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Comparative Analysis of Single and Tapering FSH Doses on Follicular and Oocyte Yield in Sahiwal Cows

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was conducted at Embryo Transfer & *In vitro* Fertilization (ET & IVF) laboratory, College of Veterinary Science, Korutla, Telangana. This study assessed the effects of single and

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multiple doses of follicle stimulating hormone (FSH) stimulation protocols on ovarian follicular development, oocyte retrieval, oocyte quality, in-vitro maturation (IVM) in Sahiwal cows. Thirty cows, irrespective of their estrous cycle stage, were categorized into three groups: Group 1 (single dose FSH, n = 10), Group 2 (tapered FSH doses, n = 10) and Group 3 (unstimulated controls, n = 10). Ovum pickup (OPU) was conducted following FSH stimulation in Groups 1 and 2, while Group 3 underwent OPU without hormonal stimulation. Retrieved oocytes were graded and subjected to IVM.

Both FSH stimulation protocols significantly increased the follicular count (Group 1: 20.51 ± 2.30; Group 2: 22.11 ± 2.21; Group 3: 5.61 ± 0.65) and the number of recovered oocytes per cow (Group 1: 11.81 ± 1.80; Group 2: 15.81 ± 2.45; Group 3: 2.81 ± 0.29). FSH stimulated groups exhibited greater number of oocytes with cumulus cell expansion and first polar body extrusion than the control group, although the rates of Cumulus cell expansion and extrusion in percentages did not differ significantly. In conclusion, both single dose and tapering doses FSH stimulation protocols effectively promoted follicular development and improved oocyte recovery in Sahiwal cows.

Keywords: Follicle; oocyte; follicle stimulating hormone; ovum pick up; in vitro maturation.

1. INTRODUCTION

Assisted Reproductive Technologies (ARTs) have transformed livestock production sector by enabling genetic improvement and enhancing reproductive efficiency. Among these, *In-Vitro* Embryo Production (IVEP) is an important tool for producing high quality embryos, particularly in cattle. However, optimizing IVEP protocols for indigenous breeds like Sahiwal cows remains challenging due to their unique reproductive physiology.

Follicle Stimulating Hormone (FSH) is widely employed to stimulate ovaries in terms of follicular development, improve oocyte recovery and enhance the yield of competent oocyte recovery for IVEP. The effectiveness of FSH in exotic breeds is well established (Gutierrez et al*.,* 2024; Hayden et al*.,* 2023). However, the choice of stimulation protocols- whether single dose or tapering doses regimens has a profound impact on oocyte quality and subsequent embryo development (Demetrio et al*.,* 2022).

Sahiwal cows, valued for their adaptability to tropical climates and contribution to India's dairy sector, exhibit lower reproductive efficiency compared to exotic breeds. This necessitates the development of specially designed ovarian stimulation protocols IVEP to explore their genetic potential effectively. Current literature indicates a significant gap in studies focusing on stimulation protocols specific to Sahiwal cows, underlining the need for indigenous breeds-specific reproductive strategies.

This research focuses on assessing FSH stimulation protocols in Sahiwal cows, comparing single dose and tapering doses regimens. The study aims to identify strategies that maximize oocyte quality and improve IVEP parameters, thereby supporting the genetic conservation and sustainable productivity of native breeds. By addressing these objectives, the findings are expected to contribute to the broader adoption of ART in indigenous livestock, ensuring both food security and the preservation of valuable genetic resources.

2. MATERIALS AND METHODS

2.1 Experimental Location

The present study was conducted at ET & IVF laboratory, College of Veterinary Science, Korutla, Jagitial district, Telangana (latitude: 18°49′36.71″N; longitude: 78° 42′50.39″E; altitude: 295.99 m above mean sea level) during the period between February and December 2024.

2.2 Experimental Animals

Thirty Sahiwal cows (Bos *indicus*) aged 3-6 years old and weighing 300-350 kg body weight were selected as oocyte and were randomly divided into three groups. The cows involved were fed adequately and ad libitum drinking water was provided all the time. Cows were kept under hygienic and optimum management conditions in loose housing system with a large, open paddock for free movement. Deworming and vaccination protocols were followed as per standard schedule.

2.3 Superstimulation Protocol and OPU Schedule

The cows were divided into three equal groups irrespective of the stage of their estrus cycle.

Cows in group 1 (FSH single injection, n = 10), on day 0, at random stage of estrous cycle were inserted with an intravaginal progesterone device (Eazi Breed CIDR – Controlled Internal Drug Release, progesterone: 1.38 gm). On day 5, Inj. GnRH was administered @ 10 µg intra muscularly for the purpose of follicular wave synchronisation (FWS) [Inj. GnRH - Buserelin acetate (Receptal, 10ml, MSD, Germany, marketed: by Intervet India Pvt. Ltd. Bhiwandi. Each ml contains 0.0042 mg of buserelin acetate)]. On day 7 Inj. follicle stimulating hormone (FSH) [Inj. Folltropin V (Vetoquinol, Canada, containing pituitary extract of porcine follicle stimulating hormone - pFSH 400mg NIH)] was administered @ 200mg in single dose intra muscularly for ovarian super stimulation, 36 hours (hr) from administration of Inj. GnRH. On day 9 CIDR was removed and Ovum Pick Up (OPU) was carried out between 48 and 56 hr after the FSH injection (coasting period). Cows in Group 2 (tapering doses of FSH stimulations, n = 10) were inserted with an intravaginal progesterone device on day 0. From day 4 to day 8, the cows in this group received 8 divided tapering doses of Inj. FSH, totally making 200 mg. The time interval between two consecutive FSH injections was 12 hr. After 36 hr of the last FSH (Coasting period), on day 10 CIDR was removed and OPU was conducted in cows. The intravaginal device and type of FSH injection used in group 1 and group 2 were same. Whereas cows in Group 3 (non-stimulated, n = 10) were subjected to ovum pick-up (OPU) once at random stage of estrous cycle**.** A total of 30 OPU sessions were performed in all 30 cows with and without FSH pre stimulation.

2.4 Follicular Aspiration and Oocyte Recovery

After the caudal epidural anaesthesia, the ovary was adjusted gently and positioned against the probe head in order to get a clear image view of the follicles on the ultra-sonography monitor (Fig. 1). By obtaining images in multiple views number of follicles per ovary was recorded (Fig. 2). All the visible follicles were aspirated, the contents were collected in a 50 ml tube and transferred to laboratory. The content of the tube was filtered and washed with 100 µm oocyte mini filter (Watanabe Applied Technology, Brazil) and OPU recovery media (BoviPlus, Ref no.19982/1281, minitube, USA).

The number of oocytes recovered per follicles aspirated per cow were recorded and were expressed as oocyte recovery rate in percentage (Goodhand et al*.,* 2000). The filtrate was collected in 60 mm petri dish. The filter was washed with 20 ml OPU media and examined under the stereo zoom microscope (SMZ-1270, Nikon, Japan) at 1x magnification to find the cumulus oocyte complexes (COCs) (Saleem et al., 2022). The collected COCs were classified into A-D grades (which takes into consideration of the presentation and number of cumulus cell layers and the ooplasm homogeneity), (Manik et al., 2003).

2.5 *In vitro* Maturation (IVM) of Oocytes

The *In vitro* maturation media (IVM) (Vitrogen, Brazil) was kept overnight equilibration in CO_2 incubator (5% CO_2 in air, 37°C temperature and humidity 98%) to stabilize the pH. The cumulus oocytes complexes (COC's) collected were first washed 2-3 times with wash media (Vitrogen, Brazil), after that rinsed twice with pre-equilibrated IVM media (Vitrogen, Brazil) and finally kept for IVM. Group of almost 20 COCs were kept in equilibrated 500 µl of IVM media overlaid with sterile mineral oil of 300µl (Vitrogen, Brazil) in 5 well dish (Minitube, Germany) and kept for IVM in a humidified CO_2 incubator (5% CO_2 in air and more than 90% RH) at 38.5 °C for 24 hr. After 24 hr of IVM, the oocytes were evaluated for their maturation status based upon their degree of expansion of the cumulus cell mass and the first polar body extrusion rate into perivitelline space.

2.6 Statistical Analysis

The collected data was statistically analyzed by using descriptive statistics and the means were tested for significance by Tukey's HSD test (Abdi & Williams, 2010) using SPSS (2009) version 16.

3. RESULTS

Thirty sessions of OPU were conducted, comparing 10 sessions in each for group 1 (FSH single injection), group 2 (FSH tapering doses) and group 3 (non-stimulated). Both the stimulated groups shown significantly ($p<0.05$) higher mean number of follicles than non-stimulated group as outline in Table 1, Figs. 1, 2, 3.

Grade A, C, D and the total number of oocytes collected were significantly higher in both group

1 and 2 than in group 3 with no significant difference between group 1 and 2. Grade B oocytes were significantly higher in group 2 than group 1 and 3 with no significant difference between group 1 and 3 which are outlined in Table 2, Figs. 4, 5.

Table 1. Mean Follicular population available per cow for OPU

Follicular distribution	Mean number of follicles per cow (mean ± SE)		
	Single dose FSH stimulated (Group 1)	Tapering doses FSH stimulated (Group 2)	Non-stimulated (Group 3)
Mean follicular population per cow	20.50 ± 2.296[a]	22.10 ± 2.213[a]	5.60 ± 0.6532[b]

Values bearing different superscripts within a row (a,b) differ significantly (p< 0.05)

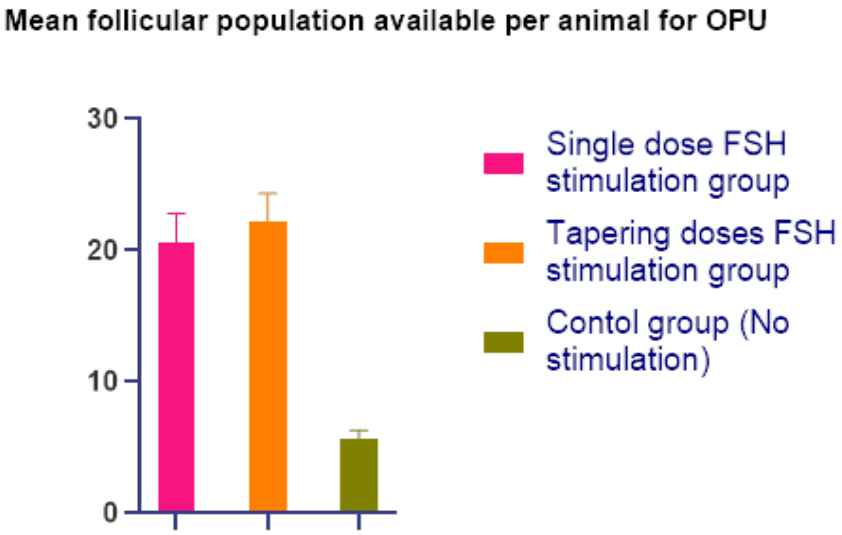

Fig. 1. Mean follicular population per cow for OPU

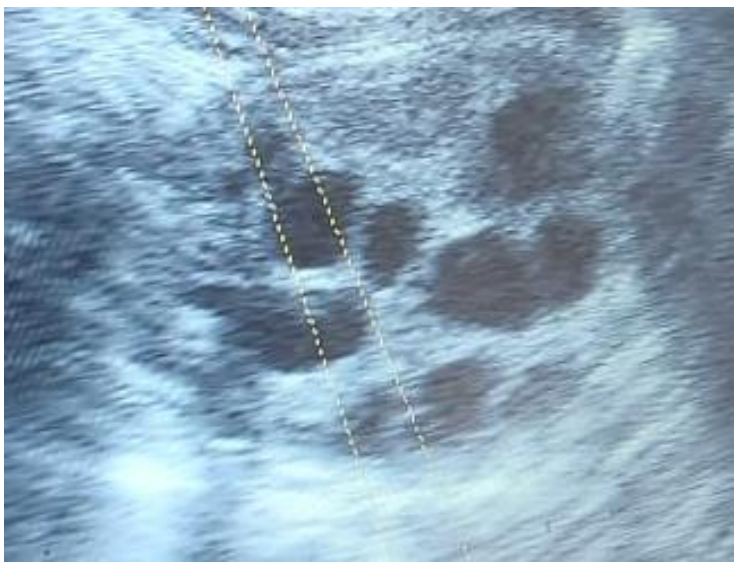

Fig. 2. Ultrasonographic image of ovary after single dose FSH stimulation

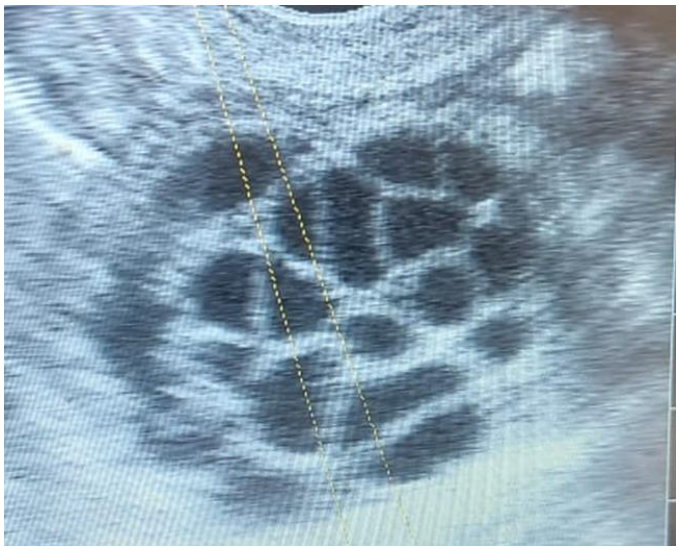

Fig. 3. Ultrasonographic image of ovary after tapering doses of FSH stimulation

Table 2. Oocyte yield and oocyte quality in FSH stimulated and non-stimulated groups

Attribute	Mean number of oocytes per animal (mean ± SE)		
	Single dose FSH stimulated (Group 1)	Tapering doses FSH stimulated (Group 2)	Non-stimulated group (Group 3)
Grade A	4.80 ± 1.133^{a}	5.90 ± 1.501^{a}	0.40 ± 0.1633^{b}
Grade B	1.80 ± 0.2906^{b}	6.00 ± 0.7454^{a}	1.00 ± 0.2108^{b}
Grade C	4.10 ± 0.9939^{a}	3.70 ± 0.6155^{ac}	1.4 ± 0.2211^{bc}
Grade D	1.10 ± 0.5859^{a}	0.20 ± 0.1333^{a}	0.00 ± 0.00^{a}
Grade A+B	6.60 ± 1.258^{b}	11.90 ± 1.923^{a}	1.40 ± 0.2211^{c}
Total oocytes recovered	11.80 ± 1.794^{a}	15.80 ± 2.444^{a}	2.80 ± 0.2906^{b}

Values bearing all superscripts different within a row (a, b, c) differ significantly (p< 0.05).

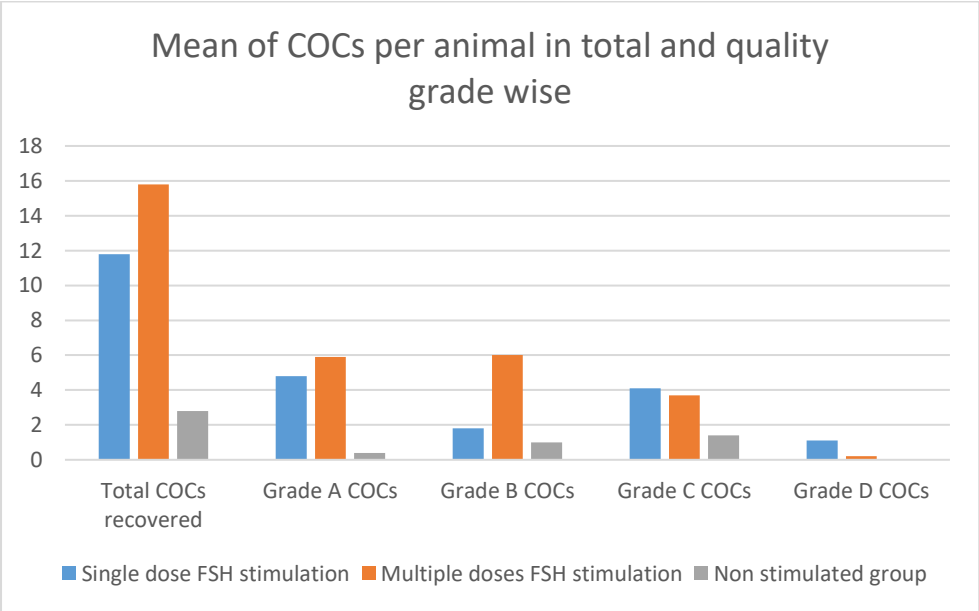

Fig. 4. Mean number of Cumulus Oocyte Complexes recovered per animal

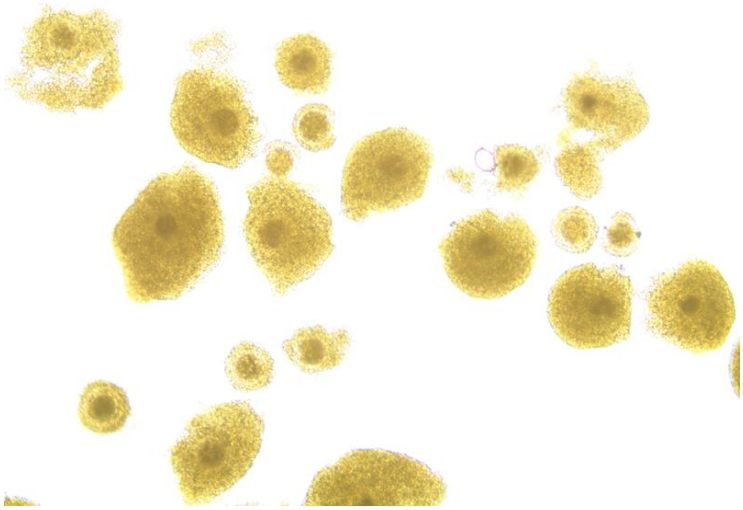

Fig. 5. Immature oocytes retrieved through OPU before IVM (under 10× of phase contrast microscope)

Table 3. Effect of FSH stimulation on cumulus cell expansion and 1st polar body extrusion of oocytes

	Group 1 Single dose FSH stimulated	Group 2 Tapering doses FSH stimulated	Group 3 Non-stimulated group
Mean of cumulus cell expansion per animal (mean ± SEM)	9.400 ± 1.759[a]	14.90 ± 2.424[a]	2.70 ± 0.300[b]
Cumulus cell expansion rate (%) (mean ± SEM)	88.93 ± 2.775[a]	95.28± 1.629[a]	96.67 ± 3.333[a]
Mean extrusion of 1st polar body per animal (Mean±SE)	6.500 ± 1.128[a]	8.900 ± 1.433[a]	1.600 ± 0.1633[b]
First polar body extrusion rate (%) (mean ± SEM)	62.65 ± 3.317 [a]	56.99 ± 1.492 [a]	60.00± 5.666 [a]

Values bearing different superscripts within a row (a,b) differ significantly (p< 0.05).

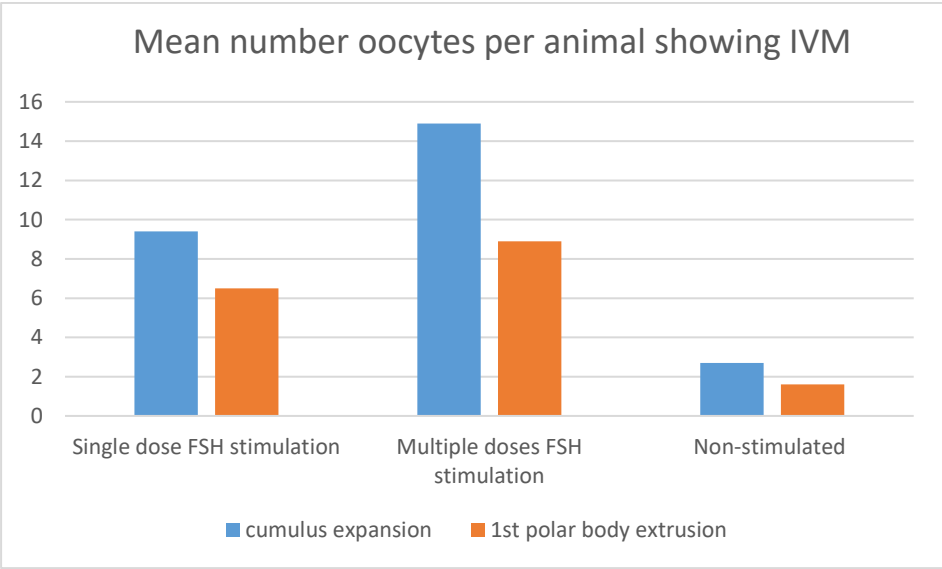

Fig. 6. Mean number of oocytes per cow showing cumulus cell expansion and 1stpolar body extrusion in FSH stimulated and non-stimulated groups

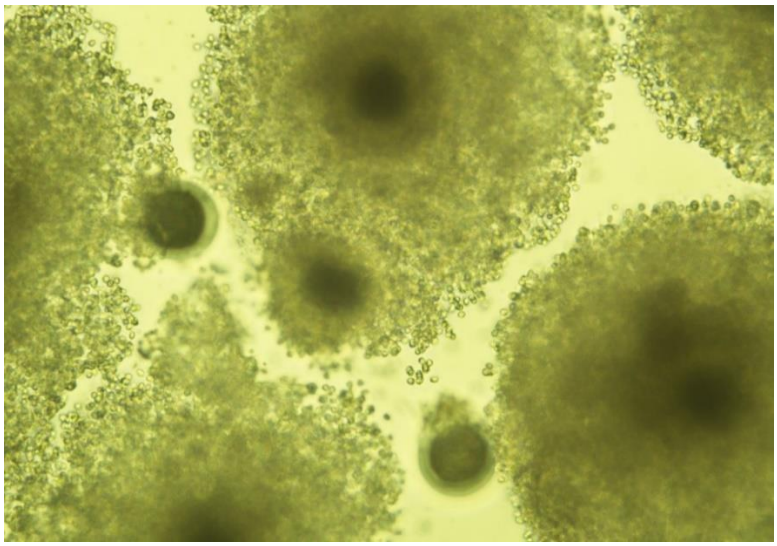

Fig. 7. Cumulus Oocyte Complexes showing cumulus cell expansion after 24hrs of IVM (under 20× of phase contrast microscope)

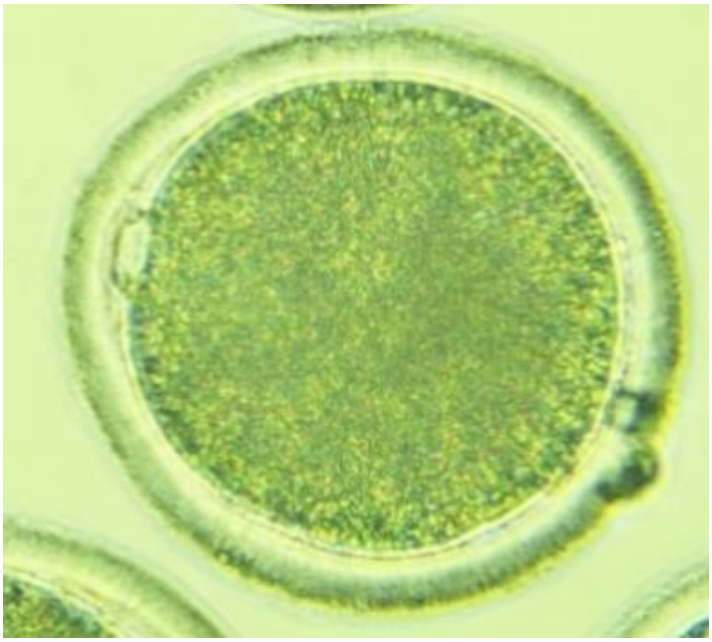

Fig. 8. Oocyte showing first polar body extrusion after 24hrs of IVM (under 20× of phase contrast microscope)

The capacity of immature oocytes retrieved through Ovum Pick-Up (OPU) for rates of cumulus cell expansion and first polar body extrusion during IVM reveals no significant difference statistically ($p<0.05$) among groups as mentioned in Table 3, Figs. 6, 7, 8.

4. DISCUSSION

The current study aimed to assess the impact of ovarian super stimulation using single and tapering doses of FSH on follicular development, oocyte yield, quality and *In vitro* Maturation (IVM) rates in Sahiwal cows. (Farheen et al*.,* 2023)

4.1 Follicular Development

FSH-stimulated cows demonstrated significantly higher ($p < 0.05$) numbers of aspirable follicles compared to non-stimulated controls. Group 1 (20.50 ± 2.296) and Group 2 (22.10 ± 2.213) had substantially higher follicular counts than Group

3 (5.60 ± 0.6532), though no significant differences were observed between the stimulated groups. These findings highlight FSH's role in promoting follicular growth and mitigating atresia in subordinate follicles, thereby enhancing follicular availability for aspiration. Previous studies (Vieira et al., 2016; Carvalho et al., 2019; Vennapureddy et al., 2022) corroborate these observations.

The absence of a significant difference between single and tapering FSH doses in follicular populations aligns with Goodhand et al. (2000), who reported comparable results. Conversely, studies by Meintjes et al. (1995) and Silva et al. (2017) indicated no discernible impact of FSH stimulation on follicular numbers at ovum pick-up (OPU). Variations across studies may be attributed to differences in FSH protocols, dosage, coasting periods, animal age, breed, and individual variability.

4.2 Oocyte Yield and Recovery Rate

FSH-stimulated groups (Group 1: 11.80 ± 1.794; Group 2: 15.80 ± 2.444) had significantly ($p < 0.05$) higher oocyte recovery than the non-stimulated group (2.80 ± 0.2906), though no significant differences were observed between stimulated groups. Recovery rates of 58.31 ± 4.877% (Group 1), 69.61 ± 4.169% (Group 2), and 57.20 ± 8.510% (Group 3) revealed no significant inter-group differences.

These findings diverge from reports by Jeyakumar (2004) and Ongaratto et al. (2020), where vacuum pressure, needle gauge, operator proficiency, follicular population, and OPU timing were identified as influencing factors.

4.3 Oocyte Quality

Oocyte quality, a pivotal determinant of developmental competence, was significantly enhanced in FSH-stimulated groups. Group 2 (4.80 ± 1.133) and Group 1 (5.90 ± 1.501) produced more Grade A oocytes compared to Group 3 (0.40 ± 0.1633). The tapering FSH protocol (Group 2) yielded the highest proportion of Grade A and B oocytes (75.31%), surpassing single-dose FSH (55.93%) and the non-stimulated group.

These results are consistent with findings by Demetrio et al. (2022) and Hasler et al. (1995) but contrast with Demetrio et al. (2021), where hyaluronan-based FSH injections showed no significant differences in oocyte quality between single and tapering FSH doses. Factors such as vacuum pressure, needle diameter, and environmental stressors like climate and lactation stage also influence oocyte quality (Fry et al., 1997; Silva et al., 2017).

4.4 *In vitro* Maturation

Cumulus cell expansion (CCE) rates (Group 1: 88.93 ± 2.775; Group 2: 95.28 ± 1.629; Group 3: 96.67 ± 3.333) and first polar body extrusion (PBE) rates (Group 1: 62.65 ± 3.317; Group 2: 56.99 ± 1.492; Group 3: 60.00 ± 5.666) were not significantly different among groups ($p > 0.05$). CCE facilitates nutrient channeling and germinal vesicle breakdown, while PBE serves as an indicator of nuclear maturation. Lower PBE rates in some instances may reflect oocyte ageing or damage from aspiration.

These findings align with Verma (2005), where IVM rates showed no significant differences between FSH-stimulated and non-stimulated groups. Similar results were reported by Farheen et al. (2024), with no significant differences in CCE or PBE rates between stimulated and non-stimulated groups. Discrepancies with other studies (Donnay et al., 1997) may be due to differences in animal genotype, oocyte handling, or IVM media composition.

5. CONCLUSION

FSH stimulation significantly increased follicular numbers, oocyte yield, and quality in Sahiwal cows, with tapering doses yielding slightly better results. However, IVM outcomes, including CCE and PBE rates, were not markedly influenced by FSH stimulation. These findings provide insights into optimizing FSH protocols for enhanced IVEP outcomes. Further research is warranted to explore the underlying factors affecting oocyte quality and maturation under varying stimulation regimens

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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