RESEARCH ARTICLE

Assessment of Oocyte Retrieval and its Quality from the Abattoir Ovaries of Jaffarabadi Buffaloes by Aspiration and Slicing Methods

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Abstract

Present study was carried out to assess the efficacy of aspiration and slicing methods of oocytes retrieval, retrieval rate and grades of oocytes recovered from the abattoir ovaries of culled Jaffarabadi buffaloes. The ovaries were procured from local abattoir (n=100), and the oocytes from 50 ovaries each were retrieved by slicing and aspiration method, which yielded 205 and 148 oocytes of different grades, respectively. Among the two methods of oocytes retrieval, the slicing method yielded significantly ($P < 0.01$) higher average number of oocytes (4.12 ± 0.34) than the aspiration method (2.96 ± 0.29). Recovery rate of oocytes was 3.57 and 4.18 by slicing method, and 0.65 and 3.23 in aspiration method in the presence of CL and absence of CL, respectively. The mean numbers of Grade B oocytes retrieved by slicing method in the presence and absence of CL on ovary (2.00 ± 0.44, 2.19 ± 0.23) were significantly higher ($p < 0.05$) than grade A (0.29 ± 0.19, 0.23 ± 0.08), grade C (0.43 ± 0.20, 0.35 ± 0.11) and grade D (0.86 ± 0.14, 1.42 ± 0.19, resp.). Similarly, the mean numbers of Grade B oocytes retrieved by aspiration method in the presence and absence of CL on ovary (0.50 ± 0.34, 1.59 ± 0.23) were higher than grade A (0.33 ± 0.21, 0.27 ± 0.07), grade C (0.17 ± 0.17, 0.25 ± 0.09) and grade D (0.00 ± 0.00, 1.11 ± 0.16, resp.). The better oocytes retrieval and good quality of oocytes were yielded by slicing method as compared to aspiration method in the absence of CL.

Keyword: Aspiration, Buffaloes, Oocytes grades, Ovary, Retrieval rate, Slicing.

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Introduction

Buffaloes in India are spread almost all over the country with varying density of population. Buffaloes has the potential superiority over cattle because of their distinctive qualities such as better feed conversion efficiency, more resistance to disease, higher milk fat percentage than in cows and low maintenance requirement that are profitable to farmers (Phogat *et al*., 2016). Buffaloes are contributing about 60% of milk production in India. However, the reproductive performance of buffaloes is poor owing to delayed age at sexual maturity, seasonality of breeding, silent estrus, prolonged inter-calving period, fewer primordial follicles and poor superovulatory response (Nandi *et al*., 2001). Biotechnological tools are being used in animal and agriculture to improve production and to develop specialized food and pharmaceutical products. Manipulations of reproductive processes are necessary to accomplish these goals. Production of embryo from slaughterhouse ovaries involves several sophisticated procedures such as collection and grading of oocytes followed by *in-vitro* maturation, fertilization and culture. *In-vitro* maturation, fertilization and culture of zygotes have allowed extensive research on modern reproduction techniques in farm animals. In view of the above facts, the present study was carried out to compare the retrieval rate and grades of oocytes recovered from the abattoir ovaries of culled Jaffarabadi buffaloes by aspiration and slicing methods.

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Materials and Methods

The present study was carried out at Department of Veterinary Gynaecology and Obstetrics of the College in Junagadh, Gujarat (India). The ovaries (n=100) from apparently healthy reproductive organs of culled matured Jaffarabadi buffaloes were collected within half an hour of slaughter from the local abattoir and transported within one hour to the laboratory in a vacuum flask containing normal saline (0.9%

NaCl) at pH 7.0 supplemented with gentamicin 50 µg/mL (Sigma) and the temperature of the solution maintained at 25–30°C (Totey *et al.,* 1992). In the laboratory, after removal of extraneous tissue and fat, ovaries were washed with 70% ethanol to check contamination, followed by three washes of the normal saline (39°C). Ovaries were dried with sterilized paper towel and evaluated for presence or absence of corpus luteum (CL).

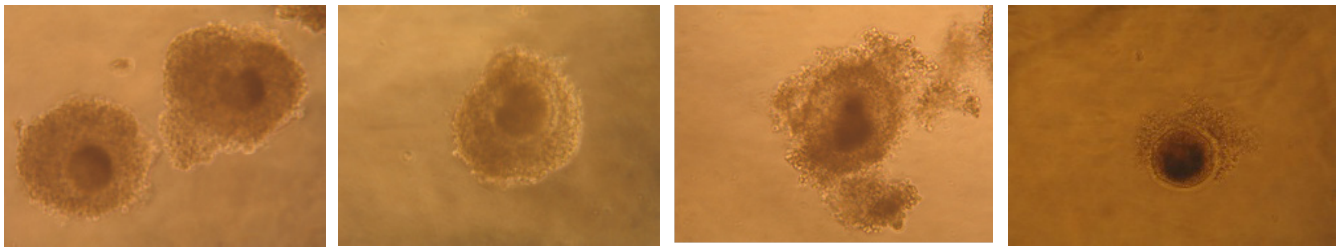
Fifty ovaries were sliced with a fine BP blade and transferred into 100 mm disposable searching petri dish with oocytes collection medium. The contents of all sliced ovarian follicles recovered were searched for Cumulus Oophorus Complexes (COCs) in 100 mm petri dish. Other 50 ovaries were used for aspiration of oocytes by using an 18-gauge needle attached to a 10 mL syringe to avoid disruption of the surrounding cumulus cells. A stereoscopic microscope (Magnus, MSZ-TR, India) was used to identify oocytes (Plate 1). The ovaries were divided into two groups, *i.e.,* with and without corpus luteum to investigate the influence of corpus luteum on the quantity and quality of oocytes recovered per ovary. The total as well as usable COCs recovered from each ovary of the two groups were recorded and grading was done as grade A, B, C & D according to criteria of COCs described by Gordon (1995).

The descriptive statistics was applied to calculate Mean ± SE among different parameters. The mean differences between the groups were compared by "t" test.

Results and Discussion

The ovaries (n=100) of slaughtered Jaffarabadi buffaloes were procured from local abattoir of Junagadh, which yielded 205 and 148 oocytes of different grades by slicing (n=50) and aspiration (n=50) method, respectively. Among the two collection methods of oocytes retrieval, the slicing method yielded significantly ($P < 0.01$) higher number of oocytes (4.12 ± 0.34) than the aspiration method (2.96 ± 0.29) (Table 1). Several methods like aspiration, puncture, slicing, dissection etc. have been used for the collection of oocytes from the ovaries of goats (Mogas *et al.,* 1992) and buffaloes (Das *et al.,* 1996), bovine (Singh *et al*., 2018) Sheep (Majeed *et al*., 2019). In the present study, significantly higher oocytes retrieval rate obtained by slicing than the aspiration method concurred with the findings of Datta *et al.* (1993) and Das *et al.* (1996), while Jamil *et al.* (2008) found that dissection yielded the highest (36.74%) oocytes, followed by puncture (32.87%) and aspiration (19.83%) methods from buffalo ovaries. However, Wani *et al.* (2013) did not find significant difference in the quality of oocyte recovered by three methods from sheep ovaries.

The recovery rate of oocytes was 3.57 and 4.18 by slicing method in the presence of CL and absence of CL, whereas it was 0.95 and 3.23 by aspiration method in the presence of CL and absence of CL, respectively. Thus, it was found that oocyte retrieval rate was higher from the ovaries in the absence of CL compared to presence of CL particularly by slicing method (Table 2). The present results very well



Grade A | Grade B | Grade C | Grade D

Plate 1: Grading (A,B,C,D) of oocytes on the basis of cumulus oophorus complex

Table 1: Retrieval rate of oocytes collected by slicing and aspiration methods

Attributes	*Slicing*	*Aspiration*	*t value*	*P value*
No. of ovaries collected	50	50	-	-
Total no. of oocytes gained	205	148	-	-
Mean ± SE	4.12 ± 0.34^{a}	2.96 ± 0.29^{b}	2.55**	0.01

Means with different superscripts within a row vary significantly ($P < 0.05$)

Table 2: Effect of CL on retrieval rate of oocytes by slicing and aspiration methods

	Slicing				*Aspiration*			
Attributes	*Presence of CL*	*Absence of CL*	*t value*	*P value*	*Presence of CL*	*Absence of CL*	*t value*	*P value*
Total (n)	7	43	-	-	6	44	-	-
No. of oocytes recovered	25	180	-	-	4	142	-	-
Oocyte retrieval rate, Mean ± SE	3.57 ± 0.72	4.18 ± 0.38	0.62	0.54	0.95 ± 0.37^{a}	3.23 ± 0.30^{b}	2.64*	0.01

Means with different superscripts within a row vary significantly ($P < 0.05$)

Table 3: Effect of presence or absence of CL on quality of Oocytes by slicing and aspiration methods

Method of oocytes collection	*Status of ovary*	*Oocytes recovered*	*Grade of oocytes*			
			A	*B*	*C*	*D*
Slicing (n=7)	Presence of CL	Mean ± SE	0.29 ± 0.19^{a}	2.00 ± 0.44^{b}	0.43 ± 0.20^{a}	0.86 ± 0.14^{a}
		No.	2	14	3	6
Slicing (n=43)	Absence of CL	Mean ± SE	0.23 ± 0.08^{a}	2.19 ± 0.23^{c}	0.35 ± 0.11^{a}	1.42 ± 0.19^{b}
		No.	10	94	15	61
Aspiration (n=6)	Presence of CL	Mean ± SE	0.33 ± 0.21	0.50 ± 0.34	0.17 ± 0.17	0.00 ± 0.00
		No.	2	3	1	0
Aspiration (n=44 s)	Absence of CL	Mean ± SE.	0.27 ± 0.07^{a}	1.59 ± 0.23^{b}	0.25 ± 0.09^{a}	1.11 ± 0.16^{b}
		No.	12	70	11	49

N= Ovaries, Means with different superscripts within a row vary significantly ($P < 0.05$).

corroborated with findings of Totey *et al*. (1992) and Das *et al*. (1996). However, Tasripoo and Kamonpatana (1997) harvested 2.2 oocytes per ovary in swamp buffalo, which is supported by the present study. On the contrary, Moreno *et al.* (1992) reported significantly higher recovery rate from pregnant cows in comparison to non-pregnant one.

The mean number of Grade B oocytes recovered by slicing method in the presence of CL on ovary (2.00 ± 0.44) was significantly higher ($P < 0.05$) than grade A (0.29 ± 0.19), grade C (0.43 ± 0.20) and grade D (0.86 ± 0.14) oocytes. The mean number of Grade B oocytes harvested by slicing method in the absence of CL on ovary (2.19 ± 0.23) was also significantly higher ($P < 0.05$) than grade D (1.42 ± 0.19) oocytes, and both were higher ($P < 0.05$) than grade C (0.35 ± 0.11) and grade A (0.23 ± 0.08) oocytes. The mean number of Grade B oocytes by aspiration method in the presence of CL on ovary (0.50 ± 0.34) was non-significantly higher than grade A (0.33 ± 0.21), grade C (0.17 ± 0.17) and grade D (0.00). The mean number of Grade B oocytes obtained by aspiration method in the absence of CL on ovary (1.59 ± 0.23) was however significantly higher than grade D (1.11 ± 0.16), and both were higher than those of grade A (0.27 ± 0.07) and grade C (0.25 ± 0.09) oocytes (Table 3).

In the present study, the effect of presence versus absence of CL on the ovary had a non-significant effect on recovery rate and quality of buffalo oocytes. In the slicing and aspiration method of oocytes recovery, Grade B oocytes were significantly ($P < 0.01$) higher than the grade A, C and D, particularly in ovaries without CL than the ovaries with the CL (Table 3).

Overall, the oocytes recovery rate was higher in the absence of CL as compared to presence of CL on the ovaries by both slicing and aspiration methods. This finding supported the earlier report of Nandi *et al*. (2000) who reported decreased recovery rate of oocytes when an ovary had a CL. This is because follicular development is restricted, as the lutein cells occupy most of the portion of the ovary (Kumar *et al*., 1997). The dominant follicle is usually observed in the CL bearing ovaries, and the other follicles are very small and remain mostly inaccessible (Gasparini *et al*., 2000). In cows (Moreno *et al*., 1992) and goats (Agrawal, 1992), ovaries containing CL yielded lower numbers of oocytes than ovaries without CL. Several researchers have reported that the presence of CL yields a lower proportion of usable oocytes (Agrawal, 1992; Moreno *et al.,* 1992). In contrast, Boedino *et al.* (1995) and Das *et al.* (1996) found no difference in the mean number of the oocytes per ovary between CL bearing and non-bearing ovaries.

The slicing method yields better oocytes recovery, as small and medium sized follicles are embedded deeply within the cortex of the ovary, which otherwise might get missed during aspiration (Pawshe *et al*., 1994). Moreover, such oocytes remained firmly attached to the small, medium sized follicles before cumulus expansion and hence cannot be aspirated. Further aspiration technique was found to be laborious and time consuming.

The quality and quantity of oocytes recovered per ovary are important considerations in successful embryo production. Therefore, appropriate method for oocytes recovery and its selection in the laboratory are vital for successful embryo production. To judge the quality of oocytes, the presence of an intact complement of cumulus cells surrounding the oocyte and homogenous appearing ooplasm remained the best criteria. The competence of buffalo oocyte *in vitro* is influenced by a wide array of biological (Nandi *et al*., 2000) and environmental (Nandi *et al*., 2001) factors such as follicular size, follicle diameter, presence or absence of corpus luteum in ovary and environmental temperature. That might be the probable reason for slight deterioration in the quality of oocytes during present study. As such, oocytes recovery was low in buffalo. The slicing method was found to be feasible technique for reasonable retrieval of buffalo follicular oocytes.

Conclusion

From the study, it is concluded that in ovaries of culled Jaffarabadi buffaloes, the oocytes retrieval rate was better by slicing method of oocytes retrieval as compared to aspiration

method. Moreover, the higher recovery rate and good quality oocytes were obtained from ovaries without CL.

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References

Agrawal, K.P. (1992). Factors affecting oocyte recovery from caprine ovaries of abattoir origin. In: *Recent Advances in Goat Production*. R.R. Lokeshwar, Ed., Proc. 5th Int. Conf. Goats, New Delhi, India, pp. 1207-1210.

Boediono A, Rajamahendran, R., Saha, S., Sumantni, C., & Suzuki, T. (1995). Effect of the presence of a CL in the ovary on oocytes number, cleavage rate and blastocyst production *in vitro* in cattle, *Theriogenology*, *43*, 169.

Das, G.K., Jain, G.C., Solanki, V.S., & Tripathi, V.N. (1996). Efficacy of various collection methods for oocyte retrieval in buffalo. *Theriogenology, 46*(8), 1403-1411.

Datta, T.K., Goswami, S.L., & Das, S.K. (1993). Comparative efficiency of three oocyte recovery methods from sheep ovaries. *Indian Journal of Animal Science*, *63*, 1178-1179.

Gasparrini, B., Neglia, G., Di Palo, R., Campanile, G., & Zicarelli, L. (2000). Effect of cysteamine during *in vitro* maturation on buffalo embryo development. *Theriogenology*, *54,* 1537-1542.

Gordon, I. (1995). Recovering the bovine oocyte. In: *Laboratory Production of Cattle Embryos*, 2nd Edn, CAB International/ Cambridge University Press, Cambridge, p. 97-112.

Jamil, Huma., Samad, HafiZ Abdus, Qureshi, Zafar Iqbal., Ur Rehman, Najeeb, & Lodhi, Laeed Akbar (2008). Harvesting and evaluation of riverine buffalo follicular oocytes. *Turkis Journal of Veterinary and Animal Science, 32*(1), 25-30.

Kumar, A., Solanki, V.S., Jindal, S.K., Tripathi, V.N., & Jain, G.C. (1997). Oocyte retrieval and histological studies of follicular population in buffalo ovaries. *Animal Reproduction Science*, *47*(3), 189-195.

Majeed, A.F., Omar, A.A., Alrawi, Saad Thabit Jasim., Al, Maadhidy., Assif, H., Ahmed, K.D., & Khudair Mohmed. (2019). Effect of collection methods on oocyte recovery rate in sheep. *Research Journal of Biotechnology*, 14(1): 262-264.

Mogas, T., Martino, A., Palomo, M.J., & Paramio, M.T. (1992). Effect of method of recovery on the number and type of oocytes obtained for IVM. *Journal of Reproduction and Fertility, 9*, 53.

Moreno, J.F., Turczynski, C.J., Flores-Foxworth, G., & Kraemer, D.C. (1992). Influence of parity of donor and presence of a CL on quantity and quality of bovine oocytes from ovarian follicles aspirated post-mortem. *Biology of Reproduction*, *46*(1), 65 (Abstr).

Nandi, S., Chauhan, M.S., & Palta, P. (2000). Effect of a corpus luteum on the recovery and developmental potential of buffalo oocytes. *Veterinary Record, 147,* 580-581.

Nandi, S., Chauhan, M.S., & Palta, P. (2001). Effect of environmental temperature on quality and developmental competence in vitro of buffalo oocytes. *Veterinary Record, 148*, 278- 279.

Pawshe, C.H., Totey, S.M., & Jain, S.K. (1994). A comparison of three methods of recovery of goat oocytes for In Vitro maturation and fertilization. *Theriogenology, 42*(1), 117-125.

Phogat, J. B., Pandey, A. K., & Singh, I. (2016). Seasonality in buffaloes reproduction. *International Journal of Plant, Animal and Environmental Sciences,* 6, 46-55.

Singh, W.L., Sonowal, J., Das, A., Barua, P.M., Geogoi, C., Mahanta, D., Deuri, N., & Sathapathy, S. (2018). Recovery of bovine oocyte in respect of quality quantity by using different techniques. Journal of Entomology and Zoology studies, 6(1), 250-253.

Tasripoo, K., & Kamonpatana, M. (1997). Assessment of factors contributing to *in vitro* maturation, fertilization and cleavage rate of swamp buffalo oocytes. *Buffalo Journal*, *13,* 325-336.

Totey, S.M., Singh, G., Taneja, M., Pawshe, C.H., & Talwar, G.P. (1992*). In Vitro* maturation, fertilization and development of follicular oocytes from buffalo (*Bubalus bubalis*). *Journal of Reproduction and Fertility*, *95*(2), 597-607.

Wani, A.R., Khan, M.Z., Sofi, K.A., Malik, A.A., Lones, F.A., & Bhat, F.A. (2013). Effect of follicular size on in vitro maturation, fertilization and culture of sheep embryos. *Iranian Journal of Veterinary Research, 14*(4), 299-304.