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Transvaginal ultrasound guided ovum pick up in cows

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Abstract

In the present study, transvaginal ultrasound guided ovum pick up was performed on 8 donor cows with good reproductive health. Mean number of large, medium and small follicles aspirated per donor cow was 2.87 ± 1.09 , 8.5 ± 2.08 and 9.63 ± 1.53 respectively. Follicles aspirated per cow per OPU session was 13.56 ± 1.91 . The mean number of COCs recovered from each donor was 9.63 ± 2.2 with the oocyte recovery rate of $47.53 \pm 6.93\%$. In conclusion, ultrasound guided ovum pick up technique is a very useful tool for oocyte aspiration and recovery from live animals.

Keywords: ultrasound, ovum pick up, recovery rate

Introduction

The ultrasound-guided trans-vaginal follicular aspiration technique for ovum pick-up (OPU) is a non-invasive method to recover oocytes from antral follicles in live animals and it can be repeatedly performed on the same animal without affecting the animal's reproductive status. The number and quality of follicles present at the time of collection determine the success rate of OPU in cattle. During an estrous cycle, two to four waves of follicles develop in cows. When ova are harvested during the recruitment or selection phase, over 70 ova per ovary per cow can be obtained (Mutembei *et al.*, 2015) [9]. Oocyte recovery rate and oocyte quality are influenced by the stage of the oestrus cycle during which the OPU is performed (Merton *et al.* 2003) [8]. Machatkova *et al.* (2004) [7] reported the greater recovery rates when the OPU was performed close to the follicular wave emergence.

Materials and Methods

Instruments used

Squeeze chute for restraining the donor, Ultrasound Scanner (Ultrasound machine veterinary, Make-IMV, France, Model- Exago, Serial number 1909EX01), trans-vaginal OPU Probe/Transducer (ECM Echo Control Medical, France), 60mm 18G Needle (ECM Echo Control Medical, France), Vacuum Pressure Pump/Suction pump (Model- BV-003D), Stereozoom Binocular microscope (Nikon, SMZ745), Silicone tube (Length-2m and internal diameter-2mm), Cell Strainer, Needle guide 18G, Scabbard, Needle holder with specific tip, Long needle brace, 60mm 18G OPU Needle.

Media used

BO-OPU media (IVF Bioscience, Bickland Industrial Park, Falmouth, Cornwall, TR11 4TA, United Kingdom), Euro Flush media (IMV Technologies, France)

Donors selection: A total of 8 donor cows were selected by thorough gynaecological examination, rectal palpation and ultrasonography. The cows having good general health, body condition score (3-3.5), larger ovaries with more number of follicular distribution, non pregnant, non lactating were selected as donors. Selected donors were dewormed and were supplemented with vitamin and mineral mixture powder before start of the study.

Ovum pick up: For the OPU procedure, the donor females were administered with an epidural anaesthesia (3-5ml of 2% lignocaine hydrochloride) at sacrococcygeal space. Rectum was evacuated by back raking and then the perineum was washed. For follicular aspiration, an 18-gauge 60 mm needle, suction pump maintained at 70-80 mmHg vacuum pressure, convex ultrasound 7.5-MHz trans-vaginal transducer were used. Follicular aspirate was collected in a 50-ml conical tube maintained at 37 °C temperature. Ivf bioscience BO-OPU media was used as

oocyte collection media. Follicle were visualized on the ovaries on the real time B mode ultrasound monitor (Veterinary Ultrasound Machine, Make-IMV, Model- Exago, France). The number and size of follicles aspirated from each ovary were determined and characterized on the basis of diameter as small (2-3 mm), medium (4-8 mm) and large (9-19 mm) size.

Recovery of Oocytes: Immediately after completing OPU from each cow, follicular fluid containing oocytes was washed and filtered using flush media 100µl cell strainer. Then the searching of oocytes was done under stereozoom microscope. Cumulus-Oocyte-Complexes (COCs) were identified and percentage of oocytes recovered was recorded.

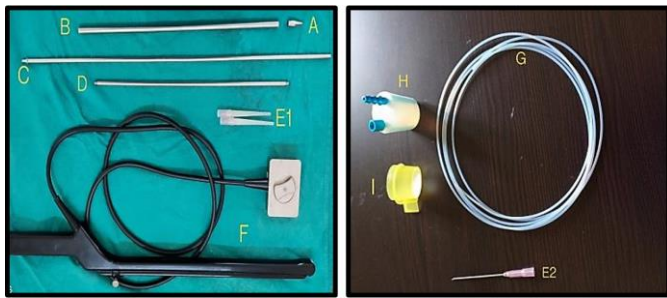


Fig 1: Instruments used for OPU procedure- A: Needle guide 18G, B: Scabbard, C: Needle holder with specific tip, D: Long needle brace, E1 & E2: 60mm 18G OPU Needle, F: Trans-vaginal OPU Probe, G: Silicone tube, H: rubber cork, I: Cell Strainer



Fig 2: Assembly for ovum pick up

Results and Discussion

In the present study, total of 13 OPU sessions were conducted. 168 follicles were aspirated which includes 23 large, 68 medium and 77 small follicles. On average, number of OPU sessions conducted per donor cow was 1.63±0.32, number of large, medium and small follicles aspirated per donor cow was 2.87±1.09, 8.5±2.08 and 9.63±1.53 respectively. The mean of total follicles aspirated per cow and follicles aspirated per cow per OPU session was 21±4.36 and 13.56±1.91, respectively, (Table 1).

The results of the present study are higher than that of Presicce *et al.* (2020) [11] who reported small follicles 3.40 ± 0.40, medium follicles 0.90 ± 0.18 and large follicles 0.70 ± 0.10 and Sakhong *et al.* (2012) [12] who reported small 2.65±0.83, medium 12.58±0.54 and large 1.00±1.09 follicles. da Silva *et al.* (2017) and Egashira *et al.* (2019) [3] aspirated significantly more number of small follicles than medium and large follicles (46.3 ± 5.1, 5.2 ± 0.5 and 1.7 ± 1.0; 47.9±8.9, 3.3±0.7 and 1.1±0.3 respectively) which were significantly higher than the present study.

The present findings are in agreement with Kang *et al.* (2019) [6] and Garcia and Salaheddine, 1998) [4] who aspirated 12.17 ± 3.47 and 12.4±6.1 follicles per OPU session respectively.

In the present study, 77 COCs were recovered. The mean number of COCs recovered from each donor was 9.63±2.2 with the oocyte recovery rate of 47.53±6.93, (Table 2).

Higher oocyte recovery rates than the present study was reported by da Silva *et al.* (2019) [1], de Moraes *et al.* (2019) [2], Egashira *et al.* (2019) [3], Kang *et al.* (2019) [6], Presicce *et al.* (2020) [11] and Nogueira *et al.* (2021) [10] who reported the oocyte recovery rates of 52.0 ± 3.9, 64.6, 69.8±5.6, 65.5 ± 1.9, 60.5 ± 4.0 and 69.22%, respectively. Greater recovery rates can be achieved when the OPU is performed close to the follicular wave emergence. Seneda *et al.* (2001) [13] recorded 50 and 72.2% oocyte recovery rates for large and small follicles aspirated. Higher oocyte recovery rates were obtained from small follicles because of, a smaller follicular fluid volume with lower intrafollicular pressure favours oocyte collection when the needle is inserted and rotated inside the small follicle. Losses during aspiration would be reduced when a smaller amount of intra-follicular material (follicular fluid and oocyte) being aspirated in these follicles. Harkal (2019) [5] recorded the higher oocyte recovery rate when follicular aspiration was done at 100 mmHg vacuum pressure than 80 and 90 mmHg vacuum pressure.

Table 1: Grading of Aspirated Follicles based on diameter

Sr. No	Donor No	No. of OPU Sessions	No. of Follicles Aspirated			Total No of Follicles Aspirated	Follicles/ Session/ Cow
			Large	Medium	Small		
			9-19 mm	4-8 mm	2-3 mm		
1	1	1	0	6	7	13	13
2	2	1	1	9	9	19	19
3	3	1	0	3	4	7	7
4	4	1	3	3	7	13	13
5	5	3	7	20	17	44	14.67
6	6	2	3	5	7	15	7.5
7	7	3	8	14	12	34	11.33
8	8	1	1	8	14	23	23
Total		13	23	68	77	168	
MEAN ± SEM		1.63±0.32	2.87±1.09	8.5±2.08	9.63±1.53	21±4.36	13.56±1.91
SD		0.92	3.09	5.88	4.34	12.34	5.42

Table 2: Total number of COCs recovered and recovery rate

Sr. No	Donor No	No of OPU Sessions	No of Follicles Aspirated	No of COCs Recovered	Oocyte Recovery %
1	1	1	13	6	46.15
2	2	1	19	13	68.42
3	3	1	7	3	42.86
4	4	1	13	10	76.92
5	5	3	44	16	36.36
6	6	2	15	5	33.33
7	7	3	34	20	58.82
8	8	1	23	4	17.39
Total		13	168	77	
MEAN ± SEM		1.63±0.32	21±4.36	9.63±2.2	47.53±6.93
SD		0.92	12.34	6.21	19.59

Conclusion

Ultrasound guided ovum pick up technique is very useful tool for aspiration and recovery of oocytes without hormonal stimulation and it can be performed repeatedly without affecting the reproductive status of donors.

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