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A comparative study of oocyte between native cattle and buffalo species in Bangladesh: a qualitative and quantitative approach

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Abstract: In vitro embryo production (IVP) can help alleviating reproductive difficulties in cattle and buffalo. This study compared qualitative and quantitative features of native cattle and buffalo ovaries and oocytes to understand them comprehensively. In addition, it was conducted to pick the ideal ovaries for oocyte collection and to assess either maturation affects oocyte diameter or not. The ovaries were collected from the local slaughterhouses of Dhaka city, Bangladesh. Next, the quantitative parameters of ovaries were measured, the follicular fluid was aspired, the oocytes were classified, and the diameters of oocytes were determined in both pre and postmaturation. Here, the results revealed that the ovaries of cattle and buffalo differed significantly ($p < 0.05$) in weight, length, width, and depth regardless of the ovary's position and the corpus luteum (CL) condition. The numbers of normal and abnormal oocytes per ovary were 1.19 and 0.96 in cattle and 1.50 and 1.13 in buffalo, respectively. Moreover, a significant difference ($p < 0.05$) was observed between the oocytes' diameter in cattle and buffalo (cattle 85.64 ± 8.16 μm vs. buffalo 99.92 ± 8.65 μm) and before and after the maturation of oocytes within the same species. It may be concluded that the buffalo ovary was greater in size, and the oocyte was larger than the cattle ovary and oocyte. Any ovaries—irrespective of the ovary's position and the CL status—can be a good source of potential oocytes. Besides, the maturation process may ultimately affect oocytes' diameter increase.

Key words: Cattle, buffalo, oocyte, diameter, in vitro maturation

1. Introduction

In Bangladesh, animal farming constituted 1.44% of the total gross domestic product, while the growth rate was 3.80% in 2020–2021[1]. Among the livestock species, cattle and buffalo are the major contributors to meat and milk production and earning foreign exchange[2]. Though the demand for meat and milk from the two species is increasing day by day, their production is problematic due to their low reproductive performance and reproductive disorders (RDs) [1–3]. Therefore, the most welcoming solution to this problem is the application of assisted reproductive technologies (ARTs), such as artificial insemination, in vitro embryo production (IVP), and multiple ovulation embryo transfer (MOET) to improve the production of animals [4]. In this study, the IVP was taken into consideration. The efficiency of IVP technologies is evaluated as the proportion of immature oocytes reaching the blastocyst stage seldom exceeds the threshold of 30%–40% in animal species, including bovine, equine, and porcine [5] and 10%–15% for buffalo [6]. Moreover, since slaughterhouse-derived animals are the most common source of ovaries for IVP [7], many significant aspects affect the quality of the oocyte, such as the age of the donor, estrous cycle stage, nutritional state, genetic potential, reproductive distress, and other characteristics are often unknown [8]. In addition, different factors can affect ovarian quantitative attributes such as animal age, size, body condition, species, heterogeneity, and pregnancy state [9–12]. According to some studies, follicular phases and hormonal profile may also play a role in this case [13,14]. Thus, knowledge about ovary and oocytes is crucial

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to mitigate these problems and produce more potential oocytes. Though some separate studies were conducted on ovarian characteristics of cattle and buffalo [9,10,15–18], a comparative analysis was not carried out, to the best of our knowledge. Moreover, little information is present related to the diameter changes of the oocytes due to maturation. Therefore, comparative studies of cattle and buffalo ovaries and oocytes are demonstrated in this research.

2. Materials and methods

The experiment was conducted at the laboratories of Animal Production and Management and Animal Genetics and Breeding departments of Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

2.1. Collection and processing of ovaries

Ovaries of native (local and cross-breed) cattle and buffalo without knowing the reproductive characteristics were collected from local slaughterhouses situated at Townhall and Krishimarket, Mohammadpur, Dhaka, Bangladesh. Ovaries were carried to the laboratory within a thermo-flask containing 0.9% physiological saline solution (Figure 1a) at 25–30 °C within 2 to 3 h after slaughtering the animals as described previously [19,20]. The cattle and buffalo ovaries (Figures 1b and 1c) were cut down from the reproductive tracts, and the unnecessary parts were trimmed.

2.2. Sample size and characteristics

A total of forty (40) cattle and twenty (20) buffalo ovaries were collected from twenty (20) and ten (10) animals, respectively. Twenty (20) left and twenty (20) right ovaries were collected for cattle, while ten (10) left and ten (10) right ovaries were collected for buffalo. For cattle, the number of corpus luteum (CL)-present ovaries was eight (8), while that of CL-absent ovaries was thirty-two (32), whereas, for buffalo, the number of CL-present ovaries was eight (8), while that of CL-absent ovaries was twelve (12). A total number of 172 cattle and 84 buffalo oocytes were aspired, while the numbers of normal oocytes (grade A + B) were 95 and 48, and the numbers of abnormal oocytes (grade C + D) were 77 and 36, respectively.

2.3. Assessment of morphology

The length, width, and depth of ovaries were measured by the slide calipers, and the ovarian weight was taken by the digital balance. The number of visible follicles was counted with the naked eye.

2.4. Method of oocyte collection and grading

The cumulus-oocyte complexes (COCs) were collected with the aspiration method [4,21]. The size of the follicle was not determined, but all the puncturable follicles were taken for the follicular fluid collection. A syringe with 18-gauge needle without filling any medium into it was used for this purpose. Those follicular fluids were considered free from blood (Figure 1d). A compound microscope (Olympus, Tokyo, Japan) at 4× scanning objective magnification was used to count the total number of oocytes [19]. The COCs were graded according to a previous study [22] (Figure 2).

2.5. Measurement of diameter of oocytes

Here a total of thirty (30) cattle and twenty (20) buffalo oocytes' diameters were noted. The COCs were taken up with a suitable micropipette glass that was prepared following the past procedure [18]. Oocytes' diameters without zona pellucida were measured using the software "Micro-measure" (Scalar Corp.; version 1.0.0.1).

2.6. In vitro maturation of oocytes

The total number of ovaries was 168 for cattle and 84 for buffalo. The 10% of it was placed aside for maturation purposes. TCM-199/EBSS (HyClone) was used as a maturation medium. The procedure was conducted by following the previous studies [17,19]. Oocyte maturation was done for 24 h in a 5% CO_2 incubator at 38.5 °C in humidified air [4,20]. A sum of twenty (20) cattle and fifteen (15) buffalo oocytes' diameters were measured after the maturation by the method mentioned above (2.4).

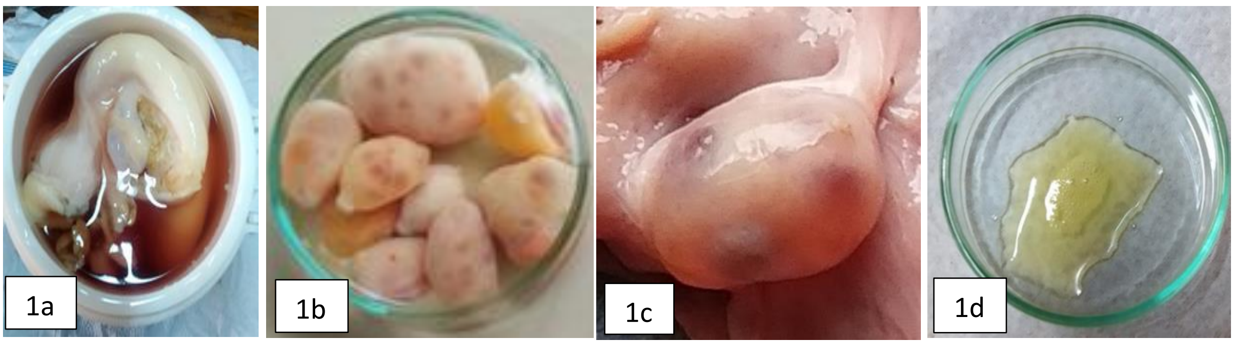

Figure 1. Reproductive tracts within thermo-flask (1a). Trimmed cattle ovaries (1b). Buffalo ovary (1c). Follicular fluid within Petridish (1d).

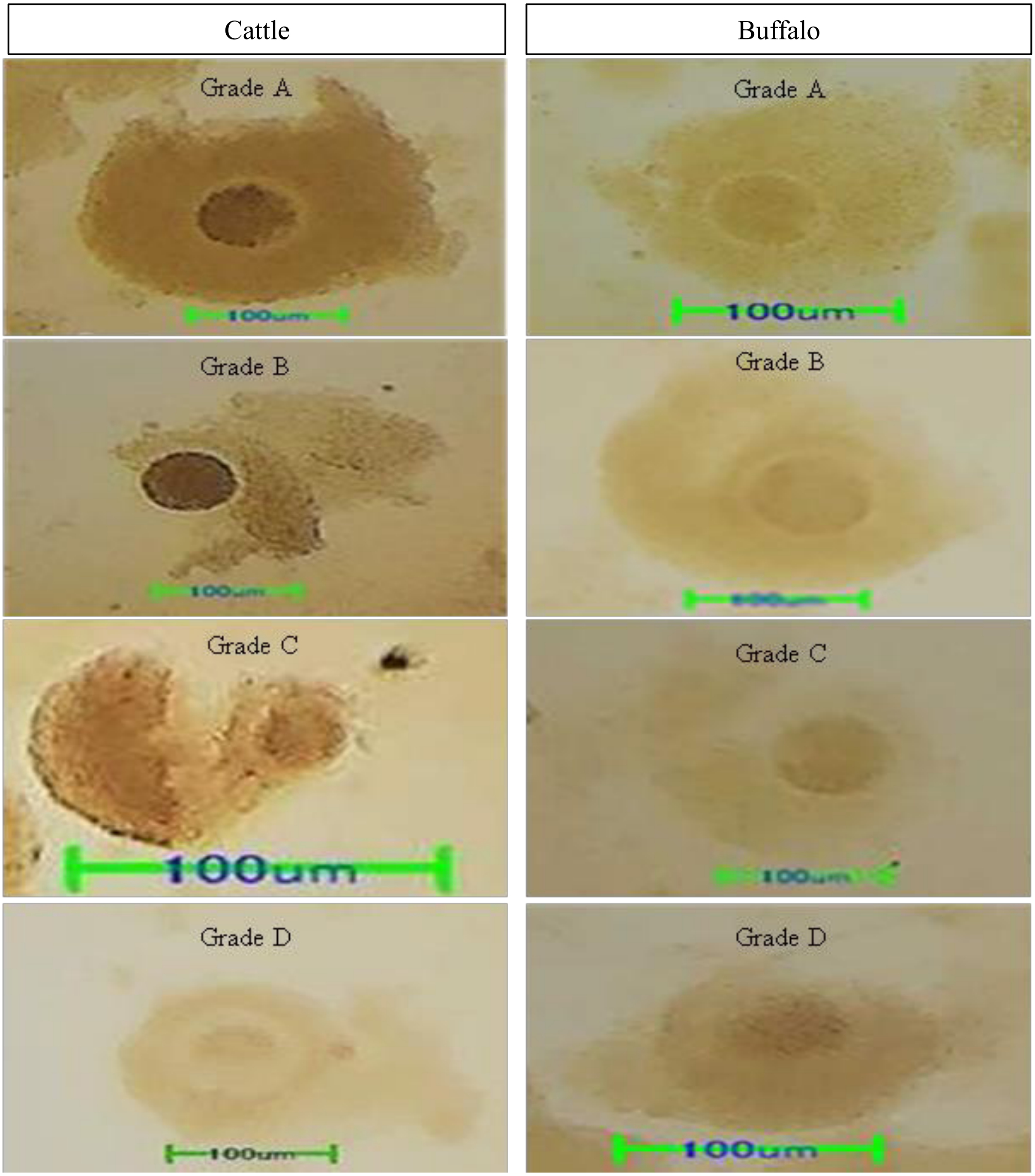

Figure 2. Grading of oocytes. The bar represents 100 µm.

2.7. Statistical analysis

Data were analyzed by an independent t-test (IBM SPSS statistics 25) to compare various parameters [23]. The difference between groups was considered significant when the p-value was < 0.05. All values were expressed as mean ± SD (standard deviation).

3. Results and discussion

3.1. Comparison between cattle and buffalo ovary

In Table 1, the weight, length, width, and depth of ovaries between the two species showed a significant difference ($p < 0.01$) which agreed with the study [10]. The buffalo's ovary demonstrated a higher value than the cattle's ovary in this study. On the contrary, the number of follicles per ovary present on the ovaries' surface had no statistical difference between the two species (Table 1). An average of 4.83 ± 4.39 follicles were found per ovary in cattle, which agreed with the goat experiment [18]. Some studies [9,10] highly contradict the bovine ovarian weight (1.75 ± 1.2 g), whereas bubaline ovarian weight (4.83 ± 2.16 g) of this research supports the findings of the past studies [11,24,25]. However, it does not support the mean value of other findings of buffalo ovarian weight [16,26]. This difference might be due to the lower body weighted and lower body conditioned breeds which were the source of ovaries in the present study. According to the literature, a positive association between the genital system weight and body size and body weight is present, proving that animals with lower body weights have smaller-sized ovaries than the animals with higher body weights [11].

Furthermore, it may be described that the population of animals had heterogeneity and individual physiological characteristics, which may differ among studies [10]. Besides, the differentiation between the two species of the present study might be caused by the species difference, age difference, and pregnancy status of the animal [9,10,12]. Moreover, follicular concentration in the ovaries reflects the ovary's propensity for antral follicles [13], various amounts of circulating hormones with hormonal receptors present in the ovary [14], and the presence of more CL containing ovaries in the buffalo sample might be the factors of differentiation. On the other hand, progesterone (P4) boosts embryonic survival and reduces embryonic losses in ruminants, and it rises at the end of the follicular phase of the ovary [27]. For these reasons, follicular growth and follicle size can be impaired by P4. The size of the follicles shrinks significantly after treatment with P4 [28], but this does not entirely prevent follicular activation [29]. In this study, the follicle size, P4, and their relationship were not considered.

3.2. Comparison of oocyte quality between cattle and buffalo

In Table 2, the comparison of oocyte numbers has been shown between the species. There was no significant difference between cattle and buffalo oocytes, though a higher mean value was shown for buffalo. The number of buffalo oocytes per ovary (1.31 ± 1.03) disagreed with some findings [26,30] where a higher number of oocytes—4.24 and 4.58 per ovary—were found. These forms of fluctuation or a lower number of COCs per ovary observed in this study can be explained by the animal's noncyclicity. Generally, animals with poor reproductive performance are slaughtered, and most of them are

Table 1. Comparison between morphometric parameters of the ovaries of cattle and buffalo.

Parameters of ovaries	Species of animal		Level of significance
	Cattle (n=84)	Buffalo (n=42)	
Weight (g)	1.75^b ± **1.20**	4.83^a ± **2.16**	*
Length (cm)	1.53^b ± **0.43**	2.29^a ± **0.28**	*
Width (cm)	0.89^b ± **0.29**	1.57^a ± **0.52**	*
Depth (cm)	0.63^b ± **0.22**	1.03^a ± **0.23**	*
Number of follicles per ovary	4.83 ± 4.39	8.25 ± 5.17	ns

The different superscripts indicated significant difference between two values in the same row; *, $p < 0.05$; ns, nonsignificant

Table 2. Comparison of oocyte quality between cattle and buffalo.

Parameters		Species of animal		Level of significance
		Cattle (n=84)	Buffalo (n=42)	
Number of COCs per ovary	Normal oocytes (Grade A+B)	1.19 ± 0.81	1.50 ± 0.89	ns
	Abnormal oocytes (Grade C+D)	0.96 ± 0.85	1.13 ± 1.15	ns
	Total	1.08 ± 0.83	1.31 ± 1.03	ns
Diameter of nucleus per oocytes (µm)		85.64^b ± **8.16**	99.92^a ± **8.65**	*

The different superscripts indicated significant difference between two values in the same row; *, $p < 0.05$; ns, nonsignificant noncyclic. Thus, the slaughterhouses may provide mostly the noncyclic ovaries, which may provide a lower number of oocytes [18].

On the other hand, the diameter of oocytes differed significantly ($p < 0.05$): 85.64 ± 8.16 µm in cattle and 99.92 ± 8.65 µm in buffalo (Table 2). This data agreed with a previous study [23] which stated that "the diameters of oocytes in buffalo seem to be greater than in cattle." The mean value of oocytes' diameters in the present study was lower than the results of other studies [23,31,32] for both cattle and buffalo. However, a wide range of diameters for buffalo oocytes (45–270 µm) was found [31,33].

The difference in oocytes' diameter among the mentioned studies might be explained by the fact that the present study sample had more CL-absent ovaries than the CL-present ovaries because the CL-present ovaries contained oocytes with larger diameter than the CL-absent ovaries [34]. Furthermore, the sample size, the different techniques of oocyte measurement, variations in follicle size (primordial, preantral, and antral) and location of oocytes (cortical and peripheral), as well as oocytes' growth stage (immature, in vivo matured) could be the major cause for this difference [23]. Besides, oocyte diameter is related to the morphology of the COCs, follicular diameter, and follicular atresia [31,35,36].

3.3. Comparative assessment of ovaries and oocytes according to the position of the ovary in animal

Here, the data of twenty (20) left and twenty (20) right ovaries for cattle, and ten (10) left and ten (10) right ovaries for buffalo are presented (Table 3). A statistically significant difference ($p < 0.05$) was observed between cattle and buffalo ovaries—right vs. right and left vs. left—in the aspect of quantitative characteristics, but the significant difference was absent in terms of oocyte quality. The biometrical values of the ovary in the present study agreed with the findings of others whereas the ovarian weight showed a lower value for buffalo species than in the present study [16,37]. The data (Table 3) disagreed with [38,39] those for zebu cattle in the case of ovarian weight, length, width, and thickness, which were higher than these findings. This might be due to the different breeds, body conditions, reproductive status, and regional differences in animal rearing among these studies. On the contrary, the average number of different graded oocytes per ovary did not differ significantly between the two species (Table 3). Moreover, it showed that the right ovary was heavier and more prominent in size than the left ovary in both species, which corresponded with other studies [15,40].

3.4. Comparative assessment of ovaries and oocytes according to the corpus luteum status in the ovary

Among the 40 cattle ovaries, eight (8) ovaries had CL, while thirty-two (32) ovaries had no CL. In addition, among the twenty (20) buffalo ovaries, eight (8) ovaries were recorded as CL-present, whereas twelve (12) as CL-absent ovary. A statistically significant difference ($p < 0.05$) was found between CL-present ovaries and between CL-absent ovaries in terms of weight, length, width, and depth for cattle and buffalo (Table 4). The findings of cattle (Table 4) were related to the ovarian weight and length of [41] and ovarian length, follicle, and COCs number of [18] with goat

Table 3. Comparison between qualitative and quantitative characteristics of ovaries and oocytes between cattle and buffalo according to the ovary's position.

Parameters		Right Ovary		LS	Left Ovary		LS
		Cattle (n=20)	Buffalo (n=10)		Cattle (n=20)	Buffalo (n=10)	
Weight (g)		1.98^{b} ± **1.40**	6.17^{a} ± **1.86**	*	1.54^{b} ± **0.92**	3.50^{a} ± **1.62**	*
Length (cm)		1.57^{b} ± **0.45**	2.41^{a} ± **0.23**	*	1.50^{b} ± **0.42**	2.17^{a} ± **0.29**	*
Width (cm)		0.91^{b} ± **0.25**	1.85^{a} ± **0.54**	*	0.88^{b} ± **0.33**	1.30^{a} ± **0.38**	*
Depth (cm)		0.67^{b} ± **0.14**	1.16^{a} ± **0.23**	*	0.59^{b} ± **0.27**	0.90^{a} ± **0.16**	*
No. of follicles per ovary		5.90 ± 5.09	9.75 ± 6.18	ns	3.75 ± 3.33	6.75 ± 4.27	ns
No. of COCs per ovary	Grade A	1.30 ± 0.86	1.50 ± 0.57	ns	1.30 ± 0.68	2.00 ± 0.81	ns
	Grade B	1.40 ± 0.82	1.00 ± 0.81	ns	0.75 ± 0.78	1.50 ± 1.29	ns
	Grade C	1.15 ± 0.81	1.00 ± 0.81	ns	0.55^{a} ± **0.60**	1.25^{b} ± **0.50**	*
	Grade D	1.50 ± 0.82	1.00 ± 1.41	ns	0.65 ± 0.81	1.25 ± 1.89	ns
	Total	1.34 ± 0.82	1.13 ± 0.88	ns	0.81 ± 0.76	1.50 ± 1.15	ns

The different superscripts indicated significant difference between two values in the same row; *, $p < 0.05$; ns, nonsignificant; LS, level of significance.

experiment. Different mean values of surface follicles were found in the buffalo and the cattle ovary (Tables 3 and 4), which can be explained by the fact that the buffalo ovary had a small-sized but higher number of follicles than the bovine ovary. The cow ovary has more primordial follicles than the bubaline ovary—from 60 to 100 thousand in cows and 12 to 20 thousand in buffalo cows [25]. Moreover, the number of follicles highly depends on the estrous cycle, progesterone production, and CL status of the animal. The number of big follicles in the ovaries of early pregnant buffalo cows reduces dramatically. At the same time, the developing follicles are smaller in size and have a lower chance of continuing into the later stages [10,42].

The variation in the ovary weight and size may be attributed to the presence of CL [43] and the number of primordial follicles, which are dependent on the cyclicity of the animal and implantation of the zygote in the uterus of the animal [42]. The fresh weight and DNA content of CL rose linearly from days 2–12 of the estrous cycle, and the ovine CL grows hyperplasia, which may create a higher weight [43]. A higher number of oocytes were recovered from the CL+ ovary than CL– ovary (Table 4). Similar results for different species of animals were observed [44]. This study proved that the CL-present ovaries produce more oocytes due to the higher progesterone level in the blood and the constant follicular changes occur due to the follicular degradation in the ovary [42]. On the other hand, according to a previous study [45], CL dramatically decreased the number of ovarian follicles in buffaloes and produced fewer oocytes, contradicting this study (Table 4). These scientists attributed the contradiction to the breed or genotypic differences in ovarian function between the river- and the swamp-type buffaloes.

3.5 Comparison of oocyte diameters before and after maturation

The diameters of oocytes excluding zona pellucida in pre- and postmaturation are presented in Table 5. The diameter changes of the oocytes due to maturation were compared

Table 4. Comparison between qualitative and quantitative characteristics of ovaries and oocytes between cattle and buffalo according to corpus luteum status.

Parameters		CL-present (CL+)		LS	CL-absent (CL–)		LS
		Cattle (n=8)	Buffalo (n=8)		Cattle (n=32)	Buffalo (n=12)	
Weight (g)		2.91^b ± **1.66**	5.96^a ± **2.23**	*	1.47^b ± **0.85**	4.16^a ± **2.04**	*
Length (cm)		1.78^b ± **0.43**	2.33^a ± **0.20**	*	1.47^b ± **0.41**	2.27^a ± **0.34**	*
Width (cm)		1.08^b ± **0.29**	1.83^a ± **0.60**	*	0.85^b ± **0.28**	1.42^a ± **0.42**	*
Depth (cm)		0.80^b ± **0.21**	1.23^a ± **0.23**	*	0.59^b ± **0.20**	0.91^a ± **0.14**	*
No. of follicles per ovary		7.38 ± 3.66	11.00 ± 6.92	ns	4.19 ± 4.37	6.60 ± 3.70	ns
No. of COCs per ovary	Grade A	1.38 ± 0.74	1.67 ± 0.57	ns	1.28 ± 0.77	1.80 ± 0.83	ns
	Grade B	1.67 ± 0.57	1.00 ± 1.00	ns	1.00 ± 0.70	1.40 ± 1.14	ns
	Grade C	1.33 ± 0.57	1.33 ± 0.57	ns	1.40 ± 0.54	1.00 ± 0.70	ns
	Grade D	1.00 ± 1.00	1.33 ± 1.50	ns	1.80 ± 0.83	1.00 ± 1.73	ns
	Total	1.13 ± 0.75	1.33 ± 0.88	ns	1.06 ± 0.85	1.30 ± 1.12	ns

The different superscripts indicated significant difference between two values in the same row; *, $p < 0.05$; ns, nonsignificant; LS, level of significance.

Table 5. Comparison between diameter of oocytes before and after maturation.

Species of animal	Diameter of nucleus per oocytes (µm)		Level of significance
	Before maturation	After maturation	
Cattle (n=28)	85.64^b ± **8.16**	94.09^a ± **11.23**	*
Buffalo (n=23)	99.92^b ± **8.65**	109.20^a ± **8.65**	*

The different superscripts indicated significant difference between two values in the same row; *, $p < 0.05$.

so that it could help to understand more about the oocyte's structure and developmental competence. The diameter of oocytes indicated a value of a significant difference ($p < 0.05$) that showed an increasing trend of diameter changes after the maturation of oocytes. The rise in oocyte diameter during 24 of culturing is consistent with the past findings [32,36] and could be a result of RNA production during oocyte growth [46]. This result contradicts another study where any significant difference between the two diameters of oocytes were not found [34], and a larger diameter of oocytes in both pre- and postmaturation were observed than this study [32]. This contrast might be made due to the different methods of diameter measurement, various maturation media, and time and sample size differences among the mentioned studies. In addition, the ability to continue and complete the meiosis process and developmental competence of oocytes may differ in the diameter measurements [31,36].

In conclusion, the present study showed that the buffalo ovary was more prominent in size and heavier in weight than the cattle ovary, and both the right, left, and the CL+, CL– ovaries would be a good source of potential oocytes. Additionally, it may assert that the maturation process increased the diameter of oocytes. Further research could determine how oocyte diameters change during maturation and the factors contributing to this shift.

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