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Research Article

TRANSVAGINAL ULTRASOUND-GUIDED OVUM PICK-UP AND *IN VITRO* EMBRYO PRODUCTION IN COWS

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ABSTRACT

In this study, 198 oocytes were aspirated from six different donor cows (Sahiwal × HF) in 21 OPU sessions, averaging 9.42 oocytes per Ovum Pick Up (OPU) session. The oocyte recovery rate was 1.29 ± 0.43, 2.62 ± 0.53, 3.24 ± 0.64, 1.67 ± 0.28 and 0.62 ± 0.16 for A, B, C, D and E grade embryos, respectively. Out of total 198 collected oocytes 185 carefully selected oocytes with grades A, B, C and D underwent further *in vitro* maturation (IVM). It was observed that a total of 75.67 ± 6.31% of oocytes (147 out of 185) were matured. Out of 147 oocytes kept for *in vitro* fertilization (IVF), 86 oocytes were cleaved. The average cleaved oocyte was 4.096 ± 0.78 out of the mean fertilized oocytes of 7 ± 1.33, yielding a cleavage rate of 59.18 ± 6.83%. Out of 147 oocytes kept for *in vitro* fertilization, 55 fertilized oocytes reached the blastocyst stage. The blastocyst production rate was 37.53 ± 6.81 %.41 *in vitro* produced embryos were transferred to recipient cows and a conception rate of 24.39% (10/41) was recorded.

Keywords: Cattle, IVF, IVM, , Ultrasound-guided ovum pickup

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Over the past two decades, the ultrasound-guided transvaginal follicular aspiration technique for ovum pickup (OPU) has become increasingly popular in the dairy industry for producing embryos from elite cows in India. However, this process is more complex than artificial insemination as it includes steps like OPU, IVM, IVF, *in vitro* Culture (IVC), and then transferring of the embryo in the appropriate recipient. But despite the complexity, these procedures are widely practiced and yield good results in embryo transfer programs. The number, and the quality of follicles present at the time of aspiration determine the success rate of OPU in cows. The first objective of this experiment was to evaluate oocyte recovery by OPU and embryo production rate in cows. The second objective was to study the conception rate in 'recipients' cows (Sahiwal x HF).

MATERIALS AND METHODS

The current study was carried out at ET-IVF Laboratory, Bull Mother Farm, Animal Husbandry (Govt. of Maharashtra), Tathawade, Pune and KNP College of Veterinary Science, Shirwal, Dist. Satara. All cow-related procedures and protocols were preapproved by the IAEC of the college. The experimental cows (Sahiwal × HF) were intensively housed with stall feeding and grazed during the day. The study was carried out from October 2023 to March 2024. Six elite donor cows with a known history of good lactational yield and breeding value were screened and selected by thorough clinico-gynecological examination, rectal palpation, and ultrasonographic examination of genital organs. The animals with good general health, a good body condition score (3-3.5), larger cyclic ovaries, and non-pregnant donors were selected as donors. Donors were dewormed and supplemented with a good quality mineral mixture powder and a well-balanced ration before the start of the investigation.

Donors were subjected to the OPU a day before when the recipients were detected in estrus as per the synchronization protocol (Double PGF2α). For the OPU procedure, the donor animals were given with epidural anesthesia (3-5 ml of 2% lignocaine hydrochloride) at the sacrococcygeal space. The rectum was emptied by back raking and then the perineum was washed with mild antiseptic solution. An 18-gauge 60 mm needle, suction pump maintained at 70-80 mm Hg vacuum pressure and a convex ultrasound 7.5 MHz trans-vaginal ultrasound guided OPU probe were used for follicular aspiration. After the follicle aspiration was done, follicular aspirate was collected into the Euro Flush media (IMV Technologies, France) 50 ml conical tube maintained at 37º C temperature. Oocytes were searched under a Stereo zoom binocular microscope (Nikon, SMZ745). The recovered oocytes were divided into five grades (A, B, C, D, E) according to the nature of ooplasm and the number of layers of cumulus cells. The COCs were further graded and then transferred to the maturation medium BO-IVM in 4 Well plates and incubated at 38.5º C at 6% CO_2, 5% O_2 and 89% N_2 in the bench top incubator for 20 to 22 hours. After IVM, COCs

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Table 1. Grading of recovered oocytes

Sr. No.	Donor cow Tag No.	Total	A	B	C	D	E
1.	410806	7	2	3	1	1	0
2.	416008	25	8	7	6	4	0
3.	410806	6	3	3	0	0	0
4.	410806	3	0	0	0	2	1
5.	416008	24	3	6	13	2	0
6.	600363	15	2	6	2	3	2
7.	410806	5	0	0	2	1	2
8.	410806	3	0	0	3	0	0
9.	640024	12	0	4	6	1	1
10.	640024	9	0	6	2	1	0
11.	410806	9	1	3	3	2	0
12.	640024	5	0	2	3	0	0
13.	413472	5	0	2	2	1	0
14.	268900	15	1	4	5	4	1
15.	410806	6	1	0	3	1	1
16.	640024	10	0	2	3	3	2
17.	268900	10	0	0	5	4	1
18.	413472	4	0	1	1	1	1
19.	410806	5	2	0	2	0	1
20.	640024	2	0	0	0	2	0
21.	268900	18	4	6	6	2	0
	Mean ± SE	198	1.29 ± 0.43^{c}	2.62 ± 0.53^{ab}	3.24 ± 0.64^{a}	1.67 ± 0.28^{bc}	0.62 ± 0.16^{c}

were washed and transferred in the fertilization medium BO-IVF in 4 well plates. The thawed semen samples were layered on the percoll gradient. After centrifugation, the resultant sperm pellet was used for *in vitro* fertilization (IVF). The fertilization was accomplished by adding semen pellets in 4 well plates containing matured COCs. The gametes were incubated for 20-22 hat 38.5° C at 6% CO_2, 5% O_2 and 89% N_2 in the bench top incubator. Zygotes produced after the fertilization with unsexed sperm were placed in culture droplets BO-IVC in 4 Well Plates under mineral oil overlay and incubated at 38.5º C at 6% CO_2, 5% O_2 and 89% N_2 in the benchtop incubator. The *in vitro* produced embryos were evaluated and assessed for quality on Day 7. The dishes containing embryos were screened under a stereo zoom microscope at lower magnification (40x) 3-4 times in the laboratory. The embryos were then washed, graded with the help of the micropipette, and transferred into the small petri dish (35 × 10 mm, Falcon Becton Dickinson, Labware, New Jersey, USA) containing 1.5-2.0 ml of embryo holding medium for further use. The embryos were examined, evaluated and graded according to the International Embryo Transfer Society criteria given by (BO and Mapletoft, 2018). To remove operator variation, all procedures, *viz*., OPU and laboratory procedures for IVF and *in vitro* embryo production (IVEP), were performed by the same worker.

RESULTS AND DISCUSSION

198 Oocytes were collected from six different donor cows in 21 OPU sessions, averaging 9.42 Oocytes per OPU session. Oocyte recovery, according to the grading system, was 1.29 ± 0.43 for grade A, 2.62 ± 0.53 for grade B, 3.24 ± 0.64 for grade C, 1.67 ± 0.28 for grade D and 0.62 ± 0.16 for grade E. The A, B, C, D and E grade oocyte recovery percentages were 13.64, 27.78, 34.34, 17.68 and 6.57%, respectively. The present study's findings indicated that the recovery percentage for C-grade oocytes was significantly higher than for all other grades. In contrast, A and E oocytes were lower than other grades. Grades B and D were found at the intermediate level, where grade B was numerically higher than grade D, as depicted in Table 1. Some what similar results were reported by Looney *et al.* (1994), Goodhand *et al.* (2000), Sakhong *et al.* (2012) and Cebrian-Serrano *et al.* (2013), where C grade oocytes were more in numbers with variable numbers of other grade oocytes. In this study, a total of 75.67 ± 6.31% of oocytes (147 out of 185) matured, on average, 8.81 ± 1.46 oocytes were kept for IVM, out of this, a mean number of oocytes that matured and were subsequently transferred for IVF was 7 ± 1.32 (Table 2). An almost similar oocyte maturation rate, i.e., 75.53%, was reported by Pontes *et al.* (2011). Lonergan *et al.* (1994) reported a slightly lower oocyte maturation rate of 70.20%. Kumar *et al.* (2020) and

Table 2. Oocyte maturation rate, cleavage rate, blastocyst production rate

Sr. No.	Donorcow Tag No.	Oocytes used for IVM	Oocytes matured and transferred for IVF	Oocytes maturation rate (%)	Oocytes cleaved	Cleavage rate (%)	Blastocyst	Blastocyst production rate (%)
1	410806	7	6	85.71	5	83.34	0	0
2	416008	25	22	88	16	72.72	10	45.45
3	410806	6	7	116.67	6	85.71	4	57.14
4	410806	2	0	0	0	0	0	0
5	416008	24	22	91.67	7	31.82	4	18.18
6	600363	13	12	92.31	6	50	3	25
7	410806	3	2	66.67	0	0	0	0
8	410806	3	3	100	1	33.34	1	33.34
9	640024	11	10	90.91	6	60	3	30
10	640024	9	8	88.89	5	62.5	2	25
11	410806	9	7	77.78	6	85.71	5	71.43
12	640024	5	5	100	3	60	2	40
13	413472	5	4	80	1	25	0	0
14	268900	14	13	92.86	5	38.46	5	38.46
15	410806	5	2	40	1	50	1	50
16	640024	8	8	100	3	37.5	3	37.5
17	268900	9	4	44.44	4	100	4	100
18	413472	3	3	100	2	66.67	1	33.34
19	410806	4	2	50	2	100	2	100
20	640024	2	1	50	1	100	0	0
21	268900	18	6	33.33	6	100	5	83.34
	Total	185	147		86		55	
	Mean ± SE	8.81 ±1.46	7 ± 1.32	75.67± 6.31	4.09 ±0.78	59.18 ±6.83	2.62±0.54	37.53 ±6.81
	S. D.	6.71	6.075	8.92	0.78	6.83	0.54	6.81

Lonergan *et al.* (1996) reported the oocyte maturation rate higher than the present study, which is 91.30 ± 1.27 and 91%, respectively. Out of 147 oocytes kept for *in vitro* fertilization, 86 oocytes were cleaved. The average cleaved oocyte was 4.096 ± 0.78, yielding a cleavage rate of 59.18 ± 6.83%. Looney *et al.* (1994) reported 44.7 % cleaved oocytes, while Presicce *et al.* (2020) reported 49.4% cleaved oocytes. These results are lower than those of the present research work. Nogueira *et al.* (2021) discovered a 61.19% cleavage rate, which is almost similar to the present study. Higher Cleavage rates, i.e., 78% and 68%, than the present study was reported by Pontes *et al.* (2010) in Holstein cattle and Gir cattle, respectively. Egashira *et al.* (2019) and Da Silva *et al.* (2017) reported 83% and 63% cleavage rate, respectively which was higher than the present study. Out of 147 oocytes kept for *in vitro* fertilization, 55 fertilized oocytes reached the blastocyst stage on day 7th of the culture. The blastocyst production rate was 37.53 ± 6.81%. There was variability in the development of embryos among different donors. Several publications reported lower blastocyst production rates than the current study: Looney *et al.* (1994) found 16.4%, Nogueira *et al.* (2021) found 21.13%, DaSilva *et al.* (2017) found 23.5%, and Presicce *et al.* (2020) found 23% blastocyst production rate. A slightly higher blastocyst production rate, i.e. 46% and 45%, than the present study, was recorded by Pontes *et al.* (2010) in Holstein and Gir cattle, respectively. DeMoraes *et al.* (2019) recorded 41.5 ± 4.2% while Egashira *et al.* (2019) recorded a 60.2% blastocyst production rate. In this study, 55 embryos are classified into four distinct codes *viz*., 1, 2, 3, and 4. During each OPU session, on average, 2.61 embryos were yielded. The number of embryos with code 1 (1.71±0.37) was more than codes 2 (0.571 ± 0.20), 3 (0.142 ± 0.10) and 4 (0.19 ± 0.11). Different authors recorded variable results of various grades of embryos in their study, which may be due to the physiological and nutritional status of animals, breed variation, season, etc. Fourteen in vitro produced embryos were transferred to recipient cows, and a conception rate of 24.39% (10/41) was recorded. Pregnancy diagnosis was

made by ultrasound examination of genitalia on days 25-30, which was further monitored by per rectal examination on day 45 post embryo transfer. Thus, successful transvaginal ultrasound-guided OPU and in vitro embryo production and transfer was done in cows in the present study. (Sahiwal x HF)

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