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Influence of Season on the Ovarian Biometry, Oocyte Yield and Quality in Buffaloes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present experiment was designed to study the effect of seasonal variations on ovarian biometry, ovarian surface visible follicle number, oocyte recovery rate, and quality of recovered oocytes in buffaloes. Ovaries were collected from slaughtered buffaloes during the winter and summer seasons. The size (length and width) and weight of each ovary were measured. Grossly visible follicles on the surface of the ovary were counted, aspirated and oocytes were retrieved and evaluated. The biometrical studies revealed that the length and weight of the right ovaries were significantly higher ($p<0.05$) during the winter than in the summer season. However, the effect of season on the length, width, and weight of left ovaries and ovaries with corpus luteum and without corpus luteum was non-significant. The average surface follicle count per ovary was significantly ($p<0.05$) higher in ovaries collected during winter season than in summer. Furthermore, a significantly higher ($p<0.05$) oocyte recovery rate was obtained from the ovaries collected during

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the winter season. Ovaries collected during the winter and summer season had a higher ($p<0.05$) proportion of grade-A and grade-D oocytes, respectively. The results indicated that summer can adversely affect the functional activity of the ovaries to produce follicles suitable to give a high incidence of good quality oocytes.

Keywords: Buffalo; cumulus oocyte complexes; ovarian size; season.

1. INTRODUCTION

Buffalo (*Bubalis bubalis*) is a multi-purpose livestock species in many countries particularly in South Asia. It plays a prominent role in economy of Bangladesh [1]. Buffalo productivity depends upon successful reproduction, which in turn is affected by late maturity, poor expression of the estrous signs particularly during summer, irregular estrous cycle, silent heat, seasonality in breeding, poor conception rate/early embryonic mortality, and prolonged inter-calving interval [2,3,4]. Moreover, the reproductive efficiency of buffalo is greatly and adversely influenced by biometeorological factors viz: ambient temperature, relative humidity, day length and photoperiod either alone or in combination [5,6]. In addition, the reproductive problems are aggravated during the hot season, when fertility decreases drastically [7]. Variation in overt estrus and conception rate over the year has led to a general recognition that buffalo reproduce in a seasonal pattern [3]. The breeding season begins during the rainy season, with winter being the most favorable and summer to be the least favorable time of the year [3]. High ambient temperature has a negative impact on the oocyte yield, quality and developmental competency of oocytes retrieved from slaughterhouse buffalo ovaries [8,9]. High environmental temperature and humidity impair reproductive events by reducing estrous behavior expression, affecting follicular development, compromising oocyte competence, and limiting embryonic development [10,11]. Ovaries are mostly inactive in buffaloes during hot seasons [12] as compared to cold season owing to reduction in number of antral follicles [13]. Further, heat stress has been reported to degrade oocyte quality by altering follicular growth, steroid synthesis, and gene expression patterns [14].

The majority of farmers in Bangladesh raise buffaloes on grazing systems. During the winter and summer season, the temperature ranges from 5 to 15ºC and 27 to 38ºC, respectively [15]. In tropical areas like Bangladesh, buffaloes are subjected to harsh environmental conditions such as direct sunlight, high temperature, humidity, rains, and cool weather. Buffalo are generally slaughtered when they become infertile owing to subfertility or inadequate feed circumstances, both of which decrease the amount and quality of ovarian follicles, therefore resulting in reduced fertility [16]. However, the seasonal influence was assumed to be an essential factor [16] affecting reproductive performance of buffaloes. Although season plays an important role in the reproductive efficiency of buffaloes, the potential impact of environmental change, specifically season, on buffaloes has not been adequately assessed; very few references exist addressing the seasonal effects on ovarian biometry, as well as recovery and quality of buffalo follicular oocytes. Therefore, the current study was designed to investigate the effects of seasonal changes on ovarian biometry, surface follicle number, oocyte yield, and quality of recovered oocytes in buffaloes.

2. MATERIALS AND METHODS

The study was conducted in the laboratory of the Department of Animal Production & Management and Department of Animal Nutrition, Genetics & Breeding, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

2.1 Collection and Processing of Ovaries

Ovaries of sexually mature buffaloes with unknown reproductive history were collected immediately after slaughtering from local slaughterhouses during winter (December and January; n=14) and summer (April and May, n=16) seasons. They were then transported within 4-5 hours of slaughter to the laboratory in a thermos flask containing 0.9% physiological saline at 25-30°C. In the laboratory, the ovaries were shifted to sterilized Petri dishes and thoroughly rinsed with physiological saline at a temperature of 25°C. Each ovary was trimmed to remove extraneous tissues. The collected ovaries were categorized as right and left ovary that was previously marked during slaughter with the assistance of the butcher. After that, the ovaries were examined and labeled as corpus

luteum absent (CL-) and corpus luteum present (CL+) groups.

2.2 Biometrical Studies of Ovaries

The length and width of the ovaries were measured with a digital slide caliper and represented in centimeter (cm). Individual ovary weight in gram (gm) was recorded in a tabular form after being weighed on a digital balance. The number of visible follicles on the surface of the ovaries was counted and data were recorded accordingly.

2.3 Oocyte Aspiration and Grading

The ovaries were washed 2-3 times in physiological saline solution (0.9% NaCl) at room temperature. After that, they were kept in a beaker and placed in a water bath at 30°C. The cumulus-oocyte-complexes (COCs) were recovered by aspiration of visible follicles (2-8 mm) using an 18-gauge needle connected to a 10 ml disposable syringe containing 1.0-1.5 ml of Dulbecco's phosphate-buffered saline (DPBS) (Sigma, USA). The recovered COCs were evaluated under a microscope and classified into 4 grades on the basis of cumulus cells and nucleus as described by Khandoker et al. [17]: 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. COCs in grades A and B were regarded as normal, whereas COCs in grades C and D were considered abnormal. The number of oocytes with different grades (A, B, C, and D) was recorded.

2.4 Statistical Analysis

Data were presented as mean ± SE (Standard Error). Data were analyzed using one-way analyses of variance (ANOVA) with the SPSS program (Version 16.0; SPSS Inc., Chicago, IL, USA). Differences at $p < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Seasonal Effect on Ovarian Biometry

The biometrical studies of buffalo ovaries as related to seasons have been presented in Table 1. The obtained results showed variations between seasons in the biometrical data of the ovaries but only right ovarian weight and length were significantly ($p<0.05$) higher in the winter season than in summer. However, the size (length and width) and weight of all categories of ovaries tended to be higher in winter than in the summer season, but the differences were not significant (Table 1). The higher ovarian biometric measurements in winter may be attributed to the presence of more ovarian structure, more and bigger follicles or perhaps corpora lutea, during the winter than in the summer season. In ovine, the breeding season is connected with an increase in ovarian measurements [18], whereas ovaries obtained during the breeding season were bigger with larger follicles and corpora lutea than those collected during the non-breeding season [19]. Osman and Shehata [20] reported that there were no significant variations in the dimensions (length, breadth, and thickness) and weight of the ovaries between seasons. The corpus luteum is an extra cellular material within the ovary which made the differences of its length, width, and weight [21]. However, the significant changes in ovarian weight found between seasons confirm the findings of Farrag [22] and Osman [23] that ovaries containing corpus luteum and follicles were larger than ovaries without either. In general, the values of ovarian length, width and weight in the camel were higher during the peak than the low breeding season for both ovaries as mentioned by Ali et al. [24]. According to Tingari [25], the corpora lutea are formed by ruptured follicles that are usually found towards the ovary's poles, resulting in increased ovarian length and possibly width, but not thickness.

3.2 Effect of Season on Ovarian Surface Follicle Number

In concern with the number of surface follicles per ovary, the obtained results showed a marked ($p<0.05$) reduction in the incidence of follicles during summer (Table 2) which coincide with the reports of Osman and Shehata [20] and Abdoon et al. [13]. This may be due to the relatively inactive status of ovaries during the hot season that often renders buffalo as seasonal breeders [26]. Similar findings were reported in a study in which the recovery rate of large pre-antral follicles from buffalo ovaries during peak breeding season was greater compared to low-breeding season [27]. According to Li et al. [28] high environmental temperature suppresses ovarian follicular development, therefore, resulting in lower reproductive efficiency in dairy

cows during summer. Summer season influences ovarian follicular development with lower rate of follicular growth in goats [15]. As per Abdoon et al. [29], the season has a detrimental effect on the development of ovarian follicles, with a significantly ($p<0.05$) reduced number of ovarian follicles documented during the hot season compared to the cold season. During the hot season, the incidence of cyclic animals was low, whereas the rate of smooth inactive ovaries was higher in buffaloes [29]. Hot season reduced follicular growth and dynamics [30], and decrease the number of ovarian follicles in cattle [31].

3.3 Seasonal Effect on the Recovery Rate and Quality of Buffalo Oocytes

The average number of oocytes recovered per ovary was significantly higher ($p<0.05$) during the winter season compared to the summer season (Table 2). The season had a significant effect on the quality and gradation of oocytes, with poor quality oocytes being more prevalent in the summer, which could be related to physiological heat stress disrupting hormonal balance and causing ovarian dysfunction. The current findings were similar to those of Jamil et al. [32], who found a significantly higher ($p<0.05$) oocyte recovery rate from ovaries sampled during peak breeding season. Datta and Goswami [33] found that the average number, as well as, the percentage of good quality oocytes recovered from buffalo ovaries during cool months (<25°C) was significantly higher than the corresponding values obtained during moderately hot (25-30°C) and hot (>30°C) months. It could be suggested that oocytes were compromised during development and differentiation when the environmental conditions were not favorable for reproduction. Furthermore, Zohier et al. [34] noted that the proportion of good buffalo oocytes collected during winter was substantially higher than that obtained during summer (74.6 vs. 50). The breeding season yielded more category (I) and total oocytes per ovary in camels than the non-breeding season [35]. Singla et al. [36] stated that buffaloes exposed to heat stress produce fewer good quality oocytes than their unstressed counterparts. However, the lower yields of usable oocytes during the hot season may be attributed to heat stress, which alters endocrine rhythms and reduces follicular development Nandi et al. [37]. In contrast to our findings, Di Francesco et al. [38] and Tasripoo et al. [39] observed that the quantity of oocytes recovered per ovary, as well as the number and incidence of good quality oocytes, were not affected by season in buffaloes. Variations in oocyte yield and quality between studies could be attributed to differences in breeds, agro-climatic conditions, the status of animals slaughtered, the season of ovary collection, the number of ovaries processed, the techniques used, and the criteria for selecting ovaries.

Table 1. Biometry of buffalo ovaries under two different seasons

Ovarian category	Parameter	Summer season	Winter season	Level of significance
Left	Weight (g)	3.47±0.12	3.78±0.14	NS
	Length (cm)	2.37±0.06	2.44±0.05	NS
	Width (cm)	1.65±0.04	1.71±0.06	NS
Right	Weight (g)	3.89^{b}±0.12	4.53^{a}±0.17	*
	Length (cm)	2.47^{b}±0.07	2.64^{a}±0.06	*
	Width (cm)	1.86±0.06	1.97±0.05	NS
CL present	Weight (g)	4.29±0.17	4.66±0.19	NS
	Length (cm)	2.54±0.07	2.65±0.06	NS
	Width (cm)	1.83±0.05	2.02±0.05	NS
CL absent	Weight (g)	3.59±0.13	3.75±0.16	NS
	Length (cm)	2.42±0.06	2.43±0.04	NS
	Width (cm)	1.63±0.03	1.70±0.05	NS

*CL, corpus luteum; Data were presented as mean ± standard error; Means with different superscripts in the same row differ significantly; *, p<0.05; NS, non-significant*

Table 2. Ovarian surface follicles count, oocyte yield and quality in buffalo ovaries collected during summer and winter season

Parameter		Summer season	Winter season	Level of significance
Number of ovaries		16	14	
Total no. of follicles		77	99	
No. of follicles/ovary		4.75[b]±0.29	7.07[a]±0.43	*
Total oocytes recovered		28	32	NS
Oocyte recovered/ovary		1.75[b]±0.17	2.29[a]±0.19	*
Normal oocyte	Grade A	25.00%[b] (7)	37.5%[a] (12)	*
	Grade B	28.57% (8)	28.13% (9)	NS
Abnormal oocyte	Grade C	17.86% (5)	15.62% (5)	NS
	Grade D	28.57%[a] (8)	18.75%[b] (6)	*

*Values in the parentheses indicate the number of observation; Data were presented as mean ± standard error; Means with different superscripts in the same row differ significantly; *, p<0.05; NS, non-significant*

4. CONCLUSION

In conclusion, the number of ovarian surface follicles and recovered oocytes were higher during the winter season, and consequently, the quality of oocytes was also better. Thus, the study on ovarian biometry and other parameters under varying seasonal conditions could help for better reproductive management of buffaloes during heat stress conditions. Along with various biotechnological studies in the laboratory, this study may be quite worthy for further study on in-vitro maturation, fertilization and embryo production of buffaloes.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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