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ORIGINAL ARTICLE

Evaluation of relative efficiency of oocyte collection techniques from abattoir-derived goat ovaries

Md. Abdur Raihan Ratul[1*], Md. Roknuzzaman Khan[2], Md. Lokman Hossain[1], Md. Harun-Or- Rashid [1], Muhammad Rakibul Islam[1]

[1]Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207; [2]Department of Medicine and Public Health, Sher-e-Bangla Agricultural University, Dhaka-1207

Background: This study aimed to compare different parameters between right and left ovaries, ovaries with corpus luteum, and without corpus luteum, and to determine the most effective oocyte collection technique.

Methods: The experiment was conducted at the Department of Animal Nutrition, Genetics, and Breeding at Sher-e-Bangla Agricultural University, Dhaka- 1207, from January 2019 to December 2019. A total of 431 ovaries were collected from different abattoirs in Dhaka. Oocytes were harvested through different oocyte collection techniques like aspiration, slicing, and puncture. Data on parameters like weight, length, width, total number of visible follicles, number of follicles aspirated, collected grade A, grade B, grade C, and grade D cumulus-oocyte- complexes (COCs) per ovary, total number of COCS were recorded following standard procedures.

Results: The length (cm) of the right ovaries (1.31±0.04) was found to be significantly ($p<0.05$) greater than that of the left ovaries (1.18±0.04). The number of normal COCs (Grade A and Grade B) was significantly ($p<0.01$) higher in left ovaries (2.14±0.08 and 1.65±0.08) compared to right ovaries (0.36±0.08 and 0.23±0.08), respectively. Additionally, ovaries without a corpus luteum (CL) had a significantly ($p<0.01$) higher number of normal COCs (Grade A and Grade B) (1.21±0.07 and 0.90±0.07) compared to those with a CL (0.32±0.09 and 0.29±0.09), respectively. The number of normal COCs (Grade A and Grade B) was significantly ($p<0.05$) higher using aspiration (3.80±0.60 and 3.33±0.42) compared to slicing (3.67±0.78 and 3.20±0.54) and puncture (2.20±0.60 and 1.40±0.42), respectively.

Conclusion: The highest yield of normal COCs was obtained through aspiration, followed by slicing and puncture. Left ovaries contained more normal COCs and a higher number of follicles compared to right ovaries. Additionally, ovaries without a corpus luteum had a higher number of follicles and normal COCs than those with a corpus luteum. Therefore, it can be concluded that left ovaries, ovaries without a corpus luteum, and the aspiration technique are optimal for obtaining quality COCs.

Key words: Ovary, follicle, corpus luteum, aspiration, slicing, puncture

*Correspondence: raihanraz37@sau.edu.bd

Introduction

The genetic improvement of livestock can be achieved by properly utilizing proven sires and dams by following artificial insemination (AI) with frozen semen and Embryo Transfer Technology (ETT). Follicular oocytes could be matured in vitro and used for in vitro fertilization for producing great quantity of embryos (Agarawal and Suzuki, 1992). To produce good embryos, quality oocyte is obligatory. Oocytes are the raw material for in vitro production (IVP) experiments. Ovaries from slaughtered animals are the cheapest and the most abundant source of primary oocytes. Some research works have been performed to compare the efficiency of the oocyte collection techniques in cattle (Katska, 2007, Lonergan *et al*., 1991), sheep (Wahid *et al*., 1992, Wani *et al*., 2000) and goat (Mogas *et al*., 1992, Wang *et al*., 2007) in abroad.

In vitro production (IVP) of embryos indicates using laboratory techniques to generate embryos. The average number of good quality oocytes recovered from ovaries without corpus lutea, which can be effectively used for IVF (Kumar *et al*., 2004). According to Salim (2004) the average numbers of normal follicles reported significantly higher in normal breeder than acyclic, cyclic but not conceived postpartum anestrous in Black Bengal does. Majeed *et al*. (2019) studied found that the recovery rate of oocyte by using aspiration and puncture methods were significantly ($P<0.05$) higher than the oocyte recovery rate via slicing. Among the three collection methods, aspiration (0.966±0.139) and puncture (0.966±0664) methods recorded a high recovery rate due to the aspiration and puncture considered as the applicable technique for obtaining perfect oocytes production (quality and quantity), while the presence of the ovarian tissue debris in the slicing (0.571±0.320) due to destruction the ova during the examination. Kulasekhar *et al*. (2012) reported that the oocyte recovery rate per ovary was 2.91, 1.53 and 1.89 in slicing, aspiration and post aspiration slicing, respectively, in primiparous goats. A total of 1092 oocytes were retrieved from 498 ovaries with an average of 2.19 oocytes per ovary. Mehmood *et al*. (2011) compared oocyte recovery methods i.e., aspiration vs. slicing in goats. They recorded better ($P<0.05$) COCs recovery with the slicing method (2.2 COCs/ovary) than with aspiration (0.9 COCs/ovary). Jamil *et al*. (2008) evaluated the comparative efficacy of oocyte collection methods on the recovery rate of oocytes in native goats. They reported significantly ($P<0.05$) higher number of oocytes recovered/ovary in dissection (2.31) than puncture (1.46) and aspiration (1.21) methods. The objective of this study is to compare different parameters between the right and left ovaries, ovaries with corpus luteum and without corpus luteum, and to observe the oocyte recovery rate between the ovaries with corpus luteum and without corpus luteum. The objective of this study is to compare different parameters between the right and left ovaries and find out the best oocyte collection technique in goat. In Bangladesh, some works are done for the analysis of qualitative and quantitative parameters of goat ovaries, but little work is done on finding the best oocyte collection technique, which triggers me to do such work for further genetic improvement of our local goat which has poor reproductive characteristics.

Materials and methods

Ovary collection

Goat ovaries were collected from different abattoirs in Dhaka. Goats of Black Bengal and Jamunapari breeds were available mainly in the abattoir. The age of the goat varied from 1 year to 3 years. We collected the goat reproductive system from different markets of Dhaka North City Corporation, like Krishi market, town hall market, and Kaptan Bazar market (Figure 1).

Figure 1. Ovaries after collection

Relative efficiency of oocyte collection techniques

Harvesting Oocyte

After fundamental washing (Figure 2), each ovary was treated individually, and the oocytes were harvested by the following three techniques (Wani *et al*.,2000).

Figure 2. Trimming of an ovary

Puncture

Ovaries were kept in a 90-mm petridish with 5 ml of oocyte harvesting medium, held with forceps, the entire ovarian surface punctured with an 18-gauge hypodermic needle (Figure 4-a).

Slicing

Ovaries were put in a 90-mm petridish holding 5 ml of the oocyte harvesting medium, done with forceps support. Using a scalpel blade, incisions were made along the entire ovarian surface (Figure 4-b).

Aspiration

The 10-ml syringe was filled with D-PBS (1.0-1.5ml) and the needle (18 G) was kept in the ovarian parenchyma close to the vesicular follicles and all 2-6 mm diameter follicles were aspirated near the point at the same time (Figure 4-c).

Grading of Cumulus-oocyte-complexes (COCs)

Excess media was removed by using a syringe without hampering the oocytes at bottom of petridish and observed under an inverted digital microscope at 10x magnification. After counting total number of oocytes which were harvested. The COCs were classified into four grades on the basis of cumulus cells and nucleus (Khandoker *et al*., 2011).

Grade A: Oocytes completely surrounded by cumulus cells.

Grade B: Oocytes partially surrounded by cumulus cells.

Grade C: Oocytes not surrounded by cumulus cells.

Grade D: Degeneration observed both in oocytes and cumulus cells.

Grades A and B were considered normal COCs, and grades C and D were considered abnormal COCs.

Measuring length, width, and weight of ovary

The length and width of ovaries (right and left ovaries; ovaries with CL and without CL) were measured with the help of slide calipers and expressed in cm. The weight (Figure 3) of individual ovaries was measured by placing them on a digital balance.

Figure 3. Measuring width of a on ovary

Statistical analysis

The data generated from various aspects with and without CL, viz., ovarian weights, ovarian lengths and widths, follicular counts, and different oocyte collection techniques, viz., oocyte retrieval rate, oocyte recovery rate, and grading of oocytes, were suitably tabulated and analyzed using SAS (Statistical Analysis System) statistics software. The differences among the parameter means were compared using DNMRT (Duncan's New Multiple Range Test).

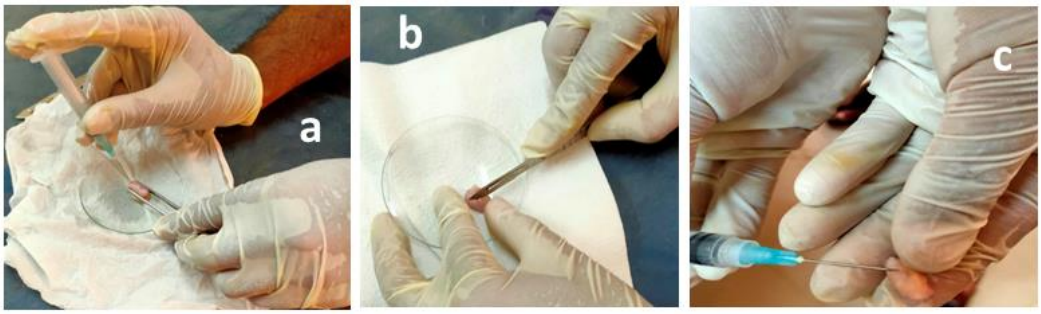

Figure 4. Different oocyte aspiration techniques: a) Puncure, b) Slicing, c) Aspiration

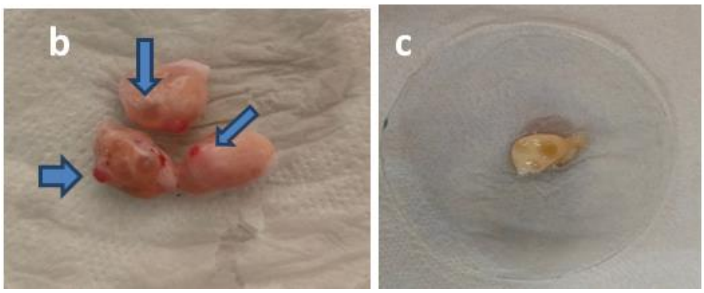

Figure 5. Ovaries with corpus luteum (b)_and without corpus luteum (c)

Results and Discussion

Left and right ovary

Length (cm), width (cm) and weight (g) of ovary

Among different parameters obtained from different categories of ovaries, the mean weight (g) and width (cm) were non-significant between right and left ovaries, and the mean length (cm) was significantly different between right and left ovaries (Table 1). The mean weight (g), length (cm), and width (cm) were clearly higher in the case of right ovaries (1.14, 1.31, and 0.90, respectively) compared to left ovaries (1.13, 1.18, 0.86, respectively). Results support the previous studies of Asad *et al*. (2016), who reported that the length (cm) of right ovaries (1.19±0.09) was found to be significantly ($p<0.05$) higher than left ones (1.15±0.04). Other, including width, weight and total number of COCs aspirated per ovary did not differ significantly ($P<0.05$) between right and left ovaries (Table 1).

Number of follicles in total and aspirated

Variation on the number of follicles (total and aspirated) was significant regarding follicle count between right and left ovaries (Table 1). The highest number of follicles in total was observed in left ovary with a mean of 6.83 compared to right ovary with a mean of 6.54. The highest number of follicles was aspirated in left ovary with a mean of 4.77, compared to right ovary with a mean of 4.74.

Oocyte grades

The presence of total COCs with normal and abnormal was significant in the left and right ovaries (Table 1). Grade A and grade B were considered normal COCs. The grade C and grade D were considered as abnormal COCs. The number of normal COCs was significantly higher ($p<0.01$) in left than right ovary. Results explained that the highest number of normal COCs (Grade A and Grade B) were found in the left ovary with a mean of 2.14 and 1.65, respectively. Distinctly, the lowest number of normal COCs (Grade A and Grade B) were found in the right ovary, with a mean of

Relative efficiency of oocyte collection techniques

0.36 and 0.23, respectively. The number of abnormal COCs was significantly higher ($p<0.01$) in the right than in the left ovary. The number of abnormal COCs (Grade C and Grade D) were found in the right ovary with a mean of 1.67 and 2.20, respectively, whereas the lowest abnormal COCs (Grade C and Grade D) were observed in the left ovary with a mean of 0.33 and 0.34 respectively. The total COCs were almost the same in the left and right ovaries, with a mean of 4.46. The highest numbers of normal COCs were found in left than right ovary, which supports the previous result of Khandoker *et al*. (2011), who reported that the collected normal COCs were higher in left ovaries (2.42 ± 0.14 per ovary) compared to right ovaries (2.32 ± 0.12 per ovary).

Ovaries with or without corpus luteum

The less reproductive performer goats are usually slaughtered, and most of them might be non-cyclic. So, there was the possibility of getting more non-cyclic ovaries from the abattoir during random sampling. The cause of the highest number of follicles found in the without CL (Figure 5) group ovaries than those of the CL group due to the absence of hormonal influence during the estrus cycle.

Table 1. Qualitative and quantitative parameters in right and left ovaries

Ovary (*n*)	Weight(g) (mean±SE)	Length (cm) (mean±SE)	Width (cm) (mean±SE)	Visible follicles (mean±SE)	Aspirated follicles (mean±SE)	Collected COCs per Ovary (mean±SE)				Total
						Normal			Abnormal	
						GradeA	GradeB	GradeC	GradeD	
Total(136)	1.14±0.26	1.24±0.04	0.88±0.03	6.68±0.26 (929)	4.75±0.21 (687)	1.25±0.08 (173)	0.89±0.08 (122)	0.99±0.09 (243)	1.27±0.08 (174)	4.46±0.17 (712)
Right(68)	1.14±0.26	1.31^{a}±0.04	0.90±0.03	6.54±0.26 (451)	4.74±0.21 (327)	0.36^{b}±0.08 (25)	0.23^{b}±0.08 (15)	1.67^{a}±0.09 (117)	2.20^{a}±0.08 (151)	4.46±0.19 (308)
Left(68)	1.13±0.26	1.18^{b}±0.04	0.86±0.03	6.83±0.26 (478)	4.77±0.21 (360)	2.14^{a}±0.08 (148)	1.65^{a}±0.08 (107)	0.33^{b}±0.09(126)	0.34^{b}±0.08 (23)	4.46±0.15 (404)

Mean values in the same column with different superscripts (a, b) differ significantly at $p<0.05$. SE= Standard error. The figure in the parenthesis indicates the total number.

Number of follicles in total and aspirated

Variation in the number of follicles (total and aspirated) was significant regarding follicle count (Table 2). The total follicles were significantly higher ($p<0.01$) observed in ovaries without CL with a mean of 6.79 compared to ovaries with CL with a mean of 5.84. The number of follicles was aspirated significantly higher ($p<0.01$) in ovaries without CL with a mean of 3.79 compared to ovaries with CL with a mean of 3.62. Those results support the previous study of Asad *et al*., (2016) who reported that total number of follicles and total number of follicles aspirated were higher in ovaries without CL with the mean (5.21 and 2.74 respectively) than ovaries with CL with the mean (5.11 and 2.69 respectively).

Oocyte grades

The presence of total COCs with normal and abnormal was significant in ovaries with CL and without CL (Table 4). Grade A and grade B were considered normal COCs. The grade C and grade D were considered as abnormal COCs. Results explained that the highest number of normal COCs (Grade A and Grade B) were found in ovaries without CL.

Length, width and weight of ovary

Significant variation was found on different parameters on the ovary with CL and without CL (Table 2). Results explained that the mean weight (g) and width (cm) were clearly higher in case of ovaries with CL (0.74 and 1.25 respectively) than ovaries without CL (0.72 and 0.96 respectively). On the other hand, the mean length (cm) was found higher in the ovaries without CL (1.47) than the ovaries

with CL (1.34). Those results support the previous study of Asad *et al*., (2016), who reported that the mean weight (g) and width (cm) were clearly higher in case of ovaries with CL (0.72 and 0.81 respectively) than ovaries without CL (0.66 and 0.76 respectively). They also reported that the mean length (cm) was found to be higher in the ovaries without CL (1.17) than in the ovaries with CL (1.16).

Effect of oocyte collection techniques on COCs recovery

Presence of total COCs with normal and abnormal was significant in different techniques of slicing, puncture and aspiration (Table 3). The grade A and B were considered as normal COCs. The grade C and D were considered as abnormal COCs. Total number of 67, 66, 51 COCs were collected by slicing, puncture and aspiration techniques respectively from each of 64 ovaries. The results revealed that slicing and puncture yielded significantly higher ($p<0.01$) number of total COCs per ovary (22.2 and 13.20 respectively) than aspiration (10.37). But the highest number of normal COCs (Grade A and B) were found ($p<0.05$) in aspiration (3.80 and 3.33 respectively) than in slicing (3.67 and 3.20 respectively) and puncture (2.20 and 1.40 respectively) techniques. The total number of abnormal COCs including Grade C and D were found significantly lower ($p<0.01$) in aspiration (1.40 and 1.80 respectively) compared to puncture (5.00 and 4.60 respectively) and slicing (7.00 and 8.33 respectively) techniques.

Table 2. Qualitative and quantitative parameters in With CL and without CL groups ovaries

Ovary (*n*)	Weight(g) (mean±SE)	Length (cm) (mean±SE)	Width (cm) (mean±SE)	Total number of follicles (mean±SE)	Number of follicles aspirated (mean±SE)	Collected COCs per ovary (mean±SE)				
						Normal		Abnormal		Total
						GradeA	GradeB	GradeC	GradeD	
Total(103)	0.73±0.03	1.38±0.08	1.05±0.07	6.15±0.17 (682)	3.73±0.11 (420)	0.92±0.05 (104)	0.70±0.05 (79)	0.48±0.05 (52)	0.64±0.06 (69)	2.75±0.09 (300)
With CL(34)	0.74±0.05	1.34±0.14	1.25^{b}±0.13	5.84^{b}±0.21 (273)	3.62^{b}±0.20 (154)	0.32^{b}±0.09 (19)	0.29^{b}±0.09 (16)	0.82^{a}±0.09 (30)	1.18^{a}±0.09 (43)	2.62±0.16 (109)
Without CL(69)	0.72±0.04	1.47±0.09	0.96^{a}±0.09	6.79^{a}±0.29 (409)	3.79^{a}±0.14 (266)	1.21^{a}±0.07 (85)	0.90^{a}±0.07 (63)	0.31^{b}±0.06 (22)	0.39^{b}±0.07 (26)	2.81±0.11 (191)

Mean values in the same column with different superscripts (a, b) differ significantly at $p<0.05$. CL- Corpus Luteum, SE= Standard error. The parenthesis indicates the total number

Table 3. Oocyte collection techniques, number of cumulus-oocyte-complexes (COCs), and types of COCs harvested

Oocyte collection techniques	Total number of oocytes	Collected COCs/ovary (mean±SE)				Total number of COCs (mean±SE)
		Grade A	Grade B	GradeC	GradeD	
Slicing	64	3.67^{a}±0.78	3.20^{a}±0.54	7.00^{a}±1.02	8.33^{a}±0.98	22.20^{a}±2.40
		(11)	(10)	(21)	(25)	(67)
Puncture	64	2.20^{b}±0.60	1.40^{b}±0.42	5.00^{a}±0.79	4.60^{b}±0.76	13.20^{b}±1.86
		(11)	(7)	(25)	(23)	(66)
Aspiration	64	3.80^{a}±0.60	3.33^{a}±0.42	1.40^{b}±0.79	1.80^{c}±0.76	10.37^{b}±1.86
		(19)	(16)	(7)	(9)	(51)

In the aspiration technique, COCs were collected from 2-6 mm diameter of surface follicles using a hypodermic needle with 10 ml syringe. In case of puncture, the whole ovarian surface was punctured by hypodermic needle. In slicing technique, incisions were given along the whole ovarian surface using a scalpel blade. Ferdous (2006) reported that normal COCs were found to be significantly higher ($p<0.05$) in 2-6 mm diameter follicles than others. Moreover, puncture and slicing techniques involve producing more debris which might interfere with the searching of oocytes under the microscope and also require more washing than aspiration. As a result, a number of COCs were deprived of cumulus cells due to repeated washing, resulting in a lower number of normal COCs compared to aspiration at the final observation. The lower number of normal COCs with a higher number of abnormal COCs in case of puncture and slicing than those of aspiration (Table 3) might be due to this reason. Those results also support the previous study of Mehmood *et al*,. (2011) who reported that compared oocyte recovery methods i.e., aspiration vs. slicing in goats. They recorded better ($P<0.05$) COCs recovery with the slicing method (2.2 COCs/ovary) than with aspiration (0.9 COCs/ovary). Those results also support the previous study of Jamil *et al*., (2008) who reported that significantly ($P<0.05$) higher number of oocytes recovered/ovary in dissection (2.31) than puncture (1.46) and aspiration (1.21) methods. The result of this study was comparable with the observation of Wang *et al*. (2007) who harvested oocytes from ovary of Boer goat by one of the four collection techniques (slicing, puncture, aspiration I and aspiration II) and graded COCs as good, fair, poor. They reported that slicing and puncture of the ovaries yielded a higher ($p<0.05$) number of oocytes per ovary (6.3 and 5.8, respectively) compared to aspiration I (2.9) and aspiration II (3.1). Still, the good quality COCs per ovary were significantly higher ($p<0.05$) in aspiration I (3.9) and aspiration II (3.6) than slicing (2.4) and puncture (2.1). Wani *et al*. (2000) reported that slicing (9.5±0.4) and puncture (9.5±0.4) yielded significantly ($p<0.05$) more COCs per ovary than aspiration (6.8±0.3) in sheep but the percentage of good quality oocytes was higher in the aspiration method (64.4%), compared to the puncture (54.7%) or slicing (54.3%) was also similar with the results of present study.

Conclusions

The aspiration method proved to be the most effective technique for recovering normal cumulus-oocyte complexes (COCs) from abattoir goat ovaries, surpassing both slicing and puncture methods. While slicing yielded the highest number of abnormal COCs, aspiration consistently produced the highest yield of viable, normal oocytes. These findings suggest that for optimal oocyte recovery, aspiration should be the preferred method in this context.

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Conflict of Interest

The authors declare no conflict of interest.

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