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Effect of Media on *In vitro* Maturation Rate of Dromedary Camel Oocytes

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Abstract: A total of 2127 oocytes were collected from 196 ovaries obtained from slaughter house. Two experiments were carried out. Experiment 1, aimed to investigate the recovery rate and oocyte quality during the breeding season. Experiment2, was designed to define the effect of *in vitro* maturation (IVM) media (TCM-199 and $CR1_{aa}$ with or without epidermal growth factor, EGF) on the *in vitro* maturation rate of dromedary camel oocytes. The quality of camel oocytes were classified according to their cumulus investment and evenly granulated ooplasm into excellent, good, fair and denuded. Excellent and good quality oocytes were cultured in TCM-199 (n=254), TCM+EGF (n=212), $CR1_{aa}$ (n=213) and CR1aa+EGF (n= 206) media in 5% CO_2 at 38.5°C for 40 h. Maturation was conducted by assessment of cumulus cell expansion and appearance of first polar body. Results revealed that the mean number of aspirated oocytes/ovary was 10.85. During breeding season a significant (P<0.01) higher percentage of excellent and good quality oocytes were recovered from ovary of dromedary camel. Cumulus expansion G2 was significantly higher (P<0.05) in oocytes *in vitro* matured in TCM-199 when compared with CR1aa media. Addition of EGF on *in vitro* maturation media (TCM199 or CR1aa) significantly increased (P<0.01 or P<0.05) maturation rate (87 and 83 % respectively) when compared with the same media without EGF (75 and 70 % respectively) of dromedary camel oocytes. In conclusion, breeding season was characterized by significantly higher recovery rate of excellent and good quality oocytes that suitable for *in vitro* embryo production in dromedary camel. Addition of EGF to maturation media improve maturation rate in dromedary camel oocytes.

Key words: *Camelus dromedarius* · Ovary · Oocytes quality · *in vitro* maturation · TCM-199 · $CR1_{aa}$ · EGF

INTRODUCTION

Camels are gaining popularity in many countries because of its ability to survive and perform well under arid and semi-arid climatic condition [1]. However, reproductive efficiency of camel under natural conditions is considered to be poor. The reasons for this low reproductive efficiency include the short breeding season; late age of reaching puberty and the long gestation period (thirteen months) long inter calving interval and higher incidence of early embryonic death [2]. Maintenance of high levels of reproduction in the camel is essential not only for profitable production but also to provide ample opportunities for selection and genetic improvement. The application of assisted reproductive technologies such as artificial insemination (AI), embryo transfer (ET) and *in vitro* embryo production (IVEP), would offer an opportunity to explore factors regulating developmental competence of camel oocytes and could improve the reproduction rate and genetic performance in camel[3]. The ultimate goal of an oocyte *in vitro* maturation and *in vitro* fertilization program (IVM/IVF) is to produce high-quality embryos, capable of normal pregnancies and live births following transfer to recipients on dromedary camels [4].

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Oocyte quality is one of the crucial factors affecting the success of *in vitro* embryo production programs. To a certain extent, oocyte morphology is correlated with developmental competence. Oocytes with fewer than five layers of cumulus cells and homogenous cytoplasm had lower cleavage rate than oocytes with more than five layers of cumulus cells [5]. Oocyte quality [6] and culture medium [7] are most important factors in *in vitro* maturation of follicular oocytes. Maturation conditions play an important part in embryo quality after *in vitro* fertilization (IVF) of oocyte [8, 9]. Several media have been used for *in vitro* embryo culture in a variety of species. There are few reports on *in vitro* maturation media in dromedary camel [3, 10, 11]. One of the most important factors regulating *in vitro* maturation rate is the culture system used for IVM. The components of the culture media and culture conditions can affect even modulate the meiotic regulation of mammalian oocytes [12-14]. It is therefore necessary to devise and optimize culture system that takes into account all the factors essential for the completion of oocyte maturation *in vitro*. Culture conditions for *in vitro* maturation in other domestic species have been improved in previous years such that a large percentage of oocytes successfully complete nuclear maturation [15-17]. Supplementation of *in vitro* maturation media with EGF enhanced nuclear and cytoplasmic maturation in bovine oocytes [18-20]. The degree of cumulus cell expansion and meiotic maturation rate had a relative importance in evaluating *in vitro* maturation [21, 14].

The aim of this study is to investigate the oocyte quality during breeding season and effect of maturation media (TCM-199 and $CR1_{aa}$) and supplementation with epidermal growth factor on cumulus expansion (cytoplasmic maturation) and maturation rate of Dromedary camel oocytes.

MATERIALS AND METHODS

The present study was carried out in the Department of Animal Reproduction and Artificial Insemination, Veterinary Division, National Research Centre, Giza.

All chemicals and media used in the present work were purchased from Sigma Company (Sant, Louis, MO, USA).

Experiment I: Oocyte Yield and Quality

Ovaries Collection: Camel ovaries were collected from El-Warraq slaughter house in Giza, in breeding season of 2010 to 2012 (October – March from each year). The ovaries were placed into a thermo container containing warm normal saline solution (NSS, 0.9% NaCl) at 35°C and transported to the laboratory within 2-3 h. At the laboratory, the ovaries were washed once with 70% ethanol and at least three times in NSS supplemented with 100 IU/ml penicillin and 100 µg/ml streptomycin.

Cumulus Oocytes Complex (COCs) Aspiration: The cumulus oocytes complex (COCs) were aspirated from follicles of 2 – 8 mm diameter using sterile 18 gauge needle containing 1 ml of aspiration medium (1 ml PBS plus 3 mg/ml bovine serum albumin BSA, Sigma, USA and 50 µg/ml gentamycin). Aspirated oocytes washed three times in maturation media and classified according to quality under stereomicroscope (Olympus, Japan) at 90 x.

Oocytes Yield and Quality: Camel oocytes quality (COCs) was classified under stereomicroscope (90 X) into four categories based on their cumulus investment and evenly granulated ooplasm [3].

Excellent Quality: Oocytes with five or more layers of complete cumulus-cells and evenly granulated dark ooplasm.

Good Quality: Oocytes with 1-4 layers of cumulus-cells and evenly granulated dark ooplasm.

Fair Quality: Oocytes with cumulus-cells incompletely surrounding the oocyte and little granulation in ooplasm.

Denuded: Oocytes without cumulus cells and covered by zona pellucida.

Experiment II: Effect of Media on *in vitro* Maturation of Camel Oocytes: The excellent and good oocytes were matured in four maturation media (five trials for each media). The *in vitro* maturation media were (TCM-199 or $CR1_{aa}$ with or without 20 ug/ml epidermal growth factor (EGF, Sigma)) +10 % fetal calf serum (Sigma) + 10µg/ml FSH (Sigma) + 50 mg/ml gentamycin. Oocytes were cultured in 5% CO_2, 95% humidity at 38.5C° for 40 h. The total number of oocytes that *in vitro* cultured was 254 in TCM-199, 212 in TCM-199 + EGF, 213 in $CR1_{aa}$ and 206 in CR-1aa + EGF media. Then assessment of cytoplasmic and was carried out by the degree of cumulus-cell expansion after 40 h of IVM. The criteria used for assessing the degree of cumulus expansion were as follow:

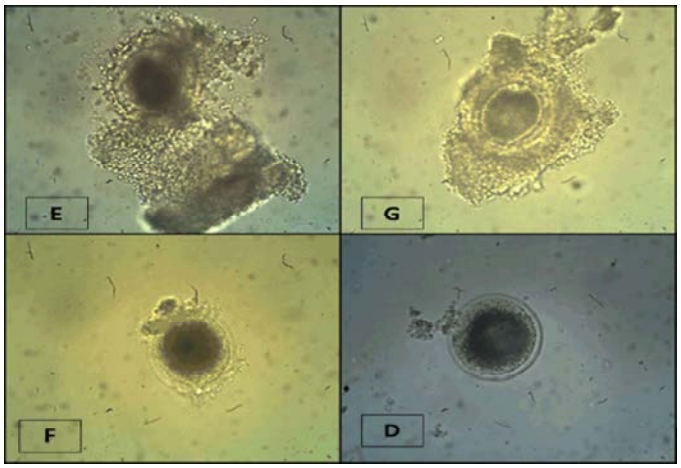

Fig. 1: Oocyte quality of dromedary camel during breeding season
E: Excellent G: Good F: fair D: Denuded

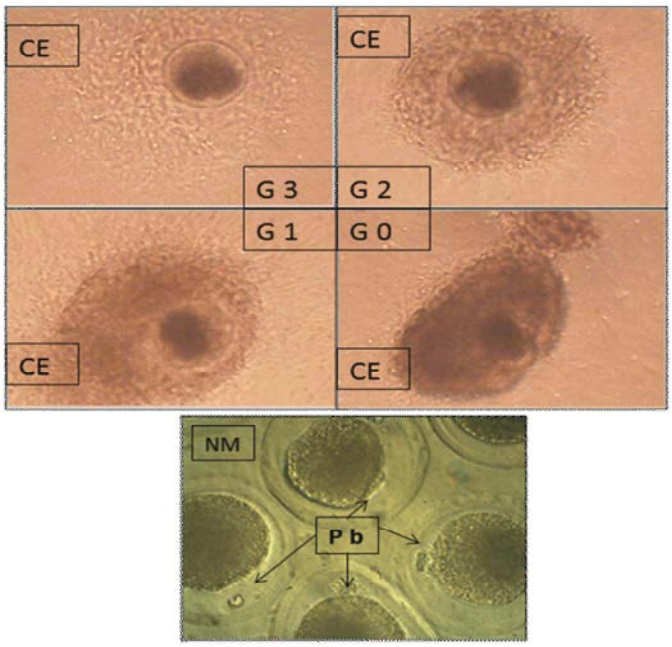

Fig. 2: Degree of cumulus expansion (CE, cytoplasmic maturation) and nuclear maturation (NM) with polar body (Pb) of in vitro matured dromedary camel
G3: Full CE G2: Moderate CE G1: Slight CE G0: No CE

- **Grade 0 (G0):** with no expansion.
- **Grade 1 (G1):** with slight expansion in the outer layer of cumulus-cells.
- **Grade 2 (G2):** with moderate expansion.
- **Grade 3 (G3):** with full expansion.

(2) The nuclear maturation: The presence of first polar body in the perivetteline space (M II) in the decomulated matured oocytes (using pipette) was the criteria for maturation of the oocyte.

Nuclear maturation rate (MII) was calculated as the number of matured oocytes (with polar body, MII) divided by the total number of oocytes multiplies by 100.

Statistical Analysis: Data were expressed as mean ± standard error (SE). The significance of differences was tested by paired t-test and analysis of variance (ANOVA) followed by post hoc test Statistical analyses were performed using SPSS (SPSS, 2008). The percentage differences were carried out by using decision analyst STATS™ 2.0.

Table 1: Oocyte yield and quality in dromedary camel during breeding season

				Oocyte quality			
Items	Ovary no.	Oocytes	No. of oocytes/ovary	E	G	F	D
N	196	2127	10.85	1043	544	276	264
Mean ± SE	5.16 ±1.17	55.16±9.91		27.45±1.01	14.32±0.33	7.26±0.29	6.95±0.72
%				49%	26%	13%	12%

Table 2: Effect of different media on cumulus expansion rate (%) and maturation rate (%) in dromedary camel oocytes during breeding season

	Cumulus expansion rate (%)				
Medium	G0	G1	G2	G3	MII (%)
$CR1_{aa}$+EGF	9 (18/206)	10 (20/2206)	29[a] (59/206)	53 (110/206)	83[a] (170/206)
CR1aa	15[a] (31/213)	15 (33/213)	18[c] (38/213)	52 (111/213)	70[c] (149/213)
TCM199+EGF	8[b] (16/212)	10 (22/212)	25 (54/212)	57 (120/212)	87[a] (184/212)
TCM199	10 (25/254)	12 (31/254)	28[a] (70/254)	50 (128/254)	75[b,d] (191/254)

a- c, a-d P<0.01 a-b P<0.05 within the column

Fig. 3: Oocyte quality in dromedary camel during breeding season

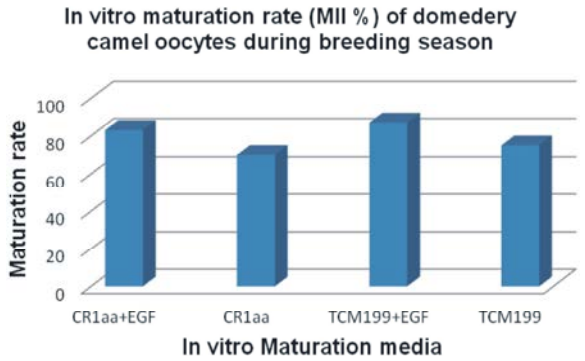

Fig. 4: Effect of different media on maturation rate in dromedary camel oocytes during breeding season.

RESULTS

Oocyte Yield and Quality: Ovaries (n=196) were collected during breeding season (Table 1), the total number of aspirated oocytes was 2127 and the mean number of aspirated oocytes/ovary was 10.85. Excellent and good quality oocytes showed highly significance (P<0.01) in mean ± SE and percentage (27.45±1.01, 49% and 14.32±0.33, 26% respectively) in breeding season when compared with fair and denuded oocytes (7.26±0.29, 13% and 6.95±0.27, 12 % respectively).

Effect of Media on *in vitro* Maturation of Dromedary Camel Oocytes: Table 2, revealed that cumulus expansion G2 was significantly higher (P<0.01) in CR1aa+EGF (29%) and TCM199 (28%) when compared with CR1aa media (18%) and CR1aa showed significant higher percent (15%) of matured oocytes without expansion (G0) than that observed in oocytes matured in TCM199+EGF (8%). Maturation rate (MII) was highly significant (P<0.01 or P <0.05) in media supplemented with EGF either in TCM199+EGF (87%) or CR1aa+EGF (83%) when compared with the same media without EGF (TCM199,75% and CR1aa, 70%).

DISCUSSION

Results obtained in this study showed that the mean number of oocytes recovered per ovary was 10.85. The results showed higher number of oocytes than that reported by Mahmoud *et al*. [22] who show that the total number of oocytes collected from the ovary during breeding season was in the average number of 5.3 oocytes /ovary. In the literature there are wide variations in number of oocytes per camel ovary, being 3.99 [23], 5.3 [22], 7.64 [3], 6.67[24], 4.1 [25], 3.5 [4] and 12.4 [26]. The high number of oocytes/ovary obtained in this study as compared to the others previously reported on camel may be attributed to pronounced differences in animal

ages, seasonal differences [3, 27, 28] reproductive status [3, 10], site of the ovary [23, 29] and method of oocytes collection [30, 31].

The results of the present study (Table 1) showed that significant higher percent of excellent and good quality oocytes than fair and denuded oocytes during breeding season of dromedary camel. Thus seasonal effect may induce complete follicular waves in which the growing follicles succeeded to reach maturity during breeding season [29]. The effect of different environmental and nutritional factors or techniques used may play a role [28].

The current results showed that cumulus expansion rate (G2) was highly significant in using TCM-199 when compared with $CR1_{aa}$ in camel oocyte maturation for 40 h. The expansion of cumulus cells in our results depends largely on the culture media (TCM-199 & $CR1_{aa}$) used for maturation of the oocytes and supplements in this media that enhance cumulus expansion. In mammals, oocytes are ovulated in metaphase II (MII) due to the presence of greater amount of maturation promoting factor (MPF) and cytostatic factor (CSF). The differences in maturation percentage may be attributed to the composition of the media itself and the expansion of cumulus cells depends largely on the media used [32].

The results of our experiment show that a higher nuclear maturation rate (MII) can be achieved when camel oocytes are cultured in media (TCM199 or CR1aa) supplemented with EGF. Similar results were previously reported in buffalo [33]. The action of EGF is likely to be on the oocyte itself, as was suggested by Lonergan *et al*. [20] in bovine oocytes. The zona pellucida allows the passage of molecules as large as 150 kDa in the mouse [34], the camel zona may be somewhat similar in this regard. Hence, the relatively small EGF molecules (~6kDa) are likely to traverse easily through the zona of camel oocytes to exert its effect directly on the oocyte. The presence of EGF receptors on the camel oocyte and their up-regulation during the mid follicular and the pre-ovulatory period further strengthen this possibility [35]. Thus, EGF may have exerted its positive effects directly on the oocyte after binding to its receptor and activating the tyrosine kinase[36]. EGF increased proteoglycan synthesis [37] and production of tissue plasminogen activator and urokinase plasminogen activator by cumulus cells and oocytes stimulated by EGF [38] and it may also be due to stimulation of DNA synthesis in cumulus cells by EGF and FSH [39].

In conclusion, breeding season was characterized by significantly higher recovery rate of excellent and good quality oocytes that suitable for in vitro embryo production in dromedary camel. Moreover, addition of EGF to maturation media improve maturation rate in dromatery camel oocytes.

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