Ovarian category, follicles and oocytes analysis of Goat ovaries in view of *in vitro* production of embryos

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ABSTRACT

The study was undertaken to evaluate the slaughter house goat ovaries, follicle and cumulus-oocyte-complexes (COCs) with the view of *in vitro* production of embryos. Goat ovaries were categorized as right, left, with corpus leutum (CL) and without CL group. Ovaries were then evaluated on the basis of weight (gm), length (cm), width (cm), total number of follicle on the surface of each categorized ovaries, number of follicle aspirated, total number of COCs, normal COCs and abnormal COCs. The length (cm) of right ovaries (1.19±0.09) was found significantly ($p<0.05$) higher than left ones (1.15±0.04). The number of normal oocyte was found significantly higher ($p<0.05$) in left ovaries (1.18±0.10) then right ovaries (1.11±0.09). Other parameters, including width, weight and total number of COCs aspirated per ovary did not differ significantly ($P<0.05$) between right and left ovaries. When compared the ovaries in between with-CL and without-CL group, significantly ($p<0.05$) higher number of normal COCs (1.12±0.07) were found in without-CL group with an increase of length (1.17±0.01), total number of follicles (5.21±0.03), number of aspirated follicles (2.74±0.12) and total number of COCs (1.99±0.14), but decrease in weight (0.66±0.03), width (0.76±0.02) and abnormal COCs (0.87±0.15) per ovary. Therefore, it could conclude that, both right and left ovaries is great source for large number of oocyte and without-CL ovaries is considered as a suitable source to supply quality oocyte for *in vitro* embryo production of goat.

Keywords: Goat, Corpus luteum, Follicles, Cumulus-oocyte-complexes

INTRODUCTION

The domestic goat *Capra hircus* is an important livestock species in Bangladesh and other developing countries especially in Asia and Africa. Since it provides a good source of meat, milk, fiber and skin, it is popularly known as the "poor man's cow" (MacHugh and Bradley, 2001). Out of 920.60 million world goat population, Asia itself possesses about 551.23 million which is almost 59.8% of the total world population (FAO, 2010). Goat population in Bangladesh constitutes nearly 7.05% of the total population in Asia (FAOSTAT, 2009). Goat is numerically and economically very important and promising animal genetic resources in the developing countries like Bangladesh. Thus, genetic improvement of goat in Bangladesh could be made by planned artificial insemination (AI) with frozen semen, multiple ovulation and embryo transfer (MOET) and *in vitro* production (IVP) of embryos. To produce good embryos, quality oocyte is obligatory. Though lot of ovaries are waste in slaughter house but it may be a good source of quality livestock production which can full fill the existing scarcity of meat, milk and skin. Ovary collection, evaluation and grading technique results in rapid genetic gain of outstanding females. It complements the exploitation of superior males through artificial insemination program. So, *in vitro* production of embryo from slaughterhouse ovaries might be considered as a low cost and sustainable technique in Bangladesh condition (Rahman, 2003). Embryos can be produced from the oocytes collected

from the ovaries of the females that are usually being slaughtered in slaughterhouse for meat purpose, and the embryos thus produced can be transferred to the recipient females. In Bangladesh, *in vitro* techniques in goat is a recent concept but a great deal of work has been, still going on to standardize IVEP techniques followed by IVM and IVF (Ferdous, 2006; Islam et al., 2007; Mondal et al., 2008; Hoque, 2009). A critical goal for mass production of goat embryo production is the recovery of a large number of oocyte with high developmental competence. With some other factors successful embryo production is also depend on oocyte quality. So, the present study was undertaken with a view to collection and evaluation of goat slaughter house ovaries, follicles and COCs to create vast opportunities to conduct the research work in the area of IVP of goat embryos.

MATERIALS AND METHODS

The experiment was conducted from July 2014 to June 2015 at the Animal Nutrition, Genetics and Breeding Laboratory of Sher-e-Bangla Agricultural University, Dhaka.

Ovary collection and processing

Before starting the experiment, all the necessary electrical power driven or digital equipment were properly installed and checked for good condition. These were repaired, reinstalled and finally cleaned and sterilized with 70% alcohol. Unless otherwise mentioned, all chemicals and media were purchased from Sigma Chemical co. (St. Louis, MO, USA).Goat ovaries were collected from slaughter house and were kept in collection vial containing 0.9% physiological saline in a thermo flask at 25°C to 30°C and transported to the laboratory within 5 to 6 hours of slaughter. The ovaries were then transferred to the sterilized petridishes containing same saline. During collection, ovary were marked whether it is right or left. The ovaries were rinsed thoroughly by physiological saline solution for two times at 25°C temperature. In the laboratory each ovary was trimmed to remove the surrounding tissues and overlying bursa. Each ovary was treated to three washings in D-PBS and two washings in oocyte harvesting medium (DPBS+4mg/ml BSA+1.50 IU/ml Penicillin) as described by Wani et al. (2000). After collection and trimming, ovaries were evaluated on the basis of presence and absence of CL. There are numerous follicles on the surface of all ovaries. The number of visible follicles on the surface of different category of ovaries were counted and recorded.

Evaluation of ovary

After collection and trimming ovaries were evaluated on the basis of the following parameters-

Measurement of weight, length and width of ovaries

Individually right, left, CL-present and CL-absent ovaries were weighed in gm in a digital balance and the weight was recorded in tabular form. The length and width in cm of right, left, CL- present and CL-absent ovaries measured with the help of a digital slide calipers.

Counting of follicle on the surface of the ovary

Different size of follicles on the surface of both ovaries are present. The number of visible follicles on the surface of different category (with or without CL) of ovaries were counted and recorded for further analysis.

Oocyte collection and COCs evaluation

The ovaries were washed 2-3 times in saline solution at 30°C. They were then placed in a beaker and kept in a water bath at 30°C. One ovary was picked up in hand. The 10 ml syringe was loaded with D-PBS (1.0 -1.5ml), and the needle (18G) was put in the ovary parenchyma near the vesicular follicles (2 to 6 mm diameter) and all 2 to 6 mm diameter follicles were aspirated near the point at the same time. After aspirating the follicles from one ovary, the aspirated follicular materials were transferred slowly into a 90-mm Petridis, avoiding damage to the cumulus cells and the COCs were searched and graded under microscope (Olympus, Tokyo, Japan) at low magnification. The COCs were then classified into 4 grades as described by Khandoker et al. (2001). Briefly, grade A: oocytes completely surrounded by cumulus cells; grade B: oocytes partially surrounded by cumulus cells; grade C: oocytes not surrounded by cumulus cells and grade D: degeneration observed both in oocytes and cumulus cells, where grade A and B will be considered as normal COCs and grade C and D as abnormal COCs.

Statistical Analysis

All values were expressed as Mean±SE. Statistical significance of differences between different parameters was evaluated by using student's t-test. The statistical analysis was done by SPSS program (Version 16.0; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Goat ovaries were collected from local slaughter house. Among (N=160) ovaries; 80 on each side (left and right) was classified into the ovaries with and without CL. A number of 130 belonged without CL and 30 recorded as with CL ovaries. The results of the different parameters are summarized in Table 1 and Table 2. The length (cm) of right ovary (1.19±0.09) was significantly ($p<0.05$) higher than the left (1.15±0.04) ones but no significant ($p<0.05$) differences were found in the width (cm) and weight (gm) of left (0.75±0.02 and 0.68±0.03) and right ovaries (0.82±0.02 and 0.70±0.03), respectively (Table 1). Similar results were found in goat (Islam et al., 2007). Normal physiological explanation of ovarian activity is that right ovaries are more active than left ones (Rahman et al., 1977) and (Sarker, 1993). On the other hand, the width and weight were significantly ($p<0.05$) higher in ovaries with CL than those of ovaries without CL (Table 2). The CL is an extra cellular material within the ovary which made the differences of its width and weight. The mean length (cm) was found reverse in the ovaries without CL (1.17±0.01).

Table1: Ovarian classification (right and left) and other parameters of goat ovary

Classification (No. ovaries)	Weight(g) (Mean±SE)	Length (cm) (Mean±SE)	Width (cm) (Mean±SE)	Total number of follicle (Mean±SE)	Total number of follicle aspirated (Mean±SE)	Collected COCs per ovary (Mean±SE)		
						Total	Normal	Abnormal
Total (160)	0.69±0.01	1.17±0.04	0.78±0.01	5.15±0.19	2.7±0.06	1.97±0.13	1.13±0.07	0.83±0.04
Right (80)	0.70 ±0.03	1.19^{a}±0.09	0.82 ±0.02	5.10 ±0.12	2.6 ±0.16	1.97 ±0.09	1.11^{b}±0.09	0.86 ^{b}±0.07
Left (80)	0.68 ±0.03	1.15^{b}±0.04	0.75 ±0.02	5.20 ±0.22	2.8 ±0.11	1.97 ±0.15	1.18^{a}±0.10	0.79 ^{a} ±0.07

Means with different superscripts within the same column differ significantly (P<0.05)
Figure in the parenthesis indicates the total number

Table 2: Ovarian classification (with and without CL) and other parameters of goat ovary

Class (No. ovaries)	Weight(g) (Mean±SE)	Length (cm) (Mean±SE)	Width (cm) (Mean±SE)	Total number of follicle (Mean±SE)	Total number of follicle aspirated (Mean±SE)	Collected COCs per ovary (Mean±SE)		
						Total	Normal	Abnormal
Total (160)	0.69±0.02	1.17±0.01	0.78±0.03	5.15±0.04	2.7±0.17	1.95±0.06	1.02±0.05	0.94±0.11
With CL (30)	0.72^{a}±0.03	1.16±0.03	0.81^{a}±0.02	5.11^{b}±0.02	2.69^{b}±0.19	1.90^{b}±0.09	0.88^{b}±0.17	1.02^{a}± 0.21
Without CL (130)	0.66^{b}±0.03	1.17±0.01	0.76^{b}±0.02	5.21^{a}±0.03	2.74^{a}±0.12	1.99^{a}±0.14	1.12^{a}±0.07	0. 87^{b}±0.15

Means with different superscripts within the same column differ significantly (P<0.05)
Figure in the parenthesis indicates the total number

This result contradicts with the previous result of Singh et al.(1974), Rahman et al. (1977) and Sarker et al. (1993) and it might be due to less number of ovaries were processed. The total of 675 follicles were recorded on the surface of the ovaries and 395 follicles were aspirated from the surface of both (right and left) ovaries and among them 196 were obtained with a mean of 2.6±0.16 per ovary from right and 199 from left ovaries with a mean of 2.8±0.11(Table 1). This result found no significant difference between rights and left one. The collected COCs were found almost same in right and left ovaries. When the COCs were classified as normal and abnormal groups, the highest numbers of normal COCs were found in left (1.18) than that of right (1.11) ovary which supports the previous result of Islam et al. (2007) performed in goat. This may be occurred due to presence of more CL on the surface of

right ovaries which released more oocyte in estrus cycle and control the FSH secretion for oocyte development. In other case, 395 follicles were aspirated out of 675 follicles on the surface of both ovaries from CL group and without CL ovaries. The number of follicle aspirated in this experiment was lower because of collection techniques. Only aspiration technique was applied to harvest the COCs as a result, the oocytes remain firmly attached to the small and medium sized follicles before cumulus expansion and cannot be aspired. Thus, the lower number of COCs recovered by the aspiration method in this experiment may be attributed to the presence of some follicles released by other collection method. In this study, the significantly higher ($p<0.05$) number of follicles were aspirated per ovary in ovaries without CL (2.74±0.12) than that of CL containing ovaries (2.69±0.19) (Table 2). This result was comparable with the observation of Wang et al. (2007) who harvested oocytes from ovary of Boer goat by aspiration (2.9) collection techniques. It also supports the findings of Singh et al. (2013) in goat. The causes of higher number of follicles found in ovaries without CL than those of CL containing group were understood well as it fits the endocrinological explanation. Various factors that might influence oocyte recovery revealed that non-luteal phase ovaries yielded significantly higher number of oocytes compared to luteal phase ovaries. 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 initiated 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). Growth initiated of follicles has variously been attributed with hormonal triggers (gonadotropins), ochastic process (fluctuation in the internal signal follicle) and external inhibitory control growing follicles (Webb et al., 1999). The balance between the gonadotropins (FSH and LH) and steroid (estrogen and progesterone) might be the important criteria in this process. The highest number of follicles that are found in ovaries without CL in the present study might reflect the optimum level of gonadotropins and steroid. Again, when the COCs were classified in normal and abnormal groups, the significantly higher ($P<0.05$) number of normal COCs was found in ovaries without CL than those ovaries with CL with the mean of 1.12±0.07 and 0.88±0.17 follicles per ovary respectively. The reverse trend was found in abnormal group 0.87±0.15 and 1.02±0.21 follicles per ovary respectively. Age, season, nutritional status (body condition) and cyclicity of animals at the time of slaughter, size and functional status of follicles, method of oocyte retrieval are some of the factors that might contribute to recorded variation in oocyte quality (Nandi et al., 2001; Zoheir et al., 2007; Amer et al., 2008). In terms of quality of oocytes, Ferdous (2006) reported that the numbers of normal COCs were found to be significantly higher ($p < 0.05$) in 2 to 6 mm diameter follicles than others. In the ovaries without CL, the negative effect of progesterone on anterior was not functional in this types of ovaries. As the ovaries are collected from slaughterhouse, it was impossible to confirm the cyclic state of the ovaries. So, there might have some discrepancies in the present result. The cause of low number of oocytes per ovary with a CL is likely because of the follicular development is restricted, as lutein cells occupy a great portion of the ovary and also attributed that CL may inhibits the growth of follicles and increase their atresia (Hafez, 1993). The presence of CL in cyclic female's ovary produces a higher level of

progesterone hormone that signals a negative response to anterior pituitary gland for the restriction of gonadotropin secretion and ultimately follicular degeneration occurs (Webb et al., 1999). But due to the absence of CL in non-cyclic female, the negative effect of progesterone might not be functional and estrogen-progesterone remains in balanced level which allows follicular growth and oocytes maturation. The highest number of COCs in CL absent group of ovaries than that of CL present group as found in this study explains the role of hormonal balance on goat folliculogenesis. The findings of CL-present group of ovaries explain the role of progesterone on goat follicular degeneration and further strengthening the previous statement. Hafez (1993) reported that progesterone secreted by the luteal cells of the CL inhibits estrus and gives the negative feedback on the anterior pituitary to secrete FSH. As a result, the growing follicles regressed and became antretic. The effect of progesterone on follicular growth could not be investigated in the present study. But it can be assumed that the highest number of normal grade in ovaries without CL and the lowest number of normal grade in ovaries with CL might arise from the activity of CL.

CONCLUSION

The present findings revealed that right and left ovaries both have a great potentiality to provide good number of oocytes for *in vitro* studies. Considering with the effects of CL on ovaries; highest number and normal grade oocyte would be collected from without CL contains ovaries. Moreover, this result creates a great opportunity of conducting further research on goat embryo production in Bangladesh.

CONFLICT OF INTEREST STATEMENT

The author declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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