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Effect of corpus luteum on the number and classification of follicles and Oocytes in the ovaries of local ewes (*Ovis aries*)

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Abstract. *The aim of current study to estimate effect of corpus luteum (CL) on the number and classification of follicles and oocytes in the ovaries of local ewes (Ovis aries) during September 2021 to February 2022. The genital organs of 524 for adult non-gravid ewes slaughtered in the slaughterhouse in Al-Najaf government were collected and transferred to the laboratory within two hours. The ovaries (n=1048) were removed and divided in to the ovaries bearing CL (n=166) and without CL (n=882). Follicles were calculated, measured and categorized in to three categories, small (<3mm), medium (3-5mm), and large (>5mm). The oocytes were collected, examined, calculated, evaluated and graded into three degrees depending on the number of cumulus cells layers, homogenous of cytoplasm and oocyte morphology. The results showed that the number of follicles (n=4422) and the average number of follicles per ovary (5.01±0.04) were significantly (p<0.01) increased in the ovaries without CL when than the ovaries with CL.A Significantly (P<0.01) higher the number of follicles for all follicle sizes in the ovaries non-bearing CL compared to those bearing CL. A greater number of oocytes (n=2771) and the average number of oocytes per ovary (3.14±0.06) were found in non-bearing CL ovaries than those bearing CL ovaries (n=406 and 2.44±0.04 respectively). The number of first and second degrees of oocytes were significantly (p<0.01) increased in the ovaries without CL than those with CL. Thus on the basis of our results it can be concluded that the presence of CL have negatively and harmful effect on the number of follicles and oocytes and oocytes quality.*

Keywords: *Ovary, corpus luteum, follicles, follicular fluid, Oocytes*

1. INTRODUCTION

Reproductive and Sheep breeding are an important part of the national economy as it is inexpensive to raise and has the ability to transform the lowest to highest valuable foodstuffs, also considered as an important source of meat, wool and leather and is characterized by its ability to offered the sever environmental conditions [1] .For a purpose to improve the productive and reproductive qualities owned by sheep in terms of quantity and quality, breeding and reproduction means must be promoted through the application of assisted reproductive techniques. (ART), Which includes oocytes collection, maturation and fertilization in vitro [2]. For applying these techniques, it is necessary to obtain oocytes at low prices and

at the lowest costs, and the ovaries obtained from slaughterhouses are an abundant and important source for collecting these oocytes [3].

The corpus luteum (CL) acts permanently which provides endocrine conditions that are necessary and sufficient for the establishment and survival of pregnancy [4]. The (CL) is formed from the remains of the follicle after ovulation and with the help of the luteinizing hormone [5]. Many studies have shown the difference in the number and quality of oocytes with the presence and absence of the CL in the ovary, as the absence of the CL led to an increase in the number and quality of oocytes, while the presence of it will decrease the number and quality of oocytes [6], [7].Follicular fluid contents are similar to plasma with some differences which are vital for ovarian functions such as follicle growth, oocytes maturation, ovulation and oocytes transport through the oviduct [8]. Investigation and analysis of follicular fluid contents and the size of the follicles gives a clear picture of the important and necessary needs during the laboratory maturation and fertilization of the oocytes [9]. growing of oocytes and maturation with biochemical conditions that change with the growth and development of the follicle, and these substances have a modulating effect on the development of the follicle and oocytes [10]. The morphological characteristics of the oocytes, including cumulus cells, and ooplasm state and Zona pellucida, give the ability to classify the oocytes as good, medium and poor [11] Based on the above information, the aim of this study is to estimate effect of CL on the number and classification of follicles and oocytes, in ovaries of local ewes (ovis aries).

2. MATERIALS AND METHODS

2.1. Place of study

This study was completed in reproductive physiology and artificial insemination laboratory, Technical Animal production department, Al-Musaib Technical College, Al- Furat Al-wast Technical University (AUT), for the period from September 2021 to February2022.

2.2. Collection of genital organs

Genital organs (n=524) seemingly normal adult ewes of obscure reproductive history slaughtered at Al-Najaf abattoir were collected instantly after slaughtering. Genital organs were transferred to the laboratory in a thermos flask containing 0.9% physiological saline genitive with penicillin and streptomycin as antibiotic at 25-30°C temperature within 2 hours. The genital organs were collected, on rate 20 organs weekly (Fig. 1. A & B).

2.3. Removed and processing of ovaries

The ovaries (n=1048) were removed from ovarian ligament, and cleaned from suspended tissues and ligament by surgical blade. The ovaries were washed twice with cooled physiological saline and dried by placing them on filter paper. The ovaries were then divided into ovaries bearing CL and without CL (Fig. 1. C & D).

2.4. Measurement and classification of follicles

The diameters of the follicles for each ovary were measured by using Vernier clippers. The follicles were classified in to three categories, small with diameter less than 3mm (<3mm), medium with diameter from 3 to 5mm (3-5mm), and large with diameter more than 5mm (>5mm). The follicles were calculated and recorded [7] (Fig. 1. E).

2.5. Follicular fluid processing

The follicular fluid was aspirated from the visible follicles (2-6mm) on the surface of the ovary using a 18-20 gauge needle attached to a disposable plastic syringe of 1,2 and 5ml containing a phosphate buffer saline with heparin. The follicular fluid obtained from different sized follicles were transferred slowly into a 90mm Petri dish obviading harm to oocytes. Samples were left for 10 minutes in the petri dish to allow the oocytes to settle to the button (Fig. 1. F).

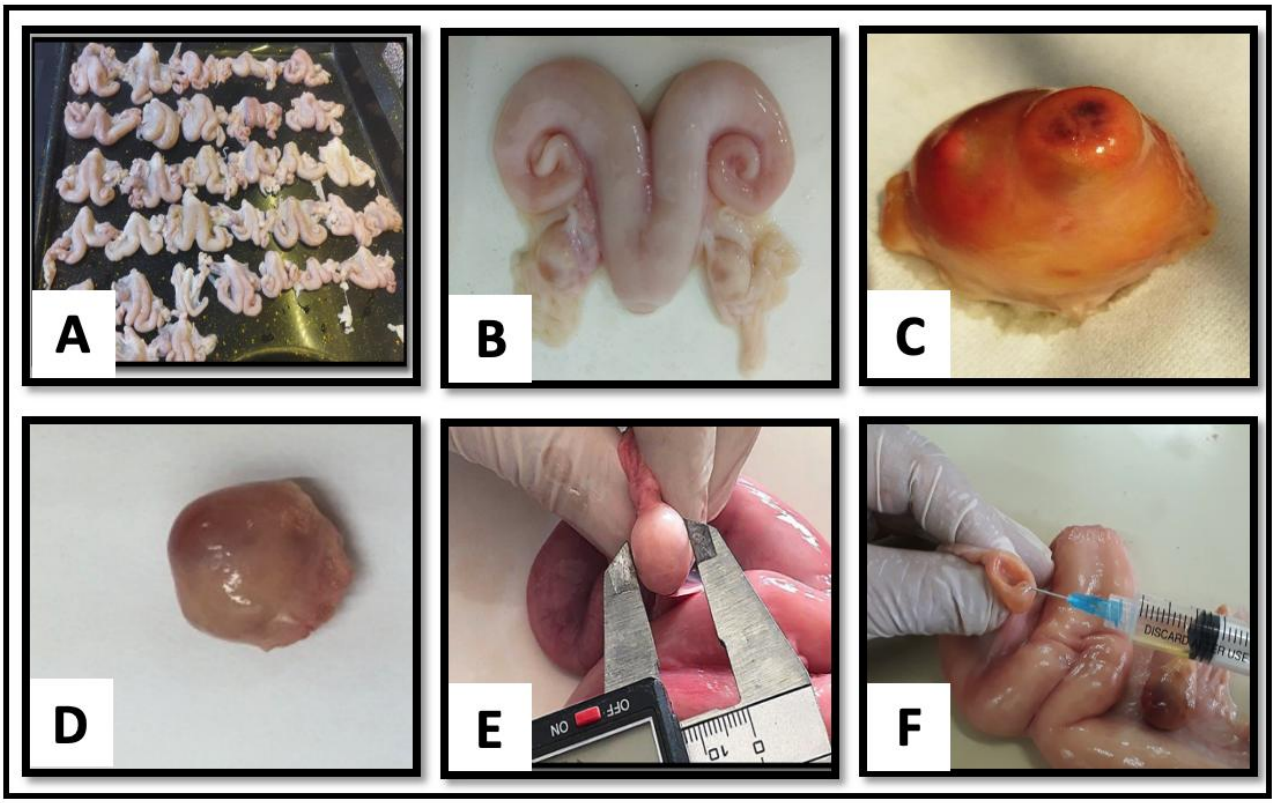

Figure 2. A) Genital organs B) Genital organ C) Ovary with CL D) Ovary without CL E) measuring the diameter of follicle F) Aspiration of follicular fluid

2.6. Oocytes grading

After deposition and settling of the oocytes to the buttom, the oocytes were searched for using microscope with magnification of 40x. The oocytes were photographed using a camera attached to the microscope and connected to the computer. The number of oocytes for each ovary were calculated and recorded. The number of layers of cumulus cells, the homogeneity of the cytoplasm, and the shape of the oocytes were recorded. The oocytes were graded depending on these characteristic. The oocytes were then divided into four digress according to [12] and as follows:

- First degree: The oocyte is surrounded by more than 4 larges of compact cumulus cells with smooth and granular cytoplasm and normal shape of oocyte (Fig. 2. a).
- Second degree: The oocyte is surrounded by at least 2-4 layers of compact cumulus cells with smooth and granular cytoplasm and normal shape of oocyte. (Fig. 2. b).
- Third degree: The oocyte is denuded or incomplete layers of cumulus cells with black and incomplete granular cytoplasm. (Fig. 2. c).

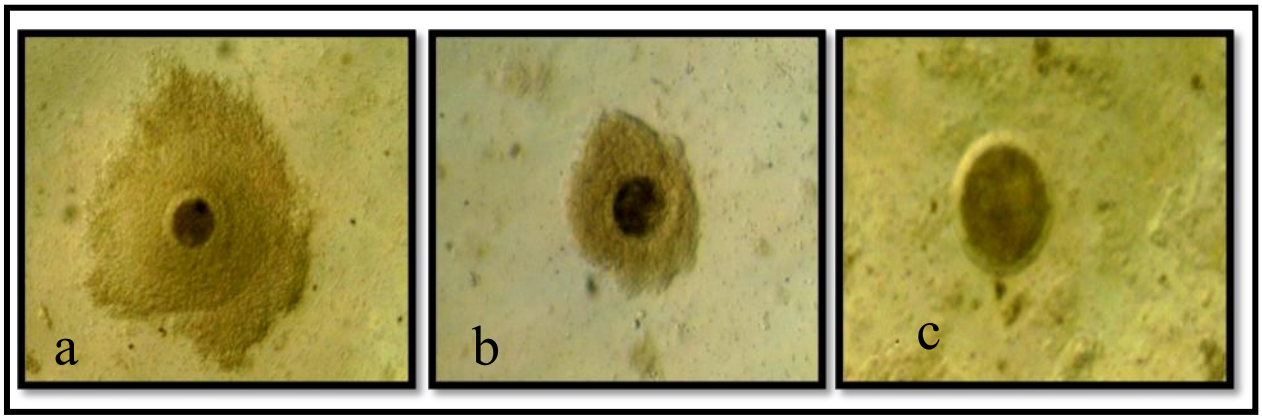

Figure 2. a) oocyte first degree b) oocyte second degree c) oocyte third degree

2.7. Statistical analysis

Data were analyzed using Statistical Analysis System [13] to study significant of the differences in the average values (Mean± S.E.) according to completely randomized design (CRD). The significant differences were performed using Duncan's multiple range test [14].

3. RESULT AND DISCUSSION

3.1. The follicles

Data offered in Table 1 showed a highly significant ($p< 0.01$) increased of the number of follicles (n= 4422) and the average number of follicles per ovary (5.01±0.04 follicle/ovary) for the ovaries without CL compared with the number of follicles, (n=721) and average number of follicles per ovary (4.34±0.02).The table indicated a significantly ($p< 0.01$) higher number of follicles and average number of follicles per ovary for small, medium and large follicles of the ovaries without CL than those of CL bearing ovaries.

This significant increase of the ovarian follicles that without CL compared with ovarian bearing CL, perhaps due to hormonal changes. As the progesterone hormone, which is produced by the CL inhibits the follicle stimulating hormone (FSH), which is secreted from anterior lobe of pituitary gland and is responsible for the growth and development of the follicles, through negative feedback, this preventing the growth new follicles and decay of the follicles on the ovaries[15], or might also be due to that the progesterone hormone inhibits the lutinizing hormone (LH),which work on the continuity of the growth of the follicle and its arrival to stage of ovulation [16].

The results of our study agree in high number of follicles per ovary for ovaries without CL than those bearing CL with [7] study on Bengali ewes, as the showed that the average number of follicles per ovary for ovaries without CL was 3.30±0.09, which with CL was 2.50±0.13, and with [17] study on Egyptian ewes, they found that the average number of follicles per ovary for ovaries without CL was 5.00±0.97, while was 3.00±0.08 for ovaries bearing CL, and with [18] study on indian ewes they concluded that the

number of follicles per ovary for ovaries without CL was 4.06±0.08, whereas was 3.40±0.06 for ovaries bearing CL.

The results of our study differ from what [19] stated in their study on Bengali ewes, as they stated that the average number of follicles per ovary for ovaries non-bearing CL was 4.78±0.27compared with the ovaries bearing CL was 5.49±0.15, and with the study of [12] on Cameroonian ewes, the showed that there was non-significant difference in the average number of follicles per ovary between the ovaries without CL(8.81±2.98) and the ovaries bearing CL (8.90±3.08).

The difference between the results of our study and results of other studies regarding the effect of the CL on the number of follicles on the ovaries of ewes, may be due to the difference of animal breed, climatic influence, animal age, and number of samples [20].

Table 1. Effect of corpus luteum (CL) on the number and classification of follicles in local ewes (Mean ±S.E.)

Corpus luteum (CL)	No. of follicles	Classification of follicles		
		< 3mm	3-5 mm	>5 mm
Without CL (882 ovaries)	4422 5.01 ±0.04 A	3216 a 3.65 ±0.11 A	1044 b 1.18 ±0.02 A	162 c 0.18 ±0.04 A
With CL (166 ovaries)	721 4.43 ±0.02 B	428 a 2.57 ±0.01 B	260 b 1.56 ±0.02 B	28 c 0.17 ±0.04 B
Values with different small letters in same row differ significantly (p< 0.01) Values with different capital letters in same row differ significantly(p<0.01)				

3.2. The Oocytes

The results in table 2 showed a significantly higher (p< 0.01) number of oocytes (n=2771) and the average number of oocytes per ovary (3.14±0.06) for ovaries without CL than the ovaries bearing CL, Which amounted to 406 oocytes and 2.44±0.04 respectively. The table also indicated that a highly significant (p< 0.01) increase in number (n=1251) and the average number of oocytes per ovary (1.41±0.03) of first degree oocyte for ovaries without CL compared with the ovaries with CL which reached 162 oocytes and 0.97±0.05 respectively.

The decrease in the number of oocytes in the ovaries bearing CL may be due the fact that the growth and development of the follicles are restricted by the luteal cells that occupy a large part of the surface area of the ovary. Also, the CL inhibits the growth of the follicles and increased their degeneration [6].

The absence of CL in the ovaries means that the negative effect of progesterone hormone may be disabled and that the ratio of progesterone to estrogen hormones remains at a balanced level that allow the growth and development of the follicle and oocyte [21], or perhaps when the CL is absent, it creates better conditions for the formation of a follicular fluid that nourishes follicle and the oocyte, and as a result leads to an increase in the number of first degree oocytes and a decrease in third degree oocytes than to the ovaries bearing CL.

The results of our study agree with the study of [17] on Egyptian ewes, as they found that the average number of good oocytes per ovary for the ovaries bearing CL was 0.80±0.17, while in the ovaries without CL was 1.25±0.09, and with [7] study on Bengali ewes, they showed a significant increase in the average number of oocyte per ovary for ovaries without CL than those bearing CL, and with [15] study on Iranian ewes, and with [6] study on indian ewes.

The results of our study differed from results of the [22] study on Indonesian ewes and the [12] study on Cameroonian ewes, as they concluded that there was no significant difference in the number and percentage of good and fair oocytes between the ovaries without CL and the ovaries bearing CL.

Table 2. Effect of corpus luteum on number and gradation of Oocytes in local ewes (Mean ± S.E.)

Corpus luteum (CL)	No. of oocytes	Gradation of oocytes		
		First degree	Second degree	Third degree
Without CL (882 ovaries)	2771 3.14±0.06 A	1251 a 1.41±0.03 A	1022 b 1.02±0.06 A	498 c 0.56±0.03 A
With CL (166 ovaries)	406 2.44 ±0.04 B	162 a 0.97 ±0.05 B	89 c 0.54 ±0.03 B	155 b 0.93 ±0.07 B
Values with different small letters in same row differ significantly ($p< 0.01$) Values with different capital letters in same row differ significantly($p<0.01$)				

4. CONCLUSIONS

The presence of CL in the ovaries negatively effects on the number of follicles and oocytes and oocytes quality. The concentration of the metabolic and hormonal compounds of follicular fluid vary with the change in follicular size and presence or absence of the CL.

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