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Recovery of bovine oocytes in respect of quality and quantity by using different techniques

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Abstract
The study evaluated the best techniques (aspiration/ slicing) for the recovery of oocytes from the bovine ovary. Total 601 numbers of bovine ovaries were divided as group-I (aspiration) and group-II (Slicing). The oocytes collected from the groups were classified into 3 categories separately as Type A, B and C in respect of the morphology of cumulus cell layers tightly adhered with the zonapellucida of oocytes and cytoplasmic appearance of oocyte. The rate of recovery in aspiration technique was found to be the highest for grade A (62.27±1.60) and the least in grade C (13.98±1.41) type of oocytes; while for slicing technique, highest in grade B (51.36±2.01) and the least in grade C (18.23±1.31) type of oocytes. The rate of recovery of culturable oocytes (grade A+B) was 86.01±1.41 and 81.76±1.31, by aspiration and slicing technique, respectively. Aspiration technique is the better method for recovery of oocytes for further (*in-vitro*) studies.

Keywords: aspiration, slicing, bovine, ovary, COCs

1. Introduction
The technique of *in-vitro* maturation (IVM) is a part of assisted reproductive technology (ART) to conduct *in-vitro* fertilization of female gametes [1]. Oocytes are female germ cells involved in reproduction and these are the largest cells (almost 0.12-0.50 mm in diameter) of mammalian body, which are typically visible to naked eyes without use of the magnification device [2]. A cluster of cells surrounding the oocytes are known as cumulus oophorus cells. It gives protection to the oocytes as well as helps in the follicular development and maturation of oocytes [3, 4]. Glycosaminoglycans, cytokines, steroid hormones, growth factors and other nutrients secreted by the cumulus cells are important in the nourishment of oocytes during various stages of nuclear and cytoplasmic maturation and further development [3]. A thick layer of cumulus cells may provide an efficient level of nutrients and other factors to the oocyte in the stages of development [5, 6]. Basically, the oocytes having more than 4-5 layers of cumulus cells surrounding the zona pellucida along with homogenous cytoplasmic appearance are considered as good quality oocytes [5, 6]. Recovery of oocytes with a proper technique is the first and very important step in the *in-vitro* production of oocytes in respect of quality and quantity.
So, the present study is planned to determine the best techniques for recovery of bovine oocytes in respect of quality and quantity oocytes.

2. Materials and methods
This study was conducted for duration of 8 months in the Department of ARGO, CVSC, AAU, Khanapara, 781022 from November 2016 to June 2017.
A total of 601 numbers of bovine ovaries were divided as group-I (Aspiration) and group-II (Slicing). In the slicing technique, the whole ovary was sliced into small pieces with a BP blade on a Petridish containing base media (TCM-199). In the aspiration technique, the follicles (3-8 mm) on the surface of the bovine ovary were punctured with an 18-gauge needle containing aspiration media and aspirated the fluids containing cumulus oocyte complexes (COCs). The oocytes were searched under steriozoom microscope. The oocytes collected from the groups were classified into 3 categories separately as Type A, B and C in respect of the morphology of cumulus cells layers tightly adhered with the zonapellucida of the oocytes and cytoplasmic appearance of oocyte. Grade A+B oocytes were considered as good quality of oocytes.

2.1 Preparation of Media for Aspiration and Washing of Oocytes

The aspiration and washing media were prepared for smooth aspiration of the oocytes from the follicles and for washing the cumulus-oocyte-complexes (COCs) to make them free from debris. After thorough mixing of the ingredients, the medium was sterilized by filtration using a 0.22 µm syringe filter and then kept in a CO2 incubator maintaining 5 per cent CO2 at 38.5 ^{0}C with 90%-95% relative humidity for 2 hours, prior to use. The composition of aspiration media were TCM-199 (40 ml), BSA (0.15g), Gentamicin (50 µg/ml), L-glutamine (0.004g). Washing media was consisting of TCM-199 (36 ml), 10% (v/v) FBS (4ml), L- glutamine (0.004g), Gentamicin (50 µg/ml), Sodium Pyruvate (0.0036g) and Cystamine (50 µM/ml).

2.2 Recovery of Oocytes

A total 601 numbers of Bovine ovaries were collected from the local abattoirs soon after the animals were slaughtered. Ovaries were carried to the laboratory in a thermos flask containing warm (37 ^{0}C) normal saline solution (NSS) with antibiotic. In the laboratory, extraneous tissues were removed from the ovaries with the help of a pair of scissors. Then the ovaries were washed 3-4 times with NSS containing antibiotic prior to further processing. Bovine ovaries were classified into two groups as a group-I (aspiration) and group-II (slicing) for recovery of the oocytes with different techniques.

2.2.1 Aspiration technique

Follicles on the bovine ovary measuring 3 to 8 mm diameter were selected for the collection of oocytes by aspiration technique. A 10-ml disposable syringe attached to an 18-gauge needle was loaded with 1ml of the sterilized aspiration medium. The needle was then introduced at the base of the follicle through the ovarian stroma to aspirate cumulus oocytes complexes (COCs) along with follicular fluid. The aspirated fluids were then placed in a searching petridish and examined under the stereo zoom microscope.

2.2.2 Slicing technique

The ovaries were chopped off into small pieces in a graded glass petridish containing aspiration media with the help of a sterilized surgical blade. After keeping the petridish without disturbing for 10-15 minutes, the sliced stromal tissues were discarded with the help of a pair of toothed forceps. The content of the petridish was then examined under a stereo zoom microscope and the oocytes were collected with the help of a lifter, and placed in a petridish (35 mm) containing washing media (2-3 ml).

2.3 Searching, grading & selection of oocytes

The recovered bovine oocytes were washed 5-6 times in the washing medium and graded into three categories based on their gross morphology and integrity of cumulus cells surrounded it as follows:

Grade A: COCs with a compact cumulus mass having at least 4-5 layers of cumulus cells, and with homogenous cytoplasm.

Grade B: COCs with 2-3 layers of cumulus cells adhered to the zona pellucid and with homogenous cytoplasm.

Grade C: Oocytes partially denuded of cumulus cells and / or with irregular shrunken cytoplasm.

2.4 Statistical Analysis

The statically analysed data was prepared by using SAS enterprise guided 4.3.

3. Results and Discussions

In the present study, the rate of recovery of A, B and C grade oocytes was 62.27±1.60, 23.73±1.35 and 13.98±1.41 per cent, and 30.40±1.74, 51.36±2.01 and 18.23±1.31 per cent, respectively by aspiration and slicing technique. Davachi *et al.* (2014) [7] also reported recovery of 60.00±2.1 per cent of grade A oocytes by aspiration technique as similar to the present study. Contrary to the present finding, Ahmed *et al.* (2015) [8] reported lower recovery rates of grade A (38.77) and B (27.02) per cent, using aspiration technique. Wang *et al.* (2007) [9] reported slicing technique yielded significantly more oocytes per ovary than follicle by aspiration technique. Hoque *et al.* (2011) [10] reported that slicing yielded a significantly higher number of total COCs per ovary than that of aspiration, however, a significantly higher number of normal COCs per ovary was observed in aspiration than those of slicing techniques. Wani *et al.* (2000) [11] reported that slicing more COCs per ovary than aspiration but the percentage of good quality oocytes was higher in the aspiration method when compared with slicing. However, Katska (1984) [12] pointed out that one of the difficulties by the aspiration method is that only 30-60% oocytes may be retrieved from the punctured follicles. Gordon (2003) [13] also opined that aspiration method could lead to greater disruption of surrounding cumulus cells and a possibility of retention because of cumulus cells being firmly attached to the stratum granulosum.

The recovery of A+B grade oocytes was recorded to be 86.01±1.41 and 81.76±1.31 per cent by aspiration and slicing technique, respectively. Ahmed *et al.* (2015) [8] reported that the average culture grade (Grade A and B COC's together) recovery was 65.79 per cent. Zarcula *et al.* (2012) [14] reported slicing method (81.70 per cent) yield better culturable oocytes than aspiration method (74.47 per cent), quality based on morphological aspects.

The findings of the present study could be subjective owing to many governing factors like season, breed, age and reproductive variability of animals, etc. Another imperative influence is the presence of a corpus luteum (CL) that impede on the ovarian space but also otherwise known to affect follicular development and hence recovery of COCs. Moreover, it is to be borne in mind that the source of ovaries collected at slaughter comprises of a heterogeneous group of cattle with varying ages and reproductive status, and equally likely that most may have been selected for culling owing to their decreased fertility. Actual yield in quantifiable and quality terms can be assessed in a more homogenous group within a fairly similar age, breed and reproductive abilities.

Analysis of variance revealed that there was a significant difference (P<0.01) in the recovery rate of different grades of oocytes among the techniques. DMRT (Duncan's Multiple Range Test) indicated that the percentages of the recovered oocytes were significantly (P<0.01) higher in grade A than grade B and C oocytes in aspiration while percentages of the recovered oocytes were significantly (P<0.01) higher for grade B than grade A and C oocytes in slicing technique. However, there was no significant difference in overall recovery rate between the techniques. Also, revealed significant differences in interaction between grades and technique. This shows that there is no independent variable in between technique and grades (Table 1).

Aspiration method is the most used technique in retrieving bovine cumulus-oocyte complexes from slaughterhouse ovaries (Shirazi *et al*, 2012) [15]. It is efficient for recovery of ample amount of COCs and yield good quality culturable oocytes for subsequent *in vitro* studies. The aspiration method

provides greater visual assessment of follicles as well as better selection and assortment for quality oocytes yield than slicing method. Moreover, slicing techniques produce more debris which might interfere with the searching of oocytes under the microscope and also required more washing when compared to aspiration (Shahid *et al*, 2014) [16]. As a result, a number of COCs were denuded from cumulus cells due to repeated washing and ultimately resulted in a lower number of normal COCs when compared to aspiration at the final observation.

Table 1: recovery rate (mean±se) of different grades of bovine follicular oocytes by aspiration and slicing technique.

Technique	Ovary	Oocytes	No. of observation	Oocytes		Rate of recovery (%) (Mean±SE)	No. of oocytes recovered per ovary
				Grades	Number		
Aspiration	310	664	32	A	408	62.27^{a}±1.60	1.32
				B	155	23.73^{b}±1.55	0.50
				C	101	13.98^{c}±1.41	0.33
Slicing	291	661	30	A	206	30.40^{b}±1.74	0.71
				B	331	51.36^{a}±2.01	1.14
				C	124	18.23^{c}±1.31	0.43

Means with the different superscripts in a column differ significantly (P<0.01)

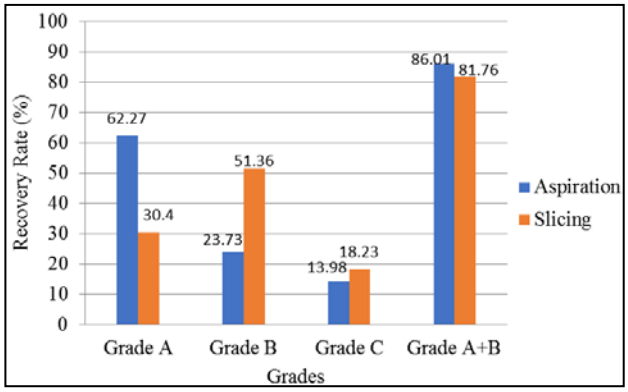

Fig 1: Recovery rate (%) of different grades of bovine follicular oocytes by aspiration and slicing technique

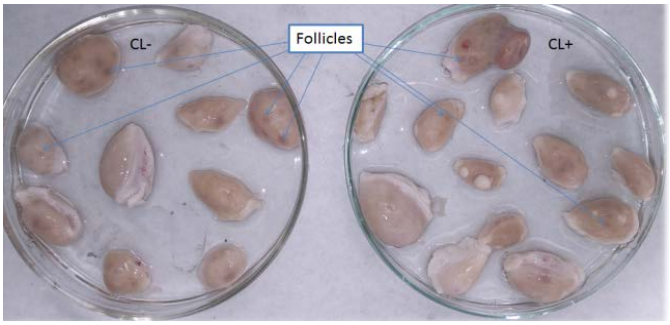

Fig 2: Corpus luteum (cl) present and absent group of bovine ovaries

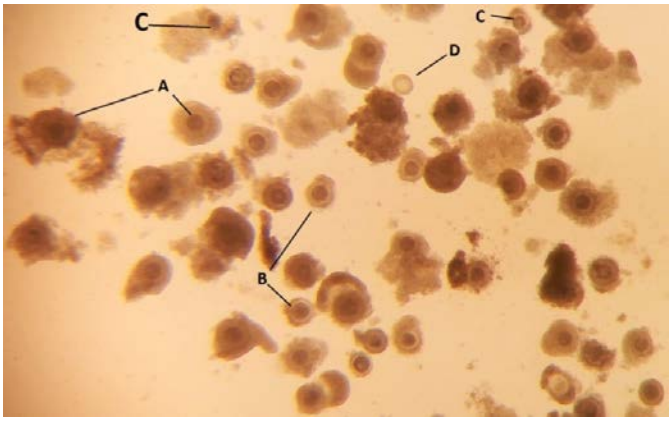

Fig 3: Different grades of bovine follicular oocytes

4. Conclusion

The recovery rate of "A" grade oocytes in aspiration technique was significantly higher than the B and C grade among the techniques. The rate of recovery of 'A+B' grade oocytes was 86.01±1.41 per cent in aspiration technique which was higher than slicing (81.76±1.31) technique. Aspiration technique could be considered as the best technique for harvesting the quality and quantity of bovine follicular oocytes.

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