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Quantitative and qualitative study of buffalo oocytes recovered by different oocyte harvesting techniques

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ABSTRACT

Present study was undertaken to assess the comparative efficacy of oocyte harvesting techniques, viz. aspiration and slicing methods on oocyte retrieval, recovery rate and quality of oocytes recovered in buffalo ovaries obtained from local slaughterhouse. Oocyte retrieval rate was significantly higher in slicing (67.75%) than aspiration (41.58%) technique. The oocyte recovery rate from ovaries by slicing (3.05 ± 0.24) was significantly higher than those by aspiration (1.76 ± 0.13) technique. The mean number of oocytes/ovary of grade I (0.93 ± 0.03) was significantly higher than grade II (0.55 ± 0.04) and grade III (0.34 ± 0.05) in aspiration technique. The reverse trend was observed in slicing technique, where the mean number of oocytes/ovary of grade III (1.32 ± 0.05) was significantly higher than grade II (1.07 ± 0.06) and grade I (0.81 ± 0.06). In both the techniques of oocyte collection, viz. aspiration and slicing the percentage of BCB+ oocytes (68.39% and 53.51%, respectively) were significantly higher than BCB– oocytes (31.61% and 46.49%, respectively). Overall higher numbers of oocytes were in BCB+ (60.29%) than BCB– (39.71%). In conclusion, quantitatively slicing was better over aspiration, while qualitatively aspiration technique was found to be superior over slicing technique.

Key words: Aspiration, BCB, Recovery rate, Retrieval rate, Slicing

Abattoir-derived ovaries are the most important source of oocytes for embryologists working with domestic animals (Gonzalez-Bulnes *et al.* 2005), but their quality is highly variable (Gandolfi *et al.* 1997), as it depends on oocyte recovery methods (Totey *et al.* 1992, Das *et al.* 1996). Recovery of oocytes by aspiration of vesicular follicles is the most commonly used method in terms of speed of operation compared to recovery of oocytes by follicular dissection/slicing method which is 3 times slower (Sianturi *et al.* 2002). However, the slicing or dissection method yields a better oocyte recovery rate than aspiration. The number of high quality oocytes recovered per ovary is an important consideration in the *in vitro* production of embryos. Hence, an appropriate oocyte collection method is to be developed to recover the highest number of good quality oocytes from a given ovary. There is a considerable variability among oocytes and morphological criteria are not sufficient for the identification of competent oocytes appropriate for *in vitro* development to the blastocyst stage (Alm *et al.* 2005, Han *et al.* 2006). With urgent need for establishing non-invasive and non--perturbing means for selecting more homogenous and competent oocytes, the brilliant cresyl blue (BCB) stain test was evaluated in cattle (Alm *et al.* 2005), buffaloes (Manjunatha *et al.* 2007), goats (Rodriguez-Gonzalez *et al.* 2002) and pigs (Wongsrikeao *et al.* 2006, Ishizaki *et al.* 2009). Very little systematic study on above aspects in indigenous buffaloes has been done. Therefore, the present work aimed to assess the comparative efficacy of oocyte harvesting techniques on quantitative and qualitative aspects of oocytes recovered from slaughterhouse derived ovaries.

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MATERIALS AND METHODS

Collection of ovaries: Buffalo ovaries (202) of unknown reproductive status were collected from the local slaughterhouse and transported within 2h of slaughter to the laboratory at 37° to 38°C in a pre-warmed thermos flask containing 0.9 % normal saline solution supplemented with penicillin G (100 IU/ml) and streptomycin sulphate (100 µg/ml).

Recovery of oocytes: To study the efficiency of 2 recovery methods, the ovaries were grouped for aspiration (104) method and slicing (98) method. In aspiration method, the cumulus oocyte complexes (COCs) were recovered from all 2 – 8 mm visible non-atretic follicles by aspiration through 18-guage needle attached to a 5 ml sterile plastic syringe containing 1 ml of pre-warmed oocyte collection

medium (OCM) composed of OCM stock solution, estrus buffalo serum and albumin from bovine serum (fraction V). While, in slicing method, collected ovaries were chopped into small pieces with sterile surgical blades in 90 mm sterile petri dish containing 10 ml OCM to harvest the oocytes.

Oocyte retrieval and recovery rates: Oocyte retrieval and recovery rates in different oocyte collection methods were calculated by following formulae:

$$\text{Oocyte retrieval rate (\%)} = \frac{\text{No. of oocytes collected}}{\text{No. of visible follicles counted}} \times 100$$

$$\text{Oocyte recovery rate/ovary} = \frac{\text{Sum of average oocytes/ ovary in batches}}{\text{Total no. of batches}} \times 100$$

Grading of oocytes: COCs were evaluated under stereo-zoom microscope using morphological criteria and graded in 3 categories in both these methods (Leibfried and First 1979).

- Grade I: Good oocytes with a homogenous evenly granular ooplasm, surrounded by compact and dense 3–5 cumulus cell layers.
- Grade II: Fair oocytes, surrounded by 1-3 layers of cumulus cells.
- Grade III: Denuded oocytes with uneven ooplasm and completely devoid of cumulus cells around them.

Brilliant Cresyl Blue (BCB) staining: The selected COCs were washed 3 times in Dulbecco's phosphate buffer saline (DPBS) without bovine serum albumin and exposed to 26μM of BCB diluted in DPBS for at least 90 min at 38.5°C in humidified air atmosphere at 5% CO_2. Following exposure to BCB, the COCs were washed 3 times in DPBS. The COCs were examined under stereo-zoom microscope, and according to their cytoplasm coloration they were divided into 2 groups, viz. oocytes with varying degree of blue coloration to the cytoplasm (BCB+, grown oocytes) and oocytes without blue cytoplasm (BCB-, growing oocytes).

Statistical analysis: The data pertaining to various aspects were suitably analyzed using SPSS statistics (version 20) software. The differences among the parameter means were performed using statistical methods, viz. chi-square test, t-test. The mean differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Oocyte retrieval rate: The per cent oocyte retrieved out of total visible follicles was 41.58% in aspiration technique; whereas, it was 67.75% in slicing technique. The oocyte retrieval rate was significantly ($P<0.01$) higher in slicing technique as compared to aspiration technique. The findings were in accordance with Kulasekhar *et al.* (2012) who reported 73.68, 43.61 and 47.91 % oocyte retrieval rate in slicing, aspiration and post aspiration slicing methods, respectively, in pluriparous buffaloes. However, as compared to present findings, Hiroshi (1979) recovered lower per cent of unfertilized ova by aspiration from slaughtered cattle. Whereas, Aziz *et al.* (2012) retrieved higher percentage of immature buffalo oocytes through follicular aspiration compared to present study.

Oocyte recovery rate: Significantly ($P<0.01$) higher oocyte recovery rate was obtained from ovaries by slicing technique (3.05 ± 0.24) as compared to oocyte recovery rate from ovaries by aspiration technique (1.76 ± 0.13). Singh *et al.* (2012) reported lower oocyte recovery rate from buffalo ovaries by slicing method compared to present findings. While, the recovery rate/ovary obtained by Singh and Dhanda (2007) from buffalo ovaries by mechanical aspiration of oocytes was at par with present study. Whereas, Kalleshwarappa *et al.* (2009) reported very low number of oocytes recovered per buffalo ovary in aspiration method. However, the recovery rate per ovary obtained by Leal *et al.* (2007) by aspiration of buffalo ovaries was higher than the present findings. Similarly, Aziz *et al.* (2012) also recovered higher immature buffalo oocytes through follicular aspiration than the present study.

Das *et al*. (1996) obtained significantly more oocytes in slicing technique than aspiration technique from buffalo ovaries. The oocyte recovery rate in aspiration method was almost the same, however, better recovery rate was observed in slicing method when compared to results of present experiment. Similarly, Jamil *et al*. (2008) reported significantly ($P<0.05$) higher number of oocytes recovered/ ovary in dissection than aspiration methods in buffaloes. Palanisamy *et al.* (2009) also found significantly higher ($P<0.01$) average yield of oocytes per ovary by slicing than by follicle aspiration method in buffalo. Our results are in agreement with the above reports.

However, as compared to temperate cattle (Greve and Madison 1991) the number of good COCs/ovary in buffalo is lower, which may be due to an inherently smaller number of primordial follicles and a higher frequency of atresia in buffalo (Drost 2007). The low proportion of *in vitro* recovery of the oocytes in the present study might be due to the poor body condition and reproductive status of the slaughtered buffaloes, as the female buffalo appeared for slaughtering are usually poor reproductive performer and most of them might be non-cyclic. Further, poor recruitment of primordial follicles into growing Graafian follicles and more atresia in acyclic buffalo ovaries may be other reasons for the low number of oocyte recovery per ovary in buffaloes.

Oocyte grading: The mean number of oocytes per ovary of grade I (0.93 ± 0.03) was significantly higher ($P<0.01$) than grade II (0.55 ± 0.04) and grade III (0.34 ± 0.05) in aspiration technique. However, the reverse trend was observed in slicing technique of oocyte collection, where the mean number of oocytes per ovary of grade III (1.32 ± 0.05) was significantly higher ($P<0.01$) than grade II (1.07 ± 0.06) and grade I (0.81 ± 0.06). Hoque *et al.* (2011) observed similar results as they obtained significantly higher ($P<0.01$) number of abnormal COCs/ovary in slicing followed by aspiration technique. Whereas, the number of

normal COCs/ovary was significantly higher (P<0.01) in aspiration followed by slicing techniques. However, contrary to the present findings, Das *et al*. (1996), Wang *et al.* (2007) and Palanisamy *et al.* (2009) recovered better quality oocytes by slicing than by aspiration method.

BCB staining: The present study was conducted to use the brilliant cresyl blue test as a selection criteria of developmentally competent buffalo oocytes, before IVM; thereby increasing the efficiency of embryo development after IVM-IVF. Out of total oocytes recovered only grade I and grade II oocytes were stained by BCB stain. In aspiration technique, the percentage of BCB+ oocytes (68.39%) was significantly higher (P<0.01) than BCB- oocytes (31.61%). Similarly, in slicing technique also, the percentage of BCB+ oocytes (53.51%) was significantly higher (P<0.01) than BCB- oocytes (46.49%). Overall higher numbers of oocytes were found to be BCB+ (60.29%) than BCB– (39.71%). Shanthi *et al.* (2012) also found significantly (P<0.05) higher mean number of BCB+ oocytes than BCB– oocytes by slicing technique. In the present findings 60.29 % oocytes were BCB+ after exposure to 26 µM BCB which was in agreement with Manjunatha *et al*. (2007) who reported 57.2% BCB+ oocytes after exposure to 26 µM BCB in buffalo.

The BCB stain is an electron acceptor, which can be used to measure the level of G6PDH activity semi-quantitatively in the oocytes, by modification of a visual colour (Tian *et al.* 1998). The BCB test is based on the capability of G6PDH to convert the BCB stain from blue to colorless (active G6PDH: BCB—, inactive G6PDH: BCB+) (Rodriguez-Gonzalez *et al.* 2002). During the oocyte growth phase, immature oocytes synthesize a variety of protein, including G6PDH (Manjunatha *et al.* 2007). The G6PDH enzyme is thus active in the growing oocyte, but has decreased activity in oocytes that have finished their growth phase (Rodriguez-Gonzalez *et al.* 2002).

Our results suggested that on quantitative aspects of oocyte studies *in vitro,* slicing was better over aspiration, while qualitatively aspiration technique was superior over slicing technique.

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