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Original Article

Qualitative and quantitative assessment of bovine oocytes

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A B S T R A C T

The reproductive development of cattle is must to hasten the production of milk and meat. It relies mostly on the quality of oocytes. Thus the present study was carried out to sort out the superior ovary to be used in further research. Cow ovaries were collected from slaughter house, processed and categorized as right or left and ovaries with or without corpus luteum (CL). Morphology of ovaries were studied on the basis of length (cm), width (cm), weight (g), total number of follicles on the surface of each category ovaries, number of follicles aspirated, total number of cumulus-oocyte-complex (COCs) and then graded as normal and abnormal. Significantly higher mean weight (3.47±1.85 vs 2.44±1.76), length (1.94±0.34 vs 1.71±0.39) and width (1.36±0.34 vs 1.09±0.29) were found in right ovaries than left. Forty seven of right and 20% of left ovaries were found with CL. Numerically higher number of normal COCs was found in left ovaries than right ovaries whereas total and abnormal COCs were higher in right ovaries. On the other hand, in comparison between the ovaries with and without CL group, significantly (p<0.05) higher mean weight (3.81±1.68 vs 2.53±1.83), length (2.04±0.30 vs 1.72±0.38) and width (1.45±0.25 vs 1.11±0.32) were found in with CL ovaries than without CL. The average number of normal COCs of with CL ovary (0.75±0.85) was lower than that of without CL (0.85±0.83) but they are not significant. Finally, it can be concluded that, oocytes from left ovaries in comparison to right and without CL in comparison to with CL are superior in the context of selection of oocytes for embryo development.

Introduction

Cattle are the major member of Farm Animal Genetic Resources (FAnGR) of Bangladesh. There are 24.3 million of cattle in our country that serves us milk and meat (DLS 2020). The main problem of cattle production lies in its very low productivity due to low genetic potentiality. The diseases and disorders of animals also hinder livestock development in our country (Islam *et al*., 2001). Breeding is the major technological improvement process in the dairy industry. The genetic improvement of livestock can be achieved by proper utilization of proven sires and dams by following the artificial insemination (AI) with frozen semen and Embryo Transfer Technology (ETT). The conservation of indigenous and endangered species and their faster genetic improvement has been achieved by adopting modem biotechnological tools.

Ovaries are primary reproductive organ of female. It supplies germ cells and oocytes as well as maintains reproductive health by producing hormones. Remarkably lower numbers of ovarian follicles is considered as one of the major causes of infertility (Amin *et al*., 2005). The degree of reproductive performance relies upon the interaction of genetic and environmental factors however this performance is particularly susceptible to latter, for example, the seasonal availability of nutrients can affect reproduction considerably (Forcada and Abecia, 2006).

In abroad, quantitative and histological aspects of ovary and ovarian follicles have been studied in sheep (Draincourt *et al*., 1993), bovine (Singh and Adams, 2000), mouse (Satosi and Motoalci, 2004), wapiti (McCorkel *et al*., 2004) and Iranian Lori-Bakhtian Sheep and native goat (Mohammadpour, 2007). However, until today, there is no complete study on qualitative and quantitative analysis on the ovary, ovarian follicle and oocytes in cattle.

Therefore, the objectives of the present research work was to assess the quality and quantity of oocytes collected from

bovine ovaries and to find out the superior quality of ovary and oocytes to use in further study with a view to help in the making genetic improvement of cattle through *in vitro* embryo production

Materials and Methods

Experimental site

The experiment was conducted at the Laboratory of the Department of Animal Production and Management at Sher-e-Bangla Agricultural University, Dhaka-1207 from January 2019 to December 2019.

Collection and Processing of Ovaries

Collection and processing of ovaries were performed as described previously by Jamil *et al.*, (2008) with minor modification. At early morning, ovaries along with female genital tract of mature cows having at least 2 pairs of permanent teeth with their unknown reproductive history were collected within 30 min of slaughter from the slaughter house at Geneva Camp, Mohammadpur, Dhaka. They were then transported within 2 h of slaughter to the laboratory in a vacuum flask containing sterilized phosphate buffered saline (PBS) (pH 7.35) supplemented with 100 IU penicillin G and 100 mg/ml streptomycin at 25-30°C.

At the laboratory, ovaries were separated from other parts by cutting. All the excess materials like adipose tissue, surrounding bursa etc were removed from ovarian surface. Right and left ovaries were kept in different petri dishes. According to Wani *et al.* (2000) each ovary was washed five times i.e. three washings in D-PBS and two washings in oocytes harvesting medium (DPBS+4mg/ml BSA+1.50 IU/ml Penicillin). After all five washings, ovaries were transferred to sterilized petri dishes and rinsed thoroughly by physiological saline at 25°C before further processing as described by Haque *et al.*, (2016).

Categories of ovary

The ovaries were divided into two groups, i.e. the ovaries with and without a corpus luteum to investigate the influence of the corpus luteum on the quantity and the quality of cumulus-oocyte complexes (COCs) recovered per ovary. COCs recovered from each ovary of the two ovary groups were recorded. Some of them were usable and some of them were not.

Measurement of weight, length and width of ovaries

All the ovaries were weighed individually irrespective of grouping in g in a digital balance. Then the length and width of each ovary were measured in cm with the help of a slide calipers. All data were recorded in tabular form individually and separately.

Counting of follicle on the surface of the ovary

Ovarian surface contains many follicles of different sizes. All the visible follicles were counted manually irrespective of grouping i.e. CL present or absent, left or right ovary. Number of follicles on each ovarian surface was recorded individually in tabular form.

Oocytes 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. After fundamental washing, each ovary was treated individually and the oocytes harvested by aspiration techniques as illustrated by Wani *et al.*, (2000). Each ovary was individually handled, and oocytes were recovered by following method.

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 with 22 G hypodermic needle fixed to 5 ml disposable syringe containing 1-2 ml of DPBS. The cattle oocyte was aspirated from individual ovary and placed in petri dish containing 1 ml of DPBS. Number of collected oocytes was recorded after grading.

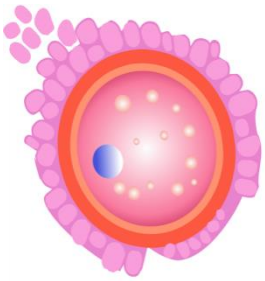
Microscopic study and grading of COCs

The petri dish was kept undisturbed for 5 minutes after collection to settle down the oocytes. Unexpected media was discarded by using a syringe without hampering the oocytes at the bottom of the petri dish. The 10 ml syringe was loaded with D-PBS (1.0 -1.5 ml), and the needle (18G) was used to transfer oocytes from petri dish to glass slide. Then it was observed under an inverted digital microscope at 10x magnification and the total number of harvested oocytes were counted.

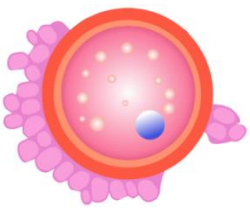
According to Khandoker *et al.* (2011), the COCs were classified into four grades on the basis of cumulus cells and nucleus. In brief, he categorized oocytes completely surrounded by cumulus cells as Grade A, oocytes partially surrounded by cumulus cells as Grade B, oocytes not surrounded by cumulus cells as Grade C and degenerated oocytes and cumulus cells as Grade D. The grade A and B were considered as normal COCs where oocyte was surrounded by cumulus cell. On the other hand, the grade C and D were considered as abnormal COCs where oocyte was not surrounded by cumulus cell. The graphical representation of the COCs classification is shown in Figure 1.

The grade A and B were considered as normal COCs where oocyte was surrounded by cumulus cell. On the other hand, the grade C and D were considered as abnormal COCs where oocyte was not surrounded by cumulus cell. The number of different grades of COCs in each category noted.

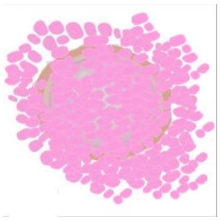
Grade A: Oocytes completely surrounded by cumulus cells



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

Figure 1. Graphical representation of oocyte grading adopted from Khandoker *et al.* (2001).

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

Ovaries from 30 cows were collected where 20 ovaries found with CL whereas 40 were without CL. A total of 381 follicles were counted on the surface of ovaries. Two hundred and eighty follicles were aspired to get 90 oocytes including 49 from normal and 41 from abnormal category. The average length, width and weight were noted. The result of the different parameters is summarized in this chapter.

Gross Study of the Ovary

Almond-shaped, pale colored ovaries were found at the edge of the mesovarium near the lateral margin of the pelvic inlet. May (1970) was reported like this. Their report also supported this study in the statement that, follicles of different sizes projected from the surface of each ovary to make the surface irregular (Figure 2).

The uterine extremity of the ovaries was connected with the extremity of the horn of uterus by a proper ligament of the ovary. There was no demarcation between the horn of the uterus and the flexuous uterine tubes.

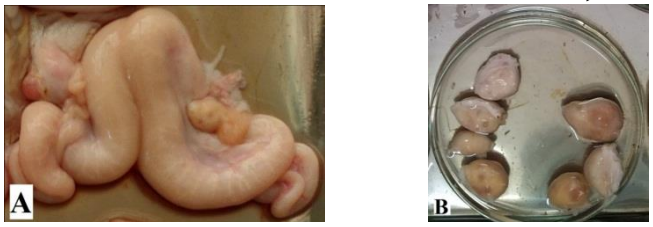


Figure 2. Reproductive tract of cow (A) and collected ovaries (B).

Ovarian Categories Regarding Right and Left Ovary

i. Morphology of ovary

Different parameters of the morphology of the ovaries under the current study were found significantly different between right and left ovaries ($p<0.05$). Higher mean weight (g) (3.47±1.85 vs 2.44±1.76), length (cm) (1.94±0.34 vs 1.71±0.39) and width (cm) (1.36±0.34 vs 1.09±0.29) were found in right ovaries than that of left ovaries (Table 1).

Right ovaries are more active than left ovaries. Thus they are much bigger than left part in terms of length and width as well as weight (Dangudubiyyam and Ginther (2019), Rahman *et al*. (1977) and Sarkar (1993). Similar results were found in goat (Islam *et al*., 2007).

But the results of previous study of Singh *et al.* (1974) did not support this result. They claimed that, there was no significant difference ($p>0.05$) in the parameters of left and right ovaries of goat. Asad *et al* (2016) found significantly ($p>0.05$) larger right ovaries in terms of length in goat whereas weight and width were found insignificant.

Table 1. Ovarian categories in respect to morphology of right and left ovaries

Ovary (*n*)	Weight (g) (mean±SE)	Length (cm) (mean±SE)	Width (cm) (mean±SE)	Total number of visible follicles (mean±SE)	Number of follicles aspirated (mean±SE)	Collected COCs per Ovary (mean±SE)		
						Normal	Abnormal	Total
Right (30)	3.47^{a}±1.85	1.94^{a}±0.34	1.36^{a}±0.34	6.60±3.45 (198)	5.00±2.65 (150)	0.67±0.80 (20)	0.73±0.78 (22)	1.40±1.16 (42)
Left (30)	2.44^{b}±1.76	1.71^{b}±0.39	1.09^{b}±0.29	6.13±3.72 (184)	4.33±2.59 (130)	0.97±0.85 (29)	0.63±0.72 (19)	1.60±0.97 (48)
	*	*	*	NS	NS	NS	NS	NS

Mean values in the same column with different superscripts (a, b) differ significantly at $p<0.05$.
Figure in the parenthesis indicates the total number of ovaries.
n= number of ovary

Table 2. Ovarian categories in respect to morphology of with and without CL ovaries

Ovary (*n*)	Weight (g) (mean±SE)	Length (cm) (mean±SE)	Width (cm) (mean±SE)	Total number of visible follicles (mean±SE)	Number of follicles aspirated (mean±SE)	Collected COCs per Ovary (mean±SE)		
						Normal	Abnormal	Total
With CL (20)	3.81^{a}±1.68	2.04^{a}±0.30	1.45^{a}±0.25	5.05^{a}±2.95 (101)	3.90±2.22 (78)	0.75±0.85 (15)	0.70±0.80 (14)	1.45±1.19 (29)
Without CL (40)	2.53^{b}±1.83	1.72^{b}±0.38	1.11^{b}±0.32	7.03^{b}±3.70 (281)	5.05±2.75 (202)	0.85±0.83 (34)	0.68±0.73 (27)	1.53±1.01 (61)
	*	*	*	*	NS	NS	NS	NS

Mean values in the same column with different superscripts (a, b) differ significantly at $p<0.05$.
Figure in the parenthesis indicates the total number of ovaries.
n= number of ovary

ii. Presence and absence of CL

Significant variation was found on corpus luteum (CL) in terms of presence or absence in left and right ovaries. A total of 20 ovaries were found with presence of corpus luteum among the total of 60 ovaries. In which, higher portion (24, 70%) was from right ovaries in comparison to left (6, 30%)

ovaries. On the other hand, higher without-CL ovaries were found in left (16, 60%) than right (14, 40%) among total 40 without-CL ovaries.

As the right ovaries are more active than the left, right ovaries produce more ova than left. So, more ovulation as well as more CL is a normal phenomenon.

The result was similar to Casida *et al.* (1935), Clark (1936), Schram (1937), Reece and Turner (1938), Erdheim (1942) and Spriggs (1945).

iii. Number of follicles

Variation on number of follicles (total and aspirated) was not significant in terms of follicles count in left and right ovaries (Table 1). The highest number of total follicles was observed in right ovary (6.60±3.45) with highest aspirated follicles (5.00±2.65). Again, the lowest total follicles count was found in left ovary (6.13±3.72) with lowest aspirated follicles (4.33±2.59). Right ovaries are more active than left according to Stalfors (1916) who found the right ovary to be more active than the left one. So finding of higher number of follicles in right ovary is a normal phenomenon.

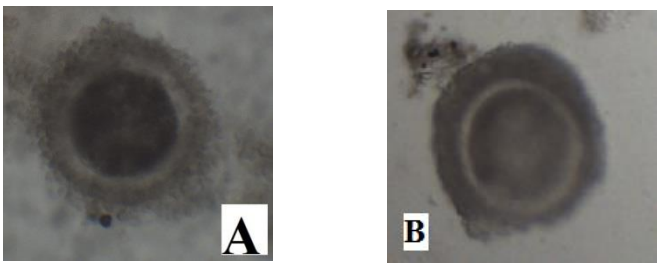
iv. Grading of COCs

Grading of COC's was done on the basis of cumulus cell diameter. Khandoker *et al*. (2011) classified the COCs into four grades on the basis of cumulus cells and nucleus. Oocytes completely and partially surrounded by cumulus cells were graded as Grade A and Grade B respectively and commonly as normal oocytes. Whereas, oocytes not surrounded by cumulus cells were classified as Grade C and degenerated oocytes and cumulus cells were classified into Grade D. Last two are commonly named as abnormal oocyte. Presence of total COCs with normal and abnormal was not significant in left and right ovaries (Table 1). The mean count of normal and total COCs was higher in left ovaries than that of right ovaries numerically. On the other hand, abnormal COCs were higher in right ovaries. But the differences were not statistically significant in cattle (p<0.05). The average number of normal and abnormal COCs of right ovary was recorded to be 0.67±0.80 and 0.73±0.78 respectively with a total of 1.40±1.16, similarly the left ovary possessed 0.97±0.85 and 0.63±0.72 normal and abnormal COCs with a total of 1.60±0.97 (Figure 3).

The cumulus cells (CCs) surrounding the oocyte plays a key role in oocyte maturation, and they are known to supply nutrients, energy substrates (Sutton *et al*., 2003).

This result 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).

The result of the study also supports with the report of the study of Patra *et al.* (2013) and Islam (2005).



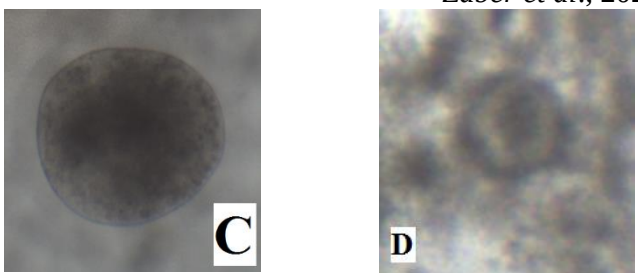


Figure 3. Normal oocyte [Grade-A (A) and Grade B (B)] and abnormal oocyte [Grade C (C) and Grade D (D)]

Ovarian Categories Regarding with CL or without CL

i. Morphology of ovary

Different parameters of the morphology of the ovaries under the current study were found significantly different between presence and absence group of ovaries (p<0.05). Higher mean weight (g) (3.81±1.68 vs 2.53±1.83), length (cm) (2.04±0.30 vs 1.72±0.38) and width (cm) (1.45±0.25 vs 1.11±0.32) were found in with-CL ovaries than that of without-CL ovaries (Table 2).

After ovulation, if the ovum fails to contact with sperm a fleshy structure is formed known as corpus luteum, which might give rise to the weight, length and width of ovaries. This result supports with the previous result of Singh *et al.* (1974), Rahman *et al.* (1977) and Sarkar *et al.* (1993). The result is very usual as the hypertrophy of luteinized granulosa cells, hyperplasty of fibroblasts of the connective tissues and vascularity contribute to an increase in size of the CL (Jablonka-Shariff *et al*., 1993). The maximum diameter of CL is reached 6~9 d after ovulation and then regression starts between days 13 and 16 if maternal recognition does not occur (Jablonka-Shariff *et al*., 1993). Asad *et al* (2016) found significantly (p<0.05) higher width and weight in with CL group whereas length was found insignificant.

ii. Presence and absence of CL

A total of 20 ovaries were found with presence of corpus luteum among 60 ovaries. Significant variation on was found on corpus luteum (CL) in terms of presence or absence. Results showed that the CL was present in 33% ovaries where CL was absent in 67% ovaries. 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 caused due to the less reproductive performer cows are usually slaughtered and most of them might be non-cyclic. So, there had been the possibility to get more non-cyclic ovaries from the slaughterhouse during random sampling. The cause of highest number of follicles found in without CL group ovaries than those of with CL group due to absence of hormonal influence during estrus cycle.

Islam (2019) found the similar result in buffalo whereas Hoque (2009), Ferdous (2006) and Islam (2005) found in goat. All of them studied on slaughterhouse derived ovaries of respective species.

iii. Number of follicles

Variation on number of total follicles was significant in terms of follicles count in with and without CL ovaries (Table 2). The highest number of total follicles was observed in CL-absent ovary (7.03±3.70) with highest aspirated

follicles (5.05±2.75). The difference was statistically significant (p<0.05). Again, the lowest total follicles count was found in CL-present ovary (5.05±2.95) with lowest aspirated follicles (3.90±2.22). Aspired follicle count was higher in CL-absent ovary than CL-present numerically but they was no statistically significant difference (p<0.05).

A study was reported that the presence of a CL stimulates the development of significantly higher (p<0.01) number of ovarian follicles which produced a significantly higher (p<0.05) number of good quality oocytes by Abdoon and Kandil (2001).

As follicle bears oocyte, so we can say that more follicle contains more oocyte and less follicle contains less oocyte. According to Nandi *et al*. (2000) the oocyte recovery rate decreased when ovaries had a corpus luteum. This is because follicular development is restricted, as lutein cells occupy most of the ovary (Kumar *et al*., 2004). The dominant follicle is usually observed in the corpus luteum-bearing ovary, and the other follicles are very small and remain mostly inaccessible (Gasparrini *et al.,* 2000). Cow (Moreno *et al.,* 1993) and goat (Agarwal *et al.,* 1995) ovaries containing a corpus luteum yielded a lower number of oocytes than ovaries without a corpus luteum. Several researchers have reported that the presence of a corpus luteum yields a lower number of oocytes per ovary and a lower proportion of usable oocytes (Moreno *al.,* 1993). In contrast, Boediono *et al*., (1995) and Das *et al.,* (2010) found no difference in the mean number of oocytes per ovary between corpus luteum-bearing and non-bearing ovaries.

iv. Grading of COCs

Grading of COC's was done in two types as normal and abnormal. Presence of total COCs with normal and abnormal was not significant in with and without CL ovaries (Table 2). The mean count of normal and total COCs was higher in CL-absent ovaries than that of CL-present ovaries numerically. On the other hand, abnormal COCs were higher in CL-present ovaries. But the differences were not statistically significant in cattle (p<0.05). The average number of normal and abnormal COCs of CL-present ovary was recorded to be 0.75±0.85 and 0.70±0.80 respectively with a total of 1.45±1.19, similarly the CL-absent ovary possessed 0.85±0.83 and 0.68±0.73 normal and abnormal COCs with a total of 1.53±1.01. Those results support the previous study of Mondal *et al.* (2008).

The presence of CL in cyclic female's ovary produces a higher level of progesterone hormone that signals negative response to anterior pituitary gland for the restriction of gonadotrophin secretion and ultimately follicular degeneration occurs by 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 oocyte maturation. The higher number of COCs in ovaries without CL than that of ovaries with CL 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 cattle follicular degeneration and further strengthening the previous statement. The results of the number of total and normal COCs per ovary strongly supports the result of the study of Mondal *et al.* (2008), who reported that significantly higher (p<0.01) number of total COCs (3.94 vs 1.30 per ovary) and normal COCs (1.54 vs 0.68 per ovary) were found in CL-absent group than those of CL-present group of ovaries. In addition, significantly higher (p<0.05) number of abnormal COCs per ovary in CL-present group (0.86) than absent group (0.66) as reported by Mondal *et al.* (2008) is also similar to the present results. Ferdous (2006) reported that normal COCs were found to be significantly higher (p<0.05) in 2-6 mm diameter. Since Mondal *et al.* (2008) collected COCs by aspiration of 2-6 mm diameter follicles, they obtained lower number of abnormal COCs.

According to Webb *et al.* 1999, growth initiation of follicles has variously been attributed to i) hormonal triggers (gonadotropins). ii) stochastic processes (fluctuation in internal signal molecule) and iii) external inhibitory control from growing follicles. 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 in the process rather than the causes. The higher number of follicles that were found in CL absent group of ovaries in the present study, might reflect the optimum level of gonadotropins and steroids.

Above all, the number and quality of COCs recovered per ovary is a significant consideration for *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) of COCs, *in vitro* production (IVP) of embryos, multiple ovulation and embryo transfer (MOET).

Conclusions

Finally, it can be concluded that, left ovaries contain more normal COCs and higher number of follicles than right ovaries and without CL ovaries contain higher number of follicles and normal COCs than with CL ovaries. Higher number of normal COCs recovered in left ovaries without CL of slaughter house cattle. So, right ovaries having no corpus luteum are suitable for obtaining good quality cumulus-oocyte-complexes (COCs) in experiment for IVM and might for IVF and subsequent embryo culture.

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