'et.Med.J.,Giza. Vol.51, No.3. (2003) :411-420.

:HROMOSOME CONFIGURATION DURING *IN VITRO* MATURATION)F DROMEDARY CAMEL OOCYTES

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teceived: 26.5.2003.

ıccepted: 30.6.2003.

SUMMARY

The aim of the present study was to examine the changes in chromosomal configuration of camel oocyte during in vitro maturation (IVM) at different culture periods of 24, 30, 36 and 48 hours and to compare the influence of pregnant camel serum and fetal calf serum on IVM of camel oocytes at 24 – 30 hours.

A total of 1978 oocytes were collected from 390 ovaries of slaughtered camels by aspiration method. The average number of camel oocytes per ovary was 5.30 ± 0.22. The proportion of COC, POC and DO oocytes was 29.27, 32.90 and 32.61% respectively. In addition 5.21% of oocytes were degenerated.

A high percentage of maturation was obtained at 24 – 30 hours (47.70% and 48.35% respectively). At 48 hours post-maturation, all metaphases were undefined due to degeneration of chromatin. it was found that, addition of pregnant camel serum had no beneficial effect on IVM of camel oocytes in comparison to fetal calf serum (40.10 vs. 45.34% respectively).

INTRODUCTION

The capacity for fertilization and embryonic development depends on nuclear and cytoplasmic maturation of the oocytes (Crister et al., 1986). Mammalian oocytes can spontaneously undergo meiotic maturation in vitro (Edwards, 1965). Nuclear events such as germinal vesicle breakdown and polar body formation appear to occur normally during this spontaneous maturation (A'arabi et al., 1997). The changes that take place within an oocyte during maturation, help in the completion of meiotic division (Parrish et al., 1992 and Downs, 1993) and formation of metaphase II stage at which the oocytes are competent to

undergo normal fertilization and development (Mahmoud, 2001). Information available on in vitro maturation (IVM) of camel oocytes are scanty and incomplete. Elucidating details of chronology of maturation events will help in optimizing the protocol for IVF in camels. Also, the available literature does not offer a ready comparison of beneficial effect of fetal calf serum and pregnant camel serum additives on IVM of camel oocytes.

The present study therefore was undertaken to determine 1- The basis of sequential changes in chromosome configuration of camel oocytes nucleus during IVM at the different periods (24, 30, 36 and 48 hours). 2- The suitable period of maturation of camel oocytes. 3- The effect of unheated pregnant camel serum and fetal calf serum on maturation at 24 – 30 hours.

MATERIALS AND METHODS

Collection and classification of oocytes.

Ovaries were collected from camels of unknown reproductive history (4-20 years old) slaughtered in Cairo abattoir during breeding season (October to March 2001). Within 2 hours of slaughter, the ovaries were transported in physiological normal saline (0.9%, w/v, NaCl) with antibiotic maintained at 30°C to the lab. The ovaries were washed three times in phosphate buffered saline (PBS). The oocytes were aspirated from small antral follicles 1 to 5 mm in diameter using an 20-gauge needle attached to a 5 ml syringe containing PBS with 3% Bovine Serum Albumin (BSA) and antibiotics (100 µg/ml streptomycin and 100 IU/ml penicillin). The oocytes were counted and classified according to their quality into four categories:- COC (Cumulus oocytes complex), POC (Partial oocytes complex), DO (Denuded oocytes) and degenerated (fragmented denuded oocytes). The oocytes were washed three times in TCM-199 with Earle's Salt and Hepes (Sigma, USA) plus 0.2% L-glutamine and antibiotic.

Maturation of oocytes

Recovered oocytes with completely or partially investing cumulus cell layers were matured in vitro using TCM-199 supplemented with 10% sera according to the design of the experiments at 39°C in atmosphere containing 5% CO2 and 95% relative humidity.

Experimental design

1- Oocytes were cultured in TCM-199 + 10% FCS for 24, 30, 36, 48 hours.
2- Oocytes were cultured in TCM-199 + FCS and TCM-199 + 10% pregnant camel serum for 24 – 30 hours.

Preparation of pregnant camel serum (PCS)

Twenty ml of blood were obtained from pregnant camels slaughtered at a local slaughterhouse. The pregnancy was proved by presence of conceptus in different stages in the uterus after slaughter according to El-Wishy et al. (1981). The fetal age

ranged from 6-7 months. The blood was centrifuged at 500 g for 10 min. The serum was separated and stored at -20°C until required for the culture of the oocytes.

Chromosome preparation:

Cytogenetic analysis was attempted in 395, 306, 209 and 200 oocytes at 24, 30, 36 and 48 hours after culture respectively. Chromosomal preparations of oocytes were performed using a modification of the air-drying technique as described by Tarkowski (1966). Each oocyte was transferred to 1% hypotonic sodium citrate solution for 10 minutes and then placed on a microscope slide with a minimal amount of hypotonic solution. Three drops of fixative (methanol: acetic acid, 3:1) were dropped onto the oocytes. Subsequently, the fixed material was stained with 1% orcin stain. The different stages of nuclear configuration were classified according to Datta and Goswami (1999) into metaphase I, anaphase I, telophase I and metaphase II. The oocytes, that reach telophase I and metaphase II stages, were considered as matured oocytes.

Statistical analysis

All data were transformed to arcsin and analyzed using ANOVA and Student's t-tests at a confidence limit of 95% using SAS statistical computed program (SAS user's guide, 1988).

RESULTS

A total of 1978 oocytes were collected after aspiration from 390 camel's ovaries during breeding season. Fig. 1 demonstrates the distribution of different morphological classes of camel oocytes obtained in breeding season. The average number of recovered oocytes per ovary was 5.30 ± 0.22. Perusal of table 1 indicated that, about one third (32.61%) of collected oocytes was denuded, while 5.21% of the oocytes was fragmented. The percentage of compact and partially denuded cumulus cells was 29.27 and 32.90 % respectively.

Table 2 and fig. 2 illustrated chromatin configuration of 1110 camel oocytes at different culture periods (24, 30, 36 and 48 hrs). Of the examined oocyte 14.80, 11.72, 7.81 and 35.93% had undergone germinal vesicle breakdown (GVBD), metaphase I, telophase I and metaphase II at 24 hours post IVM respectively. The proportion of oocytes in the GVBD, anaphase I and telophase I stages was found to decrease gradually from 24 to 30 hours post IVM followed by degeneration at 48 hours. Maximal proportions of metaphase II was observed at 30 – 36 hours (42.37 and 41.66%, respectively). The frequency of metaphase I stage was nearly constant at 24 – 30 hours (11.72 and 11.86%, respectively) and degeneration occurred at 48 hours. After 48 hours all metaphases were undefined due to degeneration of chromatin. No

diploid oocytes were observed in the present study.

The data in table 3 showed that the maturation rate (telophase I and metaphase II) was 43.68 ± 2.67, 44.06 ± 3.43, 41.20 ± 2.44 at 24, 30 and 36 hours respectively and none was recorded at 48 hours. No significant difference in maturation rate was observed among the different culture periods.

Concerning the effect of fetal calf serum and pregnant camel serum on the percentage of telophase I and metaphase II, the maturation rate of camel oocytes cultured in FCS and PCS was 45.34 and 40.10% respectively, at 24-30 hours (Table 4). Statistical analysis showed no significant differences between the two sera used for in vitro maturation.

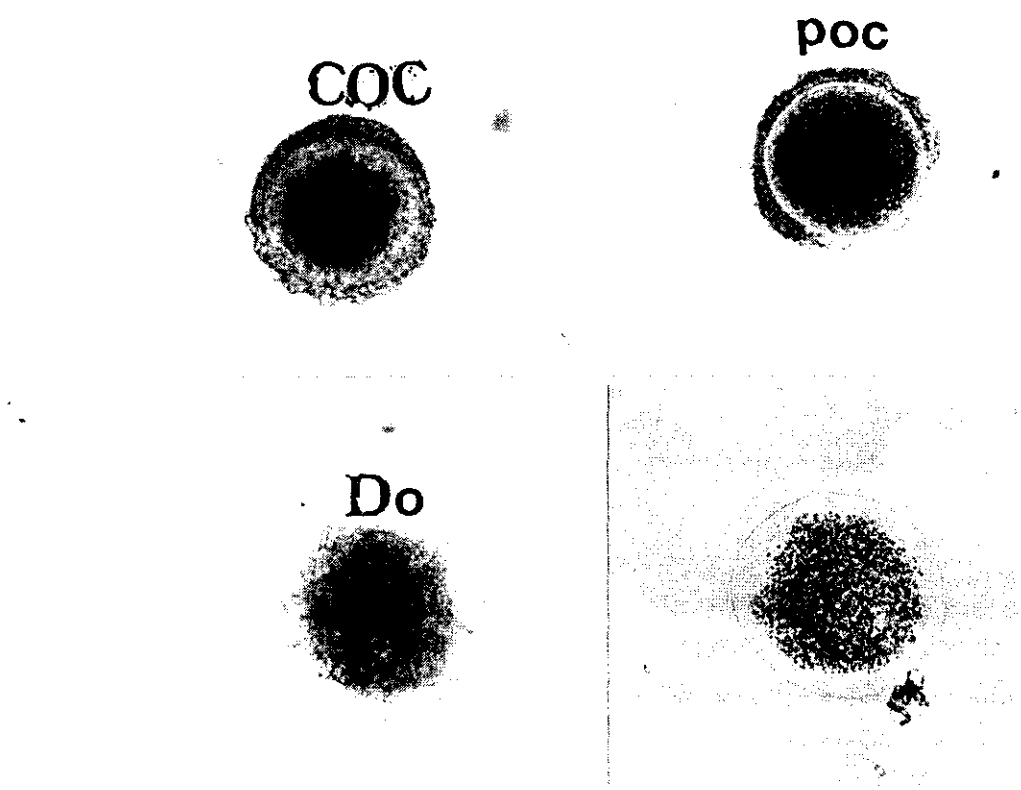

Fig. 1. Quality of recovered immature camel oocytes: COC (compact oocyte complexes); POC (Partially denuded oocyte), DO (denuded oocyte) and degenerated oocytes (x 400).

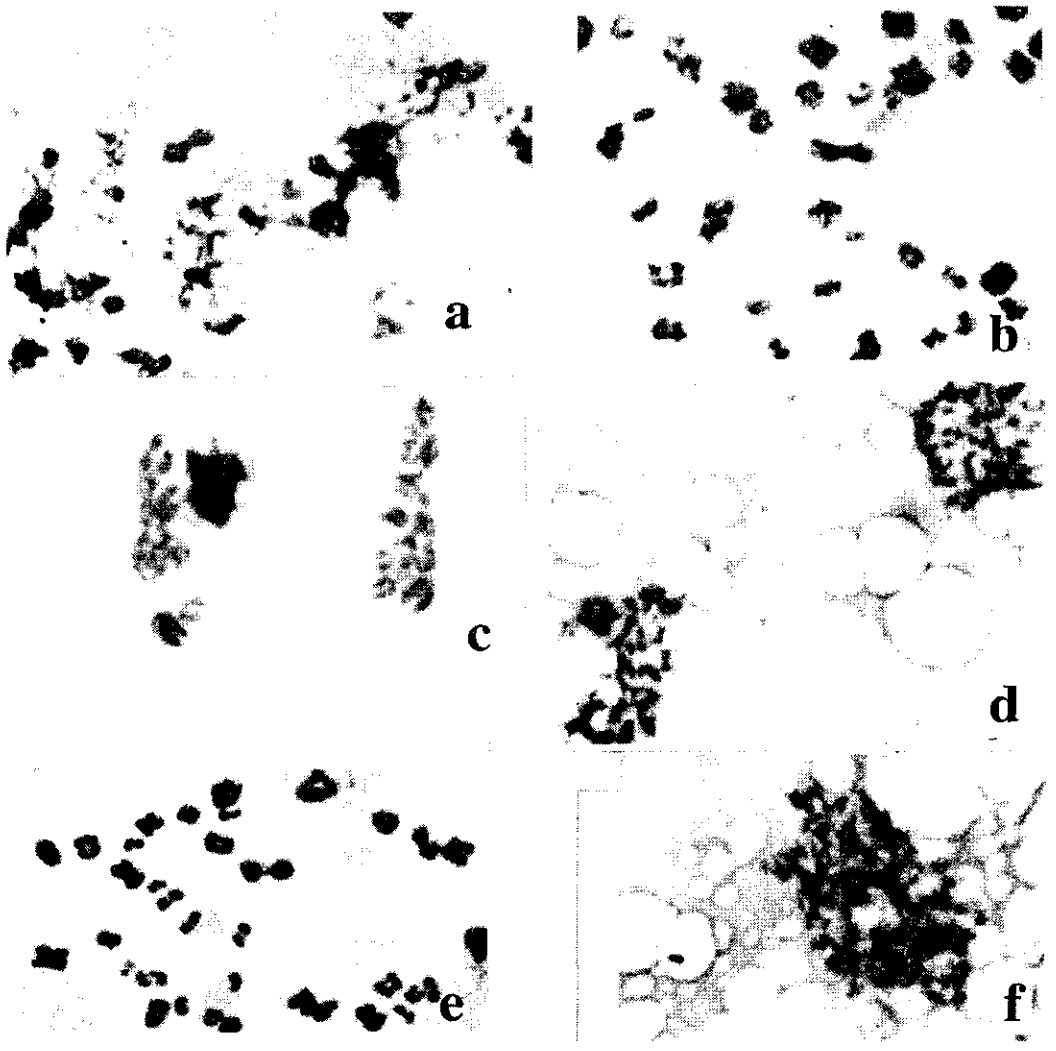

Fig. 2. Chromosome configurations of camel oocytes (x 400).

(a) Post germinal vesicle breakdown stage showing isolated chromatin mass.

(b) Oocyte at metaphase I stage of meiosis, note the bivalent chromosome.

(c) Complete homologous segregation of chromosomes at anaphase I.

(d) Oocyte at telophase I stage of meiosis showing two groups of equally spread homologous chromosomes.

(e) Oocyte at metaphase II. Note the normal haploid number.

(f) Undefined metaphase. Note chromatin degeneration.

Table (1): Recovery and quality of camel oocytes by aspiration method during breeding season (Mean ± SEM).

No.of ovaries	No.of oocytes	No.of trials	Average no. of oocytes per ovary	Classification of oocytes								Oocytes suitable for maturaiton	
				Compact cumulus cells (COC)		Partially denuded cumulus (POC)		Completely denuded cumulus (DO) (normal morphology)		Completely denuded cumulus (DO) (abnormal morphology)			
				No.	%	No.	%	No.	%	No.	%	No.	%
390	1978	24	5.30 ± 0.22	579	29.27	651	23.90	645	32.61	103	5.21	1230	62.18

Table (2): Chromosomal analysis of in vitro matured camel oocytes at different culture periods

Cultured period (hrs)	No.of cultured oocytes	No.of fixed oocytes	No. of metaphases suitable for observation	Germinal vesicle breakdown		Metaphase I		Anaphase I		Telophase I		Metaphase II		Undefined	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
24	395	303	128	19	14.8	15	11.72	7	5.46	10	7.81	46	35.93	31	24.21
30	306	210	59	7	11.86	7	11.86	1	1.49	3	5.08	25	42.37	16	27.12
36	209	163	48	-	-	-	-	-	-	1	1.8	20	41.66	27	56.25
48	200	153	47	-	-	-	-	-	-	-	-	-	-	47	100
Total	1110	829	282	26	9.21	22	7.80	8	2.83	14	4.96	91	32.26	121	42.90

Table (3): In vitro maturation rate (Telophase I+ Metaphase II) of camel oocytes at different culture periods

Time (hrs)	24	30	36	48
Maturation rate	43.68 ± 2.67^a (47.70%)	44.06 ± 3.43^a (48.35%)	41.20 ± 2.44^a (43.40%)	00.00*

Mean ± SEM (according to C.I. Bliss, Angle = Arc Sin$\sqrt{\text{percentage}}$)

(the return % value)

Same superscript indicates non significant difference in the row at ($P<0.05$).

* The metaphases were undefined due to degenerating chromatin

Table (4): Effect of fetal calf serum and pregnant camel serum on in vitro maturation rate of camel oocytes at 24-30 hours.

Type of serum	No. of cultured oocytes	Maturation rate (Telophase + Metaphase II)
Fetal calf serum (FCS)	485	41.34 ± 1.53^a (45.34%)
Pregnant camel sèrum (PCS)	271	39.29 ± 1.60^a (40.10%)

Mean ± SEM (according to C.I. Bliss, Angle = Arc Sin $\sqrt{\text{percentage}}$)
(the return % value); Same superscript indicates non significant difference between values in the column at ($P<0.05$).

DISCUSSION

The number and the quality of oocytes are influenced by the method of oocyte recovery (Alm et al., 1997). In our study, 5.30 oocytes/ovary were recovered by aspiration method. However, Abdoon (2001) recovered 8.90 oocytes/camel ovary by the same method. In our study, the lower number of oocytes recovered could, perhaps, be attributed to the beginning of the breeding season (October; marked with lower follicular growth). This is also reported by Torner et al. (2003). In the present study, the percentages of COCs, POC and DO oocytes were 29.27; 32.90 and 32.61% respectively. These results are nearly similar with the report of Torner et al. (2003) who also found that the percentage of denuded oocytes was less than 7% by slicing method. This variation in the rate of denuded oocytes may be attributed to difference in the method of recovery and presence of corpus luteum. About 5.21% of camel oocytes showed different sizes of fragments called degenerated oocytes in the current work. This type of oocytes was not recorded in other animals as buffalo (Mahmoud, 2001), cattle (Hegab, 1991) and sheep (El-Shahat, 2001). The percentage of oocytes suitable for maturation, in the current work, was similar to that reported in buffaloes by Hamam et al. (2001).

The in-vitro matured camel oocytes demonstrated wide range of meiotic configurations namely metaphase I, anaphase I, telophase I and metaphase II. This is in agreement with finding previously reported for cattle and horse (Ectors et al., 1995 and Sosnowski et al., 1997). There were no diploid metaphases recorded in the present study as compared to cattle and buffaloes (Mahmoud, 2003). The low proportion of oocytes showing metaphases with increased incidence of undefined metaphases may be attributed to the fact that most of degenerative changes of chromatin as a result of old age of slaughtered camels that consequently affect oocyte quality. In this respect, Ghoneim et al. (1999) reported that higher percentage of apoptotic cumulus cells after in vitro maturation was observed in in vitro maturation of camel oocytes. Moreover, Moor et al. (1998) suggested that the key to maturation and embryo viability in vitro resides in the follicular compartment rather than the oocyte.

Our investigation explored that a high in vitro maturation rate was achieved at 30 hours. These findings coincide with those previously reported in camel (Kafi et al., 2002) and in llama (Del-Campo et al., 1994). In contrast, Abdoon (2001) and Torner et al. (2003) reported that 36 hours produced a higher percentage of cumulus expansion and metaphase II stage.

In bovine, Ibrahim (1993) recorded that pregnant cow serum at day 20 was superior to fetal calf serum for embryo supporting. Similar findings were not recorded in the present investigation of in vitro maturation for camel oocytes.

In conclusion, the optimal duration of in vitro maturation for camel oocytes is 24 to 30 hours. The maturation rate does not improve with the addition of pregnant camel serum instead of fetal calf serum.

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