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# EVALUATION OF CORPORA LUTEA EFFECT ON OVARIAN MORPHOMETRY, FOLLICULAR POPULATION AND BIOCHEMICAL PROFILE IN FOLLICULAR FLUID AND BLOOD OF SLAUGHTERED COWS

MERVAT, S.H. [1] and MARWA EL-ZEFTAWY [2,3]

[1] Theriogenology Department, Faculty of Veterinary Medicine, The New Valley University, New Valley, Egypt
[2] Biochemistry Department, Faculty of Veterinary Medicine, The New Valley University, New Valley, Egypt.
[3] Biological Screening and Preclinical Trial Lab, Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.

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## ABSTRACT

Cows are occupied huge economic importance worldwide. Impaired fertility in cows due to absence of corpus luteum (CL) faces huge challenge. The aim of this study was to determine the effect of CL on ovarian biometry, follicular population, hormonal and metabolic content of serum and follicular fluid (FF) of cows. Blood samples and 48ovarieswerecollected from cows slaughtered at winter season and classified according to the presence or absence of CL into two groups, ovaries with and without CL. The diameter of antral follicles was taken and classified into three subgroups, small, medium and large follicles. FF aspirated from each follicle group. 17β-Estradiol ($E_2$), progesteron ($P_4$), glucose, total proteins, total cholesterol (TC) and nitric oxide (NO) were estimated. Result showed that the ovarian biometry was higher in right than left ovaries without significant difference. However, the total follicular populations and ovarian dimensions and their weights were significantly higher in ovaries with CL. The average number of small and medium follicles was also significantly increased in the ovaries with CL. However, the number of LF was higher in cows without CL compared to cows with CL. Glucose, Total cholesterol (TC), proteins, $E_2$ and $P_4$ concentrations were higher in serum than FF. Serum $E_2$ concentration was higher in LF and significantly reduced in ovaries had CL than without CL. On the other hand, $P_4$ concentration in FF was lower in LF and significantly increased in ovaries with CL. Ovaries had CL showed elevation of FF glucose level; however, TC, protein and NO concentrations were lower than ovaries without CL. Hence, we concluded that CL presence effects on both morphometric and metabolic conditions of cows' ovaries.

***Key words:*** Corpus luteum; Ovarian follicles; Estrogen; Progesteron; Cows.

## INTRODUCTION

Ovaries are primary organ of reproduction which responsible for gametogenesis and steroidogenesis during different stages of estrous cycle and pregnancy. Morphological and biometrical changes which occur in ovaries are related to number and size of developing follicles and developmental stages of the corpus luteum (CL) during estrus cycle, pregnancy, puerperium and lactation (Miranda-Moura *et al*., 2010).

CL is a temporary endocrine gland formed after ovulation of the ovulatory *Graffian* follicle and it is essential to regulate the estrous cycle and pregnancy maintenance (Tomac *et al*., 2011). During different stages of the estrous cycle and pregnancy, CL has several variations in size, structure and steroidogenic activities (Fields and Fields, 1996). CL has a hormonal secretory function such as $P_4$, PG, $E_2$, relaxin, oxytocin, vasopressin and inhibin secretion (Fields, 1991). $P_4$is the principal steroid hormone necessary for establishing of pregnancy in domestic mammals (Tomac *et al*., 2011). It also suppresses the secretion of the gonadotrophins which prevent behavioral estrous activity (Powell *et al.,* 2006; Shabankareh *et al.,* 2015).

Follicular fluid is a vascular compartment inside the mammalian ovary, separated from the perifollicular stroma by follicular wall which constitutes a blood-follicle barrier (Abd-Ellah *et al*., 2010; Albomohsen *et al*., 2011). FF contains locally produced substances related to the follicular cells metabolic activity and steroid hormonesas $E_2$, $P_4$, and testosterone (Blaszczyk *et al.,* 2006). These steroid hormones and metabolites are an important factor which affects oocyte maturation and early embryo development (Bender *et al*., 2010).

*Corresponding author:* Mervat, S.H.
*E-mail address:* mervat.vet@yahoo.com
*Present address:* Theriogenology Department, Faculty of Veterinary Medicine, The New Valley University, New Valley, Egypt

The aim of the current study was to evaluate the effect of CL on i: ovarian morphometry and its follicular population and ii: $E_2$ and $P_4$, glucose, TC, protein, and NO concentrations in both serum and FF from small, medium and large-sized follicles in cows.

## MATERIALS AND METHODS

### 1. Animals protocol and sample preparation

Forty-eight adult non-pregnant cows aged (5-7 years), were obtained from Bani Adi and Moesha Abattoirs, Assiut, Egypt. They were in good health condition and without any reproductive disorders.

Blood samples were collected from jugular vein of cows before slaughtering. One of blood sample was collected in sodium fluoride and potassium oxlate tube for blood glucose estimation and other sample collected in Wisterman's tubes for other parameters determination. Blood samples centrifuged at 3000 rpm for 10 min. The obtained plasma and serum were kept at -20°C till the time of biochemical analysis.

After animal slaughtered ovaries were exised immediately and washed with ice-cold saline, wrapped in plastic sheets, placed in an icebox, and transported to the laboratory within 30 minutes after slaughtered. Ovaries associated with pregnant cows and those with any pathological lesions were not included in the study. The obtained ovaries were classified according to the presence or absence of CL. Each ovaries group was transferred into two sterile separate glass beakers.

The length (from pole to pole), width (from side to side) and thickness (from attached portion or hillus to the free surface) were measured by Vernier Caliper to the nearest 0.1 mm. Also, all visible antral follicles on the ovarian surface in each group were counted, with classified according to their diameters (Driancourt *et al.*, 1991) into small follicles (SF) (<5 mm), medium follicles (MF) (5-8 mm) and large follicles (LF) (>8 mm) (Fig.1). The weight of each intact ovary was taken to the nearest 0.01 g using an electric balance (ACCULAB-V-1mg). After that, FF was aspirated from each follicle group separately using 1 ml disposable Primo syringe to the nearest 0.01 ml. The collected FF added in Eppindorf tube, centrifuged at 3000 rpm for 10 min. and stored at -20°C till the time of biochemical analysis.

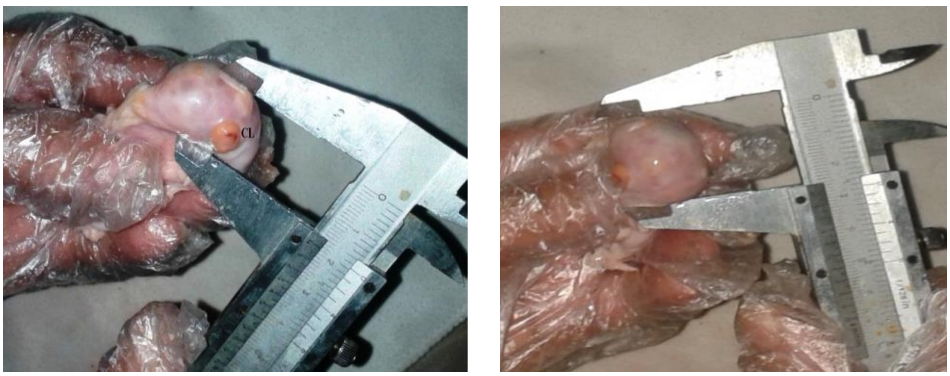


**Fig. 1:** Measuring ovarian morphometry by Vernier Calipers

### 2. Materials

ELISA kits of $P_4$ and $E_2$ were purchased from Biovision (USA). Kits of glucose, protein, TC, was obtained from Biomed (Egypt). Sulphanilamide, N-(1-naphthyl) ethylenediamine, sodium nitrite, and phosphoric acid were HPLC-grade and brought from Merck (USA).

### 3. Biochemical estimations

#### 3.1. Estimation of glucose concentration in plasma and FF

Glucose concentration was estimated by commercially available glucose assay kit that is dependent on glucose oxidase-peroxidase method (Trinder, 1969).

#### 3.2. Estimation of Total cholesterol (TC) concentration in serum and FF

TC was determined by cholesterol oxidase peroxidase (CHOD-PAP) test (Flegg, 1973).

#### 3.3. Determination of protein concentration in serum and FF

Total protein content of all assay samples (serum and FF) was estimated spectrophotometrically using commercially available kit. These values were expressed as g/dl (Wittand Trendelenburg, 1982).

#### 3.4. Determination of oxidative stress marker in serum and FF

The level of Nitric oxide (NO) was determined by using *Griess* reagent in both serum and FF. The reddish-purple azo-dye product was measured spectrophotometrically at 540 nm (Menaka *et al*., 2009).

### 4. Sandwich ELISA

### 4.1. Determination of $P_4$ concentration in serum and FF

$P_4$ was determined by ELISA method in both serum and FF according to manufacture of instruction. This assay employs the Quantitative Sandwich Enzyme Immunoassay technique. A monoclonal antibody for $P_4$ that has been pre-coated on to a microplate. $P_4$ in the sample competes with a progesterone enzyme conjugate for binding sites. Unbound $P_4$ and progesterone enzyme conjugate is washed off by wash buffer. After substrate addition, the intensity of the color is inversely proportional to the concentration of $P_4$ in the samples. A standard curve was constructed by using standard $P_4$ and the concentration of unknown samples was calculated from the standard curve. The intra- and inter-assay coefficients of variation (CVs) for $P_4$ were <10.2%.

### 4.2. Determination of $E_2$ concentration in serum and FF

$E_2$ was determined by ELISA method in serum and FF according to the instruction of manufacture. The procedure depends on Sandwich Enzyme-linked Immune-sorbent assay technology. Anti-$E_2$antibody was pre-coated on to 96-well plates and the horseraddish peroxidaseconjugated anti-$E_2$antibody was used as detection antibodies. Formation of yellow color at the end of the reaction is an indicator of enzymatic reaction occurrence. The concentration of $E_2$ was determined at 450 nm by ELISA reader. A standard curve was created by using standard $E_2$ and the concentrations of unknown samples were calculated from the standard curve. The intra- and inter-assay coefficients of variation (CVs) for $E_2$were < 4.6% and< 6.2%, respectively.

### 5. Statistical analysis

Data were analyzed using software package (SAS Institute Inc. 2000). Significance of means ± SE was detected by using Duncan's Multiple Range Test (Duncan, 1955), p≤ (0.05-0.001).

## RESULTS

### 1. Effect of CL on morphometry of ovary in cows

Dimensions of right ovaries were non-significant higher compared to left ovaries (P< 0.05) (Table 1). However, length, width and thickness of ovaries with CL were increased by 18.8%, 7.7%, and 10.7% respectively compared to ovaries without CL (Table 2). Macroscopic examination of cow ovaries showed that their shape were oval and highly changed and distorted with the presence of CL. Further, the ovarian activity of right ovaries was more significant than left ovaries (P < 0.05). The ratio between CL to ovarian weight was (58.48%; P < 0.01) (Figs. 2 and 3).

**Table 1:** Ovarian dimensions of right and left in cows (Mean ± SE).

| Ovarian parameters | Left ovary (n = 48) | Right ovary (n = 48) |
|---|---|---|
| Length (cm) | 2.44 ± 0.64[a] | 2.67 ± 0.55[a] |
| Width (cm) | 1.59 ± 0.25[a] | 1.58 ± 0.26[a] |
| Thickness (cm) | 2.04 ± 0.60[a] | 2.05 ± 0.43[a] |
| Weight (g) | 3.80 ± 0.79[a] | 4.15 ± 1.12[a] |

Values represent the mean ± SE. Data with similar superscripts in the row for same follicles are non-significant (p <0.05).

**Table 2:** Morphometry of ovaries with and without CL in cows (Mean± SE).

| Ovarian parameters | Ovaries with CL (n= 27) | Ovaries without CL (n= 69) |
|---|---|---|
| Length (cm) | 2.85 ± 0.60[a] | 2.40 ± 0.55[b] |
| Width (cm) | 1.67 ± 0.24[a] | 1.55 ± 0.25[b] |
| Thickness (cm) | 2.18 ± 0.39[a] | 1.97 ± 0.56[a] |

Values represent the mean ± SE. Data with different superscripts in the row for same follicles size are significant (p < 0.05).

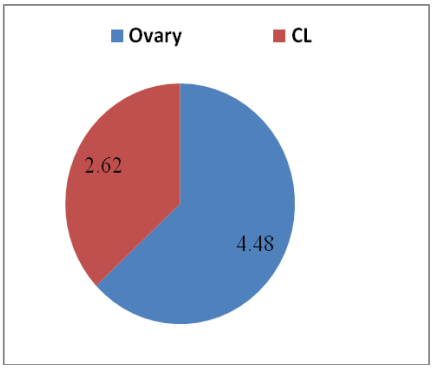


**Fig. 2:** Weight of ovaries and CL in examinedcows

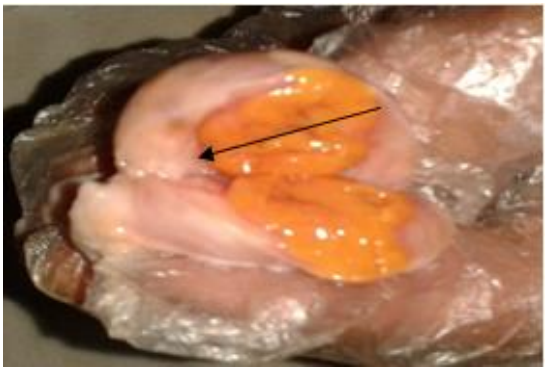

**Fig. 3.** Cross-section in CL of examined cows

### 3.3. Effect of CL on follicular populations:

The total follicular population of right ovary was increased by 7.5% than left ovary. Number of SF, MF, and LF in right ovary was significantly ($p < 0.05$) increased 0.2, 0.9 and 1.1-folds compared to the left ovary (Table 3). Marked significant ($p < 0.01$) increase of the number of SF and MF (by 20.3%, and 33.9%, respectively) of cows with CL compared to cows without CL. However, the number of LF was higher by 61% in cows without CL compared to cows with CL (Table 4).

**Table 3:** Follicular populations of right and left ovaries in cows (Mean ± SE).

| Follicular populations | Right ovaries (n= 48) | Left ovaries (n= 48) |
|---|---|---|
| SF (<5 mm) | 8.03 ± 2.79[a] | 6.97 ± 2.65[b] |
| MF (5-8 mm) | 1.68 ± 0.85[a] | 0.90 ± 0.58[b] |
| LF (>8 mm) | 0.74 ± 0.60[a] | 0.35 ± 0.47[b] |
| Total number of follicles | 3.74 ± 1.38 | 3.48 ±1.23 |

Values represent the mean ± SE. Data with different superscripts in the row for same follicles size are significant ($p < 0.05$).

**Table 4:** Follicular populations of ovaries with and without CL in cows (Mean ± SE).

| Follicular population | Ovaries with CL(n=27) | Ovaries without CL (n= 69) |
|---|---|---|
| SF (<5 mm) | 8.19 ± 2.46[a] | 6.81 ± 2.19[b] |
| MF (5-8 mm) | 1.46 ± 0.95[a] | 1.09 ± 0.64[a] |
| LF (>8 mm) | 0.66 ± 0.56[a] | 0.41 ± 0.55[a] |
| Total | 3.35±1.32 | 2.85±1.12 |

Values represent the mean ± SE. Data with different superscripts in the row for same follicles size are significant ($p < 0.01$).

### 3.3. Effect of CL on $P_4$ and $E_2$ concentrations in serum and FF of cows:

Serum $P_4$ concentration increased by 37.2% in cows with CL compared to cows without CL. Also, $P_4$ level in FF obtained from SF, MF and LF of cows with CL were elevated by 13.8%, 8.4%, and 8.6% respectively compared to cows without CL. Moreover, $P_4$ concentration decreased with increasing size of follicles. However, $E_2$ concentration increased with the increased size of the follicle. Also, $E_2$ concentration was decreased by 15%, 20.7%, 33.1% and 43.2% in serum and FF of SF, MF and LF of cows with CL compared to cows without CL (Table 5).

**Table 5:** Hormonal concentrations ($P_4$ and $E_{2)}$ in serum and FF of ovaries with and without CL in cows (Mean ± SE).

| Hormones | Ovaries status | Serum | Follicular Fluid | | |
|---|---|---|---|---|---|
| | | | SF (<5 mm) | MF (5-8 mm) | LF (>8 mm) |
| **$P_4$ (ng/ml)** | With CL | 1.29 ± 0.26[a] | 0.99 ± 0.10[a] | 0.90 ± 0.09[a] | 0.88 ± 0.07[a] |
| | Without CL | 0.94 ± 0.22[a] | 0.87 ± 0.09[b] | 0.83 ± 0.10[b] | 0.81 ± 0.14[b] |
| **$E_2$ (ng/ml)** | With CL | 1.75 ± 0.36[a] | 0.92 ± 0.09[a] | 0.93 ± 0.05[a] | 0.96 ± 0.09[a] |
| | Without CL | 2.06 ± 0.13[b] | 1.16 ± 0.11[b] | 1.39 ± 0.17[b] | 1.69 ± 0.12[b] |

Values represent the mean ± SE (n=13). Data with different superscripts in the same column are significant ($p < 0.001$).

### 3.4. Effect of CL on glucose, TC, protein and NO levels in plasma and/or serum and FF of cows:

Plasma glucose was elevated 0.9-fold in case of presence of CL. Moreover, in the case of CL presence glucose level in FF of SF, MF and LF of cows showed 0.4, 0.3 and 0.4-folds increasing than in case of absence of CL (Table 6).

However, TC showed significant ($P<0.001$) decreased in serum and FF of SF, MF and LF of cows with CL compared to cows without CL by 13.6%, 1%, 9.7%, and 11.7% respectively. Also reduction of total protein concentration 1.8%, 16.5%, 17.5% and 11.2% in serum and FF of SF, MF and LF of cows with CL compared to cows without CL was noticed. NO concentration showed a marked reduction in cows has CL than those without CL in serum and FF of SF, MF and LF by 11.5%, 13.9%, 11.6% and 12.1% respectively (Table 6).

**Table 6:** Metabolites concentrations in serum and FF of ovaries with and without CL in cows (Mean ± SE).

| Metabolites | Ovaries status | Plasma and/or serum | SF (<5 mm) | MF (5-8 mm) | LF (>8 mm) |
|---|---|---|---|---|---|
| **Glucose (mg/dl)** | With CL | 78.16 ± 10.45[a] | 36.85 ± 5.51[a] | 42.15 ± 9.65[a] | 47.93 ± 11.58[a] |
| | Without CL | 40.39 ± 11.39[b] | 25.98 ± 7.25[b] | 31.29 ± 5.33[b] | 34.49 ± 8.21[b] |
| **TC (mg/dl)** | With CL | 112.96 ± 9.44[a] | 103.65 ± 7.93[a] | 105.47 ± 9.11[a] | 108.23 ± 8.63[a] |
| | Without CL | 130.73 ± 5.34[b] | 104.72 ± 7.48[a] | 116.76 ± 9.79[b] | 122.55 ± 9.12[b] |
| **Total protein (g/dl)** | With CL | 7.55 ± 1.12[a] | 6.06 ± 0.79[a] | 6.11 ± 0.66[a] | 6.63 ± 0.62[a] |
| | Without CL | 7.69 ± 0.94[a] | 7.26 ± 0.65[b] | 7.41 ± 0.74[b] | 7.47 ± 0.90[b] |
| **NO (nm)** | With CL | 0.46 ± 0.09[a] | 0.62 ± 0.08[a] | 0.61 ± 0.10[a] | 0.58 ± 0.09[a] |
| | Without CL | 0.52 ± 0.14[a] | 0.72 ± 0.11[b] | 0.69 ± 0.11[b] | 0.66 ± 0.10[b] |

Values represent the mean ± SE (n=13). Data with different superscripts in the same column are significant ($p < 0.001$).

## DISCUSSION

In recent years reproductive disorders occupied large importance; one of the main causes contribute to the reduction of fertility in cow is low $P_4$ concentration in blood which secreted by CL. In the current study ovarian morphometry parameters were highly affected with the presence of CL which represented more than half of ovarian tissue in cows. Previous studies were done on buffaloes and showed similar results (Khandoker *et al*., 2011; Leal *et al*., 2013). Also, the present results came in accordance with previous works (S.H. Mervat, 2016; Bhajoni *et al*., 2018). Higher activity of right ovary more than left ovary in cows is attributed to the presence of the variations in the interior structures of the ovaries and

presence of CL, rather than follicles which are permanently found in the ovaries even during the early postnatal life (McEntee 1990, S.H. Mervat, 2007). It was reported that the activity of right ovary in sheep (Alsafy and EL-shahat, 2011) and cow (Rind *et al*., 1999) was more than left ovary.

The present data showed that the number of SF and MF was higher in the ovaries with CL than ovaries without CL. However, the number of LF was higher in the ovaries without CL as compared to ovaries with CL. These finding agreed with that reported in buffaloes (Acar *et al.*, 2013), however disagreed with study done in cattle which showed number of MF was significantly higher in ovary without CL as compared to that with CL (Bhajoni *et al*., 2018).

Contreras-Solis *et al. (*2008) Cited that the presence of CL affects ovarian follicular dynamics in both ovaries due to secretion of $P_4$ from CL which suppress LH pulse frequency. LH is essential for continued growth and development of LF, subsequently inhibits follicular growth (Bartlewski *et al.*, 2001).

Additionally, results of the current study showed an elevation of $E_2$ in both serum and FF of the cow without CL, these results were similar with obtained by Kor and Moradi (2013) Who found that elevation of $E_2$ in FF of the cow without CL was due to secretion of follicular androgen by granulosa cells which results in elevation of $E_2$ production and these findings were similar to previous studies (Kor *et al*., 2013; El-Moghazy *et al*., 2017).

TC and total protein concentration was elevated in serum more than FF and also increased with increasing size of follicles; these finding agreed with (Kor *et al*., 2013, Kumar *et al*., 2015). Moreover, TC and total protein in FF was reduced in cows with CL compared to cows without CL as a result of low $E_2$ level. These results were similar in buffaloes (Abd-Ellah *et al*., 2010) and sheep (Asgharimoghadam *et al*., 2015), due to a high concentration of serum $E_2$ which affect the pituitary-thyroid-adrenal axis, so serum TC was increased (Fillios and Mann, 1956). Further, $E_2$ has a direct stimulatory effect on the liver which is the main source of all plasma proteins so reduction of $E_2$ directly effects on total protein concentration in both serum and FF (Ishwar and Pandey, 1994).

On the other hand, $P_4$ concentration of both serum and FF was higher in ovaries with CL compared to ovaries without CL which attributed to secretion of $P_4$ in high concentration from both granulosa and theca cells of CL (Hunter *et al*., 2004). The current results were agreed with previous studies (Nasroallah., 2014).

Study of glucose concentration in both plasma and FF had highly significant importance. It is known that glucose is the main source of energy for all animal body and increasing the level of glucose lead to multiple metabolic disorders. The present data showed an elevation of glucose in plasma than FF, also, glucose concentration was increased with increasing the follicle size due to reduce the rate of glucose metabolism in larger follicles as compared with smaller ones, resulting in lower consumption of glucose from fluid of large follicles (Leroy *et al*., 2004). Moreover, with follicular growth, increased volume of FF and subsequently, increased permeability of the blood follicle barrier causing higher glucose levels in large follicles (Gosden *et al.*, 1988).

Also, glucose level of plasma and FF of cows with CL were higher compared to cows without CL, due to secretion of $P_4$ from CL which cause the change in body composition and higher levels of glucose (Moonmanee and Yammuen-arta2015). Current findings came in accordance with (Kumar *et al*., 2015).

Finally to evaluate the effect of CL presence on oxidative stress markers, NO determination was chosen in current study. NO is highly reactive inorganic free radical produced by many cells in the animals. Reduction of NO in both serum and FF was influenced and correlated with both $E_2$and $P_4$ concentrations (Sagar *et al*., 2012). Out of the present study, NO concentration was decreased in cows with CL than those without CL. $E_2$ induces the generation of NO in ovaries and elevation of NO levels during the follicular phase of the cycle, which essentials for follicular development, steroidogenesis, ovulation, and luteolysis, therefore $E_2$ reduction affects NO production (Bulbul *et al*. 2008). On the other hand increase, $P_4$ production by CL has an inhibitory effect of NO production (Sharma *et al*., 2016). Current results were in agreement with (Faes *et al*., 2007).

## CONCLUSION

From this study, we concluded that corpus luteum has a great effect on ovarian morphometry and its follicular populations of different size follicles. Follicular fluid content was highly affected with the presence of corpus luteum during the different growth stage of the follicle and these contents were highly related with hormones and metabolites which affecting oocyte quality. This study can be helpful in follicular dynamics, the collection of superior oocyte quality and *in vitro* embryo production.

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# تقييم تأثير وجود الجسم الأصفر علي المقاييس الحيوية للمبيض وتعداد الجريبات والمحتوي البيوكيميائي في السيرم والسائل الجريبي في الأبقار المذبوحة

***مرفت سيد حسن ، مروه الزفتاوي***

Email: mervat.vet@yahoo.com Assiut University web-site: www.aun.edu.eg

أجريت هذه الدراسة لتقيم تأثير الجسم الأصفر علي قياسات المبيض وعدد الجريبات ومستوي هرمون الإستروجين والبروجسترون ودراسة تغير مستويات الجلوكوز والكولسترول والبروتينات ومضادات الأكسدة في السائل الجريبي والمصل في الأبقار. تم استخدام ٤٨ بقرة مذبوحة وتم تقسيمها علي حسب وجود الجسم الأصفر علي المبيض إلي مجموعتين، مبايض تحتوي علي الجسم الأصفر ومبايض خالية من الجسم الأصفر. وتم قياس الجريبات وقسمت الي ثلاثة مجاميع علي حسب قياسها بالفرجار، حويصلات صغيرة ومتوسطة وكبيرة وتم شفط السائل الجريبي من كل حويصلة. أجريت الدراسات البيوكيميائية بقياس مستوي هرموني الإستروجين والبروجسترون وقياس معدل الجلوكوز والكولسترول والبروتينات وأكسيد النيتريك في كل من السائل الجريبي والدم. أظهرت النتائج أن المقاسات المختلفة للمبايض وأوزانها والعدد الكلي للجريبات أكبر معنويا في المبايض ذات الجسم الأصفر عن المبايض الخالية من الجسم الأصفر. وعدد الجريبات الكبيرة اكبر في المبايض خالية من الجسم الأصفر. وأثبتت نتائج التحاليل الكيميائية ارتفاع مستوي الاستروجين والبروجسترون ومعدلات الجلوكوز والكولسترول والبروتينات في السيرم/البلازما عن السائل الجريبي. وبمقارنة تركيز الهرمونات بحجم الحويصلات فقد لوحظ أن مستوي الإستروجين يزداد بزيادة حجم الجريبات بينما مستوي البروجستيرون يقل بزيادة حجم الحويصلات. وبمقارنة تركيز هرموني الإستروجين والبروجسترون في المبايض التي تحتوي علي الجسم الأصفر والمبايض الخالية من الجسم الأصفر فقد أثبتت النتائج أن تركيز الإستروجين أعلي في المبايض ذات الجسم الأصفر عن المبايض الخالية من الجسم الأصفر بينما تركيز البروجستيرون أعلي في المبايض الخالية من الجسم الأصفر عن المبايض ذات الجسم الأصفر في كل من السيرم والسائل الجريبي. وأيضا معدل الجلوكوز كان أعلي في المبايض ذات الجسم الأصفر ولكن تركيز الكولسترول والبروتينات وأكسيد النيتريك كان أعلي في المبايض الخالية من الجسم الأصفر.