Al-Furat Journal of Innovations in Agricultural Sciences (FJIAS)
Published by Al-Furat Al-Awsat Technical University

ISSN: 2789-6773

Effect of Presence and Absence of Corpus Luteum on Ovarian Dimensions, Follicular Numbers and Quality of Oocytes in Iraqi Buffaloes (*Bubalus Bubalis*)

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FJIAS 2025, 1(1): 30-37

Abstract: *This research aims to assess the presence and absence of corpus luteum (CL) on ovarian dimensions, follicular numbers, and quality of oocytes in Iraqi buffaloes (Bubalus bubalis). A total 300 numbers of ovaries of buffalo were collected from slaughterhouses of Babylon province from September 2020 until January 2021. The ovaries were assigned to have CL(n=110) and the ovaries not having CL (n = 190). Collected ovaries were evaluated for length, width, and thickness, and the follicles were calculated, measured, and classified as small (< 3 mm), medium (3-8mm), and Large (> 8 mm) follicles. The follicular fluid was aspirated from visible follicles (2-8mm diameter), and the oocytes were examined, counted, evaluated, and graded. The results showed that the length (24.20 ± 2.20 mm), width (16.80 ± 2.01 mm), and thickness (13.96 ± 1.80mm) were significantly increased (p ≤0.05) in ovaries bearing CL then those ovaries non – bearing CL. The average number of follicles per ovary of medium (6.58 ± 0.06) and Large (4.35 ±0.02) follicles were significantly higher (p< 0.05) numbers in ovaries without CL than the ovaries with CL. The significant higher were observed in good (1.86±0.12), fair (1.06±0.07) and poor (1.28 ± 0.07) oocytes in the average number of oocytes per ovary in the ovaries non - bearing CL compared with the ovaries bearing CL. In conclusion, based on these data, we can infer that the ovaries without CL included more obtainable good follicles and good quality of the oocytes than those obtained from ovaries with CL.*

Keywords: *Iraqi buffalo; ovary; follicles; oocytes; corpus luteum.*

1. INTRODUCTION

Water buffaloes (*Bubalus bubalis*) are a distinct species with the family of Bovidae and has distinctive place in the Iraqi agriculture system. The domestic water buffalo plays on important role in the agriculture economy , especially the rural economy and the dairy industry [1] . Buffaloes suffers from inveterate reproductive problems such as seasonality of breeding and high rate of incidence of embryonic death [2] delayed puberty, late postpartum conception, long calving interval and delayed first calving [3], low oocyte yield [4] and low number of follicles and high percentage of atretic follicles [5],[6].To vanquish these reproductive problems , must be introduced assisted reproductive technologies, like artificial insemination (AI),in vitro maturation (IVM), in vitro fertilization(IVF) and embryos transfer (ET) in genetic improvement of local buffaloes. To engage with these techniques ,it is necessary to obtain oocytes at the lowest costs, in this regard, slaughterhouses are considered low expensive and plenteous origin of the primary oocytes on a commercial scale through maturation and fertilization in the laboratory (*In vitro*) [7],[8]. Corpus luteum (CL) is in evanescent endocrine gland established after ovulation from excreted

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cells (granulose and theca cells) of follicles of ovaries. The main function of CL is the secretory of progesterone .Many researches stated that the ovaries contain CL have ability to stimulates the growth of follicles. Therefore, the ovaries that bearing of CL contains the most number of follicles [9]. Das *et al*., [10] and Kumar *et al*,[11] demonstrated that the follicular numbers and oocytes production per ovary from buffalo ovaries without CL compared with from buffalo ovaries with CL. The objective of this research to assess effect of presence and absence of corpus luteum on ovarian dimensions , follicular numbers and quality of oocytes in iraqi buffaloes *(Bubalus bubalis).*

2. MATERIALS AND METHODS

2.1 Place of experiment

This study was contacted in reproductive physiology and artificial insemination Laboratory, Department of Technical Animal Production , Al - Musaib Technical College, Al - Furat Al - Awsat Technical University (ATU) for the period from September 2020 until January 2021.

2.2.*Collection of genitals*

The genital organs with unbeknown reproductive history were removed and collected about 30 minutes after slaughter from 150 adult and non - pregnant buffaloes slaughtered at Babylon governorate. The genital organs were placed in chilled thermos flask and transferred within an hour to the laboratory.

2.3.Ovaries collection

In the laboratory the ovaries were removed from the genitals, cleaned from the stuck tissues and debris, and washed with cooled physiological solution, and finally placed on filter paper to dry them. The ovaries were divided into two classes, class bearing of Corpus luteum and Class non-bearing of Corpus Luteum. The dimensions of the ovaries were measured by measuring their length as the distance between the anterior end and posterior end along axis extending the attachment of the mesentery of the ovary [12]. The width of ovary was measured as Largest distance from the medial and Lateral borders [13]. The thickness of the ovary was recorded as Largest distance from vertical to Longitudinal axis at distance from attached to the free borders . The follicles visible on the surface of the ovaries were calculated and their diameters measured with vernier calipers according to these measurements the follicles were categories into three category Small, Less than 3mm (<3 mm), medium from 3 to 8 mm and Large Larger than 8 mm (> 8 mm) [14]**.**

2.4.Oocytes collection

The follicular fluid was aspirated from follicles visible on the surface of the ovaries (2-8 mm diameter) using 20 gauge disposable needle connected with 10 ml syringe contains 2 ml of phosphate buffer saline (PBS) with pH 7.3 [15]. The follicular fluid were put Slowly in petri dish and Leave it preserved quiet for 5 minutes to allow oocytes to settle as sediment on the bottom and oocytes were scanned under sterozoom microscope [16]. The total oocytes and the retrieved rate of oocyte for each ovary were calculated [17].

2.5.Oocytes grading

The oocyte was graded depending on the number of cumulus cells rows surrounding the oocyte , the ooplasm traits and morphology of oocyte. The oocytes were graded as : good oocyte : the oocyte contains more than 3 rows of cumulus cells with homogeneity ooplasm and normal oocyte shape, fair oocyte: contains less than 3 rows of cumulus cells with homogeneity ooplasm and normal oocyte shape and poor oocyte: the oocyte with lost of Cumulus cells (denuded oocyte) [18].

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2.6.Statistical analysis

The analysis of data were carried out by using the statistical analysis system program [19]. The significant differences were performed using the duncan multi range test [20]. The differences in means were considered significantly at ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

The results of table 1 showed that out of a total of 300 ovaries, 110 ovaries bearing CL and with percentage 33.35% and 190 ovaries non - bearing CL and with percentage 63.33%. The length (24.20±2.20 mm) width (16.80 ±2.01mm) and thickness (13.90 ± 1.80mm) were significantly increase ($p \leq 0.05$) in the ovaries having CL compared with the ovaries not having CL. Ordinarily, minimal reproductive performance buffaloes were slaughtered and a high probability of obtaining large number of ovaries non - bearing CL from slaughterhouse during samples collection The same observation was previously reported in buffaloes by [21] found among 296 ovaries were 187 ovaries not having CL (63.18) and 109 ovaries having CL (36.82). Similar results were also demonstrated in buffaloes by [22], [23]. Some Studies were showed that collected of ovaries from slaughterhouse in breeding season (winter) having more CL that those not having CL. The ratio of ovaries that contain CL to the ovaries that do not contain CL were 1-2 and half (1:2.5) Compared to one to three (1:3) [24]. The CL is an extracellular growth on the surface of the ovary, which made the differences in ovarian dimensions. The hepertrophy of luteal cells, enlargement of fibroblasts cells of connective tissues and increase blood supply accord to growth in size and dimensions of the ovaries [25]. Our study results were in agreement with previous studies in buffaloes by [26],[27], [28],[23]and [29] they stated that the length (22.96 ± 1.21mm), width (14.25±1.20 mm)and thickness (12.72 ± 0.05 mm) were significantly increased ($P \leq 0.05$) in ovaries bearing CL compared with the ovaries non-bearing CL.

Table1. Influence of presence or absence of corpus luteum on dimensions of ovaries in local buffaloes.

Attributes	Number of ovaries	Means (S.E.) dimensions of ovaries (mm)		
		Length	Width	Thick ness
Corpus luteum Present ovary	110 (33.35%)	24.20±220 A	16.8±2.01 A	13.90±1.80 A
Corpus luteum absent ovary	190 (64.67%)	20.98±1.96 B	13.25±2.31 B	11.42±1.32 B

A,B. Means denoted within the same column with defferent superscripts are significantly different at ($p \leq 0.05$).

The effects of the presence and absence of CL showed that a significant higher ($p<0.05$) in follicular number per ovary (22.40±12.70) in ovaries non - bearing CL compared to those ovaries hearing CL (18.60±8.70) (Table 2). The average number of follicle per ovary of medium (6.58 ±0.06) and Large (4.35±0.02) follicles found significantly higher ($P<0.05$) numbers in ovaries not having CL compared to the ovaries having CL (Table 2). The presence of CL significantly decrease in the number of ovarian follicles due to that follicle development is limited because the luteal Cells conquer most of the ovarian space [11]. The reason for low number of follicle can be attributed to endocrinological changes[30]. Stated that progesterone hormone produced by the luteal cells of the CL suppressing the releasing of the

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follicle stimulating hormone(FSH) from adenohypophysis (negative feedback) and thus prevents the growth of new follicles and they become atretic, which leads to a lack of retrieval of oocytes. This study demonstrated that the CL significantly reduces the number of follicle per ovary, and this is consistent with previous studies in buffaloes by [23], who found that the number of follicle per ovary from ovaries without CL was (7.84 ± 0.21) than ovaries with CL (4.35 ± 0.31) and [21] and in cattle [31].

Table 2 . Effect of presence or absence of CL on number of follicles in Iraqi buffaloes

Attributes	Average number of follicle per ovary	Means (S.E.) number of follicles		
		Small <3mm	Medium 3-8mm	Large >8mm
Corpus luteum Present ovary (110 ovaries)	18.60±8.70 (2046) B	11.19±1.80 [60.18%] B	4.73±0.04 [25.44%] B	2.68±0.03 [14.38%] B
Corpus luteum Absent ovary (190 ovaries)	22.40±12.20 (4256) A	11.47±0.04 [51.22] A	6.58±0.06 [29.36%] A	4.35±0.02 [19.42%] A

A,B. Means denoted within the same column with defferent superscripts are significantly different at ($p \leq 0.05$).

The results of this study showed that significant increased ($P<0.05$) in the rate number of oocytes for each ovary (4.20±0.12) in ovaries not having CL than the ovaries having CL (2.30± 0.16). The significant higher ($P \leq 0.05$) were observed in good (1.86±0.12) (Fig.1), fair (1.06±0-07) (Fig.2) and poor (1.28 ±0.07) (Fig.3) oocytes in average number of oocyte per ovary in the ovaries non - hearing CL than in rate number of oocyte per each ovary in the ovaries hearing CL(Table 3). The quantity of oocyte per each ovary is lower , when compared to cow, possibly because of the low number of primary follicles in the cortex of ovary and a high number of follicles are doomed to atresia [32], [33]. The ovaries containing the CL causes a decrease in the retrieval of oocytes, this is due to limiting the growth of follicles because the luteal cells grow and spread over most of the ovarian surface [34], [35]. The absence CL as a result of the lack of influence of progesterone hormone, which may lead to the formation better and more suitable follicular fluid for development of oocytes compared with those in ovaries with CL [36],[37]. The rate number of oocyte for each ovary obtained from the ovaries not having CL were significantly increase compared with the ovaries having CL. our study are similar to that found in buffaloes by [10], [38] and [21] and they stated that the CL decreases the follicular number and oocytes quality in buffaloes.

Table 3 :Effect of presence or absence of CL on number on number and grading of oocytes in Iraqi buffaloes

Attributes	Average number of oocyte per ovary	Means (S.E.) oocyte per ovary		
		Good (%)	Fair (%)	Poor (%)
Corpus luteum Present ovary (110 ovaries)	2.30±0.16 (253) B	0.97±0.12 [42.28 %] B	0.65±0.11 [28.32%] B	0.68±0.13 [29.40%] B
Corpus luteum Absent ovary (190 ovaries)	4.20±0.12 (798) A	1.86±0.12 [44.28 %] A	1.06±0.07 [25.29 %] A	1.28±0.03 [30.43 %] A

A,B. Means denoted within the same column with defferent superscripts are significantly different at ($p \leq 0.05$).

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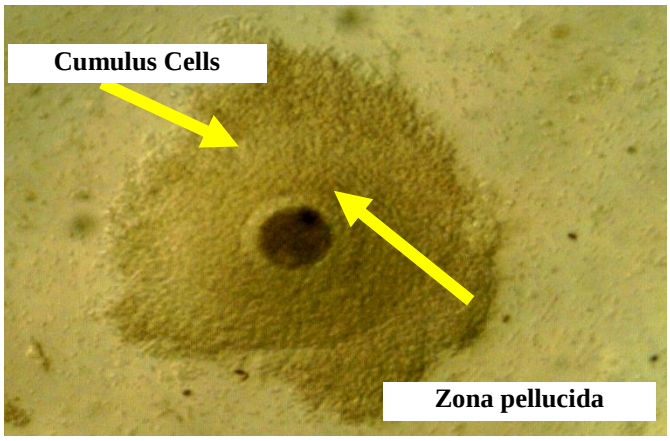

Figure1. Good oocyte with more than 3 layers of cumulus cells

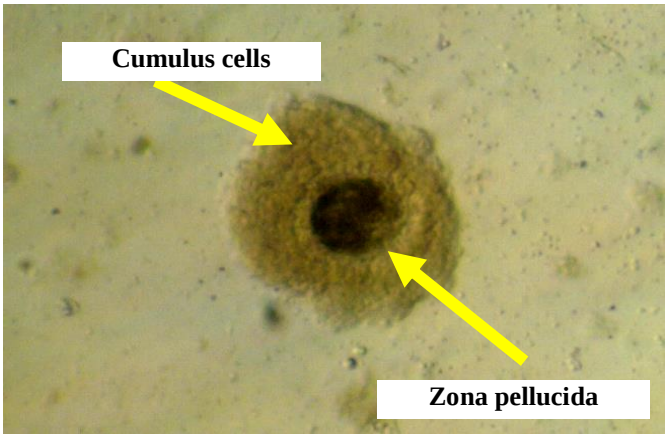

Figure 2 . Fair oocyte with less than 3 layers of cumulus cells

Figure 3. Poor oocyte without cumulus cells (denuded oocyte)

4. CONCLUSIONS

In conclusion, based on these data we can infer that the length, width and thickness of the ovaries bearing CL were significantly higher ($P \leq 0.05$) compared with those ovaries non-bearing CL. The ovaries without CL included a larger number of obtainable good follicles and good quality of oocytes Compared to those obtained from ovaries with CL.

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ISSN: 2789-6773

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