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Oocyte yield and oocyte quality following FSH stimulation in Sahiwal cattle

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Abstract
Ultrasound guided transvaginal ovum pick up and *In-Vitro* Embryo Production has opened up greater possibilities to exploit productive and reproductive potential of the superior cows. In the present study, ten Sahiwal donor cows were pre-stimulated with multiple injections of FSH prior to OPU. Total 27 OPU sessions were conducted of which 602 oocytes were recovered with an average yield of 22.37 ± 2.01 oocytes per each session of OPU. Aspirated oocytes were graded as grade A, grade B, grade C, grade D and grade E oocytes (10.78 ± 1.20, 2.93 ± 0.41, 3.00 ± 0.33, 5.26 ± 0.88 and 0.33 ± 0.15 respectively). Of the 602 COCs recovered 451 were viable (74.92%) and 151 were non-viable (25.08%). Of all the grades of oocytes the difference in the quality of Grade A and with the other Grade oocytes was significant (p<0.05).

Keywords: Ovum pick up, *in-vitro* embryo production, FSH stimulation

1. Introduction
Among 53 recognized cattle breeds (as per ICAR NBAGR) the Sahiwal cattle breed (Bos *indicus*) is native to the arid region of northern India (Joshi *et al.*, 2001) [13] and its population has been decreasing over time. In the past two decades, significant genetic advancements through Assisted Reproductive Technologies (ART) like Artificial Insemination (AI) and Embryo Transfer (ET) have improved the utilization of male gametes for overall genetic development. Meanwhile, scope for increasing the pace of enhancement of the breed through better utilization of female gametes with high genetic merit has come in to force.
Better utilization of female gametes can be achieved by Ovum Pick-Up (OPU) an ultrasound-guided transvaginal oocyte retrieval method, *In-vitro* Maturation (IVM), *In-vitro* Fertilization (IVF) and subsequent *In-vitro* Culture (IVC) of oocytes for production calves Brackett *et al.* (1982) [2] and Neglia *et al.* (2003) [19]. Oocyte quality significantly impacts early embryonic survival, developmental competency, establishment and maintenance of pregnancy, fetal growth, and adult health (Krisher, 2004) [14].

2. Materials and Methods
2.1 Experimental location: The current study was undertaken at Sri Venkateshwara Gosamrakshana shaala, Tirumala Tirupati Devasthanam, Tirupati and at IVF laboratory, College of Veterinary Science, Tirupati under SVVU-TTD project entitled "Augmentation of productivity through Assisted Reproductive Technologies in Sahiwal cows" during the period between June and October, 2023.

2.2 Experimental design
Donor cows were administered with 10µg GnRH (Receptal 2.5ml I/m) at random stage of oestrous cycle followed by FSH (Folltropin-v (Vetoquinol), 200 mg I/m in 3 divided doses @ 100mg, 60mg and 40mg) at 48, 60 and 72 h after GnRH administration. Porcine follicle stimulating hormone (pFSH) 400 mg NIH is contained in each freeze-dried Folltropin-V vial that gets reconstituted with 20 ml bacteriostatic sodium chloride injection USP so that final solution contains 20mg of FSH/ml.

OPU was carried out 24 h after the last FSH injection (coasting period). All the visible follicles were aspirated during each OPU and the oocyte yield and quality was analysed. Further the retrieved oocytes underwent the process of IVM, IVF and IVC.

2.3 Ovum pick-up technique

The ovaries were manipulated per rectum and either the right or left ovary was positioned between the fingers. The transvaginal probe with an OPU handle (WTA, So Paulo, Cravinhos, Brazil) was inserted into the anterior vagina (fornix vagina) after thorough cleaning and lubrication with paraffin. The transducer surface was placed either to the left or right of the external-os of the cervix (Fig.1)

To get an accurate view of the follicles on the ultra-sonographic monitor, the ovary was gently moved and placed up against the probe head. The number of follicles in each ovary were counted and the diameter of each follicle was measured using an internal calliper after freezing the monitor image. Later, the average of measurements obtained in two directions, i.e., vertical and horizontal, was used to compute the follicular diameters Nagai *et al.* (2015) [18].

The aspiration line-equipped needle was entered through the OPU handle, progressed to the fornix vagina, and then placed into the follicular antrum after the ovary and targeted follicle were stabilised. Each follicle's follicular fluid was aspirated by using vacuum pump having continuous negative pressure of 40 mm Hg. The follicle was curetted during aspiration by gently rotating the needle, which also helped to free any trapped or attached oocytes. The needle was removed from the ovary but left in place outside of the fornix vagina with the tip still visible on the monitor before aspirating the next follicle. Before, during and after OPU, the needle and aspiration line were thoroughly rinsed with pre heated (37ºC) OPU recovery medium (IVF Bioscience, UK) to prevent blood from clotting or oocytes from sticking to the tubing.

The number of follicles aspirated expressed as a percentage of all the follicles counted for each cow was used to calculate the aspiration rate. The number of recovered oocytes represented as a percentage of the number of aspirated follicles for each cow served as the basis for calculating the oocyte recovery rate Goodhand *et al.* (2000) [7].

All visible follicles larger than 4 mm in diameter were aspirated during each aspiration, and the aspirate was then collected in a 50 ml tube. The individual fluid-filled (non-echogenic and black) follicle picture on the screen display vanished after a successful aspiration, and the procedure was repeated until all the necessary follicles were aspirated. The process was repeated in the opposing ovary after aspirating every follicle in the first ovary. For second ovary a different collection conical centrifuge tube was utilised. Each donor received a different needle.

2.4 Screening and assessment of oocyte recovery

The 50 ml conical centrifuge tube containing the follicular aspirate was brought to the lab after both ovaries had been aspirated. In order to remove the blood tint and hazy follicular fluid, OPU media used to wash the contents and during filtration of oocytes using oocyte filter (Emcon Immuno systems Inc., Biddeford, USA) and then content of OPU tube was emptied in the filter. OPU tube was washed 3-4 times with OPU media and the contents were filtered. Filter was washed several times and excess fluid was drained out to get clear 10-15 ml colloidal OPU media with oocytes left in filter. Residual contents in the Filter were flushed twice with OPU media using 10 ml syringe in order to detach the sticky oocytes from the filter (using 10 ml syringe and 20G needle). In order to identify the cumulus oocyte complexes, the colloidal OPU fluid from the filter was then transferred to a square grid petri dish 90 x 15mm and inspected under stereo zoom microscope (SMZ800, Nikon, Japan) at 2-3X magnification. The COCs separated form the colloidal OPU media were transferred to Wash media (YVF Biotech Ltd, Brazil) and graded (Fig. 2).

2.5 Evaluation of cumulus oocyte complexes

The integrity of the oocyte, the homogeneity of the cytoplasm, and the thickness of the cumulus cell layer around the oocyte were the main criteria used for the morphological evaluation and classification of cumulus oocyte complexes. Basing on the evaluation, cumulus oocyte complexes were divided into four quality grades (A, B, C, and D) (Fig. 3) and in turn in to viable (Grade A + B + C) and non-viable (Grade D and Grade E) categories Looney *et al.* (1994) [15] and Bungartz *et al.* (1995) [3].

Table 1: Classification of oocytes retrieved by OPU

S. No	Oocyte classification	Oocyte quality description
1	A	Compact cumulus (>4 layers)
2	B	3 to 4 cumulus layers only
3	C	1 to 2 layers of cumulus
4	D	Denuded oocyte
5	E	Expanded cumulus (fluffy and fuzzy)

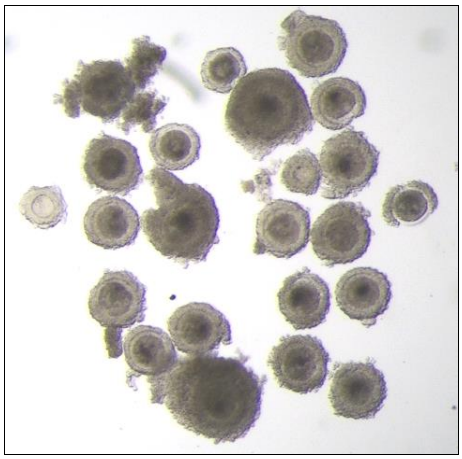

Fig 1: Tranvaginal Ovum pick up

Fig 2: Oocytes collected by OPU

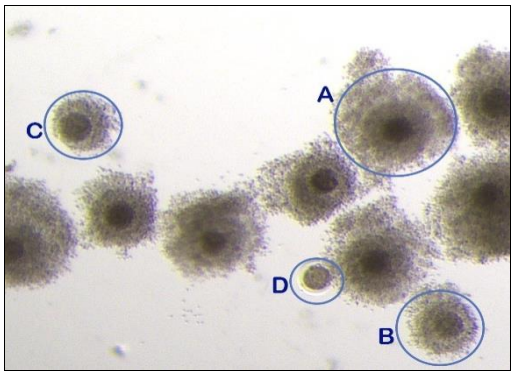

Fig 3: Grading of oocytes

3. Results

3.1 Oocyte YIELD

602 oocytes were recovered from 27 OPU sessions in Sahiwal donor cows with an average yield of 22.37 ± 2.01 oocytes per each session of OPU (ranging from 5 to 47 oocytes) per cow.

3.2 Quality of recovered oocytes and grading

All the oocytes obtained by OPU were evaluated under stereozoom microscope as Grade A, Grade B, Grade C, Grade D and Grade E. The mean number of oocytes obtained in Grade A, Grade B, Grade C, Grade D and Grade E were 10.78 ± 1.20, 2.93 ± 0.41, 3.00 ± 0.33, 5.26 ± 0.88 and 0.33 ± 0.15, respectively (Table 2). Out of all the OPU sessions a total of 451 viable COCs (grade A+B+C) were obtained from out of 602 overall oocytes. The overall mean number of viable COCs were 15.03 ± 1.49 (ranging from 2 to 40) and the overall mean number of non-viable oocytes were 5.59 ± 0.94 (ranging from 1 to 22). The difference in the quality of oocytes aspirated between grades was significant (p<0.01) but the difference in the quality between Grade B, Grade C and Grade D was not significant (p>0.05). However, the difference the quality of Grade A and with the other Grade oocytes was significant (p<0.05).

Table 2: Different Grades of oocytes obtained from 27 OPUs

Grade	Oocyte count	MEAN ± SE
Grade A	291	10.78 ± 1.20^{a}
Grade B	79	2.93 ± 0.41^{bc}
Grade C	81	3.00 ± 0.33^{bc}
Grade D	142	5.26 ± 0.88^{b}
Grade E	9	0.33 ± 0.44^{c}
TOTAL	602	22.30 ±1.99

Table 3: Total Viable and Non-viable COCs

Particulars	Viable COCs (Grade A+B+C)	Non-Viable COCs (Grade D+E)	Total
No. of COCs	451	151	602
Percentage	74.92%	25.08%	
Overall mean COCs recovered / OPU	15.3 ± 1.49	5.59 ± 0.94	22.30 ±1.99

4. Discussion

4.1 Oocyte yield

In-line with findings of this study, Patel Mayankkumar, (2020) [22] had also recorded significantly (p<0.05) higher number of oocytes per OPU comparted to other breeds in Sahiwal (22.5±3.2). Similarly, De Carvalho *et al.* (2019) [5] also recorded a total mean oocytes recovery of 12.3±1.0, 9.0±1.2 and 9.3±1.2 in Nulliparous, Primiparous and Multiparous donors cows, respectively.

In contrast to this, a higher average number of total oocytes were recovered per OPU session in Nellore (Bos *indicus*) donors (30.84±0.88). While, donors super-stimulated with CIDR and FSH prior to OPU in cows yielded 15.83±1.21 mean number of COCs Jakkali *et al.* (2023) [11].

Studies on zebu cows suggest that individual variation in the number of oocytes obtained from OPU was correlated with the expression of age Sartori *et al.* (2004) [23], parity Lucy *et al.* (1991) [16], nutritional status Oliveira *et al.* (2002) [20] and heat stress Wolfenson *et al.* (1995) [25].

4.2 Oocyte Quality

Among 22.37 ± 2.01 oocytes per each session of OPU, the mean number of oocytes obtained in Grade A, Grade B, Grade C, Grade D and Grade E were 10.78 ± 1.20, 2.93 ± 0.41, 3.00 ± 0.33, 5.26 ± 0.88 and 0.33 ± 0.15, respectively. The overall mean number of viable COCs (Grade A+ Grade B+ Grade C) were 15.03 ± 1.49 and the overall mean number of non-viable oocytes (Grade D+ Grade E) were 5.59 ± 0.94. But Seisenov *et al.* (2023) [24] reported that viable oocytes per OPU session were 8.7 ± 0.85 and 6.2 ± 0.83 in Aberdeen Angus breed and Kazakh White headed breed, respectively. While De Carvalho *et al. (*2019) [5] reported that the FSH treated donors had a greater viable oocytes rate with 7.8±0.9, 5.7±1.1 and 5.6±0.9 in Nulliparous, Primiparous and Multiparous donors, respectively when compared to non-stimulated donors. On the contrary, Da Silva *et al.* (2017) [4] reported that there was no significant improvement with FSH pre-treatment on viable oocyte yield (64.6% and 68.7% in control and FSH treated groups, respectively).

In the present study, the percentage of Grade A oocytes were higher i.e., 48.34 percent with significant difference (p<0.05) in Grade A oocytes when compared to other Grades of oocytes and the percentage of viable oocytes recovered were 74.92 percentage with mean viable COCs of 15.3 ± 1.49. Similar to the present study, higher mean viable COCs per animal (18 ± 1.5) and viable COCs % (85%) in heifers stimulated with Folltropin-v Hayden *et al.* (2022) [10]. On the contrary to the present findings, donors stimulated with Folltropin-v showed the mean viable COCs of 5.5 ± 0.5 per animal Ongaratto *et al.* (2020) [21].

The variation in the oocyte quality (normal / good / viable and abnormal / poor / non-viable) might be due to the effects of transducer type, puncture frequency, OPU regimen, treatment with FSH/PMSG, combination of needle gauge and vacuum pressure etc., Fry *et al.* (1997) [6], Hashimoto *et al.* (1999) [8], Merton *et al.* (2003) [17], Bols *et al.* (2004) [1], Jeyakumar, (2004) [12] and Da Silva *et al.* (2017) [4].

Based on cumulus cell investment around the oocyte and transparency of the oocyte cytoplasm, several researchers have classified the COC in to 3 - 5 quality grades and then grouped them as viable and non-viable. While Hasler *et al.* (1995) [9] reported 82% good quality (grade I + II) oocytes and Looney *et al.* (1994) [15] observed only 47% good (grade A + B) oocytes.

5. Conclusion

From the results, it can be concluded that gonadotrophin (FSH) treatment administered in multiple doses prior to aspiration produced more viable oocyte, and significantly more Grade A oocytes.

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