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Ovum Pick-Up and Ovaries Characterization of Black Bengal Goat from Slaughterhouse

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Abstract

Black Bengal goat is the only indigenous breed in Bangladesh. These are small-sized goats renowned for their high-quality meat, skin, and adaptability to adverse environmental conditions. To conserve the originality of this species, it is mandatory to develop an effective methodology for their germ cells to be harvested after death. Therefore, the study was conducted to assess the ovarian status and evaluate the most suitable method of ovum pick-up as well as the characterization of ovum from slaughterhouse specimens. A total of 182 ovaries were collected from local slaughterhouses from June 2022 to July 2023 and grossly examined to assess their status. Two methods were used to retrieve oocytes from the ovaries: aspiration and slicing. The grading of the ovum was done based on the germ layer present surrounding the oocytes. From 182 ovaries, the rates of corpus luteum (CL), tumor, and cyst were found to be 14.29%, 6.59%, and 27.47%, respectively. In the aspiration method, the significantly highest percentage of recovery rate (38.89%) and grade A oocytes (51.06%) were found within 1–3 hours of slaughtering. In the slicing method, the significantly highest percentage of recovery rate (19.57%) and grade A (60.61%) oocytes were found within 1–3 hours of slaughtering. The recovery rate was significantly higher (34.1%) in the aspiration method than in the slicing method (17.1%). There was no significant relationship in oocyte quality between the two methods. The recovery rate was not significantly dependent on the presence of CL. In conclusion, the findings from this study may be useful for assessing the quality of oocytes to preserve the germ cells and utilize them for in vitro fertilization for conserving Black Bengal goat.

Keywords: aspiration method, Black Bengal goat, ovum pick-up, slicing method, slaughterhouse specimen

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INTRODUCTION

The Black Bengal goat is the only indigenous breed in Bangladesh. In Bangladesh, it's worth noting that over 90% of the goat population consists of the Black Bengal breed (Husain, 1993). These are small-sized goats renowned for their high-quality meat, skin, and adaptability to adverse environmental conditions (FAO, 2011; Bhuiyan *et al*., 2021). This breed is known for its high reproductive capacity, often producing a large number of offspring. It is evident that achieving better production efficiency in goats is closely linked to high reproductive efficiency. This includes the potential for increased litter size and a shorter generation interval, especially through a higher fertility rate when compared to other farm animals (Haque *et al*. 2013). As the number of endangered species is growing, it is however essential to develop an optimum procedure for their germ cells to be grown after death and then stored with the best quality (Suhail, 2012). Various reproductive technologies have been introduced to raise the number of offspring from selected female animals and to decline the generation intervals in animals. As for example, ovarian superovulation, collection of immature oocytes, in vitro maturation (IVM), artificial insemination (AI), in vitro fertilization (IVF), and embryo transfer (ET) (Suresh *et al*., 2009).

Successful IVF in most species relies on the availability of a sufficiently large number of good-quality oocytes. Numerous researchers have

conducted studies focusing on producing embryos from oocytes recovered from the ovaries of slaughtered animals (Staigmiller and Moor, 1984; Wani *et al*., 2000).

Slaughterhouses are increasingly recognized as an economical source of oocytes, from which ovaries can be obtained. This source has proven to be conducive to the large-scale and economical production of embryos (Sianturi *et al*., 2002). In recent years, techniques such as slaughterhouse ovum pick-up (SHOPU) have emerged as valuable tools for genetic improvement and conservation efforts in various livestock species (González, 2018). The SHOPU involves the post-mortem collection of ovum from the ovaries of slaughtered animals, allowing for the assessment of genetic diversity, fertility potential, and the preservation of valuable genetic material (Ballester *et al*., 2014; Pennarossa *et al*., 2019). In buffalo, the most commonly used techniques for the recovery of oocytes from abattoir ovaries were aspiration and slicing. These methods were instrumental in obtaining oocytes for various reproductive and research applications in buffalo (Nandi *et al*., 2002).

The SHOPU technique was reviewed and adapted for application in goats, considering factors such as ovum quality and quantity (Bertolini *et al*., 2018). There was limited study on ovum pick-up and characterization of Black Bengal goats in Bangladesh; therefore, the study was undertaken to assess the ovarian status of Black Bengal goats from slaughterhouse specimens, to evaluate the more suitable method of ovum pick-up in Black Bengal goats from slaughterhouse specimens, and to characterize the cumulus-oocyte complexes (COC) and grading of ovum.

MATERIALS AND METHODS

Ethical Approval

This study did not require ethical approval because there was no treatment of animals.

Study Period and Location

The sample was collected from three different slaughterhouses i.e., Bahadurbazar, Chourongi, and Basherhat in Dinajpur district in Bangladesh. The ovum pick-up was performed in the Entomology Laboratory of the Faculty of Agriculture under the supervision of the Department of Medicine, Surgery and Obstetrics at the Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University Dinajpur, Bangladesh from July 2022 to July 2023.

Collection, Transportation, and Preparation of Samples

Samples were collected from the local slaughterhouse in the early morning by using a thermos flask. After collection, goat genitalia were transferred to the laboratory as early as possible. Then the ovaries were separated from the female genitalia and immediately placed in physiological saline. Ovarian samples were carefully separated and trimmed from the female genitalia using clean scissors. The separated ovaries were washed multiple times with normal saline. After washing, the ovaries were placed in a Petri dish containing normal saline. They were allowed to settle at 30°C for a period of 5 to 10 minutes before further processing. Then the ovaries were examined grossly to observe follicles (primary > 2–6 mm), (secondary > 2–6 mm), (tertiary > 6 mm), corpus luteum (CL), tumor, and cyst (Talukder *et al*. 2011).

Oocytes Collection using Aspiration Method

Ovaries were taken out from a thermos flask and kept in a petri dish that contained normal saline. Ovaries bearing big-size follicles (88) were subjected to aspiration by using a 5 mL syringe connected to a 19-gauge needle few saline solutions were taken into the syringe and the ovum was aspirated very carefully. The aspirated ovum was transferred into petri dishes containing saline. The Petri dishes were examined under a stereo microscope (Luxeo 6Z 6:1 Labomed) to observe and characterize the aspirated ovum (Figure 1).

Oocytes Collection using Slicing Method

A total of 99 ovaries were subjected to the slicing method as mentioned by Mehmood *et al*.,

(2011). Ovarian samples were detached smoothly and firmly from surrounding tissues and fat. Then the samples were washed with normal saline and transferred into the Petri dish. The ovaries were held firmly with the help of forceps in a sterile glass Petri dish containing 2 mL saline, then sliced into possible thin sections with a surgical blade. Saline-containing oocytes were placed in another petri dish and examined under a stereo microscope (Luxeo 6Z 6:1 Labomed) and graded appropriately. All data were recorded (Figure 2).

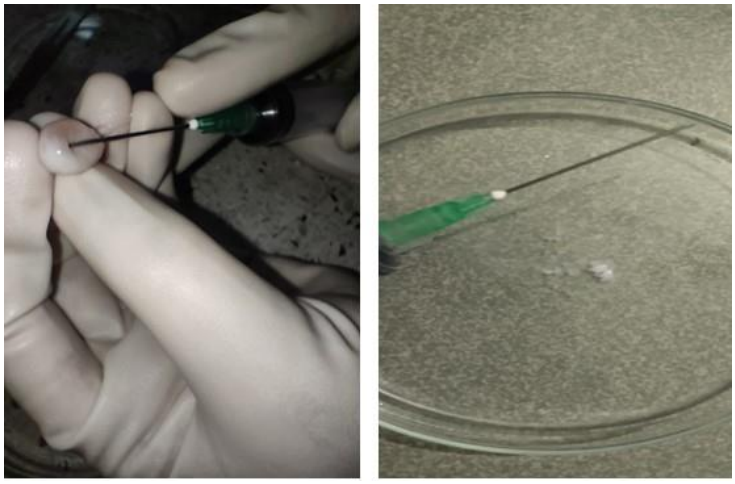

Figure 1. Aspiration method.

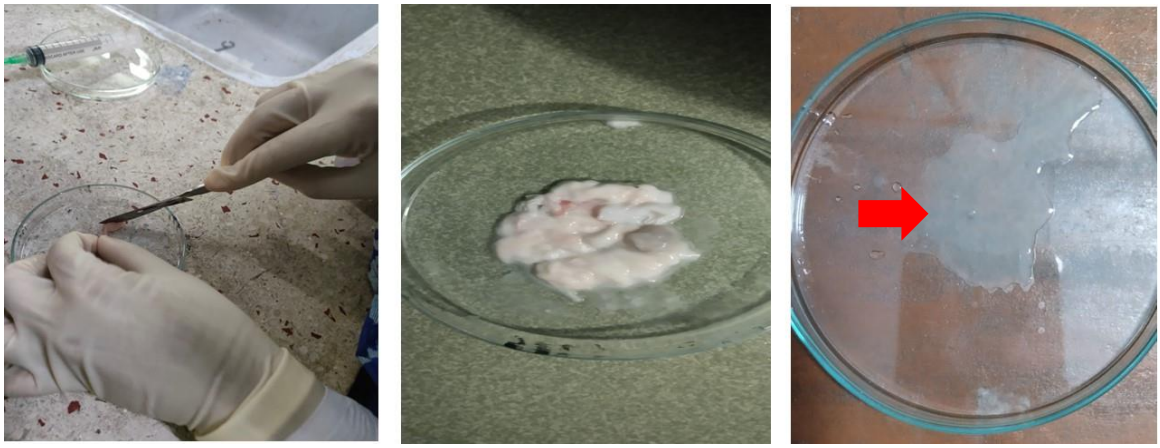

Figure 2. Slicing method. (→) Fluid containing oocytes.

Identification and Grading of Oocytes

Petri dishes containing oocytes were examined and evaluated under a stereo microscope. The recovered oocytes were graded into three categories based on the compactness of cumulus cells and the homogeneous cytoplasm present in COC (Shahid *et al*., 2014), as follows (Grade A) COC were multilayered, compact cumulus cells with finely granulated oocytes and cytoplasm, (Grade B) COC was surrounded by 1 to 3 layers of cumulus cells and finely granulated oocytes with cytoplasm, (Grade C) COC showed incomplete or no cellular vestment or heterogeneous oocytes with cytoplasm (Figure 3).

Statistical Analysis

The data were statistically analysed using the software SPSS (Version 25.0). Descriptive analysis was expressed as percentages and means. ANOVA was performed to check the effects of the ovum pick-up time after collection from the slaughterhouse on the recovery rate by two methods. Chi-square test was performed for the rest of the analysis. For all analyses, a p-value of less than 0.05 was considered as significant.

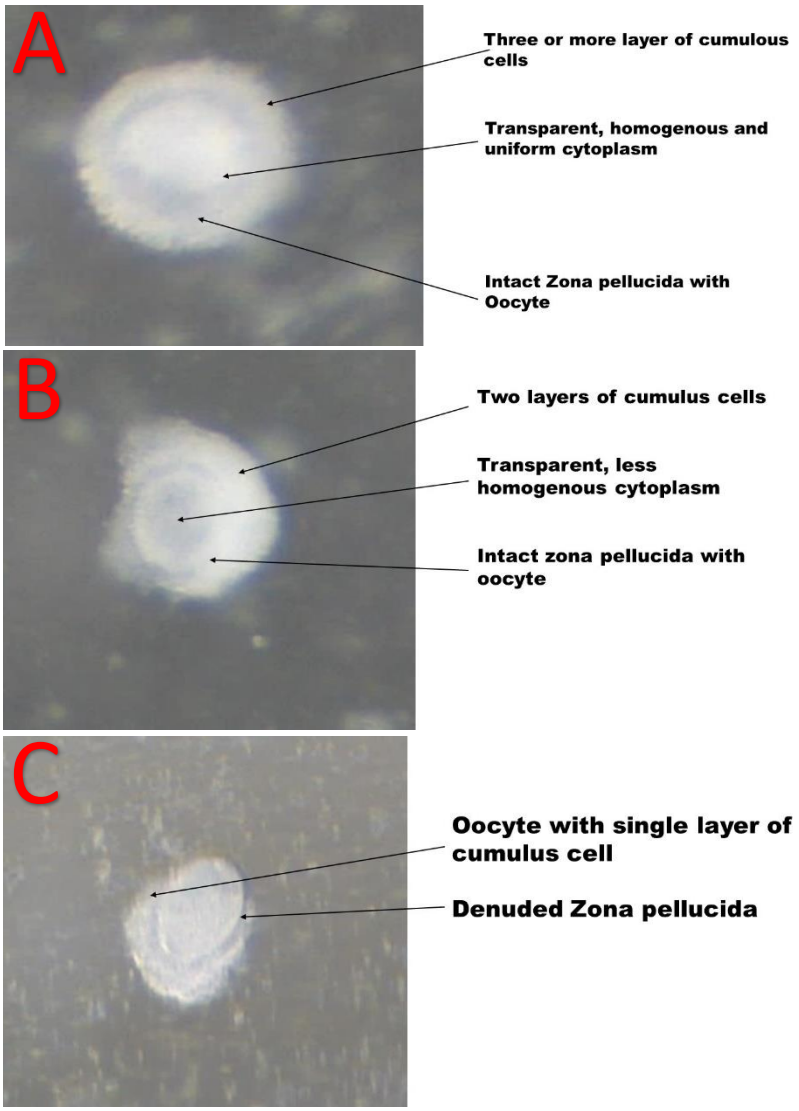

Figure 3. Grade categories are based on cumulus compactness.

RESULTS AND DISCUSSION

The Black Bengal goat is both numerically and economically significant, representing a promising animal genetic resource in developing countries such as Bangladesh. In Bangladesh, Black Bengal goats are popular for their meat quality and skin (Tarikul, 2019). To conserve indigenous and endangered species and facilitate their faster genetic improvement, modern biotechnological tools have been adopted. Substantial study has been conducted on IVM, IVF, and in vitro culture (IVC) of resulting zygotes as part of these conservation and improvement efforts (Cognie *et al*., 2003). Limited information has been available thus far on the evaluation of Black Bengal goat ovaries, particularly regarding the efficient collection and grading of oocytes. In Bangladesh, in vitro techniques for Black Bengal goats are relatively recent, but ongoing efforts are being made to standardize and advance these techniques through continued study and development (Ferdous, 2006; Islam *et al*., 2007). Therefore, the current study was designed to assess the ovarian status of Black Bengal goats in slaughterhouse specimens, evaluate the more suitable method of ovum pick-up from slaughterhouse specimens, and grade the ovum.

In this study, a total of 182 ovaries collected from slaughterhouse specimens were used for ovum pick-up. In the current study, the number of primary, secondary, and Graafian follicles was 788, 562, and 320, respectively (Table 1). According to this study, 225 oocytes were recovered from 88 ovaries by aspiration method

(34.1%) and 127 were recovered by slicing method from 94 ovaries (17.1%). The total number of follicles (n = 905) observed in another study from Black Bengal goats was lower than the findings of this study, a number of 856 follicles were observed by the aspiration method in Nili Ravi Buffalo (Shahid *et al*., 2014). According to this study, the percentage of oocyte recovery and grade A oocyte recovery was significantly ($p < 0.05$) higher within 1–3 hours of slaughtering in both aspiration (38.89%) and slicing (19.57%) methods (Table 2) and (Table 3).

Table 1. Overall ovarian assessment of Black Bengal goat

Parameters	Ovaries (n)	Total number of follicles		
		Primary	Secondary	Graafian
Total number	182	788	562	320
Percentage (%)	N/A	47.19	33.64	19.16

Table 2. Effects of time after collection on recovery rate

Time of the slaughter (hour)	Recovery of slicing method (%)	Recovery of aspiration method (%)	p-value
1–3	19.57[a]	38.89[a]	
3–6	17.61[b]	33.91[b]	0.000
6–8	13.97[c]	31.36[c]	

[a,b,c] Values with different superscript letters in the same column are significantly different ($p < 0.05$).

Table 3. Effect of time on ovum quality after collection from slaughterhouse

Time of the slaughter (hours)	Quality (%)						p-value
	Aspiration method			Slicing method			
	A	B	C	A	B	C	
1–3	51.06[a]	25.92[a]	24.60[a]	60.61[a]	24.24[a]	15.15[a]	
3–6	36.93[b]	37.93[b]	34.38[b]	43.10[b]	25.86[b]	31.03[b]	0.003
6–8	36.02[c]	32.08[c]	31.71[c]	22.22[c]	41.66[c]	36.11[c]	

[a,b,c] Values with different superscript letters in the same column are significantly different ($p < 0.05$).

Table 4. Effects of ovum collection methods on recovery rate from pooled data

Methods	Total ovaries (n)	Total number of recovery	Average recovery (%)
Aspiration method	88	225	34.1[a]
Slicing method	94	127	17.1[b]

[a,b] Values with different superscript letters in the same column are significantly different ($p < 0.05$).

Table 5. Effects of ovum collection methods on quality from pooled data

Methods	Total ovaries (n)	Total number of recovery	Quality (%)		
			A	B	C
Aspiration method	88	225	41.36[a]	31.98[b]	30.23[c]
Slicing method	94	127	41.98[a]	30.58[b]	27.43[c]

[a,b,c] Value with superscript letters in the same column are not significantly different ($p > 0.05$).

Table 6. Effects of corpus luteum (CL) on oocytes recovery rate after collection from slaughterhouse

Parameters	Total ovaries (n)	Number of recoveries	Recovery (%)	p-value
With CL	143	265	25.21[a]	0.125
Without CL	39	87	26.85[a]	

[a] Value with superscript letter in the same column are not significantly different ($p > 0.05$).

Ovum pick-up after two hours of slaughtering was the best time for the highest recovery percentage (78%) and grade A quality (31.11%) found in another study with Black Bengal goat ovary (Tarikul, 2019). The longer elapsed time between slaughter and collection correlates with a decrease in the number of viable ova, accompanied by increased damage, aging, and deterioration of the samples (Tarikul, 2019). The highest oocyte recovery rate and quality within two to four hours of slaughtering were also observed in bovine (Saleh *et al*., 2017) and buffalo (Shahid *et al*., 2014). The degree of ovum damage, aging, and deterioration increased as time elapsed from the period of slaughter to the time of collection and continued until the processing period at the laboratory (Saleh *et al*., 2017; Bahari *et al*., 2023). Research indicates that the number of oocytes and quality decrease gradually with prolonged processing time. To mitigate this effect, it is advisable to transport specimens from the abattoir to the processing facility promptly after slaughter, ideally using a cool box kept between 4–8°C (Tarikul, 2019).

The overall recovery rate of oocytes by aspiration and slicing methods was 34.1% and 17.1%, respectively. The results of this study revealed that a significantly ($p < 0.05$) higher recovery rate was found by the aspiration method than by the slicing method. The quality of oocytes had no significant within the two recovery methods (Table 4). However, other studies showed that the highest percentages (33.5%) of oocyte recovery were observed by the slicing method (Saleh *et al*., 2017).

Another study showed the slashing method outperformed the aspiration and slicing methods of oocyte recovery. The aspiration technique involved COC collecting from the surface of visible follicles on the ovary. However, this method might have missed some of the small-sized follicles deep within the cortex. On the other hand, the slicing approach can extract both surface and cortical follicles, but it generates more cell debris due to cutting the ovaries into small pieces with a surgical blade. This excess debris may pose challenges in locating oocytes and could potentially cause damage to oocytes during the chopping process (Khandoker *et al*., 2022).

Previous studies found that the average total number of oocytes recovered per ovary was significantly ($p < 0.05$) higher when using the puncture and slicing methods compared to the aspiration method (Wani *et al*., 2000; Lorenzo *et al*., 1999). The higher number of oocytes observed may be attributed to species-specific differences (Shahid *et al*., 2014). The quality of immature oocytes is influenced by the COC quality and the diameter of the oocyte (Arlotto *et al*., 1996). The results of the aspiration of ovarian follicles are known to have varying qualities of cells (Lucas *et al*., 2002). The specific cause of the variation in oocyte quality is unknown, but it may be influenced by a range of factors, including differences in follicle size and oocyte diameter. These variations are often associated with the complex process of folliculogenesis (Tarikul, 2019).

In the current study, the highest percentages of good-quality oocytes were found by the aspiration method, which was similar to other studies (Saleh *et al*., 2017; Wani *et al*., 2000). A study by Raza *et al*. (2001) found the opposite findings: the recovery of good-quality oocytes per ovary was lower in the aspiration method (1.76 oocytes per ovary) than in the scoring method (3.85 oocytes per ovary). Shaikh *et al*. (2015) recovered a higher percentage of good-quality oocytes by the slicing method (61.90%), whereas some studies showed the slashing method resulted in a significantly ($p < 0.05$) higher percentage of excellent oocytes (Akter *et al*., 2022). The observed difference may be attributed to variations in the size of the ovary and follicle from which the oocytes were recovered (Boni, 2012) (Table 5).

In the present study, among 182 ovaries, CL was found in 143 ovaries, and the rest of the 43 ovaries had no CL. Meanwhile, out of 300 observed ovaries, 90 were found to contain CL, but the remaining 210 ovaries did not have corpus luteum (Tarikul, 2019). In the present study, the oocyte recovery rate was 25.21% with the presence of CL and 26.85% with the absence of CL (Table 6).

This percentage was inconsistent with another study, where they found 75% of oocytes in the absence of CL (Khandoker *et al*., 2022). The observed differences in various aspects, such as hypertrophy of luteinized granulosa cells, hyperplasia of fibroblasts in connective tissues, and increased vascularity, may be contributing factors to the CL formation (Berliana *et al*., 2023). The corpus luteum (CL) attains its maximum diameter approximately 6 to 9 days after ovulation. Subsequently, CL regression begins between days 13 and 16 if maternal recognition does not initiate (Jablonka *et al*., 1993).

CONCLUSION

A total of 182 ovaries collected from slaughterhouses were examined. The total number of primary, secondary, and Graafian follicles assessed from these ovaries was 788, 562, and 320, respectively. The rate of collected primary, secondary, and Graafian follicles was 47.19%, 33.64%, and 19.16%, respectively. There were 14.29% of CL, 27.47% of follicular cysts, and 6.59% of tumors found in 182 ovaries. In the aspiration method, the recovery rate was significantly higher (38.89%) within 1–3 hours of slaughtering. The significantly ($p < 0.05$) highest percentage (51.06%) of grade A ovum recovery was found within 1–3 hours of slaughtering. Significantly ($p < 0.05$) higher numbers of Grade B (37.93%) and grade C (34.38%) ovum were recovered within 3–6 hours of slaughtering. In the slicing method, the recovery rate was significantly higher (19.57%) within 1–3 hours of slaughtering. The significantly higher ($p < 0.05$) percentage (60.61%) of grade A ovum recovery was found within 1–3 hours of slaughtering. Grade B (41.66%) and grade C (36.11%) ovum recovery were significantly higher ($p < 0.05$) within 6–8 hours of slaughtering. The average recovery rate was significantly higher (34.1%) in the aspiration method than in the slicing method but the ovum quality have not any significant correlation within two recovery methods. The recovery rate was not significant ($p > 0.05$) depending on the presence of CL. The results of this study will provide a holistic understanding of the application of the SHOPU method for the genetic enhancement and sustainable management of Black Bengal goats.

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AUTHORS' CONTRIBUTIONS

SN: Methodology, Investigation, Resources, Writing Original Draft. MMBM: Data Curation, Software, Writing - Review & Editing. BFZ: Conceptualization, Visualization, Project administration. MFI: Supervision, Validation, Formal Analysis. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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