STUDIES ON FOLLICULAR SIZE AND OOCYTES RECOVERY RATE FROM BUFFALO OVARIES BY SLICING METHOD

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ABSTRACT

A total of 456 ovaries of Surti buffaloes were obtained freshly from the local slaughter house and were transferred within 1-2 hours to the laboratory in normal saline at 30-35°C. The ovaries selected were without functional corpus luteum. An average number of follicles of small, medium and large size found per ovary were 0.82, 0.48 and 0.24, respectively, with an overall mean of 1.55. The distribution of small, medium and large follicles was found to be 53.18, 31.12 and 15.70 per cent, respectively. The slicing method of oocyte recovery employed yield a total of 1409 oocytes from these ovaries, with on an average recovery rate of 3.09 oocytes per ovary. The average recovery of grade A, grade B and grade C oocytes was 1.02, 1.22 and 0.85, respectively. The study demonstrated that slicing of the ovarian surface follicle is a convenient and effective method for collecting a high yield of buffalo follicular oocytes for *in vitro* maturation and embryo production.

KEY WORDS: Buffalo, Abattoir ovaries, Follicular size, Slicing, Oocyte recovery.

INTRODUCTION

An ample supply of oocytes is a pre-requisite for efficient utilization of *in vitro* embryo production (IVEP) and other allied technologies. An abattoir source for the ovaries is inexpensive and abundant for the research work. In the production of cattle and buffalo embryos from slaughter house ovaries, it becomes necessary to employ techniques that permit the recovery of good quality oocytes. Methods employed by different research workers for recovery of immature follicular oocytes and number actually recovered vary with the different methods. Slicing method of oocytes recovery has been documented as much better than puncture, dissection or aspiration in cattle (Carolan *et al.,* 1992), buffalo (Das *et al.,* 1996) and goat (Pawshe *et al.,* 1994; Dutta *et al*., 1996). In the present study, an attempt was therefore made to study the follicular size and recovery rate of immature buffalo oocytes from nonfunctional abattoir ovaries by using slicing method.

MATERIALS AND METHODS

The present study was conducted at Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, AAU, Anand, from September 2008 to February 2009. Only the ovaries (n=456) without corpus luteum from the matured Surti buffaloes slaughtered at the local abattoir were collected within half an hour and were transported to the laboratory at 30-35°C temperature in a flask containing normal saline (0.9 % NaCl) at pH 7.0 supplemented with 50 µg/ml Gentamicin (Sigma, G 3632). The extraneous tissue and fat was removed in the laboratory and ovaries were washed with 70 per cent alcohol to check contamination, followed by three washes of the normal saline (39°C). The detectable surface follicles on the ovaries were classified according to their size into three groups, viz., small (= 4 mm), medium (4-8 mm) and large (= 8 mm) size.

The washed ovaries were then sliced with a BP blade and transferred into 100 mm disposable petri-dish (Tarson® INDIA) with warm normal saline. The contents of all sliced follicles were searched for Cumulus Oophorus Complexes (COCs) in Petri-dish. A stereoscopic microscope (Nikon SMZ-2B, Tokyo, Japan) was used to identify the oocytes. The COCs were classified into grade A, B and C as under (Leibfried and First, 1979).

The number and percentage of different size of follicles observed and the grade wise oocytes recovered were recorded.

Oocyte / COCs Grade	Characteristics
Grade A	COCs with an unexpanded cumulus mass having at least five layers of cumulus cells and with homogenous cytoplasm.
Grade B	COCs with 2–4 layers of cumulus cells, and with homogenous cytoplasm
Grade C	COCs partially denuded of cumulus cells and/or with irregular shrunken cytoplasm, and /or completely denuded of cumulus cells and/or with irregular shrunken cytoplasm

RESULT AND DISCUSSION

In the present study, 456 ovaries of Surti buffaloes were collected from local slaughter house during the month of September to February. Only the ovaries which did not had any functional CL were selected. The distribution of follicles over 456 ovaries is presented in Table 1.

An average number of follicles of small, medium and large size observed per ovary were 0.82, 0.48 and 0.24, respectively. This showed 53.18, 31.12 and 15.70 per cent distribution of small, medium and large follicles, respectively. The overall average number of follicles per ovary was found to be 1.55. These results are in close conformity with the previous observations of Sarvaiya (1997).

Table 1: Distribution of follicles of different size in the ovaries of Surti buffaloes collected from the abattoir

Follicular size	Number of follicles	Average follicle/ovary	Per cent distribution
≤ 4 mm	376	0.82	53.18
4-8 mm	220	0.48	31.12
≥ 8 mm	111	0.24	15.70
Total	707	1.55	100.00

In this study, ovaries were transported to the laboratory within one hour of slaughter in a flask containing normal saline (0.9 % NaCl) at pH 7.0 supplemented with 50 µg/ml Gentamicin (Sigma, G 3632), which was useful for maintaining the normal physiology of oocytes and preventing contamination in further procedure. However, Yadav *et al.* (1996) used only normal saline solution for holding the collected ovaries and transported to laboratory at 30°-37°C.

The findings on total and usable number of COCs recovered through slicing method from the ovaries of slaughtered buffaloes are summarized in Table 2.

Table 2: Grade-wise average oocyte recovery rate by slicing method from abattoir ovaries of Surti buffalo

Grades of oocyte	Total oocytes recovered	Average oocyte per ovary
A	463	1.02
B	557	1.22
C	389	0.85
Overall	1409	3.09

Total of 1409 oocytes recovered from 456 ovaries by slicing method yielded a mean oocyte recovery rate of 3.09 per ovary. The average recovery of grade B oocyte was higher than grade A and grade C types of oocytes. Similar results were noted by Katiyar *et al.* (1988) by aspiration method. In the present study, average oocyte recovery rate was higher than 0.73 and 0.96 reported by Totey *et al.* (1992) and Dutta and Goswami (1998), respectively. While, Das *et al.* (1996) proved that slicing yielded significantly ($P<0.01$) more (5.7) oocytes per ovary than follicle puncture (2.6) or aspiration (1.7). Also the better quality oocytes (good and fair) were recovered per ovary by slicing (2.6) than by puncture (1.3) and aspiration (0.9). The slicing method of oocyte collection was reported to be the best method for recovery by Das *et al.* (1996); Dutta *et al.* (1996) and Sarvaiya (1997) also. Dutta *et al.* (1996) recovered lower number of oocytes by slicing (2.17 ± 1.28) in buffaloes. Sarvaiya (1997) recovered 1.64 oocytes per ovary by slicing method alone and 3.55 by both aspiration and slicing of abattoir ovaries of Surti buffalo. The low level of oocyte recovery may be due to the availability of the anoestrus buffaloes at the slaughter house. Danell (1987) found fewer numbers of primordial follicles in the ovaries of non-cyclic buffalo than in cyclic buffalo. Poor recruitment of primordial follicles in to growing graffian follicles and more atresia in acyclic buffalo ovaries may be other reasons for the low number of oocytes recovered per ovary.

In our study, the good, fair and poor quality oocytes recovered were 32.86, 39.51 and 27.61 per cent, respectively. Dutta *et al.* (1996) reported that the recovery of grade-I good quality oocyte was higher (0.75 ± 0.48) by slicing method. Katiyar *et al.* (1988), Yadav *et al.* (1996), Sarvaiya (1997) and Zoheir *et al.* (2007) also found higher number of good quality oocytes followed by poor and fair quality by different methods of oocyte recovery.

The quality and quantity of oocytes recovered per ovary has been an important consideration in the production of IVM-IVF embryos. The presence of cumulus cells surrounding the immature oocytes is a pre-requisite for successful *in vitro* maturation of buffalo oocytes. Appropriate methods for oocytes recovery and its selection in the laboratory are vital for successful embryo production. To judge the maturation, presence of intact complement of cumulus cells surrounding the oocyte and homogenous appearing ooplasm have been the best criteria. The competence of buffalo oocyte is influenced by a wide array of biological (Nandi *et al.*, 2000) and environmental (Nandi *et al.*, 2001) factors such as follicular size, oocyte diameter, presence or absence of corpus luteum in the ovary and environmental temperature. The numbers of culturable oocytes (usable oocytes) recovered in the present study (3.09 per ovary) were greater than 0.85 and 0.16 reported by Singh and Majumdar (1992) and Dutta and Goswami (1998), respectively. This difference may be due to selection criteria used for categorizing the oocytes in different studies.

In conclusion, the slicing method is a feasible technique for reasonable retrieval of total as well as culturable buffalo follicular oocytes.

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