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Original article

Factors affecting number of surface ovarian follicles and oocytes yield and quality in Egyptian buffaloes

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Abstract — Three experiments were conducted to evaluate factors affecting number of surface ovarian follicles and oocytes yield and quality in buffalo. In Experiment 1, ovaries (n = 126) were collected in pairs from slaughtered anoestrus, early pregnant and cyclic buffaloes. Ovarian follicles (1–3, 4–9 and ≥ 10 mm diameter) were counted, aspirated and oocytes were recovered and evaluated. In Experiment 2, ovaries were divided into 2 groups. Group 1, ovaries bearing a CL (n = 74) and Group 2 non-bearing CL (n = 74), ovarian follicles (2–8 mm) were counted, aspirated and oocytes evaluated. In Experiment 3, oocytes were recovered using aspiration or slicing methods. In all experiments, oocytes were classified into good, fair, poor and denuded. Results showed that the development of small and total ovarian follicles are continuous and independent in early pregnant or cyclic buffalo cows, however, it significantly decreased ($P < 0.01$) in the ovaries of anoestrus buffaloes. Number of medium and large size follicles was significantly increased ($P < 0.01$) in cyclic buffaloes on Days 10–16 and 17–22 of oestrous cycle, while large follicles was significantly decreased ($P < 0.01$) in the ovaries of pregnant buffaloes. A significantly higher ($P < 0.01$) percentage of poor and denuded oocytes were recovered from ovaries of anoestrus and pregnant buffalo. While, the highest ($P < 0.01$) percentage of good quality oocytes were recovered from ovaries of cyclic buffaloes on Days 1–3 and 10–16 of oestrous cycle, eliciting that the stage of oestrous cycle is affecting the quality of buffalo oocytes. In addition, the presence of a CL stimulates the development of a significantly higher ($P < 0.01$) number ovarian follicles which produced a significantly higher ($P < 0.05$) number of good quality oocytes. Slicing of buffalo ovaries produced a significantly higher number of fair, poor and denuded oocytes. In conclusion, number of ovarian follicles and yield and quality of oocytes were affected by the reproductive status, stage of the oestrous cycle, presence of a CL and the method of oocytes retrieval.

buffalo / reproductive status / follicle / oocyte quality / CL / oocyte retrieval

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1. INTRODUCTION

Better understanding of ovarian folliculogenesis is essential to deal with reproductive performance of buffalo. Counts of follicles on the ovarian surface and their classification into size categories based on diameter can be used to obtain a reasonable indication of actual population of follicles in the ovary [6]. In this respect, the number of antral follicles in swamp buffalo is 20% of those observed in cattle and the number of non atretic follicles (> 1.7 mm) average 2.9 for buffalo and 22.1 for cattle [21]. Matton et al. [17] found that small follicles decreased in number gradually from Days 3 to 18, while, medium sized follicles were more numerous on Day 13 than at other stages of oestrous cycle in cows. Pregnant cows showed fewer medium and large follicles than cyclic cows, whereas development of small follicles is not impeded during pregnancy [4]. In addition, several researchers recorded that the presence of a CL stimulates follicular development, so that the CL bearing ovary contains more follicles [19]. Very few studies have dealt with ovarian follicular development in relation to the reproductive staus in buffalo.

Selection for stage of the reproductive status that yielding a higher proportion of oocytes with optimum characteristics and appearance were not identified in buffalo. A higher proportion of good quality oocytes with higher rate of developmental competence was isolated at the end of the luteal phase of oestrous cycle in cows [15]. Other records found that the stage of oestrous cycle has no effect on bovine oocytes quality or its developmental potentials [14]. Moreover, the number of follicles and yield of oocytes per ovary were less from buffalo ovaries bearing a CL [3, 13]. In addition, methods of oocyte retrieval are directly affecting the yield and quality of oocytes. Slicing of ovary yielded a significantly more oocytes per ovary in cows [9] and buffalo cows [13].

The present investigation was undertaken to examine: (1) the effect of reproductive status and the presence of a corpus luteum (CL) on number of ovarian follicles, oocyte yields and quality in buffalo ovaries; (2) effect of methods of oocyte retrieval on yields and quality of oocytes from buffalo ovary.

2. MATERIALS AND METHODS

2.1. Experiment 1

This study aimed to examine the effect of reproductive status on the number of surface ovarian follicles and its relation to oocyte quality. After slaughtering, the genitalia of 126 buffaloes were examined carefully to determine the reproductive status either true anoestrus (n = 17) smooth ovaries without any specific structures (follicles or CL), early pregnant (n = 22, 1–2 month) or cyclic (n = 87). The ovaries were collected in pairs, placed in plastic bags containing phosphate buffered saline (PBS) and stored in thermos containing PBS at 32–36 °C. In the laboratory, identification of the stage of oestrous cycle was done using the characteristics of the ovaries based on corpus luteum color and shape according to the method adopted by Jainudeen [11]. Briefly, this combination of criteria allowed classification of pairs of ovaries into, early luteal (Days 1–3) ovulation point red and presence of red spot or small red elevation; developed CL (Days 4–9) with dark red soft protruded CL; mature CL (Days 10–16) with a sharply demarcated red to orange protrusion or embedded CL, regressing CL (Days 17–22), hard yellow to white small protrusion. Follicular diameter was measured on the ovarian surface-using caliper and follicles were classified into 3 categories: small (1 to 3 mm), medium (4 to 9 mm) and large (≥ 10 mm) according to Dominguez [4]. The content of the visible ovarian follicles was aspirated from each pair using 10-mL syringe and 18-gauge needle. Follicular contents were transferred into 6-cm glass Petri dish and oocytes were collected and evaluated under stereomicroscope (28–30 ×).

2.2. Experiment 2

This experiment was designed to evaluate the effect of presence or absence of a CL on the number of ovarian follicles and oocytes yield and quality. A total of 148 ovaries collected from cyclic or early pregnant buffalo cows. In the laboratory, ovaries were divided into Group 1 *(n* = 74) ovaries bearing a CL; Group 2 ((*n* = 74) with ovaries non-bearing CL. The number of surface ovarian follicles (2–8 mm diameter) was counted. Follicular contents were aspirated and oocytes were recovered and evaluated under stereomicroscope.

2.3. Experiment 3

This experiment was carried out to investigate the effect of methods of oocyte retrieval using aspiration or slicing on yields and quality of oocytes from buffalo ovaries. A total of 61 ovaries was collected from buffalo cows of unknown reproductive history. All visible follicles (2–8 mm diameter) on the ovarian surface were aspirated using 10-mL syringe and 18-gauge needle. Another, 67 ovaries were placed a 10 cm glass Petri dish containing 15 mL PBS and were chopped into small pieces with surgical blade. The sliced stromal tissues were discarded. For the 2 methods (aspiration or slicing) each ovary was processed and examined separately to assess total oocyte recovery and quality.

2.4. Assessment of oocyte quality

Evaluation of oocyte was carried out according to Hamano and Kuwayama [9]. Good oocytes were surrounded by more than 6 layers of cumulus cells adhering to the zona pellucida. 3–5 and 1–2 layers of cumulus cells surrounded fair and poor oocytes respectively. Necked oocytes were assigned as denuded.

2.5. Statistical analysis

The analysis of data was carried out using analysis of variance ANOVA (Tab. I), Chi-square (Tab. II) and Student “Paired-t” test (Tabs. III and IV) according to Snedecor and Cochran [20].

3. RESULTS

3.1. Experiment 1

The mean (± SEM) count of surface ovarian follicles in small (1–3), medium (4–9) and large (≥ 10 mm) size categories and total ovarian follicles are presented in Table I. Results illustrated that ovaries from anoestrous buffaloes possess a significantly low

Table I. Ovarian follicular population in relation to reproductive status in buffaloes (mean ± SEM).

	Follicular size			
Status	1–3 mm	4–9 mm	≥ 10 mm	Total no. ovarian follicle
Anoestrus (*n* = 17)	5.167 ± 0.757[b]	1.286 ± 0.338[a]	0.372 ± 0.078[b]	6.825 ± 0.916[b]
Pregnant (*n* = 22)	10.320 ± 1.713[a]	0.558 ± 0.043[b]	0.050 ± 0.039[b]	10.929 ± 1.643[a]
Days 1–3 (*n* = 14)	10.543 ± 2.565[a]	0.838 ± 0.201[b]	0.167 ± 0.153[b]	11.548 ± 2.765[a]
Days 4–9 (*n* = 22)	11.001 ± 1.594[a]	0.833 ± 0.251[b]	0.250 ± 0.112[b]	12.083 ± 1.765[a]
Days 10–16 (*n* = 35)	10.688 ± 0.939[a]	1.693 ± 0.176[a]	0.672 ± 0.125[a]	13.053 ± 1.078[a]
Days 17–22 (*n* = 16)	10.167 ± 1.167[a]	1.937 ± 0.258[a]	1.134 ± 0.224[a]	12.238 ± 1.353[a]

[a, b] Difference within column ($P < 0.01$).

Table II. Percentage of oocyte quality in relation to reproductive status in buffalo.

Status	Oocyte quality (%)			
	good	fair	poor	denuded
Anoestrus (n = 17)	11.1 (7/63)[c]	34.9 (22/63)[c]	33.3 (21/63)[c]	20.6 (13/63)[b]
Pregnant (n = 22)	31.3 (52/166)[b]	25.3 (42/166)[c]	18.7 (31/166)[a]	24.7 (41/166)[c]
Day 1–3 (n = 14)	39.6 (40/101)[a]	23.8 (24/101)[c]	17.8 (18/101)[a]	18.8 (19/101)[b]
Day 4–9 (n = 22)	14.3 (24/168)[c]	57.1 (96/168)[a]	21.4 (36/168)[a]	7.1 (12/168)[a]
Day 10–16 (n = 35)	37.9 (108/285)[a]	32.3 (92/285)[c]	17.9 (51/285)[a]	11.9 (34/285)[a]
Day 17–22 (n = 16)	30.4 (39/128)[b]	45.3 (58/128)[b]	14.8 (19/128)[a]	9.4 (12/128)[a]

[a, b] Difference within column ($P < 0.05$).
[a, c] Difference within column ($P < 0.01$).

Table III. Effect of presence corpus luteum on the number of surface ovarian follicles and oocytes quality in buffalo (mean ± SEM).

Groups		Oocyte quality			
	No. follicles/ovary	good	fair	poor	denuded
With a CL	5.832 ± 0.298**	1.74 ± 0.22*	0.98 ± 0.09	0.54 ± 0.11	0.34 ± 0.01
Without CL	4.318 ± 0.180	1.15 ± 0.32	0.78 ± 0.04	0.51 ± 0.08	0.44 ± 0.08

* $P < 0.05$; ** $P < 0.01$.

Table IV. Effect of methods of retrieval on oocytes yield and quality in buffalo ovaries (mean ± SEM).

Groups		Oocyte quality			
	No. follicles/ovary	good	fair	poor	denuded
Aspiration	3.34 ± 0.16	1.98 ± 0.14	0.76 ± 0.13	0.31 ± 0.08	0.18 ± 0.09
Slicing	8.08 ± 0.42**	2.15 ± 0.16	1.24 ± 0.18*	1.44 ± 0.11**	3.43 ± 0.32**

* $P < 0.05$; ** $P < 0.01$.

($P < 0.01$) number of small and total ovarian follicles compared with pregnant or cyclic ones. While, difference in small and total ovarian follicles lack significance between pregnant, cyclic or the stage of the cycle in buffaloes. Moreover, a significant increased ($P < 0.01$) in number of medium and large follicles was observed in ovaries of cyclic buffalo on Days 17–22 and 10–16 of the oestrous cycle respectively. While, ovaries of pregnant buffaloes had the lowest number of large follicles.

Table II summarizes the results of oocyte quality in relation to reproductive status in buffaloes. The analysis of data revealed that the percentage of good quality oocytes was significantly low ($P < 0.01$) and poor quality oocytes was high ($P < 0.01$) when oocytes were recovered from ovaries of

anoestrous buffaloes. In addition, ovaries from pregnant and anoestrus buffaloes produced significantly high ($P < 0.01$ and $P < 0.05$, respectively) percentages of denuded oocytes. The highest percentage of good quality oocytes were recovered from ovaries of cyclic buffaloes on Days 1–3 and 10–16 of the oestrous cycle. Also, significantly higher ($P < 0.01$) percentage of fair oocytes was collected from ovaries of cyclic buffaloes on Days 4–9 and 17–22 of oestrous cycle.

3.2. Experiment 2

In Experiment 3, data showed that buffalo ovaries bearing a CL had a significantly higher ($P < 0.05$) number of surface ovarian follicles and produced significantly higher ($P < 0.05$) number of good quality oocytes than ovaries non-bearing CL (Tab. III).

3.3. Experiment 3

The analysis of data (Tab. IV) indicated that slicing of buffalo ovaries produced a significantly higher ($P < 0.01$) number of oocytes per ovary compared with aspiration of follicles. In the mean time, a significantly higher number of fair and poor ($P < 0.05$) or denuded ($P < 0.01$) oocytes were recovered using slicing than aspiration.

4. DISCUSSION

Results obtained in this study indicate that reproductive status of buffalo cows significantly affect the number of ovarian follicles. In Experiment 1, number of small and total surface ovarian follicles was significantly decreased in ovaries of anoestrus compared with cyclic or pregnant buffaloes. Similar results were previously reported [3] who found poor reserves of follicles in non-cyclic buffalo. The condition was attributed to the presence of less number of primordial follicles in ovaries of non-cyclic buffalo [2]. In addition, the present work showed that numbers of small and total surface ovarian follicles lack of significance between ovaries of pregnant and different stages of the oestrous cycle in cyclic buffaloes, indicating that the development of small ovarian follicles is continue during pregnancy and independent of the stage of oestrous cycle. These results are in consistence with previous observation [18] in pregnant cows [5] and cyclic cows. In contrary, Gutierrez et al. [7] reported that the number of small size follicles increased significantly on Days 2 and 14 of oestrous cycle in cows. This discrepancy may be attributed to species difference. Concurrently, in the present study the development of medium and large sized follicle was significantly increased on Days 10–16 and 17–22 of oestrous cycle. This result concordant with previous results in cattle [17]. Moreover, the present study illustrated that the number of large follicles was significantly decreased in ovaries of early pregnant buffalo cows. The developing follicles reach only a smaller size and are less likely to continue into later stage [12].

Prediction of morphologically normal oocytes would facilitate studies, which assess factors influencing in vitro maturation (IVM) and in vitro fertilization (IVF) of buffalo oocytes. In the present study the percentage of good quality oocytes was significantly low, while, poor and denuded oocytes were significantly higher when oocytes were recovered from ovaries of anoestrus buffalo cow, indicating that most of the ovarian follicles in anoestrus buffalo may be atretic. Similar results were recorded by Jain et al. [10] in buffalo. In addition, the highest percentage of good quality oocytes was recovered from ovaries of cyclic buffalo cows on Days 1–3 and 10–16 of oestrous cycle, eliciting that the stage of oestrous cycle is influencing the quality of oocytes regardless the number of ovarian follicles. These results are similar to that previously reported in cattle [8, 15]. Those authors found that oocyte competence is

influenced by the stage of oestrous cycle, oocytes collected during days 2 and 10 produced significantly more high quality oocytes for in vitro fertilization. On the other hand, Arlotto et al. [1] observed that the stage of oestrous cycle is not influencing oocytes quality in cows. Moreover, the present work demonstrated high percentages of denuded oocytes were recovered from ovaries of pregnant buffalo. While, Domingues [4] reported that pregnancy did not seem to affect oocyte quality and the proportion of normal oocytes was similar in cyclic and pregnant cows. This discrepancy may by attributed to the stage of pregnancy or species difference.

The results obtained in Experiment 2 indicated that ovaries bearing a CL had a significantly higher number of surface ovarian follicles and yielded significantly higher number of good quality oocytes compared with ovary non-bearing CL. This finding is consistent with the observation of Savio et al. [19] in cattle. On the other hand, Das et al. [3] recorded that the presence of a CL significantly reduces the number of ovarian follicles as well as the quality of the oocytes. The reason for such difference may be attributed to breed or genotyping difference in ovarian function between the Mediterranean and swamp buffaloes.

Mean while, the present study showed that the number of oocytes recovered per buffalo ovary was significantly higher by using slicing of ovaries than aspiration of follicles. Similar observation has been reported in cattle [16] and buffalo [3, 13]. Slicing of ovary release oocytes from surface follicles and these in the deeper cortical stroma. However, the present results illustrated that slicing of ovaries produced significantly higher number of fair, poor and denuded oocytes. Martino et al. [16] attributed the condition to the fact that this technique recovers heterogeneous population of oocytes from all kinds of follicles distributed throughout the ovarian stroma, and many of these oocytes are not fully grown. In contrary, Hamano and Kuwayama [9] recorded that slicing of ovaries produced significantly higher good quality oocytes. This difference may be due to the method of slicing, apparatus used or species difference.

In conclusion, number of surface ovarian follicles is continue to develop during pregnancy and independent of the stage of oestrous cycle, but it significantly reduced in ovaries of anoestrus buffalo. Growth and dominance of follicles occurs on Days 10–16 and 17–22 in Egyptian buffalo. Higher percentage of good quality oocytes were recovered from ovaries of cyclic buffalo on Days 1–3 and 10–16 of the oestrous cycle, eliciting that stage of oestrous cycle is affecting oocytes quality. Higher percentage of poor and denuded oocytes was recovered from ovaries of anoestrus or early pregnant buffalo cows. Ovary bearing-CL had a significantly higher number of follicles and yields a higher number of good quality oocytes. Slicing of buffalo ovaries produced a significantly higher number of fair, poor and denuded oocytes.

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