Influence of Ovarian Side and Initiation Time of First Aspiration with Relation of Transvaginal Follicular Aspiration in HF x Sahiwal Cows

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Abstract

This study was carried out to observe the influence of ovarian side and initiation time of first aspiration during OPU programme in HF X Sahiwal cows. Eight cows were randomly divided in to two groups (A and B) each consisting of 4 donors. Follicular aspiration was initiated on day 4 (first follicular wave) and day 13 (second follicular wave) of estrous cycle, respectively. From group-A donors, total 200 follicles were aspirated in eight sessions with a mean of 6.25 ± 2.05 per animal per session of which 52.50 (105/200) and 47.50 (95/200) per cent follicles were from left and right ovary, respectively. The mean numbers of small, medium and large follicles aspirated were 2.06 ± 0.87, 2.65 ± 0.88 and 1.53 ± 0.91 with the recovery rate of 33.0 (66/200) (3–5 mm), 42.50 (85/200) (6–9 mm) and 24.50 (49/200) (=10 mm) per cent, respectively. From group-B donors, total 179 follicles were aspirated of which 48.60 (87/179) and 51.40 (92/179) per cent follicles were from left and right ovary, respectively. The mean number of aspirated follicles per cow per session from group-B donor cows was 5.59 ± 1.03 and the mean numbers of small, medium and large follicles aspirated 1.87 ± 0.96, 2.5 ± 0.66 and 1.25 ± 0.64 with the recovery rate of 34.07 (61/179), 44.94 (80/179) and 21.22 (38/179) per cent, respectively. The number of follicles from left and right ovaries of different categories among group A and B donor cows did not differ significantly (P>0.05). This study showed that there cows no any influence of ovarian side and initiation time on follicular aspiration.

Keywords: Ovary, Transvaginal Follicular Aspiration, Follicle, Reproductive organ.

Introduction

The mammalian ovary contains many more immature oocytes that could ever be utilized for reproductive technologies such as IVF and nuclear transfer. Ovarian oocytes are a vast resource of female gametes that could ultimately be harvested with the goal of increasing the numbers of endangered and valuable animals. If oocytes from deceased or from live animals could be rescued, the potential to produce live offspring would benefit not only researchers, but also the agricultural producers and zoological researchers attempting to save genetically superior or endangered animals. The technique of transvaginal USG guided follicle aspiration was first described in humans by Wikland and co-workers in 1987.

The technique was first employed in non-stimulated cows (Pieterse *et al*., 1988). It can be performed at any stage of the estrous cycle, two times weekly repeatedly, for a period of 3 months with no adverse effect on fertility (Pieterse *et al*., 1988). OPU is fewer dependant on the reproductive status of the donor; with use of OPU oocytes can be harvested from juvenile animals and pregnant animals in the first 3 months of pregnancy. The oocyte recovery rate is influenced by variety of factors such as, aspiration vacuum pressure, hormonal pretreatment of animals (Bungartz *et al*., 1995), frequency of puncture (Goodhand *et al*., 1999) and stage of estrous cycle (Vos *et al*., 1994). These factors make it difficult to compare recovery rate of oocytes; since different researchers follow different procedures.

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Table-1. Mean number of follicles aspirated from the ovaries of group A (First Follicular Wave) and B (Second Follicular Wave) crossbred donor cows

Group	Puncture sessions(n)	Follicle aspiration			
			Left ovary	Right ovary	Total
A First Follicular Wave	8	Number	105	95	200
		Per cent	52.50	47.5	100
		Mean± S.E	3.28± 1.11	2.96± 1.24	6.25± 2.05
B Seconc Follicular Wave	8	Number	87	92	179
		Per cent	48.60	51.40	100
		Mean± S.E	2.72± 0.62	2.87± 0.73	5.59± 1.03

This study was carried out to know whether ovarian side or initiation time of OPU i.e. during first or second follicular wave, plays any role in quantum of follicular aspiration in crossbred cows.

Materials and Methods

Oocyte Donors: Eight physiologically normal HF x Sahiwal crossbred cows to 7 years old, of SAG, Bidaj (NDDB) were selected as donor in this experiment. All selected cows were high yielder, regularly cyclic with ideal nutritional status. They were vaccinated against threatening infectious diseases regularly.

Experimental Design

Ovum Pick-up in First Follicular Wave: Group A: Four donor cows were observed for regular cyclicity and were synchronized with single dose of Prostaglandin F2α (5mL im, Illirin, Intervet GmbH, Germany). The day of oestrus after synchronization was recorded as day '0'. Oocyte aspiration from these cows was started on day 4 (first follicular wave) post-estrus (Fig 1) and subsequent OPU was made at weekly interval for 8 sessions.

Ovum Pick-up in First Follicular Wave: Group B: Four donor cows with regular cyclicity were synchronized with single dose of Prostaglandin F2α regime (as in group A) but the oocyte collection was initiated on Day 13 postestrus (second follicular wave) (Fig 2) and subsequently continued at weekly interval for 8 sessions.

Follicular Aspiration and Oocyte Collection

An epidural anaesthesia was achieved by injecting 3–4 mL of Lignocaine hydrochloride (2%) for ease of handling of the reproductive organs (mainly ovaries). All visible follicles = 3 mm were aspirated using an ultrasound machine (Aloka SSD-500, Japan) with a transvaginal convex transducer (5 MHz) having a needle guide, single lumen 18-ga 60 cm long sterile needle having an ultrasound echo tip (Cook, Veterinary Products, Qld, Australia) passed through the guide attached to the vaginal probe. The needle was connected via silicon tubing to 50 mL sterile oocyte collection tube (Tarson® INDIA) placed in electronic warmer (Cook Veterinary Products, V-FTH-2012) at 37^0C temperature on other side. The aspirants were collected in this collection tube. The vacuum pressure was maintained between 50 and 60 mmHg by vacuum pump (Cook IVF, KMAR-5100, Australia). The needle was withdrawn at intervals and rinsed with a medium Euro-Flush (IMV, France) supplemented with 12.5 µg/mL heparin (Sigma, USA).

The aspirated follicles were measured by USG facilities and categorised in three different groups as per their size; 3 to 5 mm small size, 5 to 8 mm medium size, and =10 mm large size.

The data pertaining to follicular aspiration and recovery rate of small, medium, and large follicles from left and right ovaries and in first and second follicular wave were suitably tabulated and analyzed by statistical methods (Snedecor and Cochran, 1986).

Results and Discussion

From group A donors, total 200 follicles were aspirated in eight sessions, of which 52.50 and 47.50 per cent follicles were from left and right ovary, respectively (Table 1). The mean number of aspirated follicles from group-A donor cows was 6.25 ± 2.05 per animal per session and the mean numbers of small, medium and large follicles aspirated were 2.06 ± 0.87, 2.65 ± 0.88 and 1.53 ± 0.91 with the recovery rate of 33.0, 42.50 and 24.50 per cent, respectively(Table 2).

From group-B donors, total 179 follicles were aspirated in which 48.60 and 51.40 per cent follicles were from left and right ovary, respectively (Table 1). The mean number of aspirated follicles from group-B donor cows was 5.59 ± 1.03 per animal per session and the mean numbers of small, medium and large follicles aspirated 1.87 ± 0.96, 2.5 ± 0.66 and 1.25 ± 0.64 with the recovery rate of 34.07, 44.94 and 21.22 per cent, respectively (Table 2).

The overall mean numbers of follicles aspirated per animal per session from the group A and B donors were 6.25 ± 2.05 and 5.59 ± 1.03, respectively. These results of follicle aspiration are in accordance with the study of Manik *et al*. (2003) who reported mean of 6.8 ± 0.7 follicles per session per donor in Karan fries cattle (HF X Sahiwal). However the present result was lower than the reported values of 12.4 ± 6.1 in Holstein heifers (Garcia and Salaheddine, 1998) and 12.0 ± 2.0

Table- 2: Classification of Different Size Follicles Aspirated From Group A and B Donor Cows

Groups	Follicle size			
	Small (3-5mm)	Medium (6-9mm)	Large (=10mm)	Total Follicles
Group-A	66	85	49	200
Mean ± SE	2.06± 0.87	2.65± 0.88	1.53± 0.91	6.25± 2.05
Per cent	33.00	42.50	24.50	-
Group-B	61	80	38	179
Mean ± SE	1.91± 0.46	2.50± 0.65	1.25± 0.64	5.59± 1.03
Per cent	34.08	44.69	21.23	-

in Simmental heifers (Goodhand *et al*., 1999).

The differences in the number of aspirated follicles reported by different worker could be due to influence of individual donor (Kruip *et al*., 1991), breed, physiological stresses (Draincourt, 2001), and the effect of environmental factors like humidity, hot or cold climate, season and nutrition (Boland *et al*., 2001).

Aspirated follicles were higher in group-A than group-B donor cows, however there was no significant difference between follicular aspiration initiated on day 4 (first follicular wave) and on day 13 (second follicular wave).

The variations in the number of follicles of different categories among group A and B donor cows were non-significant, although comparatively higher aspiration rates (42.50 and 44.69) of medium size follicles were observed in both the groups as compared to the small and large size follicles.

Donor Cows

As we know the right ovaries are in cows more active than the left ones but this study shows that there is no any significant influence of ovarian side on the follicular aspirations. It may be due to the individual animal performance and also the skill of the operator (Fry *et al*., 1997).

This study concluded that there is no any influence ofovarian side and initiation time of OPU on follicular aspiration in crossbred cows and that the transvaginal ultrasound guided follicular aspiration could be performed repeatedly at weekly interval for follicular aspiration without any adverse effect. In countries like India where oocytes of cattle are not easily available, which is prime requirement for IVEP, for ban on cow slaughter, this technique of transvaginal ultrasound guided follicular aspiration is one of the alternates to get developmentally competent oocytes from cattle.

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