The Haryana Veterinarian (March, 2023) **62(SI),** 1-4 ISSN 0033-4359 (P) **Research Article**

EFFECT OF DIFFERENT SYNCHRONIZATION AND SUPEROVULATION PROTOCOLS ON NUMBER AND QUALITY OF OOCYTES RETRIEVED THROUGH ULTRASOUND GUIDED TRANSVAGINAL OVUM PICK UP TECHNIQUE IN COWS

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ABSTRACT

Total 42 cows having good body condition and normal reproductive health were selected for the present study. Cows were distributed in four groups *viz*. control (n=6), synchronization (n=12), synchronization+superovulation (n=12) and superovulation (n=12). Depending upon the two different synchronization protocol and two different doses of FSH, cows were further divided into two subgroups each (n=6). Donor cows were synchronized either with GnRH-PGF2α-GnRH (GPG) and GnRH-oestradiol benzoate-GnRH (GOG). Dose of FSH *viz*. 100 mg and 200 mg was administered i/m in the cows of superovulation group. Retrieval of oocyte by ultrasound guided transvaginal follicular aspiration revealed significant increase in the number of oocytes in all treatment groups as compared to control. Furthermore, cows treated with GOG+FSH, FSH (high) and GPG+FSH recorded significantly higher number of oocytes when compared with other treatment groups. Similarly, good quality oocytes (Grade A and B) were also recovered in large numbers from these three treatment groups compared to others.

Keywords: Cows, Oestradiol benzoate, GnRH, PGF2α, Superovulation Synchronization

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The use of assisted reproductive techniques has been of great importance in livestock production. The success of several reproductive programmes is closely related to ovarian follicular development and oocyte quality. The ultrasound guided follicular aspiration technique for collection of oocytes also known as ovum pick up (OPU) was developed in the late 1980's and proved to be a useful tool for *in-vitro* production of cattle embryos (Pontes *et al.*, 2009). Transvaginal oocyte retrieval in bovines has received a major attention in the recent past due to various advantages. Ovum pickup (OPU) associated with *in vitro* embryo production (IVEP) has the potential to enhance genetic progression through the female lineage in cattle. Currently, 36.5% of embryos produced worldwide are *in vitro* derived embryos. Numerous studies have been conducted on this highly repeatable and low invasive technique with a research goal of increasing the number and quality of the retrieved oocytes. It is possible to obtain the oocytes frequently i.e. every 3 to 4 days through transvaginal follicular aspiration. (Merton *et al.*, 2003; Monteiro *et al.*, 2017). Some researchers have shown that the number of oocytes can be increased when ovaries are stimulated prior to follicular aspiration. Differences in superstimulatory therapies, such as gonadotropin preparation, total dose of gonadotropins, treatment length and timing, and the use of other hormones, have been linked to ovarian response variability and the recovery and quality of oocytes through ovum pick up. Understanding of the follicular dynamics and application of exogenous gonadotropins are two important tools that offer opportunities to maximize the number of oocytes from superior cows (Chaubal *et al*., 2006). However, variation in response to superovulation is still a problem for ovum pick-up (OPU) in cattle (Sendag *et al.*, 2008).

The aim of the present work was to study the ovarian response by applying different synchronization protocol and superovulatory treatment in terms of oocyte yield and quality of oocytes collected by USG guided OPU.

MATERIAL AND METHODS

Total forty-two donors indigenous cows of mix breed completed at least 60 days post-partumand having good body condition (Body score >2.5) and normal reproductive health were selected for the present study. Selected cows were distributed in four major groups viz. Control (without synchronization or superovulation) (n=6), Group-II (synchronization) (n=12), Group-III (synchronization + superovulation) (n-12) and group-IV (superovulation only) (n=12). Group-II cows were further divided into two subgroups (n=6) *viz*. GnRH (0 day)-PGF2α (7 day)-GnRH (9 day)(GPG)and GnRH (0 day) - Oestradiol benzoate (7 day) GnRH (9 day) (GOG). Similarly, the cows from Group III were divided into two

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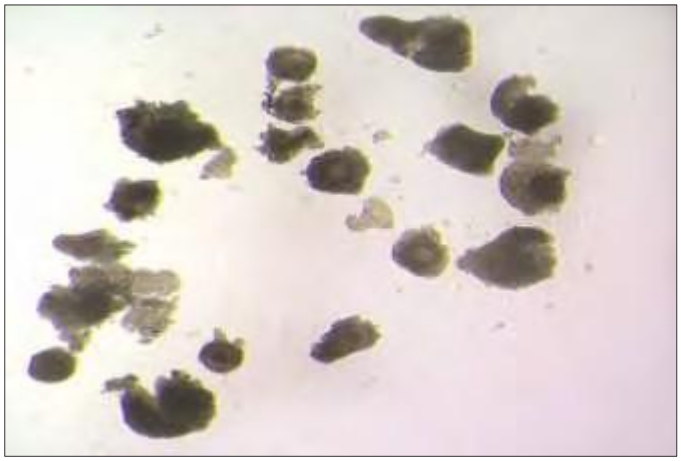
subgroups (n=6) and synchronized with similar protocol described under Group II and additionally superovulated with follicle stimulating hormone (FSH) @ 200 mg each. Group –IV cows were superstimulated with two different doses of FSH; @100 mg (low) and 200 mg (high). The synchronization protocol was initiated on day 0 (1st dose of GnRH), day 7 (PGF2α) and day 9 (2nd dose of GnRH). The superovulation with FSH was done on day 13,14 and 15 with decreasing doses and OPU was undertaken on day 17. OPU was also undertaken in the cows from Control group. All the donors cows were restrained in a Travis specially designed for OPU. Before oocyte retrieval the cows were given epidural anaesthesia with Lignocaine hydrochloride @ 5 ml/animal. A transvaginal probe of 7.5 MHz of Exago IMV (France) having oocyte aspiration assembly attached to it which was connected with aspiration pump (WTA, Brazil) and 50 ml conical aspiration tube was used for oocyte aspiration. The follicles were aspirated by using 18 Gdisposable needle in OPU media (IVF Biosciences, UK). Follicles of atleast ≥ 5 mm diameter were aspirated with pressure of 70 mm Hg. Total 250 follicles were aspirated from the Donor cows of all the groups. The data pertaining to the number of follicles aspirated is presented in Table 1.

The oocytes with OPU media was filtered through 0.75 µm embryo filter with Euroflush media (IMV, France) in petri dish and placed it under the stereo zoom microscope for counting the total number of oocytes and gradation. Depending upon the layers of cumulus cells the oocytes were graded as A (> three layers of cumulus), B (two-three layers of cumulus) and C (one-two layer of cumulus). The data generated was analyzed with standard statistical procedure.

RESULTS AND DISCUSSION

The data pertaining to the number and quality of oocytes retrieved is summarized in the Table 2 and 3. The data presented in the tables revealed that the mean number of oocytes retrieved from the cows of Group II, Group III and Group IV was 5.41±2.20, 8.00±3.26 and 6.75±2.75, respectively. Similarly, the mean number of oocytes collected from subgroups II A, II B, III A, III B, IVA and IV B were recorded as 6.33±2.58, 4.50±1.83, 7.33±2.99, 8.66±3.53, 5.16±2.10 and 8.33±3.40, respectively. However, very less number of oocytes (mean 1.33±0.54) retrieved from control group cows. Analysis of data further revealed that the mean number of oocytes recorded in treated groups was significantly more ($P<0.05$) than the cows received no treatment. Similarly, donor cows of all treated subgroups recorded significantly more number of



Trans-vaginally aspirated immature oocytes

oocytes ($P<0.01$) when compared with cows of control group.Furthermore, cows treated with GOG+FSH, FSH (high) and GPG+FSH recorded significantly higher ($P<0.05$) number of oocytes when compared with other treatment groups. On comparison between the subgroups, the number of oocytes recovered from cows treated with high dose was significantly more ($P<0.01$) than low dose. However, no significant difference was noticed between the number of oocytes of GOG and GPG synchronization subgroup and GPG+FSH and GOG+FSH subgroups. This shows that the donor cows subjected to synchronization of follicular wave and superovulation had a greater impact on recruitment and growth of ovarian follicles.

Out of total oocytes retrieved, cows synchronized with GnRH, PGF2α and OB collectively yielded 65 oocytes, cows synchronized and further superstimulated with FSH yielded 96 oocytes and those which are not synchronized but superstimulted with FSH including low and high dose recorded 81 oocytes.

Distribution of oocytes according to sub-group revealed that highest number of oocytes were retrieved from the cows treated with GOG + FSH and FSH with high dose (200 mg). Cows treated with GPG+FSH, GPG and FSH with low dose (100 mg) also showed recovery of oocytes with substantial number. However, very less number of oocytes could have retrieved from the donors of control group. Gradation of oocytes showed that more number of Grade A oocytes were obtained from the cows treated with FSH with high and low dose, GPG+FSH and GOG+FSH. However, donors treated with GPG and GOG alone yielded relatively less number of Grade A oocytes as compared to earlier groups. Interestingly more number of Grade B oocytes were recovered from GOG+FSH group cows followed by the cows treated with GPG, FSH with high dose, GPG+FSH, FSH with low dose and GOG alone.

Table 1

Number (mean±SE) of ovarian follicles in the cows of different groups before and after treatment

Groups	Sub-Group	Treatment		Pooled mean sub-group	Pooled mean groups
		Before	After		
Group I	A	2.83±1.15[a]	8.50±3.40[b]	5.66±2.31	5.12±2.09
	B	2.83±1.15[a]	6.33±2.58[b]	4.58±1.86	
Group II	A	3.00±1.22[a]	9.16±3.73[bc]	6.08±2.48	5.96±2.43
	B	2.16±0.88[a]	9.50±3.87[bc]	5.83±2.38	
Group III	A	2.17±0.88[a]	7.00±2.85[b]	4.58±1.86	5.29±2.15
	B	3.00±1.22[a]	9.00±3.67[bc]	6.00±2.44	

a, b Different superscripts in a row indicate significantly different values
b, c Different superscripts in a column indicate significantly different values

Table 2

Number of oocyte (mean, ±SE) recovered from donor cows of Controland different treatment groups (Synchronization and/or superovulation)

Groups	Pooled mean sub-group	Group Mean
Control	1.33±0.54[a]	1.33±0.54[a]
Group I A (GPG)	6.33±2.58[b]	5.41±2.20[b]
Group I B (GOG)	4.50±1.83[b]	
Group II A (GPG +FSH-P)	7.33±2.99[bc]	8.00±3.26[b]
Group II B (GOG+ FSH-P)	8.66±3.53[bc]	
Group III A (FSH-P-100 mg)	5.16±2.10[b]	6.75±2.75[b]
Group III B (FSH-P-200 mg)	8.33±3.40[bc]	

(a,b,c) Means in the same column differed significantly (P<0.05; P<0.01)

Table 3

Gradation of oocytes recovered from donor cows of Control and different treatment groups (Synchronization and/or superovulation)

Groups	OPU retrieved oocytes			Total
	Grade A	Grade B	Grade C	
Control	2	3	3	8
Group I A (GPG)	18	16	4	38
Group I B(GOG)	17	7	3	27
Group II A (GPG +FSH-P)	27	10	7	44
Group II B (GOG+ FSH-P)	22	24	6	52
Group III A (Low Dose 100 mg)	22	9	0	31
Group III B (High Dose 200 mg)	30	14	6	50

Actually gonadotropin releasing hormone is secreted by the hypothalamus which stimulate the anterior pituitary to release the FSH. Follicle stimulating hormone is a gonadotropin hormone commonly used for superovulation in cattle for embryo production in large numbers. Normally, it releases from the anterior pituitary in pulsatile manner and responsible for the follicular development. Exogenous administration of FSH stimulate the growth of more number of follicles within the estrus cycle that reaches to the stage of preovulatory follicle. Prostaglandin F2α is secreted by the myometrium in normal course when there is no fertilization. PGF2α is luteolytic in nature and causes the regression of corpus luteum. The follicular development is normally initiated after the luteolysis. Similarly, the Oestradiol 17α has also got some luteolytic activity and promote the further growth of recruited follicles.

Recovery of more number of oocytes has always remained a driving force for researchers to try various techniques and hormonal pre-stimulation. In the present study 5-6-fold increase was observed in number of oocytes after pre-stimulation of FSH and Gonadotropin releasing hormone (GnRH). The findings of the present study corroborated with Sendag *et al.* (2008) who recorded more number of oocytes (10.3±5.0) as well as grade A oocytes per Holstein cow treated with FSH-Pas compared to eCG.

Present finding is in partial agreement with Goodhand *et al.* (1999) who observed significantly more number of follicles of around 6-10 mm size and also A grade oocytes after pre-stimulation with FSH than untreated heifers. However, the oocyte recovery rate was reported as significantly lower in FSH treated heifers. Fernandes *et al.* (2020) recorded significantly more number of oocytes retrieved from control group (22.2±1.5) as compared to those treated with FSH (16.9±1.4) in Nellore breed of cows. Thus present finding is not in agreement with Fernandes *et al.* (2020) as mean number of oocyte recorded in control group cows was very poor (1.33±0.54) during present study. The existing variation may be due to difference in genetic make-up and nutritional base of the donor cows.

Malhi *et al.* (2008) studied the effect of coasting period and LH administration alongwith FSH on oocyte recovery and reported that four doses of FSH and longer coasting effect (48 hrs vs 33 hrs) resulted in greater percentage of 5-10 mm follicles with recovery of 8.5 and 11.0 mean number of oocytes with and without supplementation of LH, respectively. In present study the coasting period was 52 hrs which might have led to increase in the number of medium sized follicles and subsequently the recovery of large number of oocytes particularly in the group of cows treated with FSH. The results of present study are thus comparable with Malhi *et al.* (2008).

Petrovas *et al.* (2020) studied the effect of two different doses of FSH in buffaloes which further subjected to OPU with different coasting time and reported that the dose of 240 mg FSH was more effective than 120 mg as significantly more number of oocytes as well as good quality of oocytes were recovered with dose of 240 mg FSH. The results obtained with higher dose of FSH in the present study corroborates with Petrovas *et al.* (2020). Getz *et al.* (2011) tried different synchronization protocols *viz*. PGF2α with and without removal of dominant follicle and Crestar implant + injection along with FSH in Simmental heifers and observed no significant difference in number of follicles and recovery of oocytes. Goodhand *et al.* (2000) observed that treatment with steroid had no significant effect on number and quality of oocytes, however, administration of FSH significantly increased the number of medium and large size follicles and grade 1 oocytes. The report further stated that the total number of oocytes were also increased from no FSH to single or multiple doses of FSH. Thus the results of present study are in partial agreement with Goodhand *et al.* (2000).

In present study the number and good quality of oocytes harvested was comparatively more in the cows pre-stimulated with FSH@200 mg with or without synchronization protocol. This shows that the FSH stimulated the ovarian activity more effectively, without compromising the oocyte quality than single dose (Walsh *et al.*, 1993). The better oocyte quality may be associated with increased follicle size and development potential of oocyte following FSH treatment.The use of treatment that synchronize follicle wave emergence and superovulation with FSH is useful to increase the recovery and quality of oocyte. It is concluded that synchronization of donor cows with GPG and GOG can have increased the recovery of total no. of oocytes, however, the use of FSH @ 200 mg is much beneficial to increase the number as well as quality of oocytes.

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