Haryana Vet. (June, 2024) **63(1),** 91-93

Research Article

SUPER-STIMULATION EFFECT OF FOLLICLE STIMULATING HORMONE ON OVARIAN FOLLICULAR STATUS FOR OVUM PICK UP IN SAHIWAL CATTLE

Y. NAGENDRA REDDY*, K. VEERABRAHMAIAH[1], K. ANUSHA, N.V. KRISHNA and G. SANDEEP KUMAR[2]
Department of Veterinary Gynaecology and Obstetrics, [2]Division of Molecular Biology,
[1]Dean of Veterinary Science, COVS, SVVU, Tirupati (Andhra Pradesh)

Received: 30.10.2023; Accepted: 03.02.2024

ABSTRACT

Assisted reproductive technologies improve reproductive efficiency for enhanced productivity of cattle. Ovum Pick-Up (OPU) coupled with in vitro Fertilization (IVF) aids in the production of a greater number of embryos from superior donors. Exogenous gonadotropins are used for super stimulation to obtain greater and quality oocytes through OPU. Twelve Sahiwal cows aged 6-8 years divided into two groups. Animals in group-1 (n=6) were subjected to follicle stimulating hormone (FSH) stimulation (Folltropin-V, 200 mg i/m in 3 divided doses 100, 60, 40 mg) and animals in group 2 (n=6) also subjected to FSH stimulation (Stimufol, 250 µg i/m in 3 divided doses 125, 75, 50 µg). Ultrasound was performed after 24 hours of last FSH dose. The mean number of follicles available for aspiration was significantly ($P<0.05$) higher in the Group-1 (63.33±5.31) compared to the Group-2 (48.83±4.07), but there was no significant difference ($P>0.05$) in the number of small, medium and large sized follicles between the groups. The number of small, medium sized follicles is greater with less large folliclesin both groups which shows significantly ($P<0.05$) greater medium and small sized follicles compared to large follicles. Follicles in right ovary is comparably more than left ovary in Folltropin-V group and more in left ovary when compared to right ovary in stimufol group, but no significant difference ($P>0.05$) between the sides of ovaries. So, it may be concluded that Folltropin-V and Stimufol group cows had higher number of small, medium sized follicles which would contribute to the recovery of more oocytes through OPU. So instead of Folltropin-V, Stimufol can be used due to cost of the drug and to increase the efficacy of OPU.

Keywords: Follicle stimulating hormone, Folltropin, Stimufol, Super-stimulation

How to cite: Reddy, Y.N., Veerabrahmaiah, K., Anusha, K., Krishna, N.V. and Kumar, G.S. (2024). Super-stimulation effect of follicle stimulating hormone on ovarian follicular status for ovum pick up in Sahiwal cattle. *Haryana Vet.* **63(1)**: 91-93.

The primary focus of Assisted Reproductive Technologies (ART) is to maximize the number of offspring from genetically superior animals and disseminate germplasm worldwide (Berglund, 2008). Among that, the third generation ART's is Ovum Pick-Up coupled with *In vitro* Embryo Production which accounts for 72.7% of the total bovine embryos produced worldwide (Viana, 2019), which is possible due to the collection of a greater number of oocytes and in turn greater production of embryos per unit of time through application of procedures like super-stimulation of donor animals, sexed semen or reverse sorted semen for *in vitro* fertilisation and genomic selection of transferable Embryos (Ferre *et al.*, 2020). Among that procedures super-stimulation is the primary step so that we can increase the follicle number and subsequently oocyte yield, embryo production by using various hormones like pregnant mare serum gonadotropin (Aller *et al.*, 2012) and porcine follicle stimulating hormone (Vennapureddy *et al.*, 2022). So, present study was conducted to evaluate the super-stimulatory effect of pFSH (Folltropin-V and Stimufol) on ovarian follicular population which paves the way for collection of more oocytes through ovum pick-up in Sahiwal cattle.

MATERIAL AND METHODS

The present study was undertaken at College of Veterinary Science, Tirupati and at Sri Venkateshwara Gosamrakshanashaala, Tirumala Tirupati Devasthanam, Tirupati during the period between September and December, 2022. The climate in the region was semi-arid to sub-humid with hot summer and cool winter.

Twelve lactating/dry, multiparous, clinically normal, cyclic Sahiwal donor cows (Bos *indicus*), aged 6-8 years were selected for the study. All the selected animals were maintained with a daily ration of 2-4 kg high protein feed/animal containing 20% DCP and 70% TDN along with 10-15 kg chopped fodder and 4-5 kg paddy straw and ad libitum water. These animals were kept in a loose housing arrangement with an open paddock for free movement under sanitary management conditions.

Experimental Design

All the selected donor animals were randomly divided into two equal groups. Donor cows in group-1 (n=6) were administered with 10 µg GnRH (Receptal 2.5 ml I/M) at a random stage of oestrous cycle followed by FSH (Folltropin-V (Vetoquinol), 200 mg I/M in 3 divided doses @ 100 mg, 60 mg and 40 mg) at 48, 60 and 72 h after GnRH administration. Each vial of freeze-dried Folltropin-V contains porcine follicle stimulating hormone (pFSH) 400 mg NIH that gets reconstituted with 20 ml bacteriostatic sodium chloride injection USP so that, the final solution contains 20 mg of FSH/ml. Donor cows in group 2 (n=6) were administered with 10µg GnRH (Receptal 2.5 ml I/M) at a random stage of oestrous cycle followed by FSH (Stimufol, 250 µg I/M in 3 divided doses @ 125, 75 and 50 µg) at 48, 60 and 72 h after GnRH

*Corresponding author: nagendrareddyy93@gmail.com

administration. Each vial of freeze-dried Stimufol (Reprobiol SPRL, Belgium) contains porcine follicle stimulating hormone (pFSH) 500 µg and porcine luteinizing hormone (pLH) 100 µg that gets reconstituted with 10 ml bacteriostatic sodium chloride injection USP. The ultrasonography was performed on all the donor cows 24 hours after the last FSH treatment to evaluate the follicular population i.e., total follicles, sizes of follicles on both right and left ovary (Figs. A and B). Analysis of data was carried out by using student t-test and ANOVA. SPSS 15.0 for windows was employed.

RESULTS AND DISCUSSION

The total number of follicles available for trans-vaginal follicular aspiration in Folltropin-V and Stimufol group are 380 and 293 with mean of 63.33±5.31 (ranges from 50 to 80) and 48.83±4.07 (ranges from 39 to 68) shows significantly higher follicular population ($p<0.05$) in Folltropin-V group than in Stimufol group (Table 1). This might be presumably due to increased recruitment and growth of follicles following follicular stimulation and ovarian super stimulation with exogenous gonadotropin (pFSH) led to concomitant reduction in endogenous pulsatile secretion of LH due to an increase in the plasma progesterone, estradiol, inhibin and gonadotropin surge attenuating factor concentrations and thereby increase the availability of follicles (Sendag *et al.*, 2008).

Among the total follicles, mean number of small, medium and large sized follicles in Folltropin-V and Stimufol group are 28.50±5.08 (ranges from 12 to 48), 28.83±3.81 (ranges from 13 to 40), 6±1.52 (ranges from 1 to 10) and 19.16±1.42 (ranges from 17 to 26), 25±2.23 (ranges from 18 to 34), 4.66±0.84 (ranges from 2 to 8) (Table 1). Even though no significant difference ($p>0.05$) observed between the groups but within the groups the number of large sized follicles is significantly less when compared to small and medium sized follicles in both the groups. FSH administration to donor cows might have minimized negative effects resulting from the settling of the dominant follicle (Ginther *et al.*, 2017) by postponing the selection of the dominant follicle, atresia of the subordinate ones and increases mean diameter of the small and medium sized follicles (Demissie *et al.*, 2021). These findings were in agreement with, Hayden *et al.* (2022) recorded higher number of medium follicles (20±1.64) and small sized follicles (9.1±0.8) when compared with large sized follicles (2.7 ±0.5) when stimulated with Folltropin-V. In contrast Demissie *et al.* (2021) observed higher number of large follicles (8.20±0.96) when compared with medium follicles (7.36 ±0.57) and small follicles (4.30± 0.57) in donors stimulated with Folltropin-V. In the second group, greater number of medium sized follicles is observedwhen compared to small and large sized follicles when the donors are stimulated with stimufol (Table 1). In

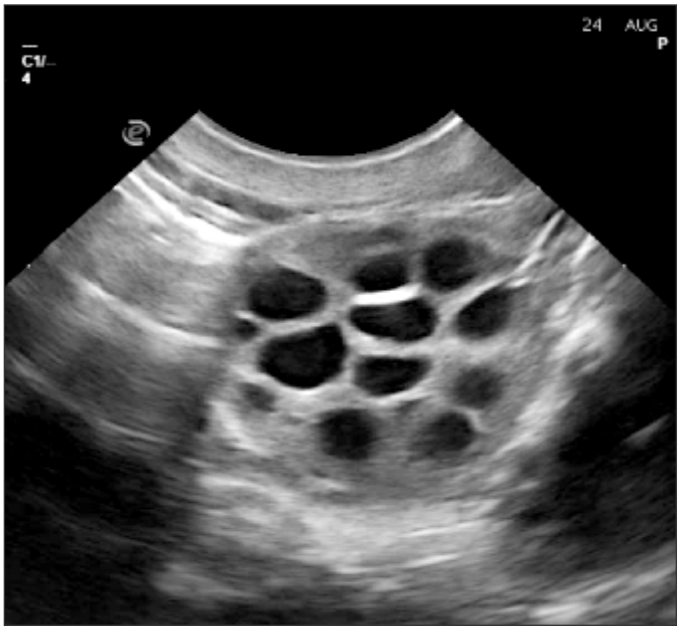

Fig. A. FSH Stimulated ovary (Folltropin-V) showing follicles of various sizes

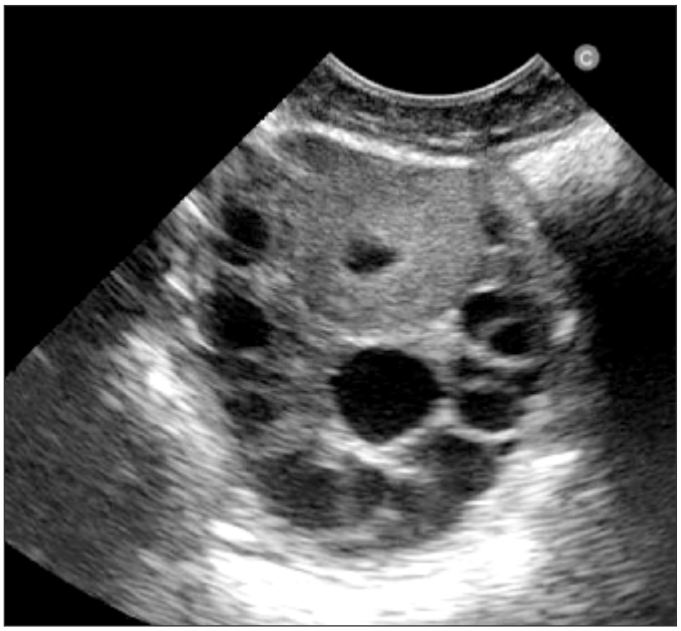

Fig. B. FSH Stimulated ovary (Stimufol) showing follicles of various sizes and corpus luteum

an agreement to our study Srimannarayana (2019) reported that a greater number of medium sized follicles (16 ±1.76) than the small (4.72 ±1.13) and large sized follicles (6.33±0.79) in donors stimulated with Stimufol. In contrast De Roover *et al.* (2005) reported that a greater number of small follicles (5.1±4.8) compared to medium (4.1±3.9) and large sized follicles (1.4±1.7) in donors stimulated with Stimufol.

In the Folltropin-V group, the total mean number of follicles on right ovary and left ovary are 34.00±2.17 (ranges from 26 to 42) and 29.33±3.56 (ranges from 20 to 42). Of the total, the mean number of small, medium and

Table 1. Total number of follicles available for trans-vaginal follicular aspiration

	Folltropin-V Group	Stimufol Group
Small	28.50 ± 5.08^{ax}	19.16 ± 1.42^{ax}
Medium	28.83 ± 3.81^{ax}	25 ± 2.23^{ax}
Large	6 ± 1.52^{abx}	4.66 ± 0.84^{abx}
Total	63.33 ± 5.31^{A}	48.83 ± 4.07^{B}

Values bearing different superscripts (a,b with in a column and x,y and A,B with in a row differ significantly ($p < 0.05$).

Table 2. Mean number of follicles on ovary

	Folltropin-V	Stimufol
Right Ovary		
Small	15.33 ± 2.29^{a}	9.16 ± 0.47^{x}
Medium	16.50 ± 2.70^{a}	12 ± 1.03^{x}
Large	2.16 ± 0.47^{ab}	2 ± 0.73^{xy}
Total	34.00 ± 2.17^{A}	23.16 ± 1.37^{A}
Left Ovary		
Small	13.16 ± 2.97^{a}	10.00 ± 1.23^{x}
Medium	12.33 ± 1.96^{a}	13.00 ± 1.59^{x}
Large	3.83 ± 1.10^{ab}	2.66 ± 0.84^{xy}
Total	29.33 ± 3.56^{A}	25.66 ± 3.24^{A}

Values bearing different superscripts (a,b and x,y with in a column and A,B with in a row differ significantly ($p < 0.05$).

large sized follicles on right ovary and left ovary are 15.33±2.29 (ranges from 8 to 22), 16.50±2.70 (ranges from 5 to 24), 2.16±0.47 (ranges from 1 to 4) and 13.16±2.97 (ranges from 4 to 26), 12.33±1.96 (ranges from 8 to 20), 3.83±1.10 (ranges from 0 to 6). Even though there is no significant ($p>0.05$) difference between the availability of follicles on right and left ovary but the follicular availability on right ovary is greater when compared to left ovary. In the Stimufol group, the total number of follicles on right ovary and left ovary are 23.16±1.37 and 25.66±3.24. Of the total, the mean number of small, medium and large sized follicles on right ovary and left ovary are 9.16±0.47 (ranges from 8 to 11), 12.00±1.03 (ranges from 9 to 16), 2±0.73 (ranges from 0 to 4) and 10.00±1.23 (ranges from 8 to 16), 13.00±1.59 (ranges from7 to 18), 2.66±0.84 (ranges from 0 to 6) (Table 2). There is no significant ($p>0.05$) difference between the availability of follicles on right and left ovary but the follicular availability on left ovary is greater when compared to right ovary. These findings were in agreement with Venkatakrishna (2021) who observed a greater number of follicles on left ovary when compared with right ovary in ongole cows. On the contrary, Sreemannanarayana (2019) observed significantly higher follicle count on right ovary than on left ovary when stimulated with Stimufol in Ongole cows.

CONCLUSION

From the results of present study, it may be concluded that Folltropin-V and Stimufol group cows had higher number of small, medium sized follicles which would contribute to the recovery of more COCs through OPU. Though, there was no significant difference in the number of small, medium and large sized follicles between Folltropin-V and Stimufol groups, we can use Stimufol instead of Folltropin-V due to cost of the drug and the cost effectiveness of *in vitro* embryo production which is the primary factor that limits the use of super-stimulation protocols with Follicle Stimulating Hormone. So super stimulation has many advantages not in terms of recruitment of more number of follicles and COCs retrieved for OPU but also the expression of certain genes that increase the efficiency of *in vitro* embryo production especially in the Bos indicus cattle.

REFERENCES

Aller, J.F., Mucci, N.C., Kaiser, G.G., Callejas, S.S. and Alberio, R.H. (2012). Effect of repeated eCG treatments and ovum pick-up on ovarian response and oocyte recovery during early pregnancy in suckling beef cows. *Anim. Reprod. Sci.* **133(1-2)**: 10-15.

Berglund (2008). Genetic improvement of dairy cow reproductive performance. *Reprod. Domest. Anim.* **43**: 89-95.

Demissie, T., Yilma, T., Degefa, T., Wirtu, G. and Lemma, A. (2021). Effect of follicular ablation and gonadotropin priming on the recovery and quality of oocytes in Boran cows. *Int. J. Vet. Sci. Res.* **7(2)**: 138-143.

De Roover, R., Genicot, G., Leonard, S., Bols, P. and Dessy, F. (2005). Ovum pick up and *in vitro* embryo production in cows superstimulated with an individually adapted super-stimulation protocol. *Anim. Reprod. Sci.* **86(1-2)**: 13-25.

Ferre, L.B., Kjelland, M.E., Strobech, L.B., Hyttel, P., Mermillod, P. and Ross, P.J. (2020). Recent advances in bovine *in vitro* embryo production: reproductive biotechnology. *Hist. Meth. Animal.* **14(5)**: 991-1004.

Ginther, O.J., Siddiqui, M.A.R., Baldrighi, J.M. and Araujo, E.R. (2017). An intra-ovarian mechanism that enhances the effect of an FSH surge on recovery of subordinate follicles in heifers. *Dom. Anim. Endo.* **59**: 67-74.

Hayden, C.B., Sala, R.V., Absalon-Medina, V.A., Motta, J.C., Pereira, D., Moreno, J.F. and Garcia Guerra, A.(2022). Synchronization of follicle wave emergence before ovarian super stimulation with FSH and ovum pick-up improves *in vitro* embryo production in pregnant heifers. *Theriogenol*. **188**: 71-78.

Sendag, S., Cetin, Y., Alan, M., Hadeler, K.G. and Niemann, H. (2008). Effects of eCG and FSH on ovarian response, recovery rate and number and quality of oocytes obtained by ovum pick-up in Holstein cows. *Anim. Reprod. Sci.* **106(1-2)**: 208-214.

Srimannarayana (2019). Optimization of oocyte retrieval through trans vaginal ovum pick-up in Ongole (Bos *indicus*) cows. M.V.Sc Thesis submitted to Sri Venkateshwara Veterinary University, Tirupati, AP.

Vennapureddy, S., Arunakumari, G. and Reddy, K.C. (2022). Effect of FSH stimulation of ovaries on *in vitro* maturation of sahiwal oocytes collected by ovum pick-up (OPU) method. *Indian J. Anim. Res.* **1**: 6.

Venkatakrishna. (2021). Comparative efficacy of ovum pick-up (OPU) protocol on *in-vitro* embryo production in Ongole cows. M.V.Sc Thesis submitted to Sri Venkateshwara Veterinary University, Tirupati, AP.

Viana, J. (2019). Statistics of embryo production and transfer in domestic farm animals. *Embryo Technol. Newsletter.* **36(4)**: 17.