Effect of gonadotropin treatment on OPU-IVEP performance in Ongole (*Bos indicus)* cattle

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Abstract

In an experiment on 36 Ongole cows, it was noticed that the gonadotropin (Follicle Stimulating Hormone) treatment significantly ($P < 0.05$) enhanced the available follicles compared to the untreated cows (59.69 ± 5.35 *vs* 44.39 ± 3.81). Also, there was a significant increase in the mean number of large and medium-sized follicles (17.47 ± 2.04 and 31.19 ± 2.86, respectively) in comparison to untreated sessions (1.89 ± 0.21 and 22.50 ± 2.21, respectively) ($P < 0.05$). Correspondingly, the small follicle population was reduced in FSH-treated animals (11.03 ± 1.26 *vs.* 20.00 ± 1.93). The aspiration rate (66.90%) and recovery rate of COCs (57.16%) were higher in unstimulated sessions compared to stimulated sessions (56.82% and 50.04%, respectively). Though more number of follicles were found growing on the ovaries of FSH-stimulated cows (59.69 ± 5.35) *vs* unstimulated (44.39 ± 3.81), it had not been reflected in the oocyte yield, presumably due to low aspiration rate and low recovery rate in stimulated cows compared to un-stimulated animals. The oocyte quality assessment revealed no significant difference ($P>0.05$), with respect to viable and non-viable oocytes between the treatment groups. FSH pre-stimulation did not evince significant impact on embryo production (un-stimulated: 2.22 ± 0.76 *vs* stimulated: 2.81 ± 0.55). In summary, the native Ongole (*Bos indicus*) breed prove to be a suitable resource for OPU-IVEP procedures, negating the requirement for stressful, costly, and laborious gonadotropin pre-stimulation.

Key words: Follicle; Ovary; Ongole cows; FSH; OPU; IVF

Introduction

Ongole cattle are known for their toughness, rapid growth rate, and natural tolerance to tropical heat and disease resistance. It was the first Indian breed of cattle to gain worldwide recognition (Kamalakar *et al.,*2015). Integration of ovum pick-up (OPU), *in-vitro* embryo production (IVEP), and embryo transfer (ET) provides a robust platform for breed preservation and the efficient amplification of desirable germplasm (Baldassarerre, 2021). The successful application of these assisted reproductive technologies relies on maximizing oocyte and embryo yield from selected donor animals within a brief time period (Chaubal *et al.,* 2006). To enhance the number of follicles available for aspiration in the donors, several studies have explored the administration of bovine somatotropin (bST) (Tripp *et al.,* 2000) and super-stimulation with gonadotropins, particularly FSH (Chaubal *et al.,* 2006; Pontes *et al.,* 2011). Accordingly, there were reports stating that the OPU-IVEP efficiency can be augmented by different super-stimulation protocols utilising FSH, which increase the population of developmentally competent oocytes (Silva *et al.,* 2017). Previous studies (Sreemannarayana *et al.,* 2024; Krishna *et al.,* 2023; Vennapureddy *et al.,* 2024; Jagadheesh Kumar *et al.,* 2025) have demonstrated the influence of gonadotropin treatment on OPU-IVEP performance in indigenous cattle breeds *viz*., Ongole and Sahiwal. Building upon these findings, this study aimed to analyse oocyte and in vitro embryo yield in stimulated and unstimulated Ongole cattle with a different FSH treatment regimen.

Materials and methods

The present study was conducted at Livestock Research Station (LRS), Sri Venkateswara Veterinary University, Lam, Guntur, Andhra Pradesh. Thirty six (36) parous, cyclic and non-pregnant Ongole cows (*Bos indicus*) weighing around 300 to 500 kg body weight and aged between 3 and 20 years were included in the current experiment. The daily ration of each animal consisted of 2-4 Kg high protein feed containing 20% DCP and 70% TDN, 20-30 kg chopped fodder and 7-8 Kg paddy straw per day. *Ad-libitum* water supply was made. Animals were maintained under hygienic and optimum management conditions in loose housing system with a large, open paddock for free movement.

All the animals underwent two OPU sessions—one with and one without gonadotropin pre-stimulation by following a crossover design. In the initial phase, half of the animals underwent OPU without any hormonal treatment, while the remaining half received a Controlled Internal Drug Release (CIDR) device (EAZI-Breed, Pfizer Animal Health, USA), containing 1.38 g of progesterone embedded in a silastic coil, inserted intravaginally at a random stage of the estrous cycle using a standard applicator. On Day 4 post-CIDR insertion, these animals received a single 200 mg dose of NIH-FSH-P1 (FOLLTROPIN®-V, Vetoquinol, Canada), with the dose administered equally via intramuscular and subcutaneous routes (Chaubal *et al.,* 2007). Following a coasting period of 36–48 hours after FSH administration, all animals were subjected to the first OPU session. After a resting interval of 3–4 weeks, the second OPU session was conducted with crossover treatment between the two groups, ensuring that each animal served as its own control.

During each OPU session, both ovaries of all animals were initially examined using a real-time B-mode ultrasound scanner (MyLab™ Gamma Vet, Esaote, Genova, Italy) equipped with a multi-frequency (4–9 MHz) transvaginal micro-convex probe (SC 3123, Esaote). The probe was fitted into a plastic vaginal probe carrier (WTA, Cravinhos, São Paulo, Brazil) to facilitate scanning and to record the number and size distribution of all follicles ≥3 mm in diameter. Subsequently, all visible follicles were aspirated using a 20-gauge disposable aspiration needle connected to an aspiration line (WTA, Brazil) and guided through a 54 cm long aspiration line carrier. Follicular aspiration was performed under negative pressure of 70–75 mmHg for unstimulated sessions and 80–85 mmHg for stimulated sessions, into a 50 ml conical centrifuge tube (Thermo Fisher Scientific, USA) containing oocyte collection medium.

Retrieved cumulus-oocyte complexes (COCs) were evaluated and graded under a zoom stereo microscope (SMZ 1000, Nikon, Japan) at 20× magnification, using a thermal stage. Oocytes were classified into Grades A, B, and C (viable), and Grades D and E (non-viable) based on cumulus cell investment and oocyte cytoplasmic morphology, as per the criteria of Looney *et al.* (1994). Subsequently, all the oocytes were subjected to *in-vitro* maturation (IVM), fertilization (IVF), and culture (IVC) following the protocols described by Krishna *et al.* (2023). All OPU and IVEP procedures were performed by the same team across both sessions to ensure consistency.

Aspiration rate (%), oocyte recovery rate (%) and Embryo production rate (%) were calculated as described by Goodhand *et al.* (2000). Aspiration rate = number of follicles aspirated/ total number of follicles. Oocyte recovery rate = number of oocytes recovered/number of follicles aspirated. Embryo production rate = number of embryos produced/number of oocytes recovered. Further, some of the embryos produced were transferred to recipients freshly and rest were cryopreserved for future transfer.

Statistical analysis was carried out as mentioned by Snedecor and Cochran (1994). SPSS 15.0 for windows was employed in the analysis. The significance of all the parameters was measured at $P<0.05$ level significance.

Results

Across 72 OPU sessions involving 36 animals, a total of 3747 follicles were found to be available for aspiration (52.04 ± 3.39), of which 1598 (42.65%) were obtained from un-stimulated sessions (average 44.39 ± 3.81) and 2149 (57.35%) from stimulated sessions (average 59.69 ± 5.35) (Fig 1 and Table 1) indicating ovarian stimulation significantly ($P < 0.05$) increased the number of follicles available for aspiration.

When the size distribution of the available follicles between treatment types were compared, the large and medium follicles were significantly higher ($P < 0.05$) in stimulated sessions and the small follicles were significantly higher ($P < 0.05$) in un-stimulated sessions (Table 1). However, the size distribution of the available follicles when compared within the groups, in un-stimulated sessions, medium and small follicles together constitute 95.75% of the total follicles and the large follicles constitute only 4.26% ($P < 0.05$). Where as in stimulated sessions medium follicles alone were significantly higher ($P < 0.05$) compared to large and small follicles, with non-significant ($P > 0.05$) difference between them (Table 1).

Table 1: Follicle size distribution within groups of cows

Follicle size	Unstimulated	Stimulated
Large	1.89 ± 0.21^{x,A} (4.26%)^{a}	7.47 ± 2.04^{y,A} (29.27%)^{b}
Medium	22.50 ± 2.21^{x,B} (50.69%)^{a}	1.19 ± 2.86^{y,B} (52.26%)^{a}
Small	20.00 ± 1.93^{x,B} (45.06%)^{a}	1.03 ± 1.26^{y,A} (18.50%)^{b}

^{a,b} Percentages bearing different superscripts with in a row different significantly ($P < 0.05$) ^{x,y} Means bearing different superscripts with in a row different significantly ($P < 0.05$). ^{A,B,C} Means bearing different superscripts with in a column different significantly ($P < 0.05$).
Figures in parenthesis indicate percentages.

Table 2: Performance of transvaginal ovum pickup (OPU) in Ongole cows

Attribute	Unstimulated	Stimulated	Overall
Number of sessions	36	36	72
No. of follicles aspirated	1069	1221	2290
Mean no. of follicles aspirated/cow/session	29.69 ± 2.85^{x} (6 -70)	33.92 ± 3.15^{x} (4 -76)	31.81 ± 2.12 (4-76)
Aspiration rate (%)	66.90^{x}	56.82^{y}	61.12
No. of COCs recovered	611	611	1222
Mean No. of COCs recovered/cow/session	16.97 ± 2.41^{x} (0-60)	16.97 ± 2.01^{x} (0-52)	16.97 ± 1.56 (0-60)
Recovery rate (COCs / aspirated follicles) (%)	57.16^{x}	50.04^{y}	53.36

^{a,b} Percentages bearing different superscripts with in a row different significantly ($P < 0.05$); ^{x,y} Means bearing different superscripts with in a row different significantly ($P < 0.05$); Figures in parenthesis indicate percentages.

Table 3: Viable and non-viable COCs recovered

Attribute	Unstimulated	Stimulated	Overall
Viable COCs (grade A+B+C)	417 (48.38%)^{x}	445 (51.62%)^{x}	862
Mean viable COCs (grade A+B+C)	11.58 ± 1.69^{x} (0 to 39)	12.36 ± 1.68^{x} (0 to 48)	11.97 ± 1.19 (0 - 48)
Viable COCs (%)	68.25^{x}	72.83^{x}	70.54
Non-viable COCs (grade D+E)	194 (53.89%)^{x}	166 (46.11%)^{x}	360
Mean non-viable COCs (grade D+E)	5.39 ± 1.01^{x} (0 to 29)	4.61 ± 0.63^{x} (0 - 15)	5.00 ± 0.59 (0 - 29)
Non-viable COCs (%)	31.75^{x}	27.17^{x}	29.46

^{a,b} Percentages bearing different superscripts with in a row different significantly ($P < 0.05$)
^{x,y} Means bearing different superscripts with in a row different significantly ($P < 0.05$).
Figures in parenthesis indicate percentages.

Table 2 presents the overall mean number of follicles aspirated per cow per session, aspiration rate (%), mean number of COCs recovered, and recovery rate (%). A total of 1222 COCs were retrieved, with an equal distribution between stimulated and unstimulated sessions. A notable finding from the transvaginal ovum pick-up (OPU) procedure in Ongole cows was that, although the mean number of aspirated follicles and COCs recovered did not differ significantly between stimulated and unstimulated sessions (P > 0.05) (Fig 1), the un-stimulated sessions demonstrated significantly higher aspiration rates (66.90% vs. 56.82%) and recovery rates (57.16% vs. 50.04%) (P < 0.05), as shown in Table 2.

A total of 862 viable oocytes were recovered across 72 OPU sessions of which 48.38% (417/852) were obtained from unstimulated sessions, while 51.62% (445/862) were derived from stimulated sessions, with no significant difference (P > 0.05) between sessions. The overall means and proportions of viable (Grades A, B, and C) and non-viable (Grades D and E) COCs are summarized in Table 3. Analysis revealed no significant difference between the treatment groups in terms of oocyte quality, indicating that the stimulation protocol had no effect on the quality of the oocytes retrieved.

Table 4: Blastocyst development in different groups of animals

Attribute	Unstimulated	Stimulated	Overall
Embryos produced	80 (44.20%)^x	101 (55.80%)^x	181
Mean no. of embryos produced / cow / session	2.22 ± 0.76^x	2.81 ± 0.55^x	2.51 ± 0.47
Blastocyst production rate (No. of embryos produced / no. of oocytes recovered) %	13.09^x (80/611)	16.53^x (101/611)	14.81 (181/1222)

^{a,b} Percentages bearing different superscripts with in a row different significantly (P < 0.05); ^{x,y} Means bearing different superscripts with in a row different significantly (P < 0.05). Figures in parenthesis indicate percentages.

Out of a total of 1,222 oocytes recovered across all the sessions, 181 embryos were produced *in-vitro*, comprising 80 (44.2%) from unstimulated sessions and 101 (55.8%) from stimulated sessions, with no significant difference between treatment groups (P > 0.05). The overall blastocyst development rate and the mean number of embryos produced per cow per session are presented in Table 4. No significant difference (P > 0.05) was observed between the treatments groups with respect to either the blastocyst development rate or the average number of embryos generated per cow per session (Fig 1 and Table 4).

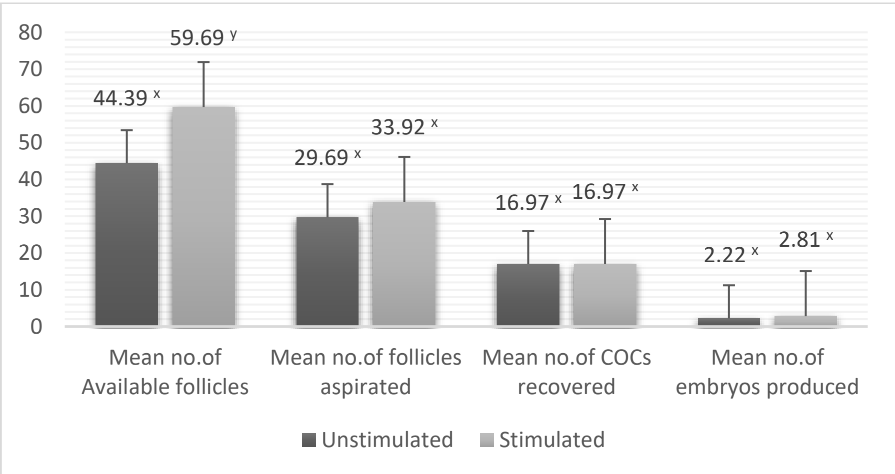

Fig 1: Comparison of OPU-IVEP performance between treatment types

Discussion

Although multiple FSH treatment increases the follicular response, in the current study the gonadotropin treatment was done by single dose to avoid the stress due to repeated handling of animals (Ooe *et al.,* 1997). A few studies have reported that usage of a single FSH treatment (im and sc) prior to OPU is adequate for optimum ovarian stimulation (Chaubal *et al.* 2007 and De Moraes *et al.* 2019). Similarly, there were reports showing the mean number of follicles aspirated and mean oocytes recovered was not different between animals receiving multiple doses of FSH and single dose of FSH (Goodhand *et al.* 2000 and Vieira *et al.* 2016).

The current findings indicate a significantly higher mean number of follicles available for aspiration in stimulated sessions (59.69 ± 5.35) compared to unstimulated sessions (44.39 ± 3.81), suggesting a positive response to FSH pre-treatment (P < 0.05). These results are consistent with earlier reports demonstrating enhanced follicular availability following FSH stimulation in Ongole cows (Goodhand *et al.,* 2000; Sreemannarayana *et al.,* 2024). However, conflicting evidence exists, as studies by Silva *et al.* (2017) in HF (*B. taurus*) donors and Krishna *et al.* (2023) in Ongole (*B. indicus*) cows observed no significant effect of FSH pre-treatment on available follicle numbers. Such variability may stem from several intrinsic factors—including follicular reserve size, recruitment dynamics, and atresia—as well as extrinsic influences such as breed, age, cyclicity, nutrition, environmental stressors, and hormonal protocol variations.

The data on follicle size distribution demonstrates that FSH treatment significantly increased the number of large (17.47 ± 2.04) and medium (31.19 ± 2.86) sized follicles, while reducing the population of small follicles (11.03 ± 1.26), compared to untreated animals (large: 1.89 ± 0.21; medium: 22.50 ± 2.21; small: 20.00 ± 1.93). Notably, nearly 82% of follicles in stimulated cows were large and medium-sized, whereas in unstimulated cows, approximately 96% were medium or small (Table 1). These findings align with those of Goodhand *et al.* (2000), who observed a marked increase in medium and large sized follicles and a concurrent reduction in small follicles following FSH treatment. Similar trends were also reported in Nelore (*Bos indicus*) cows by Viera *et al.* (2014) and Silva *et al.* (2017), indicating a consistent stimulatory effect of FSH on follicular growth dynamics across *Bos indicus* breeds.

Although FSH-stimulated cows presented greater number of available follicles, the aspiration rate was compromised compared to unstimulated sessions (56.82% *vs.* 66.90%) (P < 0.05). In contrast to the current findings, previous studies by Goodhand *et al.* (2000), Aller *et al.* (2010), and Sreemannarayana *et al.* (2024) reported higher follicle aspiration rates in FSH-treated animals. Guerreiro *et al.* (2014) highlighted considerable variation in aspirated follicle numbers across breeds, ranging from 18.6 ± 2.1 to 54.3 ± 6.1 in Nelore (*Bos indicus*) and 13.6 ± 0.9 to 20.9 ± 1.5 in Holstein (*Bos taurus*) cows. Improvements in OPU techniques—including the development of disposable needles and needle guide devices with precision vacuum pumps—have likely contributed to enhanced follicle retrieval efficiency across studies.

Similarly, the COC recovery rate was significantly higher in unstimulated sessions (57.16%) compared to stimulated sessions (50.04%) (P < 0.05; Table 2). Overall, the single-dose FSH treatment did not result in a significant improvement in oocyte yield, with both groups yielding a comparable mean number of COCs per session (unstimulated: 16.97 ± 2.41; stimulated: 16.97 ± 2.01). These findings are consistent with previous reports by Goodhand *et al.* (2000), Silva *et al.* (2017), and Krishna *et al.* (2023), which also found limited benefit of single-dose FSH protocols in enhancing oocyte recovery. The oocyte recovery rate serves as a key indicator of OPU success and is influenced by numerous factors, including hormonal pre-treatments such as FSH, PMSG, or BST, as well as technical variables like transducer type, puncture frequency, vacuum level, and operator proficiency (Bols *et al.,* 2004; Vennapureddy *et al.,* 2024; Cuervo-Arango *et al.,* 2025). As a result of these multifactorial influences, reported recovery rates vary widely—from as low as 7% to as high as 81.8% (Guerreiro *et al.,* 2014).

In the present study, considerable individual variation was observed in oocyte recovery per session, ranging from 0 to 60 in unstimulated sessions and 1 to 52 in stimulated ones. Similar variability in zebu cattle has been linked to differential expression of genes such as GDF9, BMP15, and FGF8 (Biase *et al.,* 2008). Other sources of individual variation include age, parity, nutritional status, and environmental stress, particularly heat stress (Sartori *et al.,* 2004).

Oocyte quality was assessed based on standard morphological criteria, including cumulus cell investment and cytoplasmic appearance, following grading systems previously described by Looney *et al.* (1994). In the present study, FSH pre-treatment did not significantly improve oocyte quality, as reflected by the comparable proportions of viable oocytes in unstimulated (68.25%) and stimulated (72.83%) sessions (Table 3). These findings align with Silva *et al.* (2017), who reported similar viable oocyte proportions in control (64.6%) and FSH-treated (68.7%) groups. The lack of improvement in the total as well as viable oocyte yield in FSH-treated animals may be attributed to the lower aspiration and recovery rates observed in this group. While some studies report no benefit of FSH stimulation on oocyte quality (Silva *et al.,* 2017), others have demonstrated positive effects on both quality and developmental competence (Goodhand *et al.,* 2000; Blondin *et al.,* 2012; Sreemannarayana *et al.,* 2024; Krishna *et al.,* 2023). These inconsistencies may stem from variations in factors such as transducer type, puncture frequency, OPU protocols, hormonal treatments, and technical parameters like needle gauge and vacuum pressure (Bols *et al.,* 2004; Silva *et al.,* 2017).

IVEP Performance

Mirroring the lack of significant improvement in oocyte yield and quality, FSH pre-stimulation did not enhance blastocyst development to Day 7 under the conditions of the present study. These findings are in line with the previous reports by Chaubal *et al.* (2007), Silva *et al.* (2017), and De Moraes *et al.* (2019), who also observed limited benefits of FSH treatment on embryo development. In contrast, other studies have reported improved oocyte recovery and embryo production following FSH stimulation (Blondin *et al.,* 2012; Viera *et al.,* 2014, 2016). Discrepancies among studies may stem from differences in FSH dosing protocols, including dose, regimen, and coasting period. Notably, multiple FSH injections have been shown to be more effective than single bolus administrations in promoting follicular development (Chaubal *et al.,* 2007). However, in the current study, a single 200 mg FSH dose was administered on Day 4 post-CIDR insertion, split between intramuscular and subcutaneous routes. Previous findings from MOET (Multiple Ovulation and Embryo Transfer) studies have also highlighted the superiority of multiple-dose FSH regimens over single-dose protocols in enhancing ovulation rates and embryo yield and quality (Staigmiller *et al.,* 1995).

Conclusion

In summary, while FSH pre-stimulation in Ongole cows effectively augmented follicular development, leading to an increased population of medium and large follicles and a concomitant reduction in small follicles, oocyte yield was compromised, likely attributable to reduced aspiration and recovery rates. Furthermore, FSH pre-treatment failed to enhance blastocyst development or embryo production consistent with the compromised oocyte yield. These findings underscore the complexity of follicular manipulation and suggest the need for further investigations into optimizing FSH pre-stimulation protocols to achieve tangible improvements in reproductive efficiency in this breed.

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Declaration of Competing Interest

All the authors declared no conflicts of interest to report.

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