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-Zeftawy²,³

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

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 48 ovaries were collected 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 (E2), progesteron (P4), 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, E₂ and P₄ concentrations were higher in serum than FF. Serum E₂ concentration was higher in LF and significantly reduced in ovaries had CL than without CL. On the other hand, P₄ concentration in FF was lower in LF and significantly increased in ovaries with CL. Ovaries with CF 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 P₄, PG, E₂, relaxin, oxytocin, vasopressin and inhibit secretion (Fields, 1991). P₄ 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-Elah et al., 2010; Albomohsen et al., 2011). FF contains locally produced substances related to the follicular cells metabolic activity and steroid hormones E₂, P₄, 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).
The aim of the current study was to evaluate the effect of CL on i: ovarian morphometry and its follicular population and ii: E2 and P4, 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 oxalate 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.

2. Materials
ELISA kits of P4 and E2 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 P₄ concentration in serum and FF

P₄ 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 P₄ that has been pre-coated on to a microplate. P₄ in the sample competes with a progesterone enzyme conjugate for binding sites. Unbound P₄ 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 P₄ in the samples. A standard curve was constructed by using standard P₄ and the concentration of unknown samples was calculated from the standard curve. The intra- and inter-assay coefficients of variation (CVs) for P₄ were <10.2%.

4.2. Determination of E₂ concentration in serum and FF

E₂ 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-E₂ antibody was pre-coated on to 96-well plates and the horseradish peroxidase-conjugated anti-E₂ 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 E₂ was determined at 450 nm by ELISA reader. A standard curve was created by using standard E₂ and the concentrations of unknown samples were calculated from the standard curve. The intra- and inter-assay coefficients of variation (CVs) for E₂ 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 ± 0.64ᵃ | 2.67 ± 0.55ᵃ |
| Width (cm)         | 1.59 ± 0.25ᵃ | 1.58 ± 0.26ᵃ |
| Thickness (cm)     | 2.04 ± 0.60ᵃ | 2.05 ± 0.43ᵃ |
| Weight (g)         | 3.80 ± 0.79ᵃ | 4.15 ± 1.12ᵃ |

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 ± 0.60ᵃ | 2.40 ± 0.55ᵇ |
| Width (cm)         | 1.67 ± 0.24ᵃ | 1.55 ± 0.25ᵇ |
| Thickness (cm)     | 2.18 ± 0.39ᵃ | 1.97 ± 0.56ᵃ |

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

<table>
<thead>
<tr>
<th>Follicular populations</th>
<th>Right ovaries (n= 48)</th>
<th>Left ovaries (n= 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF (&lt;5 mm)</td>
<td>8.03 ± 2.79a</td>
<td>6.97 ± 2.65b</td>
</tr>
<tr>
<td>MF (5-8 mm)</td>
<td>1.68 ± 0.85a</td>
<td>0.90 ± 0.58b</td>
</tr>
<tr>
<td>LF (&gt;8 mm)</td>
<td>0.74 ± 0.60a</td>
<td>0.35 ± 0.47b</td>
</tr>
<tr>
<td>Total number of follicles</td>
<td>3.74 ± 1.38</td>
<td>3.48 ±1.23</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Follicular population</th>
<th>Ovaries with CL(n=27)</th>
<th>Ovaries without CL (n= 69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF (&lt;5 mm)</td>
<td>8.19 ± 2.46a</td>
<td>6.81 ± 2.19b</td>
</tr>
<tr>
<td>MF (5-8 mm)</td>
<td>1.46 ± 0.95a</td>
<td>1.09 ± 0.64a</td>
</tr>
<tr>
<td>LF (&gt;8 mm)</td>
<td>0.66 ± 0.56a</td>
<td>0.41 ± 0.55a</td>
</tr>
<tr>
<td>Total</td>
<td>3.35±1.32</td>
<td>2.85±1.12</td>
</tr>
</tbody>
</table>

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 P4 and E2 concentrations in serum and FF of cows:
Serum P4 concentration increased by 37.2% in cows with CL compared to cows without CL. Also, P4 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, P4 concentration decreased with increasing size of follicles. However, E2 concentration increased with the increased size of the follicle. Also, E2 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 (P₄ and E₂) in serum and FF of ovaries with and without CL in cows (Mean ± SE).

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Ovaries status</th>
<th>Serum</th>
<th>Follicular Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SF (&lt;5 mm)</td>
<td>MF (5-8 mm)</td>
</tr>
<tr>
<td>P₄ (ng/ml)</td>
<td>With CL</td>
<td>1.29 ± 0.26ᵃ</td>
<td>0.99 ± 0.10ᵇ</td>
</tr>
<tr>
<td></td>
<td>Without CL</td>
<td>0.94 ± 0.22ᵃ</td>
<td>0.87 ± 0.09ᵇ</td>
</tr>
<tr>
<td>E₂ (ng/ml)</td>
<td>With CL</td>
<td>1.75 ± 0.36ᵃ</td>
<td>0.92 ± 0.09ᵃ</td>
</tr>
<tr>
<td></td>
<td>Without CL</td>
<td>2.06 ± 0.13ᵇ</td>
<td>1.16 ± 0.11ᵇ</td>
</tr>
</tbody>
</table>

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).

DISCUSSION

In recent years reproductive disorders occupied large importance; one of the main causes contribute to the reduction of fertility in cow is low P₄ 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...
The 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 (Alsayf 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 P4 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 E2 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 E2 in FF of the cow without CL was due to secretion of follicular androgen by granulosa cells which results in elevation of E2 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 E2 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 E2 which affect the pituitary-thyroid-adrenal axis, so serum TC was increased (Fillios and Mann, 1956). Further, E2 has a direct stimulatory effect on the liver which is the main source of all plasma proteins so reduction of E2 directly affects on total protein concentration in both serum and FF (Ishwar and Pandey, 1994).

On the other hand, P4 concentration of both serum and FF was higher in ovaries with CL compared to ovaries without CL which attributed to secretion of P4 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 P4 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 E2 and P4 concentrations (Sagar et al., 2012). Out of the present study, NO concentration was decreased in cows with CL than those without CL. E2 induces the generation of NO in ovaries and elevation of NO levels during the follicular phase of the cycle, which essentials for follicular development, steroïdogenesis, ovulation, and luteolysis, therefore E2 reduction affects NO production (Bulbul et al. 2008). On the other hand, P4 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.

ACKNOWLEDGMENTS

This work is financially supported by Biological Screening and Preclinical Trial Lab, Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.
REFERENCES


Kor, N.M. and Moradi, K. (2013): A Review of biochemical metabolites concentration and


Mervat, S.H. (2007): Some biochemical studies on follicular fluid contents of slaughtered buffaloes as related to various reproductive conditions. Master of Veterinary Science (Veterinary Theriogenology) of the Faculty of Veterinary Medicine, Assiut University.

Mervat, S.H. (2016): Biometrical and ultrasonographic studies on the reproductive genitalia of slaughtered and alive Egyptian doe. Ph.D of Veterinary Science (Veterinary Theriogenology) of the Faculty of Veterinary Medicine, Assiut University.


تقييم تأثير وجود الجسم الأصفر على المقاييس الحيوية للمبيض وتمتاز الجريبات واليونتونات 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 لتقييم تأثير الجسم الأصفر على قياسات المبيض وعدد الجريبات ومستوي هرمون الإستروجين والبروجسترون ودراسة تغير مستويات الجلوكوز والكولسترول والبروتينات ومضادات الأكسدة في السائل الجريبي والمصل في الأبقار. تم استخدام 48 بقرة مذبوحة وتم تقسيمها على حسب وجود الجسم الأصفر على المبيض إلى مجموعتين، مبيض تحتوي علي الجسم الأصفر ومبيض خالي من الجسم الأصفر. تم قياس الجريبات ومجمعتها إلى ثلاث مجاميع على حسب قياسها بالفرجار، حويصات صغيرة ومنتوزة كبيرة. تم شفط السائل الجريبي من كل حويصة. أجريت الدراسات البقوليمية بقياس مستوي هرمون الإستروجين والبروجسترون وقياس معدل الجلوكوز والكولسترول والبروتينات وأكسيد النيتراع في كل من السائل الجريبي والمصل. أظهرت النتائج أن المقاييس المختلفة للمبيض وأوزانها وعدد الكلي للجريبات أكبر معنويًا في المبيض ذات الجسم الأصفر عن المبيض الخالي من الجسم الأصفر. وعدد الجريبات الكبيرة أكبر في المبيض ناتج عن الجسم الأصفر. وتبينت نتائج التحاليل الكيميائية ارتفاع مستوي الاستروجين والبروجسترون ومعدلات الجلوكوز والكولسترول والبروتينات في السيرم/البلازما عن السائل الجريبي. وبمقارنة تركيز الهرمونات بحجم الحويصات فقد وجد أن مستوي الاستروجين وزيادة حجم الجريبات بينما مستوي البروجسترون بقل بزيادة حجم الحويصات. وبمقارنة تركيز هرمون الاستروجين والبروجسترون في المبيض الذي تحتوي علي الجسم الأصفر والمبيض الخالي من الجسم الأصفر فقد أثبت النتائج أن تركيز الاستروجين أعلى في المبيض ذات الجسم الأصفر عن المبيض الخالي من الجسم الأصفر بينما تركيز البروجسترون أعلى في المبيض الخالي من الجسم الأصفر عن المبيض ذات الجسم الأصفر في كل من السيرم والسائل الجريبي. وأيضاً معدل الجلوكوز كان أعلى في المبيض ذات الجسم الأصفر ولكن تركيز الكولسترول والبروتينات وأكسيد النيتروز كان أعلى في المبيض الخالي من الجسم الأصفر.