IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 13, Issue 12 Ser. II (December 2020), PP 40-44
www.iosrjournals.org

Morphometric assessment of oocytes recovered from ovaries of Black Bengal goat

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Abstract:*Ovary plays an important role in the reproductive biology and biotechnology of female animals. With the aim to study the oocyte morphometry of Black Bengal goat, both right and left ovaries were collected from the local slaughter houses of Dhaka city. Oocytes were collected by three methods like dissection, slicing and aspiration. The study revealed that the weights of right and left ovary were 0.79±0.17 g and 0.77±0.10 g, respectively. The length of right ovary was 1.36±0.21 cm and 1.39±0.32 cm of left ovary. The widths of right and left ovary were 1.00±0.13 cm and 0.98±0.15 cm, respectively. Slicing methods yield more oocytes with moderate quality while aspiration methods yield moderate oocytes count with good quality. The oocyte diameter was 100.21±4.84 µm and cumulus cells (CCs) diameter was 49.77±12.62 µm. The research revealed that the oocytes with larger diameter represent larger CCs diameter as well as good quality for in vitro production. These results will be helpful to manipulate ovarian functions, reproductive biology and biotechnology such as in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro embryo production (IVP) in small ruminants.*

Keywords:*Cumulus cells, goat, morphometry, oocyte, ovary*.

Date of Submission: 05-12-2020 Date of Acceptance: 20-12-2020

I. Introduction

Bangladesh is an agricultural country and livestock plays a vital role in national economy. Goat is a multi-functional animal and contributes greatly to the agrarian economy, especially in the areas where crop and dairy farming are not economic. Goats are very important livestock species in Bangladesh due to their short generation intervals, higher prolificacy rate and the ease with which the goats and their products can be marketed. The goat is called the "Poor man's cow" and it's the second important livestock in Bangladesh which plays an important role in the rural economy and earn substantial amount of foreign currency by exporting skin and others byproducts (MacHugh and Bradley, 2001). Ovary is the key reproductive organ of all the female vertebrates. Ovaries are vital organ that supplies the germ cells, oocytes and produce hormones for maintaining reproductive health. Black Bengal goat is the national pride of Bangladesh. This dwarf breed is a prolific breed, required small area for raising with the advantage of selective feeding habit and broader feed range. It is very popular to consumers for its delicious, tender meat and highly valued skin in the world market. Considering the paramount importance and bright prospects of Bangladesh, Black Bengal goat production level should be maintained properly by increasing fertility and conception rate. Therefore, the study was designed to observe the morphology and morphometry of the ovary of Black Bengal goat. Keeping the aforesaid reality in mind the research was designed with the objectives to evaluate the Black Bengal goat oocytes morphologically and morphometrically collected from Black Bengal goat ovaries in view of *invitro* production.

II. Materials And Methods

Study area

The experiment was conducted at the Laboratory of Animal Production and Management Department, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Collection of ovaries

Ovaries were collected from local slaughterhouse with their unknown reproductive history. The ovaries were then recorded as right, left and the presence or absence of corpus luteum (CL) was also recorded. They were then kept in collection vial containing 0.9% physiological saline in a thermo flask and transported to the laboratory. The ovaries were then transferred to sterilized petridishes and rinsed thoroughly by distilled water before further processing.

Processing of ovaries

The ovaries were then transferred to the petridishes which is sterilized and containing the same saline. The ovaries were rinsed for two times thoroughly by physiological saline solution. Each ovary was trimmed and the surrounding tissues, fat & overlying bursa were removed by dissection.

Morphological study of ovaries

After trimming individually, right, left, CL-present and -absent ovaries were weighed in gram by digital balance and was recorded in tabular form. The length and width of the ovaries were measured in cm with the help of a slide caliperse.

Oocyte collection

The numbers of visible follicles on the surface of different category of ovaries were counted and recorded. The ovaries were washed 2 to 3 times in saline solution. Each ovary was processed individually and the cumulus oocyte complexes (COCs) were harvested by the three (dissection, slicing and aspiration) techniques.

Oocyte grading

The petridishes were then examined under microscope, and the total number of COCs harvested was counted. The oocytes were graded into 4 grades on the basis of cumulus cells and nucleus as described by Khandoker*et al.* (2001): 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. The grade A and B were considered as normal and grade C and D as abnormal oocytes.

Morphometry of oocytes

The collected oocytes were grouped based on diameter: Group 1 (<95 µm), Group 2 (95-100 µm) and Group 3 (>100 µm). The thicknesses of the cumulus cell layer in COCs were classified into three categories: Category 1 (<30 µm), Category 2 (30-50 µm) and Category 3 (>50 µm) as described by Zhou *et al.* (2014).

Data analysis

Data were presented as mean±standard deviation (SD). The P value less than 0.05 was considered as significant. Data were analyzed with the SAS (Statistical Analysis System) software using one-way Analysis of Variance (ANOVA).

III. Results And Discussion

Gross study of the ovary

The ovaries were found almond-shaped and pale colored structures. Each ovary had an irregular surface by various sized follicles projecting from the surface. The length of right ovary was numerically higher than that of left ovary (Table 1). Comparatively a lower length for both right and left ovaries of goats was reported by Islam *et al*. (2007). However, a higher length was reported in goats (Mohammadpour, 2007; Adigwe and Fayemi, 2005; Sharma and Sharma, 2004). Non significant difference was found between the mean width of right and left ovaries (Table 1). The mean weight of the ovary recorded in the present study was higher than that of other observation (Islam *et al*., 2007). The weight, length and width of the right ovary were 0.79±0.17 g, 1.36±0.21 cm and 1.00±0.13 cm and of the left were 0.77±0.10 g, 1.39±0.32 cm and 0.98±0.15 cm respectively. This observation showed the slight difference with other report (Haque*et al*., 2012). The result expressed that both left and right ovaries were equally active to normal physiological and ovarian activity. Islam *et al*. (2007) expressed that the mean weight, length, width and ovarian activity were found to be higher in right ovaries than those of left ovaries. These observations differ to that of the present study. Talukder*et al*. (2011) observed that there was no significant (P>0.05) difference in length, width and weight between the left and right ovaries of sheep. The results of some previous study of Mohammadpour (2007), Asad*et al*. (2016) supports the findings of present study that there was no significant difference (P>0.05) in the parameters of left and right ovaries of goat.

Effect of time elapsed from slaughtering to sample processing on oocyte quality

It is better to transport quickly the samples from abattoir to the place of processing after slaughter. The degree of oocyte damaging, ageing and deterioration was elevated if the time elapsed from the period of slaughtering to processing at the laboratory, as shown in Table 2. It is noted in the present study that there was a direct effect on elapsed time from the period of slaughtering the donor animals toward time of samples processing inside the laboratory. As this time prolonged it affects the oocytes quality which interferes with final

result. This might be due to many factors affect directly the oocytes quality. Time of slaughter is the more dominant factor that influence the quality. Lihua*et al*. (2010); Saleh (2017) also approved the effect of elapsed time on the oocytes quality that might interfere (impaired) with *in vitro* oocytes maturation (IVM) that yield low quality embryos.

Effect of collection techniques on oocytes recovery

Several methods have been used for harvesting oocytes from slaughterhouse ovaries of farm animals. In the present research, the oocytes were collected by three different methods (Dissection, Slicing and Aspiration). The result of oocytes recovery per ovary by three different techniques of dissection, slicing and aspiration is summarized in Table 3. Total 33, 53 and 48 oocytes were collected by dissection, slicing and aspiration techniques; respectively, from each of 30 ovaries. The results indicate that slicing and aspiration yielded a significantly higher number of total oocytes than that of dissection method. However, a significantly higher number of normal graded (A & B) oocytes was observed in aspiration method (68.75%)) than those of dissection (33.33%) and slicing (45.28%) techniques. The most commonly practiced methods of oocytes recovery in goat are puncture and aspiration of visible follicles and follicular dissection (Wang *et al*., 2007). In the present study comparatively more oocytes per ovary was obtained by slicing method, when compared to aspiration and dissection. This is in agreement with the observations ofShirazi*et al.* (2005) and Wang *et al*. (2007) in goats. Yield of culturable quality oocyte was highest with aspiration followed by slicing and dissection. This finding is in agreement with the results of Kharche*et al*. (2006) and Wang *et al*. (2007). This research revealed that among collection techniques, slicing technique yielded maximum number of oocytes per ovary, while maximum percent yield of culturable quality oocytes obtained by aspiration method.

Interrelationship between diameter of oocytes and oocytes quality

These results showed an association between oocyte diameter and oocytes quality. During the process of folliculogenesis, the oocyte diameter and layers number of granulose cells increase. As a result, follicle size and follicular fluid accumulation will increase. During folliculogenesis, oocyte diameter growth and will continue to grow after antrum formation until to a certain diameter.The present study showed that there was a remarkable interrelationship between the oocyte diameter and quality (Table 4). The results showed a noticeable correlation between oocytes diameter with quality of oocyte. In Table 4, it is seen that oocytes group 3 (>100 µm) possess good quality oocytes when compared with group 2 (95-100 µm) and group 1 (<95 µm). On the other hand, the abnormal oocytes observed in highest number (57.14%) in the group 1 (<95 µm). These results revealed an association between oocyte diameter and oocyte quality. Research conducted in pigs (Lucas *et al.,* 2002) and in cattle (Otoi*et al.,* 1997) showed the oocytes with a large diameter resulting in a higher maturation rate when compared to oocytes with a small diameter. In other studies Haque*et al* (2012) showed that oocytes with good quality COCs have a higher success rate and embryo development after fertilization. Results of the research showed a close relationship between oocyte diameterwith oocyte quality for the ability of oocytes development.

Relationship between cumulus cells diameter and oocyte quality

The cumulus cells (CCs) surrounding the oocyte plays an important role in oocyte maturation and they are known to supply nutrients, energy substrates. To evaluate the effect of cumulus layer thickness on oocyte developmental potential, we first divided the CCs diameter into three categories according to the thicknesses of their surrounding cumulus layer (C). The CCs that were in the category of C>50 µm, had more than three layers of cumulus cells, whereas the CCs that had two or three cumulus cell layers fell into the category of 30-50 µm, and the CCs with less than two layers of cumulus cells fell into the category of C<30 µm. The Table 5 showed the relationship of CCs diameter with oocyte diameter and oocyte quality. It was seen that 48.15% of the total (27) oocyte number contain >50 µm diameter oocytes. From the Table 5, it was observed that, most of the oocytes having >100 µm diameter represent >50 µm CCs diameter. On the other hand, 57.14% of <95 µm oocytes diameter represent <30 µm. Meanwhile, less diameter CCs (<30 and 30-50 µm) are found more in oocyte diameter group (<95 µm). It was 57.14% and 28.57% than thicker Ccs diameter (>50 µm) which was only 14.29%. Zhou *et al*. (2014) found that all of the CCs with C>50 µm showed the highest rate of oocyte nuclear maturation whereas CCs with 30-50 µm showed a slightly decreased rate in comparison and the lowest oocyte nuclear maturation rate was observed in the CCs with C<30 µm. These data revealed that a thicker cumulus cell layer promotes *in vitro* oocyte maturation. The result showed that, the more diameter containing oocytes bear thicker CCs which suitable for better quality oocytes and reproduction. The oocytes that contain fewer cumulus cell layers have a lower potential to undergo maturation, fertilization, and development to an early embryo stage.

IV. Conclusion

From the study, it was observed that the length, width and weight of right and left ovaries of Black Bengal goats were not significantly different. The oocyte quality is highly influenced by timing of sample processing and more oocytes with moderate quality were obtained by slicing method while good quality oocytes were collected by aspiration collection method.So the ovaries should be collected, transported, processed and evaluated with proper temperature and duration. The larger diameter oocytes present larger diameter cumulus cells with higher percentage of good quality oocytes and these oocytes have greater contribution for *in vitro* production (IVP). The present study revealed that morphometric assessment of left and right ovaries have a great potentiality to identify the good number of oocytes for *in vitro* studies.This findings create a great opportunity for conducting further research on Black Bengal goat embryo production that may help to enhance their productivity in Bangladesh.

Acknowledgement

The authors would like to thank theSher-e-Bangla Agricultural University Research System (SAURES) and department of Animal Production and Management, Dhaka-1207 for supporting to complete the study.

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Table 1: Morphology of ovary

		Right mean±SD	Left mean±SD	Level of Significance
Morphology of Ovary	Weight(g)	0.79±0.17	0.77±0.10	NS
	Length(cm)	1.36±0.21	1.39±0.32	NS
	Width(cm)	1.00±0.13	0.98±0.15	NS

NS= Non significant

Table 2: The effect of time elapsed from slaughtering to specimens processing on oocyte quality

Time after slaughter (Hour)	Oocyte collection rate (%)	Oocyte quality
2	75%	Good
6	69%	Fair
12	62%	Poor
24	55%	Bad

Table 3: Effect of collection techniques on oocyte quality

Collection Techniques	Total no. of ovaries	Total no. of Oocytes	Oocyte Quality (%)					
			A	B	Total	C	D	Total
Dissection	30	33	4 (12.12%)	7 (21.21%)	11 (33.33%)	13 (39.39%)	9 (27.27%)	22 (66.66%)
Slicing	30	53	15 (28.30%)	9 (16.98%)	24 (45.28%)	10 (18.87%)	19 (35.85%)	29 (54.72%)
Aspiration	30	48	26 (54.17%)	7 (14.58%)	33 (68.75%)	11 (22.92%)	4 (8.33%)	15 (31.25%)

Table 4: Interrelationship between oocyte diameter and oocytes quality

Group	Oocyte Diameter	No.of Oocyte Total=75	Oocyte Quality (%)					
			A	B	Total	C	D	Total
1	<95 µm	14	2 (14.29%)	4 (28.57%)	6 (42.86%)	6 (42.85%)	2 (14.29%)	8 (57.14%)
2	95-100 µm	34	14 (41.17%)	10 (29.41%)	24 (70.58%)	5 (14.71%)	5 (14.71%)	10 (29.42%)
3	>100 µm	27	13 (48.15%)	9 (33.33%)	22 (81.48%)	3 (11.11%)	2 (7.41%)	5 (18.52%)

Table 5: Relationship between cumulus cells diameter and oocyte quality

Group	Oocyte diameter	No. of Oocyte	Cumulus cells diameter		
			<30 µm	30-50 µm	>50 µm
1	<95 µm	14	7.14% (Normal) 50% (Abnormal)	21.43% (Normal) 7.14% (Abnormal)	14.29% (Normal) 0.0% (Abnormal)
2	95-100 µm	34	14.71% (Normal) 14.71% (Abnormal)	23.53% (Normal) 5.88% (Abnormal)	32.35% (Normal) 8.82% (Abnormal)
3	>100 µm	27	11.11% (Normal) 7.41% (Abnormal)	25.93% (Normal) 7.40% (Abnormal)	44.44% (Normal) 3.71% (Abnormal)

F. Dadok, et. al. "Morphometric assessment of oocytes recovered from ovaries of Black Bengal goat." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS), 13(12), 2020, pp. 40-44.