo production in buffaloes. Journal of Advanced Veterinary Research. 10(3): 186-192.

Landry, D.A., Bellefleur, A.M., Labrecque, R., *et al*. (2016). Effect of cow age on the *in vitro* developmental competence of oocytes obtained after FSH stimulation and coasting treatments. Theriogenology. 86(5): 1240-1246.

Pontes, J.H.F., Melo Sterza, F.A., Basso, A.C., Ferreira, C.R., Sanches, B.V., Rubin, K.C.P., Seneda, M.M. (2011). Ovum pick up, *In vitro* embryo production and pregnancy rates from a large-scale commercial program using Nelore cattle (*Bos indicus*) donors. Theriogenology. 75: (9): 1640-1646.

Singh, A.P., Kumar, D., Gopalakrishna, R., Ranjan, R., Pandey, S.K. and Sarkhel, B.C. (2016). Comparison of culture media for their effects on development of caprine IVF embryos using fresh and cryopreserved semen. Indian J. Anim. Res. 50(6): 846-850 Print ISSN:0367-6722/ Online ISSN:0976-0555).

Srivastava, A.K., Patel, J.B., Ankuya, K.J., Chauhan, H.D., Pawar, M.M. and Gupta, J.P. (2019). Conservation of Indigenous Cattle Breeds. Journal of Animal Research. 9(1): 01-12. February DOI: 10.30954/2277-940X.01.

Su, L., Yang, S., He, X., *et al*. (2012). Effect of donor age on the developmental competence of bovine oocytes retrieved by ovum pick up. Reprod Domest Anim. 47(2): 184-189.

Watanabe, Y.F. (2017). Number of oocytes retrieved per donor during OPU and its relationship with *in vitro* embryo production and field fertility Following Embryo Transfer. DOI: 10.21451/1984-3143-AR1008.