QUANTITY AND QUALITY OF BUFFALO FOLLICULAR OOCYTES RECOVERED BY ASPIRATION AND SCORING METHODS FOR *IN VITRO* STUDIES

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ABSTRACT

These studies were carried out to compare two recovery methods i.e. aspiration and scoring for the total number and the yield of good quality oocytes per ovary. Buffalo ovaries were collected from local abattoir immediately after slaughter and kept in saline solution, with added antibiotic, at body temperature (37°C). The scoring method yielded a significantly ($P<0.01$) higher number (5.15 per ovary) and morphologically good oocytes (3.85 per ovary) than the aspiration method (3.30 & 1.76 per ovary). It was concluded that scoring the ovarian surface is a better method to recover oocytes for *in vitro* studies.

INTRODUCTION

Pakistani buffalo is a multipurpose animal used for milk, meat and draft purposes. There is great demand to improve the reproductive performance of this valuable animal. Development of an *in vitro* maturation, fertilization (IVM-IVF) and culture system appears to be the most economical and useful technique for the improvement of reproductive performance in the buffalo of this country. However, poor recovery of oocytes and lack of proper conditions to support IVM-IVF in buffaloes are the major impediments (Totey *et al.*, 1992).

To utilize efficiently the technology of *in vitro* embryo production from ovaries of slaughtered animals, it is important to find out a recovery method which facilitates the recovery of large number of better quality oocytes per ovary. The number of bovine oocytes obtained per ovary varies among various methods and laboratories. In cows, the conventional technique of aspiration yields about 11 oocytes per ovary (Iwasaki *et al.*, 1987; Hamano and Kuwayama, 1993). Whereas, in buffalo only 0.46 usable oocytes/ovary were recovered (Totey *et al.*, 1992). Therefore, the present studies were designed to compare the efficiency of aspiration with the scoring method in terms of total and morphologically good quality follicular oocytes recovered per ovary. Data thus obtained will be of practical importance for the researchers interested in collecting oocytes from the ovaries of slaughtered buffaloes for *in vitro* production of embryos.

MATERIALS AND METHODS

Collection of ovaries and recovery of oocytes

Buffalo ovaries were collected from local abattoir within 2 hr after slaughter and were transported to the laboratory in a thermos containing sterile normal saline with added antibiotics (100 IU/ml penicillin G, 100 μg/ml streptomycin sulphate and 0.25 μg/ml amphotericin B). Extraneous tissue was removed and the ovaries were cleaned in normal saline. Prior to oocyte collection the ovaries were rinsed in 70% ethanol to minimize the risk of contamination, followed by three rinses with sterile normal saline to remove the traces of ethanol. The recovery of oocytes from the ovaries was made by two methods i.e. the aspiration and the scoring method.

Aspiration method

Ovarian follicles (2-6mm in diameter) were aspirated using a 5ml syringe, 18-gauge needle filled with a modified tyrode-lactate medium, T1-Hepes (Bavister, 1989), supplemented with estrus buffalo serum (20%), sodium pyruvate (0.20mM) and gentamicin sulphate (10 μg/ml). The pH of the medium was adjusted to 7.3-7.4 and equilibrated at body temperature.

Scoring method

Oocytes were recovered from 2-6mm diameter follicles by scoring the surface of ovary with a sterile surgical blade, with instant rinsing and tapping the ovary to release oocytes in a sterile 60 x 15 mm Petri dish containing modified tyrode-lactate medium, T1-Hepes (Bavister, 1989). The medium was supplemented with estrus buffalo serum (20%), sodium pyruvate (0.20mM) and gentamicin sulphate (10 μg/ml) and its pH was adjusted to 7.3-7.4.

To study the efficiency of two recovery methods, individual ovaries were either randomly aspirated ($n=192$) or scored ($n=185$). The total number and

Table 1: Classification of buffalo follicular oocytes based on their morphological features

Category	Morphological features of follicular oocytes
A	Compact and dense multilayered ($\geq$5) cumulus investment with homogeneous ooplasm.
B	Compact and dense multilayered (3-4) cumulus investment with homogeneous ooplasm.
C	Less compact cumulus cell layers 1-2 with less homogenous ooplasm.
D	Denuded or naked oocytes with evenly granulated ooplasm.

number of morphologically good oocytes recovered per ovary were counted.

The following criteria for classification of buffalo follicular oocytes based on their cumulus investment and ooplasm homogencity was followed for their categorization under steriomicroscope as given in Table-1 (Deloos *et al.*, 1989; Lonergan *et al.*, 1991).

Types A, B and C oocytes were considered morphologically good/useable oocytes for *in vitro* maturation and fertilization experiments. The category "D" oocytes being poor candidates for IVM-IVF are not included for *in vitro* studies (Deloos *et al.*, 1989).

Statistical Analysis

The data obtained were subjected to statistical analysis using chi-square and the means were compared by Z test (Dunnett, 1990).

RESULTS AND DISCUSSION

The recovery rate of buffalo follicular oocytes through aspiration and scoring methods is given in Table 2.

In aspiration method 192 ovaries were aspirated and 1152 follicles (6.0/ovary) yielded 635 (55.12%) oocytes. While in the scoring method 185 ovaries were scored and 1221 follicles (6.6/ovary) yielded 954 (78.13%) oocytes. The study revealed that scoring method yielded higher number and morphologically good quality oocytes (3.85 oocytes/ovary, type A, B & C) than the aspiration method (3.30 & 1.76 oocytes/ovary).

For grading the quality of cumulus oocyte complexes (COCs), the existence of a healthy population of somatic cells surrounding the oocyte is mandatory as the cumulus cells play a supportive role by facilitating the entry of essential products and sending instructive signals to the oocyte for maturation through the gap junction (Osborn and Moor, 1982; Moor and Seamark, 1986). The type A, B and C oocytes were considered morphologically good because they were surrounded by a tight, compact, multilayered, cumulus investment with homogenous ooplasm and are most likely to be developmentally competent. The oocytes that do not possess these characters complete maturation at lower frequency (Deloos *et al.*, 1989).

Table 2: Influence of recovery method on various categories of buffalo follicular oocytes recovered for *in vitro* studies.

	Recovery Methods	
Parameters	Aspiration	Scoring
No. of ovaries	192	185
No. of follicles	1152 (6/ovary)a	1221 (6.6/ovary)a
Categories of oocytes, No. (%)		
A	117 (18.42)b	334 (35.01)a
B	98 (15.43)b	268 (28.09)a
C	123 (19.37)b	111 (11.63)a
D	297 (46.77)b	241 (25.26)a

Values with different letters in a row differ significantly ($P<0.01$).

For *in vitro* maturation, fertilization and culture of follicular oocytes, abundant recovery of good quality oocytes is the prerequisite. But unfortunately, poor recovery of usable oocytes from buffalo ovaries is a major problem for the development of a successful *in vitro* system. In the present study the scoring method yielded a total number of 5.15 oocytes/ovary and 3.85/ovary of morphologically good quality oocytes than the aspiration method which gave 3.30 and 1.76 oocytes/ovary respectively. The recovery rate of oocytes per ovary was significantly higher for the scoring method than for the aspiration method ($P<0.01$). Similarly significantly more good quality oocytes and conversely less poor quality oocytes were recovered by scoring than the aspiration method ($P<0.01$). These findings are higher than those reported by Totey *et al.* (1992), who obtained 0.46 usable oocytes per ovary by using the aspiration method. The significantly higher values of this study are attributed to the efficiency of scoring method. An average collection of 11 oocytes/ovary has been

reported in cows, about half of which were of good quality (Iwasaki *et al.*, 1987; Hamano and Kuwayama, 1993). Relatively low recovery of follicular oocytes in buffaloes than cows might be due to the lower number of primordial and Graafian follicular population in the buffalo ovaries (Danell, 1987).

These findings indicate that scoring the ovarian surface yields significantly higher proportion of total number as well as number of good quality oocytes than aspiration method, to be utilized for the production of *in vitro* embryos in the buffalo.

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