

## Effect of Ovarian Status and Follicular Size on Grade and Maturation *In Vitro* of Bovine Oocytes in Relation with Follicular Fluid Composition.

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### ABSTRACT

The aim of this study was to investigate the effect of corpus luteum (CLs), follicular size on grade and *in vitro* maturation of bovine oocytes, in relation with and follicular fluid composition. Ovaries were obtained from abattoirs and classified according to bearing CLs (CL<sup>+</sup>) or non-bearing CLs (CL<sup>-</sup>), all visible follicles were measured according to their diameters into small follicles (SF≤8 mm) and large follicles (LF>8 mm). The follicular fluids (FF) and oocytes were aspirated from SF and LF in CL<sup>+</sup> and CL<sup>-</sup> ovaries. Oocytes were classified into good, fair and poor quality. Only good quality oocytes were *in vitro* matured in TCM-199 medium. The concentration of estradiol-17β (E2), progesterone (P4), testosterone and lactic acid in FF were estimated. Results showed that CL<sup>+</sup> ovaries showed significantly (P<0.001) lower oocyte recovery rate as compared to CL<sup>-</sup> ovaries. Also, recovery rate of oocytes was significantly (P<0.001) higher for LF than SF. Proportion of good and fair oocytes derived from CL<sup>-</sup> ovaries were significantly (P<0.001) higher, while that of poor oocytes were significantly (P<0.001) lower when oocytes were recovered from CL<sup>-</sup> ovaries as compared to CL<sup>+</sup> ones. Also, proportion of good and fair oocytes were significantly (P<0.001) higher, while that of poor oocytes were significantly (P<0.001) lower when recovered from LF than from SF. Maturation rate was significantly (P<0.001) higher when oocytes were recovered from CL<sup>-</sup> ovaries as compared to CL<sup>+</sup> ones or from LF as compared to SF. Concentration of P4 and lactic acid was significantly (P<0.001) higher, while that of testosterone and E2 was significantly (P<0.001) lower in FF collected from CL<sup>-</sup> than from CL<sup>+</sup> ovaries. However, concentration of P4 and testosterone was significantly (P<0.001) higher, while lactic acid concentration was significantly (P<0.001) lower in FF collected from LF than from SF. Concentration of E2 was not affected significantly by follicular size. Effect of interaction between ovarian status and follicular size was significant (P<0.01) only on concentration of E2 and P4. In conclusion, presence of CLs and reduced follicular size showed negative effects recovery rate, quality and *in vitro* maturation of bovine oocytes. However, improving quality and *in vitro* maturation was associated with increasing P4 and testosterone concentrations.

**Keywords:** bovine, ovarian status, follicular size, follicular fluid, *in vitro* maturation

### INTRODUCTION

The competence of oocyte maturation is influenced by several biological and environmental factors such as ovarian status, follicle size, collection method, oocyte quality and culture conditions (Mahmoud and El-Naby, 2013). It is known that ovaries recovered from a slaughtered animals are at different stages of the estrous cycle, being with corpora lutea (CLs) at various functional stages or follicles at different sizes (Hosseini *et al.*, 2008) and variation in the developmental competences of oocytes quality (Pirestani *et al.*, 2012).

Previous studies confirmed the relationship between CLs development and follicles with heterogeneity in the developmental competence of oocytes, which may cause asymmetry in the reproductive organs function in dairy cattle (Contreras-Solis *et al.*, 2008; Penitente-Filho *et al.*, 2014). The evolution of the CLs is associated with viviparity in mammals and is necessary for progesterone (P4) production during the luteal phase of the estrous cycle to maintain pregnancy and also during pregnancy, to decrease gonadotrophin secretion to prevent behavioral estrous activity (Powell *et al.*, 2006 ; Karami Shabankareh *et al.*, 2015).

Follicular size is a major factor influencing the developmental capacity of bovine oocytes. During follicular development, the formation of mRNA or proteins, is accumulated in the oocyte and stored for early development of the embryo (Beker-van *et al.*, 2006; Aksu *et al.*, 2015). Follicular size was found to correlate with acquiring meiotic potential and subsequent developmental competence oocyte in pigs (Yoon *et al.*, 2001) and there was an interaction between follicular size and the phase of follicular wave on the efficiency of embryo production (Machatkova *et al.*, 2004).

The oocyte quality is determined by their ability to mature, be fertilized and give rise to normal offspring (Hussein *et al.*, 2006), and also related to stage of follicular development, follicular environment and type of media used for oocyte maturation (Keskintepe *et al.*, 1994).

Follicular fluid (FF) is a vascular compartment within the mammalian ovary, separated from the perifollicular stroma by the follicular wall that constitutes a blood-follicle barrier (Abd-Allah *et al.*, 2010). This fluid is composed of locally produced substances within the follicle, which are related to metabolism of follicular cells (Blaszczyk *et al.*, 2006). The FF is rich in steroid reproductive hormones, including testosterone, estradiol-17β (E2) and P4. Levels of these hormones in the FF are related to follicular size and bearing of CLs or no. Presence of CLs locally affects neighboring follicular composition during the luteal phase of the estrous cycle in dairy cows (Nasroallah, 2014). The FF maintains a proper environment for growth and maturation of oocytes, beside meeting the nutritional requirements of the growing oocytes (Ali *et al.*, 2008).

The ovarian morphology in relation to growth of follicles, CLs, with the view of *in vitro* recovery, quality, and *in vitro* maturation still need more investigation (Singh and Adams, 2000; Mohammadpour, 2007), therefore the aim of this study was to investigate the effect of presence or absence of CLs and follicular size on grade and *in vitro* maturation of bovine oocytes, in relation with and follicular fluid composition.

### MATERIALS AND METHODS

This study was conducted at the *in vitro* fertilization laboratory, International Livestock Management Training Center, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of

Agriculture, in cooperation with Department of Animal Production, Faculty of Agriculture, Damietta University. All chemicals used in this study were purchased from Sigma (Sant Luis, MO, USA).

**Ovaries collection and follicles classification:**

Ovaries were obtained immediately from slaughtered cows (Shebin El-kom slaughterhouse, Menofeya Governorate. They were transported to the laboratory within 2-3 h of slaughter in a thermos flask (at 25-30°C) containing fresh saline (0.9% NaCl) supplemented with 50 µg/ml gentamicin. At the laboratory, the ovarian surfaces were cleared by removing the extraneous tissues and washing with 70% ethanol, followed by washing several times in phosphate buffer saline (PBS, pH 7.3). Finally, ovaries were dried with sterilized filter paper and divided according to bearing CLs into two groups, including ovaries with CL (CL<sup>+</sup>) and without CL (CL<sup>-</sup>). Thereafter, ovaries were transferred separately into two sterile separate glass beakers. On the ovarian surface in each group, all visible antral follicles were measured and calculated according to their diameters into small follicles (SF≤8 mm) and large follicles (LF>8 mm).

**Collection of follicular fluid and oocytes:**

Based on ovarian status or follicular size, the follicular fluids (FF) and oocytes were aspirated using a sterile syringe and 18-gauge needle. Contents of syringe were placed into Petri dish (6 cm) and kept undisturbed for 1-3 min, allowing the oocytes to settle down. The Petri dishes were examined under stereomicroscopy and the oocytes were transferred to a searching dish containing PBS for grading according to Wani *et al.* (2013) into three categories, involving good quality oocytes with many complete layers of cumulus cells and uniform cytoplasm (*Cumulus oocytes-complexes COCs*), fair oocytes with thin or incomplete layers of cumulus cells and uniform cytoplasm and poor oocytes with few or no cumulus cells. Only good quality oocytes were used for *in vitro* maturation. After oocytes collection, FF collected from SF or LF of ovaries bearing or non-bearing CLs was pooled and centrifuged at 3000 rpm and the supernatant was collected (Nasroallah, 2014). The harvested FF was stored frozen at -20°C until subsequent assay of E2, P4, testosterone and lactic acid.

Concentration of steroid hormones (E2, P4 and testosterone) in FF was determined by enzyme immunoassay using commercial kits (Monobind Diagnostic Inc, Lake Forest, USA) based on method described by Nasroallah. (2014). However, concentration of lactic acid was determined using commercial kit

(Determiner LA; Kyowa-Hakkou,Osaka, Japan) after Nagai *et al.* (2007).

**In vitro maturation:**

Good quality oocytes (COCs) of SF or LF collected from ovaries with (CL<sup>+</sup>) were washed 3 times in HEPES-Tyrode's albumin-lactate-pyruvate medium (TALP medium) plus HEPES (25 mM/ml) and 3 mg/ml bovine serum albumin (BSA). A minimum of 8-10 COCs were cultured into Petri dish containing droplet of TCM-199 (50 µl) fortified with sodium pyruvate (0.2 mg/ml), fetal calf serum (10% v/v), FSH (1µg/ml), LH (1µg/ml), E2 (1µg/ml), epidermal growth factor (EGF, 20 ng/ml) and gentamicin sulphate (50 µg/ml) under sterile mineral oil. The COCs were incubated for 24 h at 38.5°C in a CO<sub>2</sub> incubator (5% CO<sub>2</sub> in air with 90–95% relative humidity).

After maturation period, expansion rate (full expanded cumulus oocyte) was recorded and considered as cytoplasmic maturation according to (El-Ratel and Fouda, 2016). The COCs were washed by PBS with hyaluronidase (1 mg/ml) and 2 times with PBS containing BSA (2%), loaded into fixation solution (3 ethanol: 1 glacial acetic acid) for 24 h and stained with in a mixture of orcein and acetic acid (1:45%). The matured oocytes at different stages were examined and percentage of oocytes having emission of first polar body (Metaphase II (MII) was considered as nuclear maturation rate.

**Statistical analysis:**

Data were analyzed by analysis of variance (ANOVA) using factorial design (2 ovarian statuses x 2 follicular sizes) using a software package (SAS, 2004). The significant differences among means were tested using Duncan's Multiple Range Test (1955).

**RESULTS AND DISCUSSION**

**Oocyte recovery rate:**

As affected by ovarian status, regardless the follicular size, CL<sup>+</sup> ovaries showed significantly (P<0.001) lower oocyte recovery rate as compared to CL<sup>-</sup> ovaries (Table 1). This finding indicated marked effect of the ovarian status on oocyte recovery rate, in term of a negative effect of CLs presence on the oocyte recovery rate in bovine. In this respect, Singh *et al.* (2012) and Nandi *et al.* (2000) reported decreased recovery rate of bovine and buffalo oocytes when ovaries had a CL<sup>+</sup>, respectively. This trend was attributed to restricted follicular development as the lutein cells of CLs occupy most of the portion of the ovary (Kumar *et al.*, 1997) and the dominant follicle is usually observed in the CL<sup>+</sup> bearing ovaries with other very small follicles (Gasparrini *et al.*, 2000).

**Table 1. Recovery rate of bovine oocytes as affected by ovarian status and follicular size.**

Item		Total number			Oocyte recovery rate%
		Ovaries	Follicles	Oocytes	
Ovarian status (OS)	CL <sup>+</sup>	355	2189	1290	58.93
	CL <sup>-</sup>	472	3345	2686	80.29
	±SEM	-	-	-	0.559***
Follicular size (FS)	SF≤8mm	355	2443	1504	61.56
	LF>8mm	472	3091	2472	79.97
	±SEM	-	-	-	0.559***

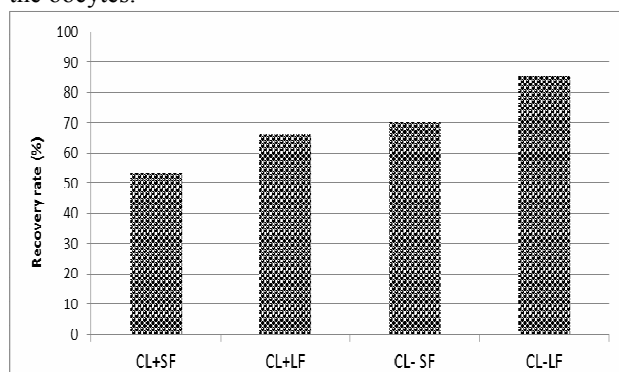
\*\*\* Significant at P<0.001.

Also, the present results cleared that oocyte recovery rate was significantly (P<0.001) higher for LF than SF, regardless the ovarian status (Table 1). Similar trend was reported in human (Triwitayakorn *et al.*, 2003),

bovine (Hendriksen *et al.*, 2000; Torner *et al.*, 2001; Lequarre *et al.*, 2005) and sheep (Majeed *et al.*, 2008). This trend may be due to the difference in processes between small and large follicles that allow the oocytecumulus cell

mass to become free-floating in the antral fluid just before follicle rupture (Triwitayakorn *et al.*, 2003). In preovulatory follicles, hyaluronic acid (synthesized by the cumulus cells and stimulated by midcycle FSH peak through its receptor) disperses the cumulus cell just before ovulation (Suchanek *et al.*, 1994) and FSH-receptor expression is associated with follicular size and FSH-receptors (Minegishi *et al.*, 1997).

Results also indicated that ovarian status did not interact with follicular size. This effect leads to the highest oocyte recovery rate from LF of CL<sup>-</sup> bovine ovaries (Fig. 1). These results are in accordance with those obtained on buffaloes by Makwana *et al.* (2012), who found that functional structure on the ovaries such as CLs and follicular size are affecting recovery rate of the oocytes.



**Fig. 1. Recovery rate of bovine oocytes as affected by interaction between ovarian status and follicular size.**

**Oocyte quality:**

Proportion of good and fair oocytes derived from CL<sup>-</sup> ovaries were significantly (P<0.001) higher, while that of poor oocytes were significantly (P<0.001) lower when oocytes were recovered from CL<sup>-</sup> ovaries as compared to CL<sup>+</sup> ones, regardless the follicular size (Table 2). In agreement with the present results, CL<sup>-</sup>

ovaries had a significantly higher number of COCs than those of CLs bearing ovaries in sheep (Alsafy and El-Shahat (2011), buffalo (Amer *et al.*, 2008; Jamil *et al.*, 2008) and goat (Kumar *et al.*, 2004). In this respect, Abdoon and Kandil (2001) recorded higher percentages of poor oocytes recovered from pregnant ovaries in buffalo. The observed reduction in quality of oocytes recovered from CL<sup>+</sup> ovaries is likely because of the restricted follicular development due to occupying the lutein cells of a great portion of the ovarian mass. Also, CLs may inhibit the follicular growth resulting follicular atresia. In this case, P4 secreted by the luteal cells of the CLs inhibits estrus due to the negative feedback of P4 on the anterior pituitary to secrete FSH.

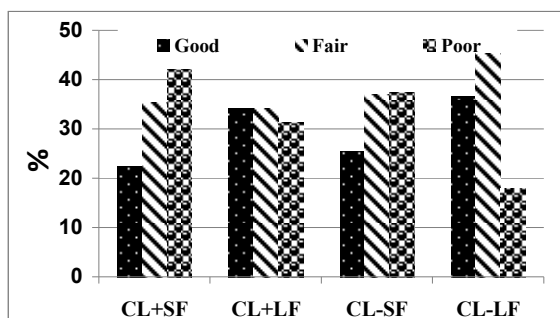
Results also revealed that proportion of good and fair oocytes were significantly (P<0.001) higher, while that of poor oocytes were significantly (P<0.001) lower when recovered from LF than from SF, regardless the ovarian status (Table 2). These results are in harmony with those early reported on bovine (Lonergan *et al.*, 1994) and sheep (Wani *et al.*, 2013). Follicular size profoundly influences the oocyte quality obtained during ovulation and (Sirard *et al.*, 2006). Improving quality of oocytes recovered from LF compared to SF might be attributed to more development of oocytes in LF than in SF (Wani *et al.*, 2013).

Effect of interaction between ovarian status and follicular size was not significant indicating the best oocyte quality for oocytes collected from LF of CL<sup>-</sup> ovaries (Fig. 2). Generally, P4 secreted by CL<sup>+</sup> ovaries inhibits follicular growth by LH pulse frequency suppression, which is critical for continued growth of LF. The P4 may also exert local effects on the growth of large antral follicles, in both luteal and non-luteal ovaries, independent of changes in gonadotrophin secretion (Bartlewski *et al.*, 2001).

**Table 2. Oocyte grades as affected by ovarian status and follicular size.**

Item		Total oocytes (n)	Oocyte grades (%)		
			Good	Fair	Poor
Ovarian status (OS)	CL <sup>+</sup>	1290	27.98	34.88	37.14
	CL <sup>-</sup>	2686	33.21	42.85	23.94
±SEM		-	0.244***	0.377***	0.290***
Follicular size (FS)	SF≤8mm	1504	24.14	36.24	39.62
	LF>8mm	2472	36.00	42.72	21.28
±SEM		-	0.244***	0.377***	0.290***

\*\*\* Significant at P<0.001.



**Fig. 2. Oocyte grades as affected by interaction between ovarian status and follicular size.**

**Cytoplasmic and nuclear maturation rate of oocytes:**

Maturation rate in term of percentage of full expanded oocytes or those reached to MII was significantly (P<0.001) higher when oocytes were recovered from CL<sup>-</sup> ovaries as compared to CL<sup>+</sup> ones or from LF as compared to SF (Table 3). Effect of interaction between ovarian status and follicular size was not significant indicating the highest cytoplasmic and nuclear maturation rate of oocytes recovered from LF of CL<sup>-</sup> ovaries (Fig. 3).

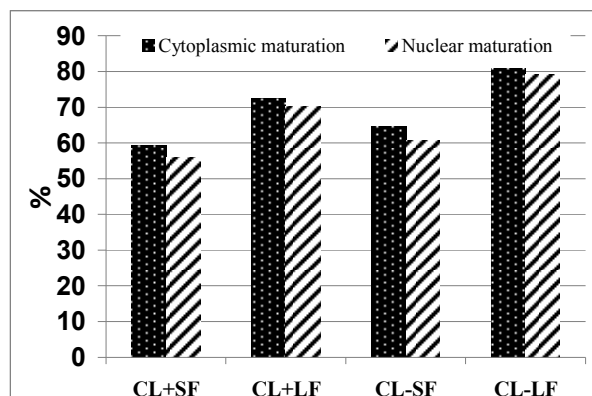
**Table 3. Maturation rate of bovine oocytes as affected by ovarian status and follicular size.**

Item		Total oocytes	Maturation rate (%)	
			Cytoplasmic	Nuclear
Ovarian status (OS)	CL <sup>+</sup>	361	67.03	64.27
	CL <sup>-</sup>	892	77.13	74.90
±SEM		-	0.332***	0.588***
Follicular size (FS)	SF≤8mm	363	62.50	58.96
	LF>8mm	890	78.99	77.08
±SEM		-	0.332***	0.588***

\*\*\* Significant at P<0.001.

In accordance with the obtained results, Mahesh *et al.* (2014) found higher percentage of MII stage oocytes recovered from the CL<sup>+</sup> buffalo ovaries as compared to CL<sup>-</sup> ovaries (Mahesh *et al.*, 2014). In this respect, Lonergan *et al.* (1994) found a reduction in developmental competence of bovine oocytes of SF, whereas Karami Shabankareh *et al.* (2015) suggested that CLs exerts negative effects on the developmental competence of bovine oocytes. In sheep, the maturation rate of LF oocytes was found to be significantly (P<0.05) higher than SF oocytes (Wani *et al.*, 2013). The maturation rates were also in accordance with those recorded by Qian *et al.* (2001) in pig, Khatir *et al.* (2007) in dromedary camel and Majeed *et al.* (2012) in goat.

Presence or absence of CLs by follicle size interactions have an effect on oocyte environment and its developmental competence as observed in the current study. After formation of the CLs, new blood vessels were formed for the development of CL. The CLs will receive the greatest rate of blood flow compared with other tissues in the ovary (Parrish *et al.*, 1986). Follicle size is an important parameter that influences oocyte competence (Martina *et al.*, 2016). The observed reduction in maturation rate of SF oocytes may be attributed to that, in viable ovarian follicles, several physiologically active non steroid such as oocyte maturation inhibitor, lutenization inhibitors, inhibitory protein, relaxin and inhibin are secreted (Hafez and Hafez, 2000). Also, nuclear events within SF oocytes are very low, and the transcripts produced during this period are critical for further development (Atanasov *et al.*, 2015).



**Fig. 3. Maturation rate of bovine oocytes as affected by interaction between ovarian status and follicular size.**

**Composition of follicular fluid:**

Concentration of P4 and lactic acid was significantly (P<0.001) higher, while that of testosterone and E2 was significantly (P<0.001) lower in FF collected from CL<sup>-</sup> than from CL<sup>+</sup> ovaries. However, concentration of P4 and testosterone was significantly (P<0.001) higher, while lactic acid concentration was significantly (P<0.001) lower in FF collected from LF than from SF. Concentration of E2 was not affected significantly by follicular size (Table 4). It is of interest to note that only the effect of interaction between ovarian status and follicular size was significant (P<0.01) only on concentration of E2 and P4. These effects reflected higher E2 and lower P4 concentrations in FF of LF than in SF only for CL<sup>-</sup> ovaries (Fig. 4).

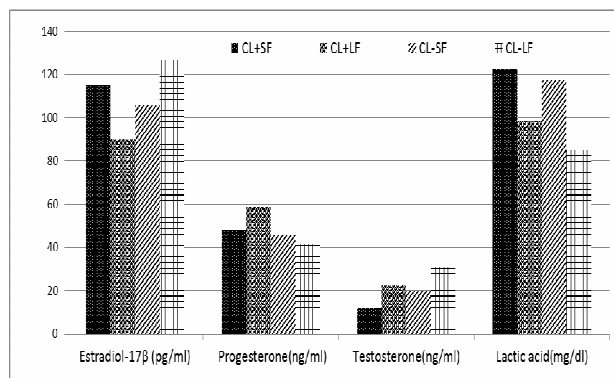
**Table 4. Concentration of some hormones and lactic acid in follicular fluid of bovine ovaries as affected by ovarian status and follicular size.**

Item		Estradiol-17β (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)	Lactic acid (mg/dl)
Ovarian status	CL <sup>+</sup>	102.50	53.00	17.00	110.33
	CL <sup>-</sup>	116.17	43.67	25.17	101.00
±SEM		1.178***	0.656***	0.513***	0.824***
Follicular size	SF≤8mm	110.17	44.67	15.67	119.83
	LF>8mm	108.50	52.00	26.50	91.50
±SEM		1.178 <sup>NS</sup>	0.656***	0.513***	0.824***

<sup>NS</sup>: Not significant. \*\*\* Significant at P<0.001.

These results were in agreement with the findings of Nasroallah. (2014), who found that non CLs bearing ovaries had significantly (P<0.01) higher concentration of E2 than those found in CLs bearing ovaries. On the other hand, P4 concentration of FF was significantly (P<0.01) higher in CL<sup>+</sup> compared to CL<sup>-</sup> ovaries. Concentration of P4 and testosterone were significantly (P≤0.05) higher in FF from the LF than SF. Also, the FF from SF contained significantly higher concentration of

lactic acid than that from LF (Nagai *et al.*, 2007). During the luteal phase, P4 is secreted in high concentrations by the CLs (Pradeep *et al.*, 2009) from the granulosa and theca cells of bovine follicles (Hunter *et al.*, 2004). Presence of CLs affects ovarian follicular dynamics in both ovaries (Contreras-Solis *et al.*, 2008) and P4 had a suppressive effect on follicular growth during the ovine estrous cycle (Khera, 1989).



**Fig. 4. Concentration of some hormones and lactic acid in follicular fluid of bovine ovaries as affected by interaction between ovarian status and follicular size.**

In disagreement with the obtained results, Kor *et al.* (2013) who showed that as the follicular size increased, FF concentration of E2 increased. The noted slight reduction in E2 and increasing testosterone concentration recorded in our study may be attributed to that, increasing follicular size lead to increasing testosterone secretion from the theca cells under the stimulus of LH, which is converted into E2 by granulosa cells under the influence of FSH (Hafez and Hafez, 2000).

In the present study, enhancing quality and *in vitro* maturation of oocytes recovered from LF was matched with increasing P4 and testosterone, and decreasing lactic acid concentrations in their FF. This may suggest an important role of P4 on quality and maturation of oocytes within the follicles.

In conclusion, knowledge on bearing or non-bearing CLs and follicles at different developmental stages of ovaries collected from slaughtered cows can be helpful in *in vitro* embryo production. Based on the foregoing results, presence of CLs and reduced follicular size showed negative effects recovery rate, quality and *in vitro* maturation of bovine oocytes. However, improving quality and *in vitro* maturation was associated with increasing P4 and testosterone concentrations.

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## تأثير حالة المبيض والحجم الحويصلي على جودة وإنضاج بويضات الأبقار معمليا والعلاقة بمكونات السائل الحويصلي

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تهدف هذه الدراسة إلى تقييم تأثير الأجسام الصفراء وحجم الحويصلات المبيضية على درجة وإنضاج بويضات الأبقار معمليا والعلاقة بمكونات السائل الحويصلي. تم جمع المبايض من المجازر وتقسيمها على حسب وجود الأجسام الصفراء من عدمها على سطح المبيض وقياس حجم الحويصلات المبيضية وتصنيفها إلى حويصلات صغيرة ( $8 \leq$  مللي) وحويصلات كبيرة ( $8 >$  مللي). تم شفط السائل الحويصلي بالبويضات من الحويصلات الصغيرة والكبيرة لكل مبيض. تم فحص البويضات وتقييمها إلى بويضات جيدة ومتوسطة وردينة و تم عمل إنضاج معملى للبويضات الجيدة فقط. تم تقدير هرمونات الاستراديول والبروجستيرون والتستوستيرون وكذلك حمض اللاكتيك فى السائل الحويصلي. وقد أظهرت النتائج ان معدل الاسترداد الكلى للبويضات من مبايض يوجد عليها أجسام صفراء اقل معنويا مقارنة بالمبايض التى لا يوجد عليها أجسام صفراء. وكان معدل الاسترداد الكلى للبويضات من الحويصلات الكبيرة أعلى معنويا مقارنة بالحويصلات الصغيرة. لوحظ أن نسبة البويضات الجيدة والمقبولة المستردة من مبايض لا يوجد عليها أجسام صفراء أعلى معنويا بينما كانت البويضات الرديئة أقل معنويا مقارنة بتلك المستردة من مبايض يوجد عليها أجسام صفراء. وكانت نسبة البويضات الجيدة والمقبولة المستردة من الحويصلات الكبيرة أعلى معنويا بينما كانت البويضات الرديئة أقل معنويا مقارنة بالحويصلات الصغيرة. وجد ان معدل الإنضاج المعملى ( سيتوبلازم أو نواه) للبويضات المستردة من مبايض لا يوجد عليها أجسام صفراء أو من حويصلات كبيرة أعلى معنويا مقارنة بتلك التى يوجد عليها أجسام صفراء أو من حويصلات صغيرة. وجد ان تركيز هرمون البروجستيرون وحمض اللاكتيك أعلى معنويا بينما كان تركيز التستوستيرون والاستراديول أقل معنويا فى السائل الحويصلي المتحصل عليه من مبايض لا يوجد عليها أجسام صفراء مقارنة بتلك التى يوجد عليها أجسام صفراء. ومع ذلك كان تركيز البروجستيرون و التستوستيرون أعلى معنويا بينما كان تركيز حمض اللاكتيك أقل معنويا فى السائل الحويصلي المتحصل عليه من حويصلات كبيرة مقارنة بالصغيرة. ولم يتأثر تركيز الاستراديول معنويا بحجم الحويصلات المبيضية. وقد لوحظ وجود فروق معنوية عند تداخل حالة المبيض مع حجم الحويصلات فى تركيز الاستراديول والبروجستيرون. وتشير النتائج المتحصل عليها أن وجود الأجسام الصفراء على سطح المبيض مع انخفاض حجم الحويصلات المبيضية لها تأثيرات سلبية على معدل الاسترداد الكلى وجودة وإنضاج بويضات الأبقار معمليا. بالرغم من أن تحسن جودة البويضات والإنضاج المعملى لها مرتبط بزيادة تركيزات هرمون البروجستيرون والتستوستيرون.