Journal of Animal Research: v.6 n.3, p. 503-507. June 2016

DOI: 10.5958/2277-940X.2016.00053.X

Retrieval and Recovery Rate of Buffalo (*Bubalus bubalis*) Oocytes Through Aspiration Technique

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Received: 30 March, 2016 **Accepted**: 05 May, 2016

ABSTRACT

The objective of the present study was to retrieve good and excellent quality of oocytes from the ovaries of buffalo having unknown reproductive history by using the aspiration technique. The retrieval as well as recovery percent was recorded. Total nine hundred and twenty ovaries were collected during the period of study, on an average 20 ovaries were collected per slaughter. Buffalo cumulus-oocyte complexes (COC's) were retrieved by aspiration technique and further graded on the basis of cellular investment and homogenicity in to grade A, B, C and D. The mean recovery rate (*i.e.* no. of oocytes/ ovary) was 2.20 ± 0.08, while that of grade A, B, A+B were 0.82 ± 0.03, 0.53 ± 0.03 and 1.35 ± 0.06, respectively. The mean retrieval percent of Grade A, B and A+B COC's were 37.10 ± 0.67, 23.74 ± 0.62 and 60.83 ± 0.84, respectively. The present finding led to the conclusion that aspiration technique seems to be a very promising technique for retrieval of developmentally competent oocytes from surface follicles and not from the deeper cortex which contained developing oocytes. By using aspiration technique, less amount of tissue debris obtained which may otherwise have adverse effect on the oocytes maturation and subsequent development of poor quality of embryos *in vitro.*

Keywords: Retrieval, cumulous oocytes complex, aspiration, recovery, COC's

Oocyte collection is the pre requisite step for *in vitro* development of embryos. Retrieval of oocytes from ovaries is very crucial step. Several methods can be used for the collection of oocytes (Ovum pick up, aspiration, slicing and puncture of follicles) for *in vitro* culture. Recovery of bovine oocytes by aspiration of vesicular follicles, using syringe and needle, has been the method most commonly employed owing to its speed of operation (Sadeesh *et al.* 2014). Though, this technique has a limitation that oocytes may only be retrieved from 30-60% of the punctured follicles (Kumar *et al.* 2013). Oocytes aspirated from 6 to 8 mm follicles have a much greater developmental potential than oocytes aspirated from smaller follicles (Nandi *et al.* 2002; Khalil *et al.* 2014). The retrieved oocytes are reported to be of good as well as excellent quality because they were obtained from the surface follicles of the ovaries. Looking to the importance of retrieval of excellent quality oocytes the present investigation was performed using the aspiration technique.

MATERIALS AND METHODS

Ovaries from apparently normal reproductive organs of adult buffaloes of unknown reproductive history slaughtered at Surat municipal abattoir were collected immediately after slaughtering. Ovaries were transported to the laboratory in a thermos flask containing sterile normal saline (NSS) fortified with antibiotic (50 µl/L Gentamicin) at 38-39°C temperature. Total nine hundred and twenty (920) ovaries were collected during the period of study, on an average 20 ovaries were collected per slaughter. Immediately after arrival at laboratory, ovaries

were cleaned by removing extraneous tissues and fat with the help of scissor and thoroughly washed twice in freshly prepared normal saline solution (NSS 0.9 %). Further, the ovaries were washed in 70% ethanol for 1 min to reduce contamination followed by washing twice in 0.9% NaCl for 1 min. After appropriate cleaning of ovaries, follicular fluid was aspirated from all the collected ovaries having surface follicles from 2-8 mm in diameter by using a 10 ml syringe (18 gauge needle attached to it). The follicular fluid was collected in working oocyte collection media (W-OCM). The follicular fluid containing different grades of oocytes was pooled in a 50 ml centrifuge tube containing working oocyte collection media as an aspiration media. The follicular fluid so collected along with aspiration media was kept in BOD incubator at 37°C for 20 minutes for settling down of oocytes. The supernatant was discarded and lower one third portion of the fluid was poured in 90 mm petridish (Axiva®India). Cumulus-oocyte complexes (COCs) were searched by using stereozoom microscope (Motic® Hong-Kong). COCs that were light in colour with an even cytoplasm and surrounded by cumulus cells (Grade A and B) were selected and considered as culturable oocytes. These culturable oocytes were collected in a petri dish containing OCM. Oocytes without cumulus cells were considered as non culturable oocytes (Grade C and D) and discarded. COCs were washed 10 to 12 times in 90 mm Petri dish containing OCM media. Washing was repeated 10 to 12 times in maturation media containing 10% fetal bovine serum (FBS).

The grading criteria of aspirated COCs have been depicted in Table 1 which was carried out on the basis of cellular investment and homogenicity as described by Chauhan *et al.* (1998).

Table: 1: Grading of cumulous oocyte complex's (COC's) retrieved through aspiration technique from buffalo ovaries

COCs Grade	COCs Characteristics
Grade A	COCs with an unexpanded cumulus cells having at least 5 layers of cumulus cells with homogenous cytoplasm
Grade B	COCs with 2-4 layers of cumulus cells and with homogenous cytoplasm
Grade C	Oocytes partially denuded of cumulus cells with irregular shrunken cytoplasm
Grade D	Oocytes completely denuded of cumulus cells with irregular cytoplasm

All the experimental data were analyzed by Sigma Software (SPSS-16.0) using one way ANOVA. The collection day wise (n=56) descriptive statistics was applied to calculate Mean ± SEM among different parameter as specified above.

RESULTS AND DISCUSSION

In the present study, one thousand nine hundred and twelve oocytes were retrieved from 920 buffalo ovaries of unknown reproductive history. Mean recovery rate of oocytes have been shown in figure no.1 and table no. 2.

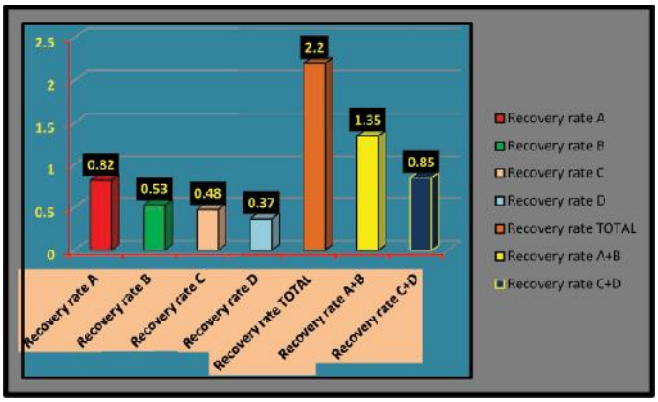


Fig. 1: Mean recovery rate of oocytes from buffalo ovaries

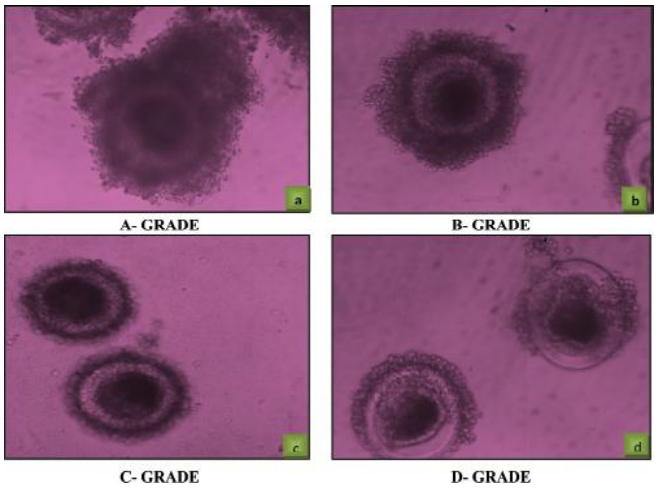
Table: 2: Recovery rate of different grades of oocytes retrieved from buffalo ovaries

Total number of ovaries collected (N)	Grade	Number of Oocytes Retrieved (X)	Overall Recovery Rate	Mean Recovery Rate (n=56)
920	A	710	0.77	0.82 ± 0.03
	B	464	0.50	0.53 ± 0.03
	C	416	0.45	0.48 ± 0.02
	D	322	0.35	0.37 ± 0.02
	(A+B)	1174	1.28	1.35 ± 0.06
	(C+D)	738	0.80	0.85 ± 0.02
	Overall	1912	2.08	2.20 ± 0.08

N= Total number of ovaries collected (920), X= Number of oocytes retrieved, n= number of collection days

The mean recovery rate obtained in the present study was 2.20±0.08. Though, several workers recovered higher rates of oocytes from ovaries of buffalo compared to present study. The higher recovery rates so obtained were 2.60 ± 0.03, 2.38 ± 0.19, 2.28 ± 0.07, 2.57 ± 0.23 and 2.50 by Barakat *et al.* 2012; Rao and Mahesh 2012; Singh *et al.* 2012; Al-shimaa *et al.* 2013 and Mahesh *et al.* 2014, respectively. Lower recovery rates than that observed in present study were 1.21, 1.53, 1.76 and 1.16 reported by Jamil *et al.* 2008; Kulasekhar *et al.* 2012; Chaudhary *et al.* 2014 and Puri *et al.* 2015, respectively in buffalo. The retrieved oocytes were classified into four grades A, B, C and D. The photographs of immature COC's of grade A, B, C and D are given in plate 1.

Plate: 1: Different grade of COC's retrieved through aspiration technique



The mean recovery rate of grade A, B, C, D and culturable oocytes (A+B) were 0.82 ± 0.03, 0.53 ± 0.03, 0.48 ± 0.02, 0.37 ± 0.02 and 1.35 ± 0.06, respectively (Table 2). Highest percent of grade A oocytes were obtained followed by B, C and D. In the present finding, the appreciable numbers of quality oocytes (recovery rate) were obtained through the aspiration technique. Though, few authors obtained higher numbers while others achieved lesser than the present findings. Mean recovery rate of grade A and B oocytes in the present study were lower than that reported by Singh *et al.* (2012). However the decreasing trend from A to D was also reported by them similar to present study. Makwana and Shah (2009) reported significantly higher ($P<0.05$) mean number of oocytes per ovary of grade A (0.82 ± 0.04), B (0.79 ± 0.04), D (0.76 ± 0.05) and C (0.64 ± 0.04) oocytes. But the Mean recovery rate of grade A was similar to present study. Grade A and B oocytes were used for further maturation and they were considered as culturable oocytes. Grade C and D were discarded and were considered as non culturable oocytes.

The mean recovery rate of culturable oocytes was 1.35 ± 0.06. Higher recovery rates for culturable oocytes than that observed in present study were 1.68, 1.61 ± 0.14 and 1.94 by Raza *et al.* 2001; Rao and Mahesh, 2012 and Mahesh *et al.* 2014, respectively. Further, the lower recovery rates so obtained for culturable oocytes than that observed in present study were 0.67±0.23 and 1.02 by Jamil *et al.* 2008 and Sharma *et al.* 2013, respectively.

In the present finding, the percent of grade A and B oocytes were 37.10±0.67 and 23.74±0.62 obtained. Mean retrieval percent of oocytes have been shown in figure no.2 and table no.3. Raza *et al*. (2001) reported lower percent of oocytes of grade A (20.62%) and B (15.62%) while Kulasekhar *et al*. (2012) reported higher percent of grade A (42.57%) and B (20.92%) oocytes as compared to the present study. However, Mahesh *et al*. (2014) graded oocytes in to three categories and reported that good, fair and poor oocytes were 43.0, 34.67 and 22.33%, respectively.

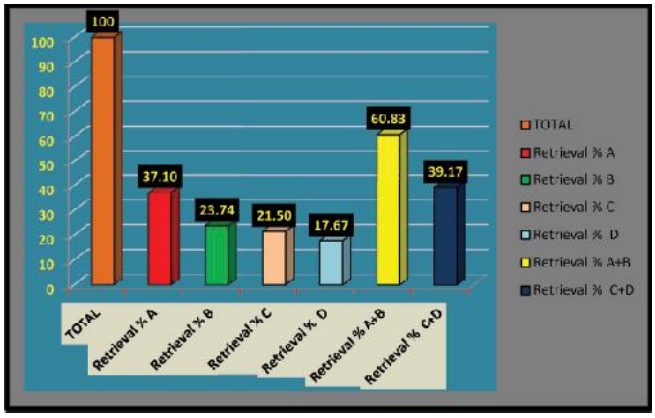


Fig. 2: Mean retrieval percent of oocytes from buffalo ovaries

In present study, the percent of culturable oocytes were 61.40%. The lower percent so obtained for culturable oocytes than that observed in present study were 52.49%, 56.19% and 41.58 by Raza *et al.* 2001; Jamil *et al.* 2008 and Chaudhary *et al.* 2014, respectively.

Table: 3: Different grade of oocytes retrieved from buffalo ovaries

Total number of ovaries collected (N)	Grade	Number of Oocytes (X)	Overall Retrieval Percent	Mean Retrieval Percent (n=56)
920	A	710	37.13	37.10 ± 0.67
	B	464	24.27	23.74 ± 0.62
	C	416	21.76	21.50 ± 0.63
	D	322	16.84	17.67 ± 0.92
	(A+B)	1174	61.40	60.83 ± 0.84
	(C+D)	738	38.60	39.17 ± 0.84

* N= total number of oocytes retrieved (1912), X= Number of oocytes grade wise, n=number of collection days

However, as compared to present findings, the higher percent of culturable grades of oocytes obtained were78.82%, 77.67% and 72.7 ± 1.59 by Prabhakar *et al.* 2012; Mahesh *et al.* 2014 and Gabr *et al.* 2015, respectively.

Proper oocyte recovery and its selection in the laboratory is vital for successful embryo production. There are number of factors that affect the recovery rate of oocytes *viz*. source of ovaries, collection technique, season and time interval between animal slaughter and the recovery of oocyte. Though, all the mentioned factors are equally important for the successful *in vitro* embryo development, but the retrieval technique plays a very crucial role in recovering the appreciable number of competent oocytes from the surface follicles of the ovaries of buffalo. However, in the present study aspiration technique was used and that is reported to be the best technique (Das *et al.* 1996; Mehmood *et al.* 2011). The aspiration technique opted in the present study retrieved competent oocytes with a less amount of tissue debris. The debris in the culture medium may have an adverse effect on the oocytes maturation and subsequent development of poor quality of embryos *in vitro*. Another reason for considering this technique is retrieval of superior quality COC's from surface follicles because these follicles reside most competent oocytes with proper development while the oocytes located in the deeper cortex are in developing stage.

CONCLUSION

The present finding led to the conclusion that aspiration technique seems to be a very promising technique for retrieval of developmentally competent oocytes from surface follicles. Less amount of tissue debris were obtained when the oocytes retrieved through aspiration technique, which may have an adverse effect on the oocytes maturation and subsequent development of poor quality of embryos *in vitro.*

ACKNOWLEDGEMENTS

Authors are thankful to Dean and Principal, Vanbandhu College of Veterinary Science and Animal Husbandry, NAU, Navsari for providing facilities and financial assistance for conducting this research work.

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