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Assessment of the Impact of Corpus luteum on *in Vitro* Maturation and Developmental Competence of Dromedary Camel Oocytes

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TO ASCERTAIN the effect of corpus luteum (CL) on the oocytes retrieval, quality and *in vitro* developmental competence in camels, ovaries were collected from apparent healthy slaughtered she-camel during breeding season and categorized according to the presence (group I) or absence (Group II) of CL. Oocytes were aspirated from 2-5 mm antral follicles on the surface of pathological-free-ovaries, quantified and morphologically graded depending upon the cumulus investment and cytoplasmic distribution. Oocytes from each group were matured *in vitro* and examined for nuclear maturation in fixed slides after 30 hours of incubation. *In vitro* matured oocytes from each group were fertilized with epididymal sperm suspension and assessed for the cleavage and development to morula and blastocyst stages at 3rd, 5th and 7th day, respectively. Under the present experimental conditions, the accessible data showed a significant influence of CL presence on the quantity, quality and *in vitro* nuclear maturation of retrieved oocytes in camels. The number of all retrieved oocytes classes was higher in the absence of CL. While the rate of excellent and poor quality oocytes retrieval was higher ($p < 0.05$) in the absence of CL, the good quality oocytes were retrieved at higher rate ($p < 0.001$) in the presence of CL. The highest rate of metaphase II (MII) was recorded in oocytes harvested from ovaries had no CL. Nevertheless, CL had no marked influence on the rate of cleavage, morula and blastocyst formation. It could be concluded that the presence of CL is not a barrier against embryo developmental competence, though its marked influence on ovarian follicular activity.

Keywords: Camel, Corpus luteum, Embryo developmental competence,
In-vitro oocytes maturation, *In-vitro* fertilization.

Introduction

Camels are seasonal breeders, induced ovulator animals [1], displaying their maximum reproductive efficiency during the period from December to April in Egypt [2]. The reproductive efficiency of camels under natural conditions is generally low, due to the relatively short breeding season, long pre-pubertal period, long gestation period, long calving interval, and the lack of use of assisted reproductive techniques such as embryo transfer and artificial insemination [3]. In Egypt, there are few attempts to establish *in vitro* fertilization (IVF) techniques in dromedary camel [4-6].

For *in vitro* oocyte maturation, it has been reported that the rate and the quality of oocyte yield from slaughtered animals' ovaries were affected by several factors including the ovarian morphology [7], methods of oocyte retrieval [8], season [9] and ovarian status [10].

Former studies reported many inconsistencies about the influence of corpus luteum (CL) on the oocytes developmental competence. In camel, it was noticed that the presence of CL during early pregnancy did not inhibit the growth and development of ovarian follicles [11]. In bovines, it has been reported that the oocytes recovered from ovaries having CL showed high maturation

rate, and the absence of this structures was associated with poor *in vitro* oocytes development [12]. On the other hand, Shabankareh *et al.*[13] clearly proved that CL exerts negative effects on the developmental competence of bovine oocytes *in vitro*. So, the present study was designed to verify the impact of corpus luteum on the oocytes retrieval, quality and *in vitro* developmental competence in camels.

Material and Methods

All Chemicals were purchased from Sigma-Aldrich Co., except tissue culture medium (TCM-199) and fetal calf serum (FCS) were purchased from Gibico BRL (Grand Island, New York, USA), and ringer lactate solution (ADWIA Pharmaceuticals Co.) was bought from the pharmacy.

Ovaries collection, grouping and oocyte harvesting

Ovaries were collected from apparent healthy slaughtered she-camel of unknown reproductive history at El-Warrak slaughter house, Cairo, within 2 hours (h) of slaughter according to Lee and Fukui [14]. Ovaries, free from pathological lesions or abnormalities, were transported to the laboratory in 0.9% NaCl supplemented with gentamicin (10 µg/mL) and maintained at 30-38°C in thermos-container. Collected ovaries were washed three times by warm saline and were categorized according to the presence (group I) or absence (Group II) of corpus luteum.

Oocytes were aspirated from 2-5 mm antral follicles on the surface of ovaries with syringe fitted with 18G needle according to Ali and Abdel-Razek [15]. The harvested oocytes were washed 2-3 times in maturation medium, examined morphologically with stereo-microscope and graded depending upon the cumulus investment and cytoplasmic distribution into excellent (A), good (B), fair (C) and poor (D) according to Kharche *et al.* [16].

In vitro maturation and nuclear maturation evaluation

Oocytes maturation was carried out according to Mahmoud *et al.* [4]. Briefly, oocytes (n=10-15/100 µl of maturation medium) were cultured in 4-well plastic Petri-dishes (pre-incubated for a minimum of 2 h before culturing of oocytes), covered with mineral oil layer (to

prevent evaporation and contamination with microorganisms) and incubated for 30 h. At the end of the culture period, slides were prepared for the evaluation of nuclear maturation according to the procedure described by Tarkowski[17]. Briefly, cumulus cells were mechanically removed by gentle pipetting, oocytes were transferred to 1% hypotonic sodium citrate solution for 10 min., placed on a microscope slide with a minimal amount of hypotonic solution and fixed with methanol/glacial acetic acid (3:1) before being stained with 1% orcein stain. The state of nuclear maturation, Teleophase-I (TI) and/or MII stages, was determined as described earlier by Mahmoud [18]. TI was defined as completion of separation of two chromosome sets and MII was haploid set of chromosomes after emission of first polar body.

In vitro fertilization and embryo culture

Freshly harvested epididymal spermatozoa for IVF were prepared as described by Moawad *et al.* [19]. Camel epididymis, collected from a local abattoir (Toukh, Qalyoubia), was washed with sterile saline, isolated, incised longitudinally, rinsed 3-4 times with 2 ml of bovine serum albumin (BSA) free BO medium [20] containing 10 mg/ml heparin and 2.5 mM caffeine, and centrifuged at 800 ×g for 10 min. Sperm pellets were re-diluted with BO medium containing 20 mg/ml BSA to adjust spermatozoa concentration to 2.5 × 10⁶ sperm/ml.

Matured oocytes were washed three times in BO medium containing 10 mg/ml BSA and were co-cultured (n= 5-10 oocytes/droplet) with 100 µl droplets of sperm suspension under mineral oil for 5 h. After that the oocytes were washed in TCM-199 to remove attached spermatozoa and cumulus cells by vortex. Presumptive fertilized oocytes were transferred to TCM-199 culture droplets and incubated for 7-8 days with renewal of the culture media every other day. The cleavage was assessed after 72 h of culture (Day 0=day of insemination). The number of embryos developed to morula and blastocyst stages was assessed on the 5th and 7th day, respectively. The morphological appearance of embryos was evaluated under an inverted microscope. The cleavage and blastocyst rates were calculated from the total number of inseminated oocytes.

Statistical analysis

Data were presented as mean (±S.E.) and

analyzed for significance by Student t-test and Chi-square test using SPSS (ver.16) statistical software. Differences were considered to be significant at $P < 0.05$.

Results and Discussion

Effect of reproductive status on oocyte retrieval

The mean number of retrieved oocytes per ovary per conducted trial ($n=13$) significantly ($p < 0.01$) differed between CL and non-CL groups (2.98 ± 0.31 vs. 5.84 ± 0.59 , respectively). Also, the overall oocyte retrieval rate per ovary markedly ($p < 0.0001$) varied between evaluated

groups (244/84 ovaries (2.91) and 648/114 (5.68), respectively) (Table 1).

The number of all retrieved oocytes classes was higher in the absence of CL (group II). The excellent and poor quality oocytes retrieval rate was higher ($p < 0.05$) in the second group (CL absent), while the good quality oocytes were retrieved in higher rate ($p < 0.001$) in the first group (CL present) (Fig. 1). Oocytes were classified into excellent, good, fair and poor quality depending upon the cumulus investment and cytoplasmic distribution. The rate of retrieved oocyte in each class was analyzed by Chi-square test.

TABLE 1. Effect of corpus luteum presence on oocyte retrieval rate from camel ovaries during the breeding season.

Parameter	Group I (CL present)	Group II (CL absent)	P value
Number of ovaries	84	114	
Total number of retrieved oocytes	244	648	
Number of retrieved oocytes per ovary (Mean \pm SEM)	2.98 ± 0.31	$5.84 \pm 0.59^{**}$	> 0.01
Overall retrieval rate (%)	2.91	5.68^{****}	> 0.0001

Differences in retrieval rate among groups were analyzed with Chi-Square analysis. Means of groups were analyzed with Student t-test.

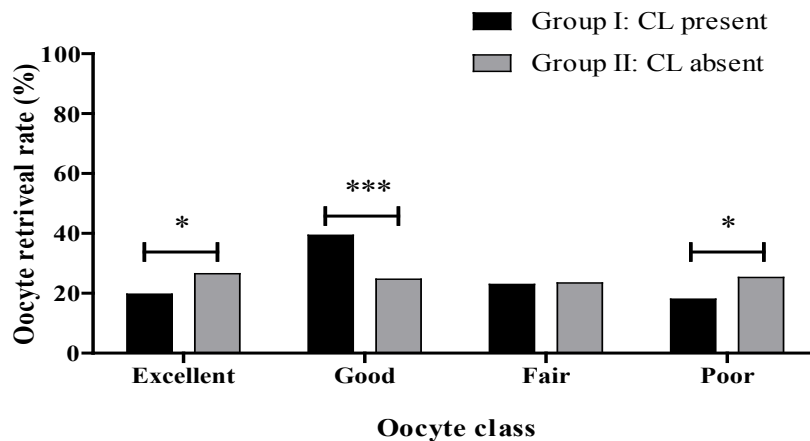


Fig. 1. Effect of corpus luteum presence of the quality of retrieved camel oocytes during the breeding season.

A clear relation has been found between the follicular growth and CL development [21]. It has been reported that the number of oocytes per ovary in camel is affected by ovarian status, animal ages, site of the ovary, breeding season and/or their interactions [22]. Corpora lutea affect ovarian follicular dynamics in both ovaries by a systemic

effect with an evidence for a local ipsilateraleffect [23]. The present findings came in agreement with those reported by Abdoon [24], but disagree with studies by Torner et al. [25], Khalil [26] and Farag et al. [11], who declared that the presence of CL during early pregnancy did not inhibit the growth and development of camel ovarian follicles and

that the average number of oocytes per ovary were nearly similar in pregnant and non-pregnant camels.

Animal reproductive status has been found to influence the frequency distributions of oocytes at different stages of maturation [26]. In this respect, the data obtained in the current study is coordinated with Abdoon [24] and Amer *et al.* [27] who mentioned that the presence of CL significantly reduces the number of ovarian follicles as well as the quality of the oocytes. Khalil [26] established that a lower rate of type A and higher rate of type B and C is associated with increasing level of follicular atresia, which is tremendously high during gestation period. The reasons for the superiority of the ovaries without CL over those CL-bearing ovaries is not clear. There were many former trials to explain the mechanism of CL interference with oocyte

population and quality. Some authors denoted that the CL may alter follicles growth rate resulting in atresia [28], while others related this to the conceptus effects, as a local suppressor on follicular development in the ipsilateral ovary, to prevent luteolysis as mechanism of maternal recognition of pregnancy [29].

Effect of reproductive status on the nuclear maturation of retrieved oocyte

The effect of CL on rate of nuclear maturation in camel oocytes *in vitro* is presented in Table 2. A significant difference ($p < 0.05$) has been found between matured oocytes from ovaries with CL and without CL in terms of GV and MII stages. The highest rate of GV stage has been recorded in those retrieved from ovaries containing CL. While, the highest value of MII stage has been observed in the matured oocytes retrieved from ovaries without CL.

TABLE 2. Effect of corpus luteum presence on *in-vitro* nuclear maturation in camels.

Parameter	Group I			Group II			P value	
	(CL present)			(CL absent)			Chi-square	Student t-test
	N	%	Mean \pm SE	n	%	Mean \pm SE		
GV	26	36.62	36.35 \pm 1.53*	29	30.53	29.89 \pm 0.85	0.41	>0.05
GVBD	10	14.08	13.83 \pm 0.67	13	13.68	14.02 \pm 0.55	0.95	0.83
MI	6	8.45	8.19 \pm 0.99	7	7.37	6.70 \pm 1.62	0.79	0.48
AI	2	2.82	2.57 \pm 1.39	3	3.16	2.47 \pm 1.23	0.89	0.95
TI	8	11.27	11.25 \pm 0.90	8	8.42	8.55 \pm 1.28	0.54	0.16
MII	19	26.76	23.61 \pm 2.84	35	36.84	37.83 \pm 2.76*	0.17	>0.05
Maturation (TI+MII)	27	38.03	39.04 \pm 2.49	42	44.21	45.06 \pm 2.69	0.42	0.18

Data were presented as mean \pm SE (n=6 replicates) and were analyzed with Student t-test. Nuclear maturation rates were analyzed with Chi-square test. GV, GVBD, MI, AI, TI and MII referred to Germinal vesicle, Germinal vesicle breakdown, Anaphase-I, Telophase-I and Metaphase II stages, respectively.

To undergo a successful fertilization and embryonic development, a complete cytoplasmic and nuclear maturation is necessary [30]. The degree of cumulus cell expansion, meiotic division and evenly granulated dark ooplasm are significant in evaluating *in vitro* maturation [31]. *In vivo* and *in vitro* nuclear maturation behave the same pattern [32]. Oocyte meiotic maturation is a complex process at which germinal vesicle breakdown (GVBD) takes place, chromosomes condense, metaphase plate forms, meiosis I fulfilled, the first polar body formed and is arrest at Metaphase-II [23]. The

data currently presented verified a clear impact of CL presence on camel oocyte maturation *in vitro*. Khalil [26] has been reported that the CL presence tended to lower the number of oocytes at GV and increased the rate of oocytes at GVBD and MII stages. The difference in camel oocytes attained nuclear maturation might be interrelated with the variation in time needed for the maturation *in vitro* [25]. As the oocytes of pregnant camels require 36 h of maturation to reach levels of >50% metaphase II stage, whereas 32 h is sufficient for the oocytes from non-pregnant animals [25]. Mahmoud *et al.* [4]

that the oocyte maturation time *in vitro* was 30h in camels, regardless the presence or absence of CL. Khatir et al. [34] found high rate (63%) of the oocytes reached to metaphase II-stage after 36 h of culture *in vitro*. Noteworthy, all retrieved camel oocytes (CL-bearing and non-CL bearing) were examined for maturational changes after 36-40 hours of culture.

Effect of reproductive status on the embryonal developmental competence

The data presented in Fig. 2 revealed that the

CL was none significantly influenced on the rate of cleavage, morula and blastocyst formation. The developed camel embryos from oocytes matured *in vitro* (n=66) retrieved from CL bearing ovaries were 36 (54.55%), 18 (27.27%) and 4 (6.06%) reached to cleavage, morula and blastocyst stages, respectively. In the interim, oocytes retrieved from non-CL bearing ovaries (n=72) and *in vitro* fertilized were 38 (55.56%), 18 (25.00%) and 5 (6.94%) reached to cleavage, morula and blastocyst stages, respectively. These results concomitant with those reported formerly by Scholkamy et al. [6] in camel.

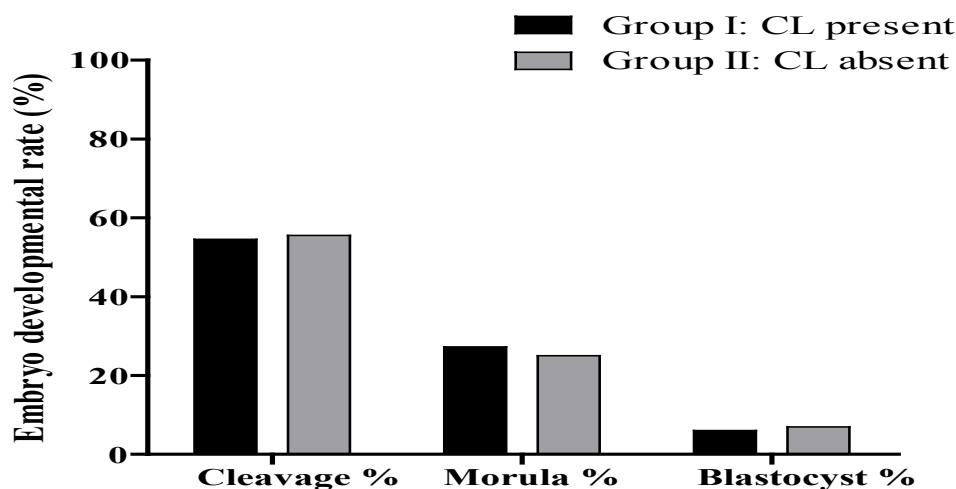


Fig. 2. Effect of corpus luteum on in-vitro embryo developmental competence in camels.

Conclusions

It could be concluded that the presence of CL, associated with pregnancy, negatively impacts on follicular growth and oocyte maturation. Nevertheless, these oocytes sustain its appropriateness for fertilization and embryo development likewise those retrieved from ovaries has no CL.

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تقييم تأثير الجسم الأصفر على النضج المعملّي وكفاءة نمو بويضات الجمل

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*قسم التوليد والتناسل والتلقيح الاصطناعي - كلية الطب البيطري - جامعة بنها **إدارة قلوب البيطرية - مديرية الطب البيطري - القليوبية و***قسم التكاثر الحيواني - المركز القومي للبحوث - القاهرة - مصر.

للتأكد من تأثير الجسم الأصفر على معدل تجميع و جودة و نمو البويضات، تم تجميع المبايض من الجمال المذبوحة أثناء موسم التكاثر و تقسيمها إلى مبايض تحتوي على الجسم الأصفر و أخرى لا تحتوي. تم تجميع البويضات من الجريبات ٢-٥ مم و تقسيمها على حسب جودتها. تم زراعة البويضات الجيدة معملياً و فحصها للنضج النووي بعد ٣٠ ساعة من التحضين. تم تخصيب البويضات بالحيامن من بربخ الجمال و تقييم انقسام و نمو الأجنة إلى مرحلة الموريولا و البلاستوسست. و أظهرت النتائج أن للجسم الأصفر تأثير معنوي على كمية و جودة النضج النووي لبويضات الجمال و كانت نسبة البويضات الجيدة و معدل نضج البويضات أعلى في غياب الجسم الأصفر. كما لم يسجل تأثير للجسم الأصفر على نسبة الانقسام و الموريولا و البلاستوسست.

و الخلاصة أن: على الرغم من تأثير الجسم الأصفر على عدد و جودة البويضات إلا أنه لم يكن هذا التأثير عائق أمام نمو الأجنة معملياً.