RESEARCH ARTICLE

Impact of Aspiration Pressure on Oocyte Quality and Retrieval Outcomes in FSH Pre-Stimulated Sahiwal Cows

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Abstract

Ovum pick-up (OPU) followed by *in vitro* embryo production (IVEP) is an efficient tool to accelerate genetic progress in cattle, particularly indigenous breeds that possess superior adaptability and disease resistance. The present study evaluated the effect of two vacuum pressures (40 vs. 50 mmHg) on oocyte recovery, quality and blastocyst rate in 16 Sahiwal donor cows that were hormonally pre-stimulated with follicle-stimulating hormone (FSH). Animals were randomly assigned to two groups (n=8 each) and subjected to OPU at either 40 mmHg or 50 mmHg. The mean number of cumulus-oocyte complexes (COCs) recovered per session was higher in the 50 mmHg group (26.13 ± 2.92) compared with the 40 mmHg group (20.63 ± 3.94). A significantly greater proportion of viable oocytes (Grades A+B+C) was recovered at 50 mmHg (74.16%) compared to 40 mmHg (71.51%), while the proportion of degenerated oocytes (Grades D+E) was lower (24.88% vs. 45.45%). Following *in vitro* maturation (IVM), an average 76.4±1.5% of recovered oocytes exhibited cumulus expansion. Of these, ~62% of total oocytes (78.7±1.6% of matured) were successfully fertilized under *in vitro* fertilization (IVF) conditions. By Day 7 of culture, ~34% of total oocytes and 43.3 ± 1.8% of matured oocytes developed into blastocysts, with a significantly higher mean blastocyst yield per OPU in the 50 mmHg group (8.5±0.9 vs 6.8±0.8). These findings demonstrate that aspiration at 50 mmHg not only enhances the yield and quality of oocytes but also improves there *in vitro* developmental competence up to the blastocyst stage. The results have practical implications for improving IVEP efficiency in elite Sahiwal cows and conserving valuable indigenous germplasm.

Key words: FSH stimulation, Oocyte quality, Ovum pick-up, Sahiwal cows, Vacuum pressure.

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Introduction

Indigenous cattle breeds such as the Sahiwal (*Bos indicus*) are vital genetic resources owing to their heat tolerance, disease resistance, and capacity to produce milk under low-input conditions (Joshi *et al.*, 2001). To accelerate genetic improvement and conserve these breeds, assisted reproductive technologies (ARTs) such as ovum pick-up (OPU) and *in vitro* embryo production (IVEP) have become essential (Pieterse *et al.*, 1988). OPU enables the repeated retrieval of oocytes from live donor cows, thereby allowing continuous embryo production even from pre-pubertal or pregnant donors (Looney *et al.*, 1994; Hasler *et al.*, 1995). The efficiency of OPU depends largely on both biological and technical factors, including donor breed and age, follicular dynamics, hormonal pre-stimulation, operator skill, aspiration needle design, and the vacuum pressure used during follicular aspiration (Arun, 2003; Ongaratto *et al.*, 2020). In a recent comprehensive review, Salek *et al.* (2025) have summarized these technical variables and their combined influence on OPU/IVEP outcomes.

Among these, vacuum pressure is particularly critical, since excessively low pressure can reduce recovery rates, while excessively high pressure can damage cumulus-oocyte complexes (Antosik *et al.*, 2007; Lakhera *et al.*, 2018). Previous studies have reported recovery rates ranging between 50 and 80% when aspiration was conducted at optimal vacuum levels (Merton *et al.*, 2003; Manik *et al.*, 2003). Vennapureddy *et al.* (2024) in Sahiwal cows showed that FSH pre-stimulation increases medium/large follicle counts and improves recovery of high-quality oocytes. Despite these advances, limited information is available on the effect of vacuum

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pressure on oocyte quality, recovery and competence for their further development upon IVM-IVF in indigenous cattle breeds of international repute such as Sahiwal. Therefore, the present study was undertaken to compare the effect of two vacuum pressures (40 vs. 50 mmHg) on the yield and quality of oocytes retrieved from FSH-pre-stimulated Sahiwal donor cows.

Materials and Methods

Experimental Animals and Hormonal Pre-Stimulation

The study was conducted at the IVF Laboratory, College of Veterinary Science, Sri Venkateswara Veterinary University (SVVU), Tirupati (India), in collaboration with the Sri Venkateswara Gosamrakshanashala, Tirupati. Sixteen healthy, pluriparous Sahiwal cows (6-8 years of age) were selected. All animals were maintained under uniform feeding and management conditions, and were clinically normal at the time of study. All these donor cows, at a random stage of the estrous cycle, received 10 µg of GnRH (Receptal®, i.m.), followed 48, 60, and 72 h later by FSH (Follitropin-V®, 200 mg, i.m.) in a step-down (125, 75, 25 mg) regimen to induce multiple follicular development prior to oocyte retrieval (Vennapureddy *et al.,* 2024).

Experimental Design and OPU

The cows were randomly divided into two equal groups (n=8 each) for OPU 24 h after the last FSH injection. Each donor cow underwent one OPU session following hormonal treatment. Donor cows were restrained in a chute, and the perineal region was cleaned and disinfected. Transvaginal follicular aspiration was carried out using an ultrasound machine fitted with a micro-convex transducer and a disposable aspiration needle connected via a tubing system to a regulated vacuum pump (Hashimoto *et al.*, 1999). In Group I follicles were aspirated at 40 mmHg with an aspiration flow rate of ~12 mL/min, while in Group II **f**ollicles were aspirated at 50 mmHg with an aspiration flow rate of ~20 mL/min. Follicular contents were collected into pre-warmed 50 mL centrifuge tubes maintained in a tube heater at 37°C to preserve oocyte viability.

Oocyte Recovery and Evaluation

Cumulus-oocyte complexes (COCs) were identified under a stereomicroscope and classified into five grades (A-E) according to morphological criteria based on cumulus cell investment and cytoplasmic appearance (Chaubal *et al.*, 2006). Grades A-C were considered viable oocytes, while Grades D and E were considered non-viable (Looney *et al.*, 1994; Bungartz *et al.*, 1995) (Fig. 1).

In Vitro Maturation (IVM) and Fertilization (IVF)

The recovered viable COCs (Grades A–C) were transferred into maturation medium (TCM-199 supplemented with 10% FBS, 0.5 µg/mL FSH, 10 µg/mL LH, and 1 µg/mL estradiol). The cultures were maintained for 24 h in a CO_2 incubator at 38.5°C, 5% CO_2, and high humidity and maturation was confirmed by cumulus expansion as per the procedure described by Gasparrini (2002) and Chaubal *et al.* (2006) (Fig. 2).

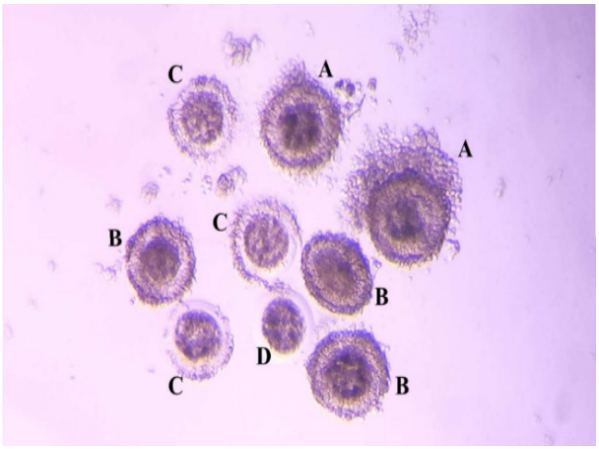

Fig. 1: Grade A, B, C, D oocytes.

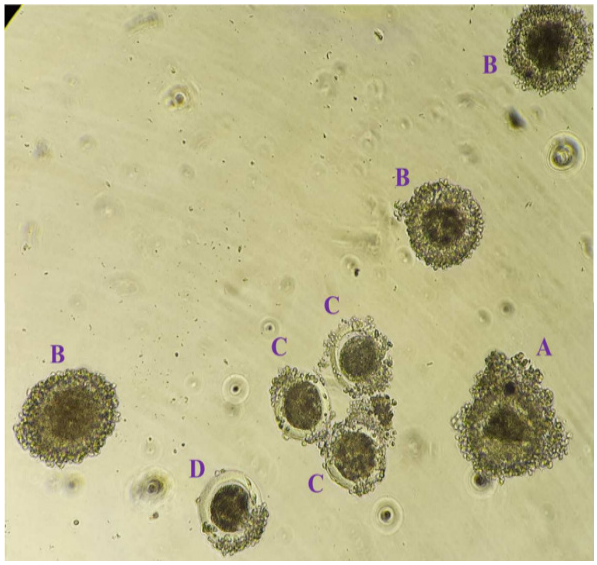

Fig. 2: Grade A, B, C oocytes; D oocytes after maturation with expanded cumulus cells.

Matured oocytes were fertilized *in vitro* using frozen-thawed sex-sorted Sahiwal semen, processed by Percoll gradient centrifugation. Oocytes showing presence of male pronuclei, extrusion of second polar body, or cleavage were considered fertilized. Fertilization assessment criteria were consistent with earlier bovine IVF studies (Looney *et al.,* 1994; Hasler *et al.,* 1995).

In Vitro Culture (IVC) and Blastocyst Development

Presumptive zygotes were denuded and cultured in SOF medium for 7 days with periodic medium renewal. By Day 7, embryos showing distinct inner cell mass, blastocoel cavity, and outer trophoblast layer were classified as blastocysts as per the established criteria of bovine IVEP protocols (Merton *et al.,* 2003; Pawshe *et al.,* 2003).

Statistical Analysis

Data were analyzed using Student's *t*-test and one-way ANOVA (SPSS 15.0). Values are expressed as mean ± standard error of the mean (SEM). A probability value of $p<0.05$ was considered statistically significant.

Results and Discussion

Follicular Aspiration and Recovery Rate

A total of 367 follicles were available for aspiration in Group I (40 mmHg) and 355 follicles in Group II (50 mmHg). The mean follicular recovery rate was higher at 50 mmHg (77.46%) compared to 40 mmHg (70.29%). The mean number of COCs recovered per donor per session was also significantly greater ($p<0.05$) in Group II (26.13 ± 2.92) than in Group I (20.63 ± 3.94). Similar improvements in recovery efficiency at higher aspiration pressures have been reported in cattle (Arun, 2003; Lakhera *et al.*, 2018) and buffaloes (Manik *et al.*, 2003). Ongaratto *et al.* (2020) also reported that maintaining vacuum pressures between 60-70 mmHg enhanced the retrieval of high-quality COCs without compromising their developmental competence. Our findings confirm that moderately higher vacuum pressure facilitates efficient follicular emptying, thereby increasing the number of oocytes collected per session. Recent studies in Sahiwal cattle have confirmed similar responses to OPU frequency and FSH pre-stimulation (Saleem *et al.*, 2022; Vennapureddy *et al.*, 2022).

Table 1: Performance of transvaginal ovum pick-up in Sahiwal cows at two vacuum pressures

Attribute	Group I (40 mmHg)	Group II (50 mmHg)	Overall
Follicles available for aspiration	367	355	722
Follicles aspirated	258	275	533
Oocyte recovery rate (%)	70.29	77.46	73.81
Mean COCs recovered per cow/session	20.63±3.94 (5-43)	26.13±2.92* (16-38)	23.38±2.47 (5-43)
No. of viable COCs (Grade A+B+C)	118	155	273
Viable COCs (Grades A+B+C) (%)	71.51	74.16*	72.99
Mean COCs (Grade A+B+C) recovered/ cow/session	4.92±0.68 (0-12)	6.46±1.1 (1-18)	5.69±0.65 (0-18)
No. of non-viable COCs (Grade D+E)	75	52	127
COCs (%)	45.45*	24.88	33.95
Mean COCs (Grade D +E) per cow per session	4.69±1.50 (0-22)	3.11±0.90 (0-9)	3.97±0.88 (0-22)

*Values differ significantly ($p < 0.05$).

Effect on Oocyte Quality

A significantly ($p<0.05$) higher percentage of viable oocytes (A+B+C) was observed at 50 mmHg (74.16%) than 40 mmHg (71.51%), while degenerated oocytes (D+E) were lower (24.88% vs 45.45%) (Table 1). The proportion of high-quality oocytes (Grade A) recovered per session was significantly greater in Group II compared to Group I (12.87 ± 1.59 vs. 7.13 ± 1.35, $p < 0.05$). Other grades showed minor, non-significant differences between groups (Table 2). This observation was consistent with earlier findings (Hasler *et al.*, 1995; Chaubal *et al.*, 2006). The results suggest that the applied vacuum pressure influences not only the number of COCs recovered but also their structural integrity, which is critical for subsequent *in vitro* maturation and embryo development (Krisher, 2004). Additionally, efficiency of retrieval is also influenced by operator experience (Scott *et al.*, 1994). The current findings highlight the importance of optimizing aspiration pressure to maximize the efficiency of *in vitro* embryo production (IVEP) programs in indigenous cattle. However, increasing aspiration pressure can increase oocyte denudation and the proportion of denuded COCs, which should be considered when selecting vacuum settings (Pawshe *et al.*, 2003).

Table 2: Grading of oocytes aspirated from the ovaries of Sahiwal donor cows subjected to transvaginal OPU under two vacuum pressure

Attribute	Group 1 (40 mmHg)	Group 2 (50 mmHg)
Grade A	7.13 ± 1.35^{aA}	12.87 ± 1.59^{aB}
Grade B	3.87 ± 1.06^{abA}	3.50 ± 0.53^{bA}
Grade C	3.75 ± 0.72^{abA}	3.00 ± 0.62^{bcA}
Grade D	8.87 ± 2.20^{abcA}	5.87 ± 1.14^{bcdA}
GradeE	0.50 ± 0.32^{abA}	0.62 ± 0.37^{bcA}

Values bearing superscripts a, b, c. within a column and those with superscripts A, B within a row differ significantly ($p<0.05$).

Earlier studies showed that while excessively low vacuum pressures may lead to incomplete aspiration of follicular fluid and reduced recovery (Bols *et al.*, 1996), very high pressures (>90 mmHg) have been associated with mechanical damage to oocytes and reduced developmental competence (Ward *et al.*, 2000; Pawshe *et al.*, 2003; Antosik *et al.*, 2007). Thus, application of OPU–IVEP using optimized parameters such as 50 mmHg vacuum pressure could serve as a vital strategy for conserving and multiplying superior germplasm of indigenous cattle breeds. Alternative strategies such as follicular flushing or double-lumen needles have shown benefits and limits and should be compared with pressure-adjustment approaches in future studies.

In Vitro Maturation, Fertilization and Blastocyst Development

Out of the total COCs recovered, a mean of 76.4 ± 1.5 underwent successful maturation as confirmed by cumulus expansion. Fertilization was achieved in ~62% of the total

Table 3: Comparative IVM, IVF, and blastocyst development of oocytes aspirated under two pressures in Sahiwal cows (Mean ± SE)

Attribute	Group I (40 mmHg)	Group II (50 mmHg)	Overall mean
Matured oocytes (%)	74.5 ± 2.1	78.3 ± 1.8	76.4 ± 1.5
Fertilized oocytes (% of matured)	77.1 ± 2.4	80.2 ± 2.1	78.7 ± 1.6
Blastocysts per OPU	6.8 ± 0.8	8.5 ± 0.9*	7.7 ± 0.6
Blastocyst rate (% of matured)	41.6 ± 2.2	45.1 ± 2.5	43.3 ± 1.8

*Significant at ($p< 0.05$).

oocytes, corresponding to 78.7 ± 1.6 of matured oocytes. Cleavage was evident within 72 h of insemination, with progression to blastocyst stage by Day 7. The blastocyst rate (43.3 ± 1.8%, 7.7 ± 0.6) was ~34% relative to total oocytes and ~44% relative to matured oocytes. Importantly, the 50 mmHg group yielded a greater number of blastocysts per OPU session compared to the 40 mmHg group (8.5 ± 0.9 vs. 6.8 ± 0.8), in agreement with the higher proportion of Grade-A oocytes (Table 3).

The blastocyst yield observed in the present study was comparable with indigenous cattle studies (Manik *et al.,* 2003; Saleem *et al.,* 2022), though slightly lower than some *Bos taurus* reports (Hasler *et al.,* 1995; Merton *et al.,* 2003). Similar trends in blastocyst development efficiency have been reported in tropical breeds, including buffaloes, where developmental competence is influenced by both breed and *in vitro* culture environment (Gasparrini, 2002). Although the present work was limited to *in vitro* blastocyst development, previous studies have demonstrated that such embryos can establish pregnancies and result in live calvings, with acceptable success rates in commercial IVEP programs (Looney *et al.,* 1994; Hasler *et al.,* 1995). This supports the practical applicability of OPU–IVF–ET pipelines in indigenous breeds.

Although the study demonstrates clear benefits of using 50 mmHg vacuum pressure, further research is needed to evaluate long-term reproductive safety of repeated OPU sessions in Sahiwal cows, as well as the developmental competence of embryos derived from these oocytes. Future studies should also consider donor age, follicular dynamics, and hormonal co-treatments, which may interact with aspiration pressure to influence OPU outcomes (Merton *et al*., 2003; Saleem *et al*., 2022). Seasonal heat stress and alterations in follicular fluid metabolites can influence oocyte competence and may confound OPU results.

Conclusion

The findings of the present study demonstrated that OPU at a vacuum pressure of 50 mmHg in FSH-pre-stimulated Sahiwal cows significantly increased both oocyte yield and the proportion of viable oocytes compared to 40 mmHg. The recovery of a higher number of Grade A oocytes and improved maturation, fertilization, and blastocyst production rates at 50 mmHg indicates that this pressure is optimal for maximizing the efficiency of *in vitro* embryo production in indigenous cattle. These findings confirm that optimizing aspiration pressure is critical for enhancing the efficiency of OPU–IVF–ET programs in indigenous cattle. Adoption of 50 mmHg aspiration settings can therefore improve both the quantity and developmental competence of oocytes, contributing to more effective genetic improvement and conservation of elite Sahiwal germplasm.

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