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Research Article

EFFECT OF CIDR AND FSH SUPERSTIMULATION ON FOLLICULAR DEVELOPMENT AND OOCYTE RECOVERY RATE BY OVUM PICK UP METHOD IN SAHIWAL COW

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ABSTRACT

The present study was conducted to assess the effect of CIDR and FSH superstimulation prior to ovum pick-up (OPU) on follicular development and oocyte recovery rate in Sahiwal cows maintained at Livestock farm complex, College of Veterinary Science, Korutla, Telangana under ET and IVF Project, Rashtriya Gokul Mission (RGM). The Sahiwal cattle were divided into two groups - Group 1 (Non-stimulated, n = 12) and Group 2 (Stimulated, n = 12). Group 1 cows were subjected to OPU at random stage of estrus cycle whereas Groups 2 cows were superstimulated with CIDR and FSH (Controlled Internal Drug Release device+Follicle Stimulating Hormone) prior to OPU. The total and mean number of follicles obtained were significantly ($p< 0.05$) higher in stimulated group (313 and 26.08±2.20, respectively) with increased mean number of medium and large sized follicles when compared to non-stimulated group (96 and 8±0.58, respectively). The total and mean number of cumulus oocyte complexes (COC) recovered were significantly ($p< 0.05$) higher in stimulated group (190 and 15.83±1.21, respectively) with increased mean number of very good, good and fair quality COCs when compared to the non-stimulated group (60 and 5 ± 0.70, respectively). However, the recovery rate was found to be similar in both the groups. Therefore, it can be concluded that super stimulation with CIDR+FSH prior to OPU in Sahiwal cows is a better alternative to obtain increased number of follicles and oocytes, further can be helpful for enhancing *in vitro* embryo production.

Keywords: CIDR, Folltropin-V, FSH, OPU, Sahiwal, Superstimulation

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Sahiwal is one of the best dairy breeds in India and Pakistan due to its unique triats like tick-resistant, heat-tolerant, high milk production and noted for its high resistance to parasites, both internal and external. There is a 6% decline in the total Indigenous (both descript and non-descript) cattle population in India over previous census (Livestock Census, 2019). This decline emphasizes the need for developing long term strategies for its conservation. Hormonal superstimulation-ovum pickup *in vitro* Embryo Production (OPU-IVEP) in combination with embryo transfer (ET) can be a viable alternative to conserve breeds, increase milk production and at the same time ensure faster multiplication of superior germplasm.

The transvaginal ultrasound-guided follicular aspiration (TVUFS) technique proves to be a useful tool in the production of cattle embryos *in vitro* (Pontes *et al.*, 2009) and help in the attainment of a large number of embryos (Galli *et al.*, 2001) and more number of calves per unit of time, which can be four to five times as large as with conventional ET (Gordon, 2004). In order to increase production rates, different protocols of hormonal superstimulation on donor animal before OPU have been described in the literature to further improve *in vitro* embryo production.

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Different hormones used for super stimulation are GnRH (Bordignon *et al.*, 1997), FSH (Vieira *et al.*, 2014; Sakaguchi and Nagano, 2020) and eCG (Jeyakumar, 2004; Aller *et al.*, 2012). The control of ovarian follicular dynamics with progesterone device and the treatment with exogenous gonadotropins provides an opportunity to increase and optimize oocyte recovery from genetically superior animals (Vieira *et al.*, 2014; Ongaratto *et al.*, 2015). Therefore, experiment was designed to test the effect of CIDR and FSH superstimulation prior to the OPU over non-stimulation on follicular development and oocyte recovery rate in Sahiwal cow.

MATERIALS AND METHODS

The present study was undertaken on Sahiwal cattle aged 3-6 years, maintained at Livestock Farm Complex under ET and IVF Project (RGM), Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Korutla, Telangana during the period between October 2020 and November 2021.

Irrespective of stage of estrus cycle, the Sahiwal cattle were divided into two groups - Group 1 (Non-stimulated, n = 12) and Group 2 (Stimulated, n = 12). Group 1 cows were subjected to ovum pick up (OPU) at random stage of estrus cycle whereas, in Group 2, CIDR

was inserted per-vaginally on day 0 in each animal at random stage of estrous cycle. After 96 hrs of CIDR insertion, all animals in Group 2 were administered with 200 mg of follicle stimulating hormone (FSH, Folltropin-V)- I/M in 8 decreasing doses (40, 40; 30, 30; 20, 20 and 10,10 mg, 12 hrs apart) on day 4, 5, 6 and 7. Each vial of freeze dried Folltropin-V (VetoquinolInc., Canada) contains porcine follicle stimulating hormone (pFSH) 400 mg, which was diluted with 20 ml of normal saline. After 36 hrs of last FSH injection (coasting period) CIDR was removed, ovum pickup was done with transvaginal

Table 1

Mean follicular population for aspiration per cow per session in non-stimulated and stimulated group of cows

S.No	Groups	No. of animals per group	Total no. of follicles for aspiration	Mean no. of follicles for aspiration (Mean ± SE)
1.	Non stimulated	12	96	8±0.58[a]
2.	Stimulated	12	313	26.08 ± 2.20[b]

Values bearing different superscripts (a,b) within a column differ significantly ($p < 0.05$). SE= Standard Error

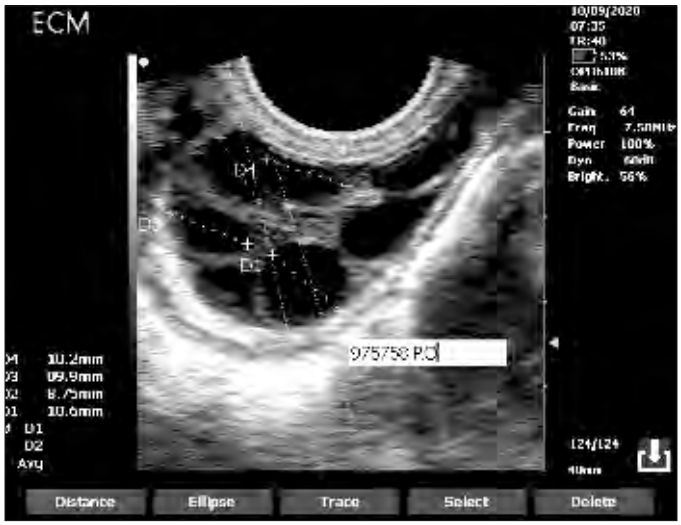

Fig. 1. CIDR + FSH stimulated ovary showing follicles of various sizes

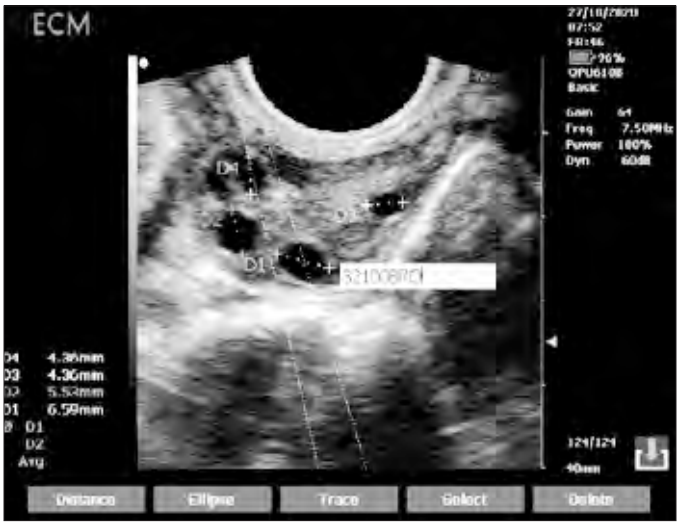

Fig. 2. Non-Stimulated ovary showing follicles of various sizes

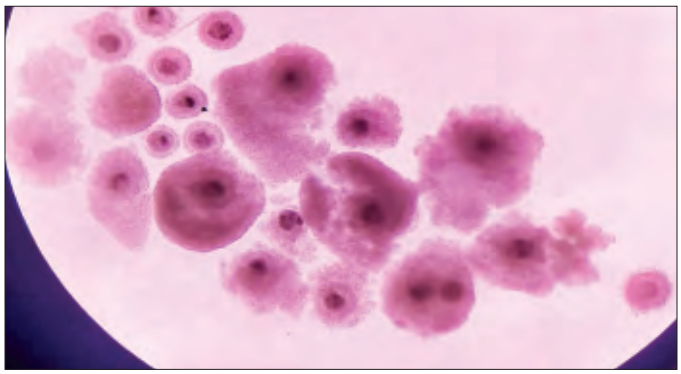

Fig. 3. COCs retrieved from stimulated ovary

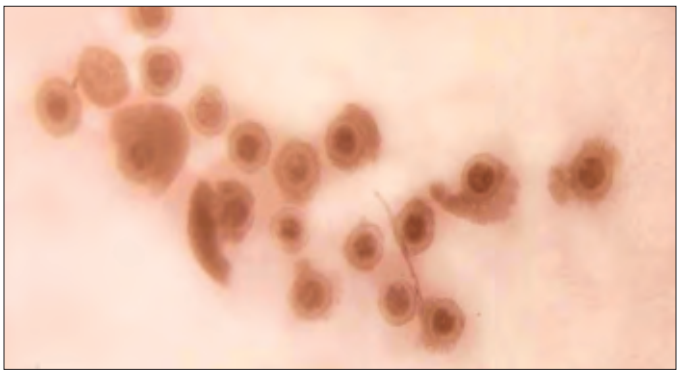

Fig. 4. COCs retrieved from non-stimulated ovary

Table2

Follicle size distribution in non-stimulated and stimulated group of cows

Follicular size	Non-stimulated		Stimulated	
	Total no. of follicles	Mean number of follicles (Mean ± SE)	Total no. of follicles	Mean number of follicles (Mean ± SE)
Small (<4 mm)	42 (43.75%)	3.5 ± 0.32[a]	49 (15.65%)	4.08 ± 0.54[a]
Medium (4-8 mm)	30 (31.25%)	2.5 ± 0.22[a]	137 (43.76%)	11.41 ± 0.95[b]
Large (>8 mm)	24 (25%)	2 ± 0.26[a]	127 (40.57%)	10.58 ± 1.08[b]

Values bearing different superscripts (a,b) within a row differ significantly ($p < 0.05$). SE= Standard Error

Table 3

Oocyte recoveryper cow per session in non-stimulated and stimulated Sahiwal cows

S.No.	Groups	No. of animals per group	Total no. of follicles for aspiration	Total no. of oocytes recovered	Mean no. of oocytes recovered (Mean ± SE)	Recovery rate (%)
1	Non stimulated	12	96	60	5 ± 0.70^{a}	62.50
2	Stimulated	12	313	190	15.83 ± 1.21^{b}	60.70

Values bearing different superscripts within a column (a,b) differ significantly ($p< 0.05$). SE= Standard Error

Table 4

Oocyte quality in non-stimulated and stimulated Sahiwal cows

Groups	Total no. of oocytes recovered	Oocyte grade							
		Very good		Good		Fair		Poor	
		Total no. of oocyte (RR%)	Mean no. of oocytes (Mean ± SE)	Total no. of oocyte (RR%)	Mean no. of oocytes (Mean ± SE)	Total no. of oocyte (RR%)	Mean no. of oocytes (Mean ± SE)	Total no. of oocyte (RR%)	Mean no. of oocytes (Mean ± SE)
Non stimulated	60	14(23.33%)	1.16 ± 0.36^{a}	24(40%)	2 ± 0.40^{a}	21 (35%)	1.75 ± 0.39^{a}	1(1.66%)	0.08 ± 0.07^{a}
Stimulated	190	72 (37.89%)	6 ± 0.94^{b}	45 (23.60%)	3.75 ± 0.61^{b}	71 (37.36%)	5.91 ± 1.03^{b}	2 (1.05%)	0.16 ± 0.15^{a}

Values bearing different superscripts within a column (a,b) differ significantly ($p< 0.05$). RR%- Recovery Rate in percentage, SE= Standard Error. Very Good- ≥ 3 layers of cumulus cells; Good-1-2 layers of cumulus cells; Fair-oocytes with partial remnants or no cumulus cells and Poor-without cumulus cells (Warriach and Chohan, 2004)

ultrasound guided (Exago, Nanuk, 930, IMV imaging, USA) follicular aspiration with a continuous negative pressure of 70-75 mm Hg under epidural anaesthesia. On ultra-sonographic monitor the number of follicles per animal and its diameter were measured and recorded before OPU.

The follicular aspirate was transferred to the laboratory, where it was filtered with 100 µm oocyte mini filter (25458, WTA, Brazil) to make it free from blood tinge. Then the filtered follicular aspirate was examined under zoom stereomicroscope (SMZ - 1270, Nikon, Japan) to identify the cumulus oocyte complexes (COCs). The oocyte recovery rate was calculated as the number of oocytes recovered from the number of follicles aspirated for each cow expressed as a percentage (Goodhand *et al.*, 2000) and grading of oocytes was done based on cumulus cells surrounding it (Warriach and Chohan, 2004) was also done.

Statistical analysis: Analysis of data was carried out using descriptive analysis and student's t-test as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The total and mean number of follicles available per aspiration (Table 1) were significantly ($p< 0.05$) higher in CIDR + FSH stimulated group (Fig. 1) when compared to the non-stimulated group (Fig. 2). On contrary to the present study, Vieira *et al.* (2014) showed no significant difference with total number of follicles among stimulated and non-stimulated group.

The mean number of medium and large follicles (Table 2) were significantly ($p< 0.05$) higher in stimulated group than non-stimulated group. However, there was no significant difference among the mean number of small follicles in both the groups. da Silva *et al.* (2017) and de Carvalho *et al.* (2019) had shown similar results, however Vieira *et al.* (2014) results were contrary to present results. CIDR used in superstimulation treatment group creates a high transient progesterone level during FSH administration and multiple dose of exogenous FSH causes ovarian stimulation and significantly increases the follicular response and oocyte recovery (Chaubal *et al.*, 2007). Srimannarayana (2019) reported that the higher follicular count in superstimulated group can be due to increased recruitment and growth of follicles from reserve pool following FSH treatment.

The total number of cumulus oocyte complexes (COCs) recovered in non-stimulated and stimulated group were 60 and 190, respectively with a recovery rate of 62.50% and 60.70%, respectively (Table 3). The mean number of oocytes recovered per animal was significantly ($p<0.05$) higher in stimulated group (Fig. 3) compared to the non-stimulated group (Fig. 4). The mean number of very good, good and fair grade oocytes recovered (Table 4) were significantly ($p< 0.05$) higher in stimulated group when compared to non-stimulated. However, the mean number of poor grade oocytes recovered was not significantly different among both the groups.

Similar to present results de Carvalho *et al.* (2019) showed that buffalo donors treated with progesterone device + FSH had a greater viable oocytes recovery rate. On contrarily, Vieira *et al.* (2014) and Ongaratto *et al.* (2015) concluded that, insertion of a CIDR device during the super stimulation treatment prior to OPU did not improve the number of COCs recovered nor their quality. The increase in very good, good and fair grade oocytes in stimulated group could be affected by transducer type, puncture frequency, OPU regimen, treatment with FSH/PMSG, combination of needle gauge and vacuum pressure etc. (Hashimoto *et al.*, 1999, Bols *et al.*, 2004, Jeyakumar, 2004; da Silva *et al.*, 2017).

CONCLUSION

In present study, superstimulation with CIDR + FSH increases the total and mean number of follicles per aspiration with increased number of medium and large follicles, also improves total number of COCs recovered with more number of very good, good and fair grade oocytes when compared to non stimulated animals. Therefore, superstimulation with CIDR + FSH prior to OPU in Sahiwal cows is a better alternative to obtain increased number of follicles; improved quality oocytes and further can be helpful for enhancing *in vitro* embryo production.

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