Full Length Article

EFFECT OF FSH PRE-STIMULATION ON OOCYTE RECOVERY IN ONGOLE (*Bos Indicus*) COWS

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ABSTRACT

The present study was aimed to assess the efficacy of oocyte retrieval through transvaginal ovum pick-up (OPU) in Ongole (Bos indicus) cows. Cows (n=18) were divided in to two equal groups; cows in group 1 cows were subjected to two OPU sessions (OPU1 and OPU2) at 96 h interval irrespective of the stage of estrus cycle. Cows of group 2 were subjected to FSH pre-stimulation before OPU 1 followed by OPU 2, 96h later. Thus, a total of 36 OPU sessions were performed on 18 animals. The number of follicles available for aspiration (17.89 ± 1.78 vs 27.06 ± 1.75), number of medium (4 – < 8 mm; 4.11 ± 0.69 vs 16.00 ± 1.76) and large follicles (≥ 8mm; 1.06 ± 0.23 vs 6.33 ± 0.79), follicles aspirated (11.95 ± 1.42 vs 17.45 ± 2.07), COCs recovered (5.72 ± 0.78 vs 10.06 ± 1.78), and viable COCs collected (4.23 ± 0.67vs 8.34 ± 1.79) were significantly higher in group 2 than in group 1.The mean number of follicles aspirated, the mean oocyte recovery and the viable oocytes collected were significantly higher at OPU 1 in both the groups than at OPU 2. It was concluded that pre-treatment with FSH increased the OPU efficiency in terms of oocyte yield and viable oocytes collected. OPU 2 performed at shorter interval after OPU 1 is not advantageous due to limited number of follicles available for aspiration at OPU 2 and consequently, reduced oocyte recovery.

Key words: *Bos indicus*, OPU, FSH pre-treatment, oocytes, Ongole

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INTRODUCTION

Ovum Pick-up – *In-vitro* Embryo Production (OPU – IVEP) in combination with Embryo Transfer (ET) is a viable technique to conserve breeds and at the same time ensure faster multiplication of superior germplasm. The technique of Transvaginal ultrasound-guided follicular aspiration for OPU originally developed for assisted reproduction in humans was suitably modified for use in bovines (Pieterse *et al*., 1988). Over the last decade, OPU and IVP for production of embryos from elite animals have become increasingly used in the cattle breeding industry (Bousquet *et al*., 1999 and Faber *et al*., 2003). Increasing the usable oocyte yield per aspiration is the key for production of more viable embryos. Efforts to enhance oocyte recovery through once vs twice weekly aspiration (Gibbons *et al*., 1994 and Li *et al*., 2007) and pre-treatment with FSH / eCG (Gibbons *et al*., 1994 and Chaubal *et al*., 2006) produced equivocal results**.** Though initial work on OPU in Indian cattle was reported by Manik *et al*. (2003), the information on the influence of hormonal pre-treatment and collection schedule on the oocyte recovery rate and their quality was scanty. The present study was therefore conducted to assess the effect of FSH pre-stimulation on OPU efficacy in Ongole (*Bos indicus*) cows. Two OPU sessions performed at 96h interval were also compared and evaluated in respect of oocyte yield and quality.

MATERIALS AND METHODS

Multiparous, lactating / dry, normally cyclic Ongole cows (*Bos indicus*) aged 6-14 years (n=18) and weighing between 400 and 500 kg body weight were selected to perform oocyte aspiration. All the animals were kept in a loose housing system with a large, open paddock for free movement. 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 with *adlibitum* of water. The cows were randomly divided into two groups. i) Group 1 (non-stimulated, n = 9); Cows were subjected to OPU twice (OPU1 and OPU2) at 96 hrs interval at random stage of estrous cycle and ii) Group 2 (Stimulated, n = 9); Cows were administered GnRH analogue (Buserelin acetate; 10 µg; i/m) at random stage of estrous cycle followed by FSH stimulation (Stimufol, 225 µg i/m in 3 divided doses - 125, 75 and 25 µg) at 48, 60 and 72 h after GnRH administration. OPU1 was carried out 24 hrs after the last FSH injection followed by OPU2, 96h later. A total of 36 OPU sessions were performed in 18 animals with and without FSH pre stimulation.

Ovum pick-up equipment consisted of ultrasound scanner (Mylab Gamma Vet, Esaote, Genova, Italy) with a multi frequency (4-9 MHz) Micro convex transducer (SC 3123, Esaote) housed in a plastic probe carrier equipped with a needle, needle guide and aspiration line (WTA, São Paulo, Cravinhos, Brazil) which was connected to a foot operated vacuum pumpin which vaccum was set at 61-65 mm Hg (V-MAR 5000, Cook Australia Pvt. Ltd., Australia). OPU was performed as per the procedure described by Vieira *et al*. (2014). Cows were restrained and epidural anaesthesia was induced (3 - 5 ml of 2% Lignocaine hydrochloride). The probe was advanced into the anterior vagina and then the ovary was positioned against the probe head to obtain a clear image of the follicle on the ultra sound monitor. The numbers of follicles per ovary were recorded and the diameters of the follicles were measured by freezing the image using the inbuilt calliper (Nagai *et al*., 2015). Based on the diameter the follicles were classified as small (<4mm), Intermediate (4 -<8mm) and large (≥8 mm) (Ginther *et al*., 1989). The aspiration needle was advanced into the antrum of the targeted follicle and the follicular fluid was aspirated into a conical tube containing prewarmed (37^{0}C) OPU recovery medium (IVF Bioscience, Denmark). Successful aspiration was confirmed by the collapse of the fluid filled follicle. The follicular aspirate was transferred to a square grid petri dish and screened for cumulus oocyte complexes (COCs) under zoom stereo microscope (SMZ - 1000, Nikon, Japan) at 20x magnification. Under 63x magnification, the quality of COCs were classified into five quality grades (A, B, C, D and E) and further grouped in to viable (grade A + B + C) and non-viable (grade D + E) categories based on the oocyte integrity, homogeneity of cytoplasm and the quantity of the cumulus cell layer surrounding the oocyte, (Looney *et al*., 1994). All the visible follicles were aspirated in each OPU session in both groups. Size distribution of follicles, aspiration rate (number of follicles aspirated / total number of follicles), oocyte recovery rate (number of oocytes recovered / number of follicles aspirated) and oocyte quality were recorded for individual cows. The data was analysed using student t-test and ANOVA as per Snedecor and Cohran (1994).

RESULTS AND DISCUSSION

The follicular aspiration parameters and oocytes recovery parameters were presented in the Table. The overall mean number of follicles available for aspiration at the time of OPU irrespective of the sessions was significantly (P<0.05) higher in Group 2 (27.06 ± 1.75) than in Group 1 (17.89 ± 1.78). The follicle count was significantly higher during OPU 1 and non-significantly higher during OPU 2 in Group 2 than in Group 1. The data indicated that the higher follicular count in group 2 is presumably due to increased recruitment and growth of follicles from reserve pool following FSH treatment, which was in agreement with the reports of Jeyakumar (2004). On the contrary Stubbings and Walton (1995) reported that the overall mean number of follicles available for aspiration was not different between unstimulated (15.7 ± 3.3) and FSH stimulated cows (14.2 ± 1.9); they observed a decline in the number of follicles to aspirate in the FSH stimulated cows and they postulated that the elevated progesterone levels after FSH treatment and an associated negative feedback could be the probable reason for the decline in the number of follicles.

The data on the follicular size distribution in the present study revealed that small follicles (<4mm) were significantly higher in unstimulated cows compared to stimulated cows. However medium and large follicles were significantly lower in unstimulated cows compared to stimulated cows. In unstimulated cows (group 1), the small, medium and large follicles did not vary between OPU 1 and OPU 2. On the other hand, in the stimulated cows (group 2) the small follicles were lower at OPU 1 than OPU 2, whereas, the medium and large follicles were higher at OPU 1 compared to OPU 2.In agreement with this study Vieira *et al.* (2014) and Silva *et al.* (2017) reported in Nelore (*Bos indicus*) donors more number of medium sized follicles and less number of small sized follicles in FSH treated cows compared to untreated animals. Good hand (2000) also reported that treatment with FSH resulted in seven fold increase in the number of medium sized follicles (1.7 ± 0.5 vs 13.7 ± 1.43, respectively, in non-stimulated vs stimulated cows) and threefold increase in number of large follicles (0.6 ± 0.13 vs 2.0 ± 0.23 respectively) with a corresponding decrease in the number of small sized follicles (15.3 ± 1.33 vs 6.9 ± 0.91 respectively).

In the present study, the oocyte recovery rate and mean oocytes recovered respectively were higher in stimulated group (57.64% and 10.06 ± 1.78) than in unstimulated group (47.91% and 5.72 ± 0.78).

More oocytes were collected at OPU 1 (7.89 ± 1.05 in group 1 and 16.89 ± 1.16 in group 2) than at OPU 2 (3.56± 0.56 in group 1 and 3.22 ± 0.62 in group 2) (Table) in both the groups. Also group 2 cows had significantly more oocytes recovered at OPU 1 (16.89 ± 1.16) than in group 1 (7.89 ± 1.05). However, the oocyte recovery did not differ between groups at OPU 2.The average yield of oocytes per cow per session was reported to be 5 – 8 (Hendriksen *et al*., 2004) but varies significantly between donor cows ranging from 0 - 26 oocytes per collection (Wagtendonk - De Leeuw, 2006). Different extrinsic (Bols, 1997) and intrinsic factors (Lonergan *et al*., 2003 and Rizos *et al*., 2005) can interfere with oocyte recovery rate. High individual donor variation in the mean oocyte recovery was observed in this study in both group 1 (2-12) and group 2 (1-24). Concomitantly, the viable oocytes collected also showed great individual variation in both the group of cows (1-11 in group 1 and 0-24 in group 2). Studies on zebu females suggest that individual variation in the number of oocytes obtained from OPU was correlated with the expression of GDF9, BMP15 and FGF8 genes (Biase *et al*., 2008). These authors reported an increase of 2.26 oocytes considering only the effect of FGF8 and increase of 7.36 oocytes when all the genes were considered together. Other factors that might cause individual variation in oocyte yield were age (Sartori *et al*., 2004), parity (Lucy *et al*., 1991 and Ginther *et al*., 1996), nutritional status (Oliveira *et al*., 2002) and heat stress (Wolfenson *et al*., 1995). In general Ongole (*Bos indicus*) donors were reported to produce more number of oocytes than *Bos taurus* cows possibly due to low rates of follicular atresia in the former breed resulting in more viable antral follicles.

Classifying the COC's in to viable and non-viable, indicate that significantly higher percentage of COC's were viable in group 2 (82.87%) than in group 1 (73.79%). The mean viable COC was 4.23 ± 0.67 in group 1 and 8.34 ± 1.79 in group 2 with significance difference between groups. Irrespective of the FSH pre stimulation, the viable oocytes were significantly higher in OPU 1 (6.23 ± 0.94 in group 1 and 15.12 ± 1.42 in group 2) than in OPU 2 (2.23 ± 0.23 in group 1 and 1.56 ± 0.34

Table: Performance of Transvaginal Ovum pick-up in Ongole cows (Group Wise)

Attribute	Group 1 (Non-stimulated)	Group 2 (stimulated)
Follicle size distribution		
Small follicles per cow in OPU 1	13.98 ± 2.72^{aA}	2.33 ± 1.59^{bA}
Small follicles per cow in OPU 2	11.56 ± 2.24^{aA}	7.11 ± 1.24^{aB}
Small follicles per cow per session	**12.72 ± 1.73^{a}**	**4.72 ± 1.13^{b}**
Medium follicles per cow in OPU 1	4.78 ± 1.24^{aA}	20.33 ± 2.63^{bA}
Medium follicles per cow in OPU 2	3.44 ± 0.65^{aA}	11.67 ± 1.25^{bB}
Medium follicles per cow per session	**4.11 ± 0.69^{a}**	**16.00 ± 1.76^{b}**
Large follicles per cow in OPU 1	1.22 ± 0.32^{aA}	8.33 ± 1.10^{bA}
Large follicles per cow in OPU 2	0.89 ± 0.35^{aA}	4.33 ± 0.64^{b}
Large follicles per cow per session	**1.06 ± 0.23^{a}**	**6.33 ± 0.79^{b}**
Mean no.of follicles/animal in OPU 1	22.33 ± 2.50^{a}	31.00 ± 2.43^{b}
Mean no.of follicles/animal in OPU 2	13.45 ± 1.91^{a}	19.23 ± 1.31^{a}
Mean no.of follicles/animal/session	17.89 ± 1.78^{a}	27.06 ± 1.75^{b}
Mean no.of follicles aspirated / cow in OPU1	16.44 ± 1.70^{aA}	24.33 ± 1.95^{bA}
Mean no.of follicles aspirated / cow in OPU2	7.44 ± 0.80^{aB}	10.55 ± 1.58^{bB}
Mean no.of follicles aspirated / cow / session	11.95 ± 1.42^{a}	17.45 ± 2.07^{b}
Mean Aspiration rate in OPU 1	0.77 ± 0.11^{a}	0.89 ± 0.19^{a}
Mean Aspiration rate in OPU 2	0.66 ± 0.16^{a}	0.54 ± 0.07^{a}
Aspiration rate (%)	0.72 ± 0.09^{a} (66.77)	0.72 ± 0.11^{a} (64.47)
Mean oocyte recovery rate in OPU 1	7.89 ± 1.05^{aA} (47.98)	16.89 ± 1.16^{bA} (69.41)
Mean oocyte recovery rate in OPU 2	3.56 ± 0.56^{aB} (47.78)	3.22 ± 0.62^{aB} (30.53)
Oocyte Recovery rate (%)	5.72± 0.78^{a} (47.91)	10.06 ± 1.78^{b} (57.64)
Mean viable COCs recovered / cow in OPU 1	6.23 ± 0.94^{aA}	15.12 ± 1.42^{bA}
Mean viable COCs recovered / cow in OPU 2	2.23 ± 0.23^{aB}	1.56 ± 0.34^{bB}
Mean viable COCs recovered / cow / session (%)	4.23 ± 0.67^{a} (73.79)	8.34 ± 1.79^{b} (82.87)

Values bearing different superscripts with in a row (a, b) and within a column (A, B) for a particular attribute differ significantly ($P < 0.05$)

Figures in parentheses indicate percentage in group 2) (Table). Also FSH pre stimulation resulted in significantly higher viable oocytes (15.12 ± 1.42) than without FSH pre stimulation (6.23 ± 0.94). However, viable oocyte yield was similar in both groups at OPU 2 in which there was no FSH pre stimulation. Looney *et al*. (1994), Meintjes *et al*. (1995) and Jeyakumar (2004) also reported that FSH pretreatment resulted in highest percentage of viable oocytes and grade 1-2 embryos than non-treated animals. On the contrary Silva *et al*. (2017) reported no significant improvement with FSH pretreatment on viable oocyte yield. Oocyte quality was reported to be affected by transducer type, puncture frequency, OPU regimen, treatment with FSH/PMSG, combination of needle gauge and vacuum pressure etc., (Fry *et al*., 1997, Hashimoto *et al*., 1999, Merton *et al*., 2003, Bols *et al*., 2004, Jeyakumar 2004 and Silva *et al*., 2017).

Irrespective of FSH treatment, the mean oocyte recovery and viable oocytes recovered were significantly higher at OPU1 than at OPU2 performed at 96 hrs (4 days) interval. In line with the present study, Reis *et al*. (2002) also reported more COCs and good quality oocytes collected during OPU1 than OPU2 performed at 6 days interval. The possible reason for lower oocyte recovery at OPU2 performed at shorter interval after OPU1, could be due to higher prevalence of blood-filled follicles also known as residual follicles that are formed during OPU1(Bergfelt *et al*., 1994, Petyim *et al*., 2000 and Yang *et al*., 2005). These blood-filled follicles may lead to inaccuracy in identifying follicles suitable for puncture due to similar echogenic characteristics and equal thickness of the wall, implying inadequate number of punctured follicles, less oocyte recovery and increased risk of blood clots in the needle and tubing system (Petyim *et al*., 2000). Generally, disappearance of residual follicles may take 10 days or even more and hence performing OPU at shorter intervals may lead to incomplete elimination of residual follicles making subsequent OPU more difficult.

It may be concluded that FSH treatment increased the number of follicles available for aspiration. Also FSH treated cows had higher number of medium and large follicles whereas untreated cows had more number of small follicles, which in turn contributed to recovery of more COCs and viable oocytes from FSH treated cows. The oocyte yield and good quality oocytes collected were higher during OPU1 than OPU2. Shorter interval of 96h between two sessions has negatively influenced the oocyte recovery in the 2nd (OPU 2) session.

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