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Collection of Bovine Cumulus-oocyte-complexes (COCs) from Slaughterhouse Ovaries in Bangladesh

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Abstract: Follicles were collected from three categories of ovary obtain from local slaughterhouse. Type-I, having functional corpus luteum (CL); type-II, CL is in almost regressed condition and type-III without CL. The average number of follicles per ovary was 4.37, 5.28 and 6.48, respectively. Significantly higher ($p<0.01$) number of follicles was obtained from type-III ovaries. The cumulus-oocyte-complexes (COCs) collected from each follicle further classified into 4 grades. The average number of grade-A COCs was 1.71, 2.85 and 3.57 for type-I, type-II and type-III, respectively. Significantly higher ($p<0.01$) number of grade-A and B COCs was obtained from type-III ovaries. The number of grade C COCs was not varied significantly ($p>0.01$) among the type. Grade-D COCs was in significantly ($p<0.01$) higher number in type-III ovaries as an exception of the usual expectation. Considering the diameter of the follicles, no significant ($p>0.01$) variation was found in the number of follicles measuring between <2 mm and >6 mm diameter in three types of ovary. Grade-A and grade-B COCs collected from 2-6 mm diameter of follicles are usually used for IVM, IVF and IVC experiment. Significantly ($p<0.01$) higher number of follicles of 2-6 mm diameter was found in type-III ovaries. On the other hand, irrespective of the types of ovaries, grade-A and B of COCs were found to be significantly higher ($p<0.01$) in follicles of 2-6 mm diameter.

Key words: Ovary, follicles, corpus luteum, cumulus-oocyte-complexes (COCs), *In vitro* production (IVP)

INTRODUCTION

Genetic improvement of cattle in Bangladesh has made little progress in the last 30 years by artificial insemination (AI). By the technique of embryo transfer (ET), outstanding cows can be used to produce remarkable number of progeny. This results in rapid genetic gain in female, which complements the AI programme. Embryo transfer technology involves the use of superior females as donors of embryos and there are three ways of embryo transfer technology, I) multiple ovulation embryo transfer (MOET), ii) *in vivo* ovum pick up (OPU) and iii) oocyte recovery at slaughter. Though the former two techniques are useful, but require huge involvement. Moreover, the success of ET mostly depends on embryo production from slaughterhouse ovaries. Embryo production from slaughterhouse ovaries involves collection and grading of COCs, *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and subsequent *in vitro* culture (IVC). *In vitro* maturation of immature oocytes from ovaries at slaughter, followed by IVF and IVC of the resulting zygotes has allowed extensive research on modern reproduction techniques in farm animals.

For *in vitro* production of embryo (IVP), the efficient method of collection and grading of COCs are the prerequisite. Several methods such as aspiration of total follicular material, dissection of individual isolated follicles with subsequent isolation of the COCs and slicing of the ovaries have been described to obtain immature oocytes from slaughterhouse ovaries. However, techniques such as slicing of the ovaries, flushing the follicles with phosphate buffer saline (PBS) or rupturing the isolated follicles may increase the number of recovered oocytes as compared with that of aspiration of follicular materials (Alm and Torner, 1994). In addition, it has been shown that a comparable higher maturation rate could be reached within 24 h of culture if the oocytes had a compact cumulus investment (Hinrich *et al.*, 1993).

However, a great deal of work has been done regarding collection of COCs from slaughterhouse ovaries, grading of collected oocytes and IVP of the embryos throughout the world. But in Bangladesh, no such work has so far been undertaken. So the present research has been undertaken for the first time in Bangladesh as a very preliminary approach and it is hoped that this work would be a base line for the future researchers who will attempt to make further contribution in this field of animal biotechnology. The following are the objectives of the present experiment:

- To know the collection and grading procedures of bovine COCs obtained from slaughterhouse ovaries

- To establish the relationship between ovarian condition and morphological quality of the collected COCs
- To establish the relationship between follicular diameter and quality of COCs

MATERIALS AND METHODS

Collection and processing of ovaries: Ovaries from cows at district city of unknown reproductive history were collected from local slaughterhouse at Mymensingh, Bangladesh. The ovaries were collected in 0.9 % saline in a thermoflask at 25 to 30°C and transported to the laboratory within 3 to 4 h of slaughter. The ovaries in the laboratory were handled aseptically wearing hand gloves. They were then categorized in three types. Type-I, having functional corpus luteum (CL), type-II have almost regressed CL and type-III without CL.

All visible antral follicles were collected by incising each ovary with scissors and blades followed by scraping the ovarian tissue with forceps and needles. After collection of all visible follicles on the surface of each ovary, the ovary was dissected to expose additional follicles within it. The follicles after collection from each ovary were stored in saline at room temperatures. For each ovary, the number of follicles collected was recorded and the diameter of each follicle was measured with the help of slide calipers.

Grading of cumulus-oocyte–complexes (COCs): The follicular materials from each follicle were harvested by blunt dissection on a sterilized glass slide. The follicular materials of each follicle were then observed under microscope and COCs were classified into 4 grades as described elsewhere, briefly, grade-A; homogeneous COCs, grade-B; COCs not homogeneous, grade-C; COCs were not found at all and grade-D; expanded COCs. The representative photograph of four grades of COCs is shown in Fig.1.

Statistical model and methods of data analysis: The experiment was designed as completely randomized design (CRD). Data were statistically analyzed using MSTAT computer package program in accordance with the principles of CRD (Steel and Torrie, 1980) and least significant difference (LSD) test was done to identify the significant differences between the mean values when analyses of variance (ANOVA) showed significant differences (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Total 34 ovaries were collected from local slaughterhouse. The ovaries were classified into type-I, type-II and type-III as described in materials and methods. Among them, 8 were categorized as type-I, 7 as type-II and 19 as type-III. Data were collected on different parameters such as number of follicle per ovary, diameter of each follicle and microscopic state of the COCs. A total of 202 follicles were obtained from all 3 types of ovary. Among them 35, 37 and 130 follicles were collected from type-I, type-II and type-III ovaries, respectively (Table 1).

Ovarian type and number of follicles and COCs per ovary: The results of the data analyzed on the number of follicles and COCs per ovary in 3 different types of ovary are presented in the Table 1. Analysis of variance on the data of the number of follicles per ovary was made to test the variation among the types of ovary and the ANOVA indicated that the variation was significant ($P<0.01$). Significantly higher ($P<0.01$) number of follicles was observed in type-III (6.48±0.38), followed by type-II (5.28±0.62) and type-I (4.37±0.58).

It is well established that all female mammals are born with a large store of follicles which rapidly declines as puberty approaches but whether this early losses represent a mechanism of physiological wastage is not definitely known. Follicle growth initiation is one of the most important and least understood aspects of ovarian biology and represents a major challenge for experimental study. Changes in the local microenvironment such as the pH and hormonal concentration probably occur as the follicles evolve into the primary stage but these are probably effects rather than causes (Webb *et al.*, 1999). In cattle, there are three wave patterns of follicular selection, although two waves or some times four waves can occur during the estrous cycle (Savio *et al.*, 1988). Each wave of follicular development is characterized by simultaneous emergence of medium sized (>4 mm in diameter) growing follicles from a pool of smaller follicles. One of these groups of follicles rapidly emerges as the dominant follicles (7-9 mm in diameter) and continues to develop; while the others undergo atresia and regress. In cattle, it usually takes 5 to 7 days for the dominant follicles to develop the ovulatory size (Fortune, 1994, Ginther *et al.*, 1996). Despite the overwhelming occurrence of follicular atresia the cellular and molecular mechanisms underlining this phenomenon still remain poorly understood.

Growth initiation of follicles has variously been attributed to I) hormonal triggers (gonadotrotopins), ii) stochastic processes (fluctuation in internal signal molecule) and iii) external inhibitory control from growing

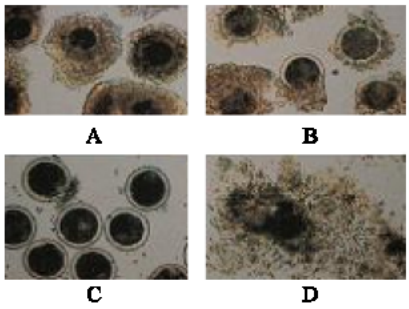

Fig. 1: Representative photographs of the four grades of COCs
Grade-A: homogeneous COCs, Grade-B: COCs not homogeneous, Grade-C: COCs were not found at all and Grade-D: expanded COCs

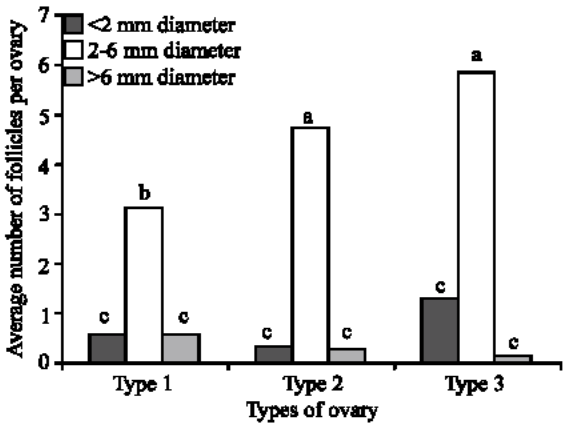

Fig. 2: Number of different size follicles recovered from different types ovaries

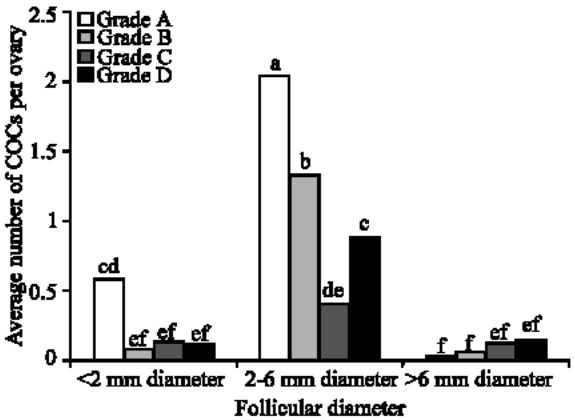

Fig. 3: Number of COCs of different grades for different follicular diameter

follicles (Webb *et al.*, 1999). The balance between the gonadotropins (FSH and LH) and steroids (estrogen and progesterone) might be the important criteria in this process. The highest number of follicles that were found in type-III ovaries in the present study might reflect the optimum level of gonadotropins and steroids. Type-III ovaries did not contain the CL and the negative effect of progesterone on anterior pituitary was not functional in this type of ovaries. Similarly, second highest number of follicles in type-II and least number in type-I ovaries further confirmed the above statement as regressed CL was found in type-II and functional CL was found in type-I ovaries. As the ovaries were collected from slaughterhouses it was impossible to confirm the cyclic state of the ovaries, so there might have some discrepancies in the present result. On the other hand, the significantly highest ($p<0.01$) number of grade A and grade B COCs was observed in type-III followed by type-II and type-I ovaries (Table 1). Hafez (1993) reported that progesterone secreted by the luteal cells of the CL inhibited estrus and gave the negative feedback on the anterior pituitary to secrete FSH. As a result, the growing follicles regressed and became atretic. The effect of progesterone on follicular growth could not be investigated in the present study. But it can be assumed that the higher number of grade A and B COCs in type-III and medium to low number of COCs in type-II and type-I ovaries might arise from the activity of CL. These results further confirmed the previous findings described in Table 1 and Fig. 1. Slightly higher number of grade-C COCs was found in type-II and medium to less number in type-I and type-III, respectively. But the differences among the types did not reach in significant level. As progesterone was reported to be responsible for follicular atresia (Hafez, 1993), the findings are very much in conformation. The less number of grade-C COCs found in type I ovary might arise from other factors causing atresia.

In respect of the number of grade-D COCs, the unexpected highest value was found in type-III and second highest was in type-I though the differences did not reach in significant level. The lowest number of this grade of COCs was found in type-II ovaries. This discrepancy might come from lack of recording of the cyclic state of the animal as slaughterhouse ovaries were used in the present study.

Number of follicles on the basis of diameter: Follicles were classified into 3 types based on their diameter: <2 mm, 2-6 mm and >6 mm diameter. Analysis of variance was done to test the differences among the ovarian types and follicular diameter and the results indicated that the difference was significant. The results on the number of follicles of different follicular diameter are presented in Fig 2. Significantly highest ($P<0.01$) number of follicles of 2-6 mm diameter was observed in type-III, but the difference between Type-III and type-II did not reach in significant

Table 1: Ovarian types, number of follicles and harvested cumulus-oocyte-complexes (COCs) per ovary

			Number of COCs			
Types of ovary	Total number of ovaries	Number of follicles per ovary (Mean ± SE)	Grade A	Grade B	Grade C	Grade D
Type-I	8	4.37± 0.58b (35)	1.71cd	0.71def	0.42ef	1.28cdef
Type-II	7	5.28±0.62ab (37)	2.85ab	1.42cde	0.57ef	0.42ef
Type-III	19	6.48± 0.38a (130)	3.57a	1.85bc	0.28f	1.71cd

Means with different superscript differ significantly from each other within third column and both in columns and rows in the remaining. The figure in the parenthesis indicates the total number

level. No significant variation was observed in the number of follicle of <2 mm diameter in three types of ovaries.

Oocytes collected from the follicles of 2-6 mm diameter were usually used for IVM and IVF and subsequent IVC study. Totey *et al.* (1993) in buffalo and Rath *et al.* (1995) in cow collected 2-6 mm diameter of follicles to determine the efficiency of *in vitro* maturation and fertilization experiment and found the desirable results. The highest number of 2-6 mm and <2 mm diameter follicles obtained in type-III ovaries indicated that the recruitment and selection of ovarian follicles were functional in usual manner due to lack of progesterone activity. Comparatively less number of <2 mm and 2-6 mm diameter follicles obtained in type-I and type-II explained the reverse effect of progesterone activity. The higher and medium number of >6 mm diameter follicles in type-I and type-II might indicate the growing follicles of the luteal phase but the further study is required to confirm the statement.

Number of COCs of different grades: The results of the number of COCs of different grades for different follicular diameter are presented in Fig 3. Significantly highest ($p<0.01$) number of grade-A COCs followed by grade-B were observed in 2-6 diameter follicles. The most notable observation was that the less number of grade-A and grade-B COCs was obtained from >6 mm diameter follilcles. Cumulus cells during maturation period supported the IVM of oocytes to the metaphase-II stage and development to the blastocyst stage in cattle (Hashimoto *et al.*, 1998). Porcine cumulus free oocytes could not be developed beyond 4-cell stage (Yamauchi *et al.*, 1999). In the current study grade-A and grade-B COCs were found in significantly highest number in type-III ovaries as well as in follicles with 2-6 mm diameter. So follicles of 2-6 mm diameter from type-III ovaries can be an important source of oocytes for IVP of embryo experiment.

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