GMR
The Original

BREED-ASSOCIATED DIFFERENCES IN OOCYTE RECOVERY AND IN VITRO EMBRYO PRODUCTION IN GAOLAO AND SAHIWAL CATTLE

Pandiri Keerthana* [1], D.S. Raghuwanshi [2], A.P. Gawande [3], M.S. Patil [3], M.S. Bawaskar[3], M. P. Kaore[4], S. A. Ingle[5], A.M. Shende[6], & A.M. Narkhede[1]

[1]PhD Scholar, Department of ARGO, NVC, Nagpur
[2]Professor & Head, Department of ARGO, NVC, Nagpur
[3]Professor, Department of ARGO, NVC, Nagpur
[4]Assistant Professor, Department of Veterinary Pathology, NVC, Nagpur
[5]Assistant Professor, Department of Veterinary Biotechnology, NVC, Nagpur
[6]Assistant Professor, Department of Veterinary Biochemistry, NVC, Nagpur
Nagpur Veterinary College, Maharashtra Animal & Fishery Sciences University-440006, Nagpur
Email ID: keerthanapandiri2307@gmail.com
dilipsinghraghuwanshi@mafsu.ac.in ; gawandeajay38@gmail.com ; mspgyn111@gmail.com
drmeenakshi.vet@gmail.com;meghakaore@mafsu.ac.in; sonalingle@mafsu.ac.in; ashishshende@mafsu.ac.in aryanarkhede123@gmail.com;

ABSTRACT

The present study was undertaken to evaluate and compare the in vitro embryo production potential of Gaolao and Sahiwal cattle using ovum pick-up (OPU) and in vitro embryo production (IVEP) techniques. A total of 16 OPU sessions were conducted, involving eight donors from each breed. Following follicular wave synchronisation and FSH stimulation, oocytes were recovered, graded morphologically, and subjected to in vitro maturation (IVM), fertilisation (IVF) and culture (IVC). A total of 126 oocytes were recovered, of which 62 developed into blastocysts, resulting in an overall blastocyst production rate of 49.20%. Breed-wise comparison revealed significantly ($P<0.05$) higher oocyte recovery in Sahiwal donors (101) than in Gaolao donors (25). Similarly, the mean number of oocytes recovered per donor was higher in Sahiwal (12.63 ± 2.96) compared to Gaolao (3.13 ± 0.67). Sahiwal donors also produced a significantly ($P<0.05$) higher mean number of blastocysts (6.63 ± 1.90) than Gaolao donors (1.13 ± 0.35). Although the blastocyst conversion rate was numerically higher in Sahiwal (43.37 ± 9.77%) than in Gaolao (31.40 ± 7.54%), the difference was not statistically significant ($P> 0.05$). A total of 62 blastocysts were produced, of which 28 were selected for embryo transfer due to the limited availability of recipient cows. Embryo transfer resulted in eight pregnancies, with an overall pregnancy rate of 28.57%. The pregnancy rate was numerically higher in Sahiwal (36.8%) than in Gaolao (11.1%), although the difference was not statistically significant. The findings indicate that Sahiwal donors exhibited better oocyte recovery and blastocyst production potential under the conditions of the present study compared to Gaolao donors.

KEYWORDS: Gaolao, Sahiwal, Ovum Pick-up (OPU), In Vitro Embryo Production (IVEP), Blastocyst Development, Pregnancy Rate.

INTRODUCTION

Assisted reproductive technologies (ARTs) have become important tools for accelerating genetic improvement in cattle. Among these, ovum pick-up (OPU) combined with in vitro embryo production (IVEP) has emerged as an effective alternative to conventional superovulation and embryo transfer program. OPU enables repeated collection of oocytes from genetically superior females, thereby increasing maternal contribution to genetic gain, enhancing selection intensity, and reducing generation intervals by using young donor animals. (Saini *et al*., 2015). Despite its widespread application, the efficiency of OPU-IVEP remains highly variable. Significant differences among donor animals in reproductive traits such as antral follicle population, follicular dynamics, oocyte quality, reproductive hormone profiles, and gene expression within the cumulus-oocyte complex contribute to variation in oocyte recovery, oocyte quality, embryo development, blastocyst production, and pregnancy rates (Lacerda *et al*., 2020; Hidalgo, 2021). Among the factors affecting IVEP success, oocyte quality is considered the most critical because it determines the oocyte's ability to mature, be fertilised, develop into a blastocyst, and ultimately establish a successful pregnancy (Oliveira *et al*., 2019).

Since embryo production is strongly associated with the number of oocytes available for culture, identifying animals with superior oocyte recovery potential is essential for improving IVEP efficiency. Moreover, high repeatability of oocyte production within individual donors suggests that donor selection based on reproductive performance can significantly enhance embryo production outcomes (Watanabe *et al*., 2017).

MATERIAL AND METHODS

The present study was carried out at the in vitro Fertilisation Embryo Transfer Technology Laboratory, Department of Animal Reproduction, Gynaecology and Obstetrics, Nagpur Veterinary College, Nagpur.

Experimental design

The cows were randomly divided into two groups: group I (Gaolao) and group II (Sahiwal), with eight replicates in each group. Both groups underwent follicular wave synchronisation before the OPU procedure. Synchronisation was initiated by administering 500mcg prostaglandin on a random day of the oestrous cycle, followed by FSH stimulation at a dose of 100mg divided into four sub-doses of 30, 30, 20 and 20 mg administered every 12h after prostaglandin administration, respectively. Ovum pick-up was performed 48h (coasting period) after the last FSH injection. A total of 16 OPU sessions were conducted in 16 animals (one session per animal).

Preparation of the donor and ovum pick-up (OPU)

Before OPU, the animals were subjected to epidural anaesthesia (3 mL of 2% lidocaine HCl), which was administered between the last sacral vertebra and the first coccygeal vertebra. After loss of tail reflexes, faeces was manually removed from the rectum, and the perineal and vulvar regions was washed with water. Then, a follicular aspiration guide with a micro convex 5.0 MHz probe (Exago, IMV, France) was introduced into the vaginal pouch. All the antral follicles in each animal were punctured with a disposable needle system (18 G, WTA Brazil) coupled to a Teflon aspiration line. The aspiration system operated at a negative pressure of 70 mmHg produced by a vacuum pump. The material from the aspirated follicles was collected through a 1.1mm internal diameter and 120cm long tubing system connected to a 50-mL conical tube (Corning) containing 15 mL of OPU collection medium that was maintained at 37 °C in a tube heater during OPU.

Searching for Oocytes

The follicular aspirates collected in conical tubes were transported to the laboratory and filtered using a 75 µm filter (WTA, Brazil) with Euroflush medium (IMV, France). The collected filtrate was placed in Petri dishes and examined under a stereo zoom microscope to identify oocytes. The recovered oocytes were evaluated morphologically under a stereomicroscope at 8–20× magnification while maintaining a temperature of 37°C.

Grading of Oocytes

Oocytes were classified according to the morphology of cumulus-oocyte complexes (COCs):

Grade A: COCs having expanded cumulus cells with four or more layers surrounding the zona pellucida and homogeneous cytoplasm.

Grade B: COCs with an unexpanded cumulus mass containing 2–3 layers of cumulus cells and homogeneous cytoplasm.

Grade C: COCs with an unexpanded cumulus mass containing 1–2 layers of cumulus cells and homogeneous cytoplasm.

Grade D: COCs with a single layer or absence of cumulus cells and homogeneous cytoplasm.

In Vitro Maturation of Oocytes

The maturation medium was equilibrated in a tri-gas incubator containing 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C and 90% relative humidity for at least 4 hours. A 100 µl maturation droplet was prepared in a 35 mm Petri dish under mineral oil overlay. After oocyte recovery and selection, Grade A, B, and C oocytes were placed into maturation droplets at a rate of 20 oocytes per 100 µl medium.

The Petri dishes were incubated for 22–24 hours in the tri-gas incubator at 38.5°C to allow maturation.

In Vitro Fertilisation and Culture of Oocytes

IVF droplets were prepared using commercially available fertilisation medium in 35-mm Petri dishes and equilibrated overnight. Following maturation, oocytes were washed in IVF wash medium and transferred into fertilisation droplets. Frozen-thawed semen with initial motility above 80% was processed using a density gradient comprising 400 µL conventional Percoll (Vitrogen, WTA, Brazil) and 400 µL diluted Percoll (Vitrogen, WTA, Brazil), prepared by mixing 200 µL sperm medium with 200 µL conventional Percoll. The semen was layered over the gradient and centrifuged (Thermofischer, USA) at 600 × g for 6 minutes at 38.5°C, after which the supernatant was carefully removed. The sperm pellet was resuspended in 400 µL pre-equilibrated IVF medium supplemented with heparin and PHE, followed by centrifugation at 150 × g for 3 minutes at 38.5°C. The supernatant was removed, leaving 200 µL of medium containing the sperm pellet, and sperm concentration and motility were assessed using a Neubauer chamber and phase-contrast microscope, respectively. Sperm exhibiting 99% fast forward progressive motility were added to the fertilisation droplets at a concentration of 10–12 × 10^6 spermatozoa per droplet (7–10 µL). The oocyte–sperm complexes were incubated for 18 hours at 38.5°C in a tri-gas incubator (Minc, Cook, Australia). Presumptive zygotes were washed in IVF culture medium, and residual cumulus cells were removed by gentle pipetting using a stripper before transferring the zygotes into culture droplets for seven days at 38.5°C. Cleavage rate was assessed after 24 hours using a stereo zoom microscope, while embryo yield was evaluated on day seven by determining blastocyst and expanded blastocyst formation.

Recipient cows were selected based on regular estrous cycles, good body condition, and the absence of reproductive tract abnormalities. The selected animals were synchronised using the Ovsynch protocol, which involved intramuscular administration of GnRH (Buserelin acetate, 2.5 mL) on Day 0, cloprostenol (2 mL) on Day 7, and a second dose of GnRH (2.5 mL) on Day 9. Estrus was detected through visual observation. On Day 8 post-estrus, recipient cows were examined by ultrasonography to assess the presence of a functional corpus luteum (CL) on the ovary. Animals with a suitable CL were selected for embryo transfer. The embryos were transferred into the uterine horn ipsilateral to the ovary bearing the CL. At 45 days post-ET, pregnancy diagnosis (PD) was performed through rectal palpation and ultrasonography (Mindray), using a linear probe. We performed statistical analysis using R (version 4.6.0; R Core Team, 2026).

RESULT & DISCUSSION

A total of 126 oocytes were recovered from 16 OPU sessions, of which 62 developed into blastocysts, resulting in an overall blastocyst production rate of 49.20%. Due to the limited availability of suitable recipient cows, only 28 (45.16%) of the produced blastocysts could be selected for embryo transfer. Following embryo transfer, eight pregnancies were established, resulting in an overall pregnancy rate of 28.57% based on the number of embryos transferred.

Of the total oocytes recovered, Sahiwal donors (101 oocytes) showed a significantly ($P < 0.05$) higher oocyte recovery than Gaolao donors (25 oocytes)(Table No.1&2). Higher mean oocyte numbers across all four quality grades (A, B, C, and D) were observed in Sahiwal (2.62 ± 0.73, 5.37 ± 1.06, 2.62 ± 0.77, and 1.87 ± 0.74) than in Gaolao donors (0.87 ± 0.39, 1.37 ± 0.46, 0.75 ± 0.25, and 0)(Table No.3&4). Statistically significant differences ($P < 0.05$) were observed in Grades B, C, and D, whereas Grade A did not differ significantly ($P > 0.05$). The present findings for Sahiwal donors were comparable with the observations reported by Deshpande *et al*. (2023), Nawaz *et al*. (2021), and Chandra *et al*. (2024); however, the recovery values for Gaolao donors were lower than those reported by Deshpande et al. (2023). The donor variations can be due to differences in the antral follicle population (Zangirolamo *et al*., 2018), follicular dynamics, plasma concentrations of AMH and FSH (Batista *et al*., 2020 & Sharma *et al.*, 2023), and the genetic composition of each donor (Yinyan *et al*., 2024; Farheen *et al*., 2024; Ciftci *et al*., 2025).

Table No.1 Oocytes recovered and Blastocyst conversion rates in Gaolao and Sahiwal donors

GROUPS	PARAMETERS	DONORS							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Gaolao	**OOCYTE RECOVERED**	2	3	4	7	4	2	2	1
	BLASTOCYST CONVERSION RATE	1(50%)	1(33%)	2(50%)	3(42%)	1(25%)	0	1(50%)	0
	TRANSFERABLE EMBRYOS	1	1	2	3	1	0	1	0
Sahiwal	**OOCYTE RECOVERED**	13	25	7	6	15	24	9	2
	BLASTOCYST CONVERSION RATE	6(46%)	13(52%)	4(57%)	0	10(52%)	14(70%)	6(60%)	0
	TRANSFERABLE EMBRYOS	6	13	4	0	10	14	6	0

Table No.2 Mean of oocytes recovered and blastocysts produced in Gaolao and Sahiwal donors

DONORS	OOCYTE RECOVERED MEAN±SEM	BLASTOCYST% MEAN±SEM	NO.OF BLASTOCYST MEAN±SEM
Gaolao	3.13±0.67	31.40±7.54	1.13±0.35
Sahiwal	12.63±2.96	43.37±9.77	6.63±1.90

The number of blastocysts produced was significantly($P<0.05$) higher in Sahiwal donors (6.62 ± 1.89) than in Gaolao donors (1.12 ± 0.35). However, the blastocyst conversion rate was numerically higher in Sahiwal donors ($42 \pm 9.52\%$) compared to Gaolao donors ($31 \pm 7.51\%$), although the difference was not statistically significant($P>0.05$). Similarly, the total number of transferable embryos was significantly ($P<0.05$) higher in Sahiwal donors (53 embryos) than in Gaolao donors (9 embryos) (Table No.1&2). The blastocyst production and conversion rates observed in Sahiwal donors were comparable with the findings of Kumar *et al*. (2022) and Nawaz *et al*. (2021), whereas the corresponding values in Gaolao donors were lower than those reported by Deshpande *et al*. (2023). The observed differences in embryo production between the two breeds may be associated with variations in the quantity and quality of oocytes recovered from donor animals. In this regard, Gimenes *et al.* (2015), Lacerda *et al*. (2020), and Patel *et al*. (2022) reported that breed-related differences in embryo production are positively associated with variations in oocyte recovery and oocyte quality. In the present study, the comparatively higher number and better quality of oocytes recovered from Sahiwal donors might have contributed to the higher blastocyst yield and total number of transferable embryos compared to Gaolao donors.

Table No.3 Morphological quality of cumulus oocyte complexes obtained from sahiwal and Gaolao donors

Breed	No. of Donors	Quality A (Excellent)	Quality B (Good)	Quality C (Fair)	Quality D (Poor)
Gaolao	8	7	11	7	0
Sahiwal	8	21	43	22	15

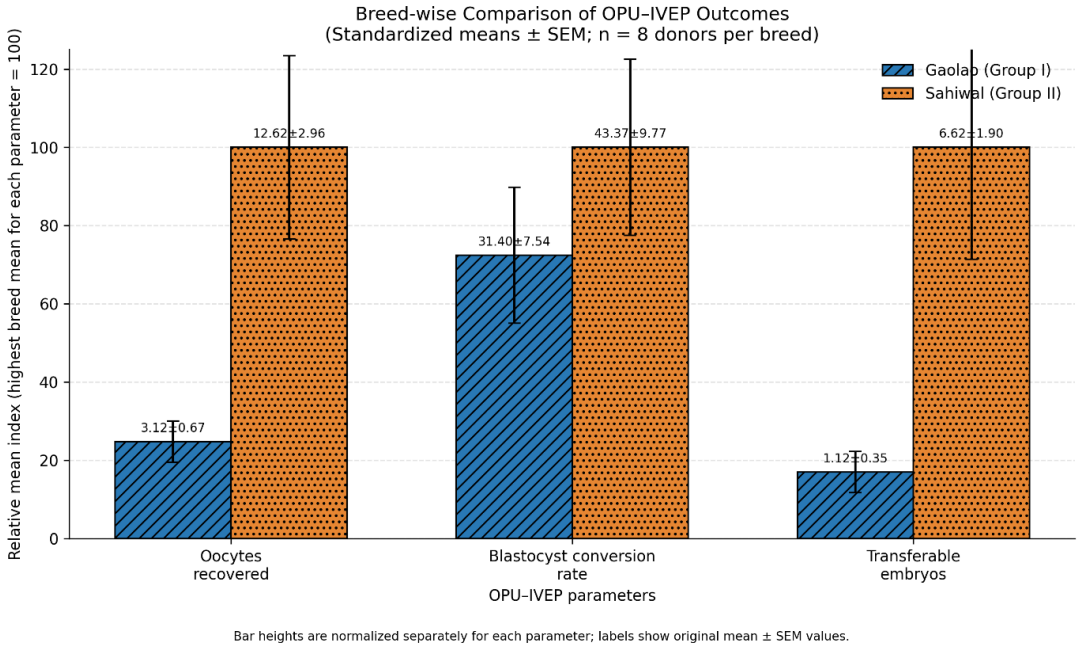
Table No.4 Mean quality of oocytes obtained from Gaolao and Sahiwal donors

Donor	Oocyte quality			
	A	**B**	**C**	**D**
Gaolao	0.88±0.40	1.38±0.46	0.75±0.25	0
Sahiwal	2.63±0.73	5.38±1.07	2.63±0.78	1.88±0.74

The pregnancy rate was numerically higher in Sahiwal donors (36.8%) than in Gaolao donors (11.1%); however, the difference was not statistically significant ($P > 0.05$) (Table No. 5). In agreement with these findings, Lacerda *et al.* (2020) reported that pregnancy rates were not significantly influenced by the breed of the donor or the quality of the recovered oocytes, but were more closely associated with the developmental stage of embryos at the time of transfer. In the present study, expanded blastocysts were transferred to recipient animals in both groups. However, out of the 62 blastocysts produced, only 28 could be transferred due to the limited availability of suitable recipient cows. Furthermore, Xie *et al.* (2024) reported that recipient-related factors, including lactation number, embryo transfer season, and the type and volume of the corpus luteum (CL), significantly influenced pregnancy rates following embryo transfer. Considering these findings, the numerical variation in pregnancy rates observed between Sahiwal and Gaolao donors may be partly associated with recipient-related factors and the limited availability of recipients rather than breed differences alone.

Table No.5 Comparative pregnancy rates in Gaolao and Sahiwal donors

Breed	Number of embryos transferred	Number of pregnancies	Pregnancy rate (%)
Gaolao	9	1	11.1
Sahiwal	19	7	36.8



produced

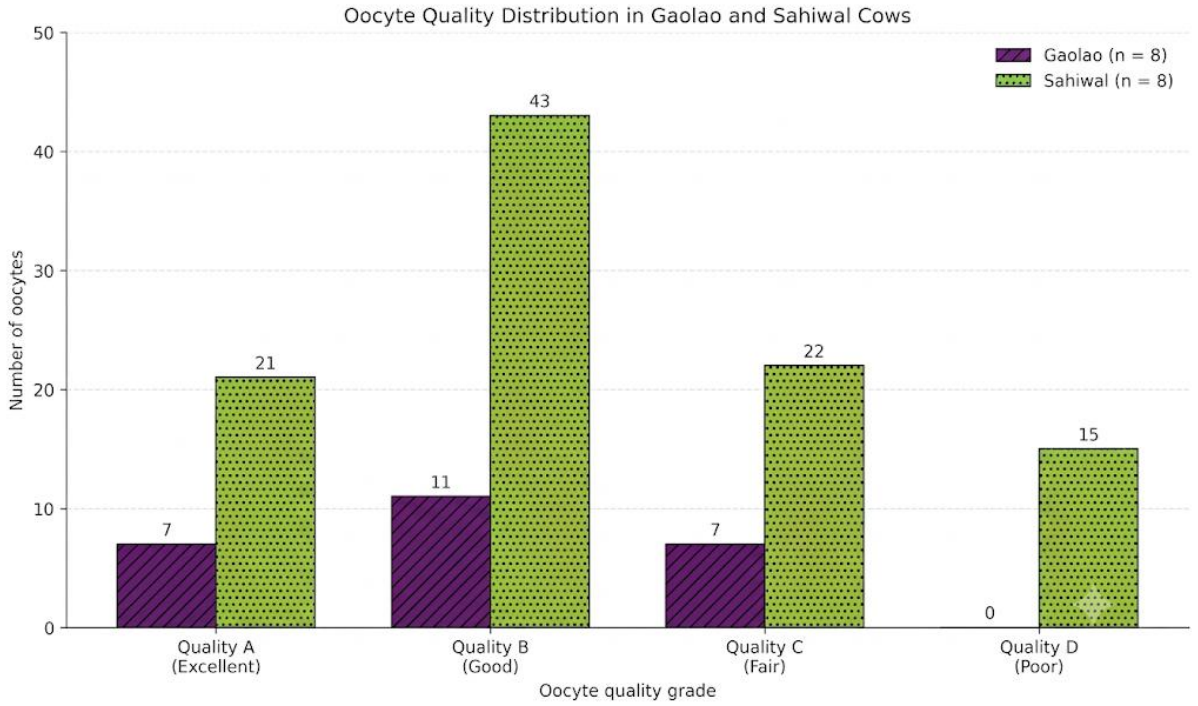


Figure 2. Comparison of the quality of oocytes obtained through OPU between Sahiwal and Gaolao donor cows.

CONCLUSION

The present study demonstrated that Sahiwal donors had significantly higher oocyte recovery and blastocyst production than Gaolao donors under the conditions investigated. These findings highlight the potential of OPU-IVEP technology for improving the reproductive utilisation of genetically valuable cattle and supporting breed improvement programmes**.**

REFERENCES

1. Chandra, P., Kumar, B., Donadkar, M. M., Bhagirathi, Jasrotia, N., Chouhan, V. S., Kharayat, N. S., & Singh, S. K. (2024). Ovum-Pick Up and In Vitro Embryo Production Responses in Stimulated and Non-Stimulated Sahiwal Cows. Indian Journal of Veterinary Sciences and Biotechnology. 20(6), 11-15.

2. Çiftçi, M. F., Gölbaşi, M., Yeşilkaya, Ö. F., Sari, A., Çizmeci, S. Ü., & Dinç, D. A. (2025). The Role of Antral Follicle Count in OPU/IVEP Donor Selection in Holstein Cattle. Kocatepe Veterinary Journal, 18(1), 1-6. https://doi.org/10.30607/kvj.1548550

3. Deshpande, P.D., Joshi, G.S., Kadam, H.D., Ghadge, V.N., Khadse, J.R. and Pande, A.B. (2023). Conservation and Multiplication of Indigenous Animals (Bos indicus) Through in vitro Embryo Production. Indian Journal of Animal Research. doi:10.18805/IJAR.B-5026.

4. Farheen, Shaik & Kumari, G & Reddy, K & Reddy, K & Nagaraj, P & Priyanka, B. (2024). Effect of follicle-stimulating hormone stimulation on in-vitro oocyte maturation in Sahiwal cattle. International Journal of Advanced Biochemistry Research. 8. 111-117. 10.33545/26174693.2024.v8.i3Sb.702.

5. Hidalgo, M. (2021). Comparison between in vitro embryo production in Bos indicus and Bos taurus cows. Research, Society and Development. https://doi.org/10.33448/RSD-V10I7.16712

6. Jessica C.L. Motta, Rodrigo V. Sala, Cameron B. Hayden, Daniela Pereira, Victor A. Absalón-Medina, Juan F. Moreno, Pablo J. Ross, Álvaro García-Guerra. Differential effects of follicle-stimulating hormone dosage on in vitro embryo production in high- and low-anti-Müllerian hormone Holstein heifers. Journal of Dairy Science. Volume 109, Issue 3,2026, Pages 3093-3107.ISSN 0022-0302.https://doi.org/10.3168/jds.2025-27587

7. Lacerda, I.P., Dode, M.A., Lima, M.M., Guerra, B.F., Costa, E., Moreira, G., & Carvalho, J.D. (2020). Cattle breed affects in vitro embryo production in a large-scale commercial program on dairy farms. *Livestock Science, 240*, 104135.

8. Lacerda, Izabelle & Dode, Margot & Lima, Marcelo & Guerra, Breno & Costa, Eduardo & Moreira, Gisele & Carvalho, José. (2020). Cattle breed affects in vitro embryo production in a large-scale commercial program on dairy farms. Livestock Science. 240. 104135. 10.1016/j.livsci.2020.104135.

9. Nawaz M, Saleem M, Yaseen M, Sagheer M, Zahoor I, Ahmad N and Riaz A, 2021. Ovarian stimulation through FSH improves follicular harvesting and blastocyst yield in Bos indicus cattle. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2021.081

10. Patel, Mayank & Hadiya, Kamlesh & Layek, Siddhartha Shankar & Gorani, Sanjay & Gupta, Rajesh & Dhami, Arjun. (2022). Oocyte recovery and relationship between quality of oocytes and embryo production in zebu and crossbred dairy cattle.

11. R Vinay Kumar, G Aruna Kumari, K Chandrashekar Reddy and P Nagaraj. Studies on *in vitro* fertilisation rates of oocytes from stimulated and non-stimulated Sahiwal cows. The Pharma Innovation Journal. 2022; 11(11S): 2637-2642.
12. Saini N, Singh MK, Shah SM, et al. Developmental competence of different quality bovine oocytes retrieved through ovum pick-up following in vitro maturation and fertilisation. *Animal*. 2015;9(12):1979-1985. doi:10.1017/S1751731115001226
13. Sarang, S., Sreekumar, D., & Sejian, V. (2024). Indigenous cattle biodiversity in India: Adaptation and conservation. *Reproduction and Breeding*. DOI:10.1016/j.repbre.2024.09.001
14. Sarwar Z, Saad M, Saleem M, Husnain A, Riaz A, Ahmad N. Effect of follicle size on oocyte recovery rate, quality, and in-vitro developmental competence in Bos indicus cows. Anim Reprod. 2020;17(3):e20200011. https://doi.org/10.1590/1984-3143-AR2020-0011
15. Sharma P, Layek SS, Hadiya KK, Gorani S, Karuppanasamy K, Patil SP, Raj S, Raval KB. (2023).Selecting donors for ovum pickup using anti-Mullerian hormone in cattle: a potential biomarker for donor selection. Reproduction Fertility and Development 36, 206. https://doi.org/10.1071/RDv36n2Ab107
16. Slade Oliveira, C., Varella Serapião, R., dos Reis Camargo, A.J., de Freitas, C., Tamy Iguma, L., Campos Carvalho, B., de Almeida Camargo, L.S., Zoccolaro Oliveira, L., & da Silva Verneque, R. (2019). Oocyte origin affects the in vitro embryo production and development of Holstein (Bos taurus taurus) - Gyr (Bos taurus indicus) reciprocal cross embryos. Animal Reproduction Science. 209, 106165.
18. Vennapureddy, S., Arunakumari, G., Reddy, K.C., Reddy, K.R. and Ambica, G. (2024). Effect of FSH Stimulation of Ovaries on in vitro Maturation of Sahiwal Oocytes Collected by Ovum Pick-up (OPU) Method. Indian Journal of Animal Research. 58(3): 395-400. doi: 10.18805/IJAR.B-4483
19. Watanabe, Y.F., Souza, A.H., Mingoti, R.D., Ferreira, R.M., Batista, E.O., Dayan, A., Watanabe, O.Y., Meirelles, F.V., Marcelo, Nogueira, F.G., Ferraz, J.B., & Baruselli, P.S. (2017). Number of oocytes retrieved per donor during OPU and its relationship with in vitro embryo production and field fertility following embryo transfer. *Animal reproduction, 14*, 635-644.
20. Xie C, Huang C, Yan L, Yao R, Xiao J, Yang M, Chen H, Tang K, Zhou D, Lin P, Wang A, Jin Y. Recipients' and Environmental Factors Affecting the Pregnancy Rates of a Large, Fresh In Vitro Fertilization-Embryo Transfer Program for Dairy Cows in a Commercial Herd in China. Vet Sci. 2024 Sep 5;11(9):410. doi: 10.3390/vetsci11090410. PMID: 39330789; PMCID: PMC11435568.
21. Yinyan Du, Yuhan Xia, Jiahui Xu, Zhihui Liu, Zhentian Liu, Meijie Zhang, Guangyong Xu, Xuesong Xing, Fuliang Du. Effects of donor age and reproductive history on developmental potential of oocyte pickup in Japanese Black cattle (Wagyu). Theriogenology. Volume 221,2024; Pages 25-30. ISSN 0093-691X, https://doi.org/10.1016/j.theriogenology.2024.03.012.
22. Zangirolamo AF, Morotti F, da Silva NC, Sanches TK, Seneda MM. Ovarian antral follicle populations and embryo production in cattle. Anim Reprod. 2018 Aug 17;15(3):310-315. doi: 10.21451/1984-3143-AR2018-0072. PMID: 34178154; PMCID: PMC8202465.