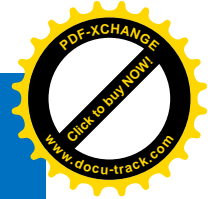
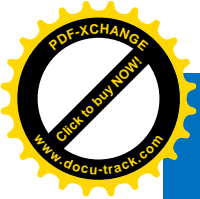


# Effect of breeding season and epidermal growth factor on the competence of camel (*Camelus dromedarius*) oocytes to mature in vitro

Ashraf El-Sayed<sup>1</sup>, El-Sayed El-Hassanein<sup>2</sup>, Hend Sayed<sup>2</sup> and Ashraf Barkawi<sup>3</sup>

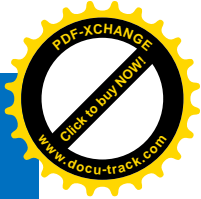
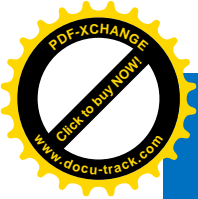
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# Introduction



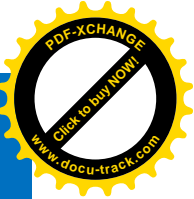
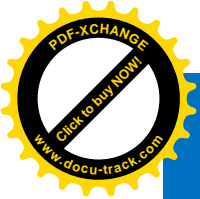
- Fertility of camels is low under natural pastoral conditions due to interaction of several environmental and genetic factors.
- Application of modern biotechnologies in camels is not only needed but also essential to improve their reproductive performance and productivity.
- Recently, a marked progress in multiple ovulation and embryo transfer (MOET) has been achieved in camels depending on natural mating or insemination with fresh semen.
- The most important step in laboratory embryo production is the maturation of the follicle with enclosed immature oocytes



# Objectives



- Evaluate the effect of hormonal additives to culture medium on in vitro oocytes maturation
- Evaluate the seasonal effect on in vitro oocyte maturation



# Material and methods



## Oocytes collection

- Ovaries were collected from dromedary camels of unknown reproductive history from slaughterhouse
- Ovaries brought to the laboratory in a thermos flask containing warm normal saline solution (NSS) at 37°C
- Ovaries were processed within 3-4h of collection



## Material and methods (cont.)

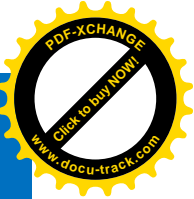
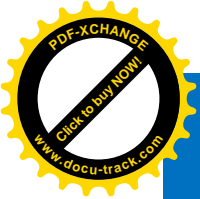
- Ovaries were washed 2- 3 times with warmed (37 °C) NSS containing 500µl/ml gentamicin to remove adhering clotted blood
- all ovaries were quickly washed once with ethanol (70%) to remove any contamination on the surface of the ovaries
- all collected ovaries were placed in glass jars containing NSS and stored in water bath



## Material and methods (cont.)

- Cumulus oocytes complexes (COCs) were harvested by slicing in warm (37°C) phosphate buffered saline (PBS) supplemented with 500 µg/ ml gentamicin
- oocytes from every group were collected and evaluated under stereomicroscope to good quality oocytes and poor quality oocytes





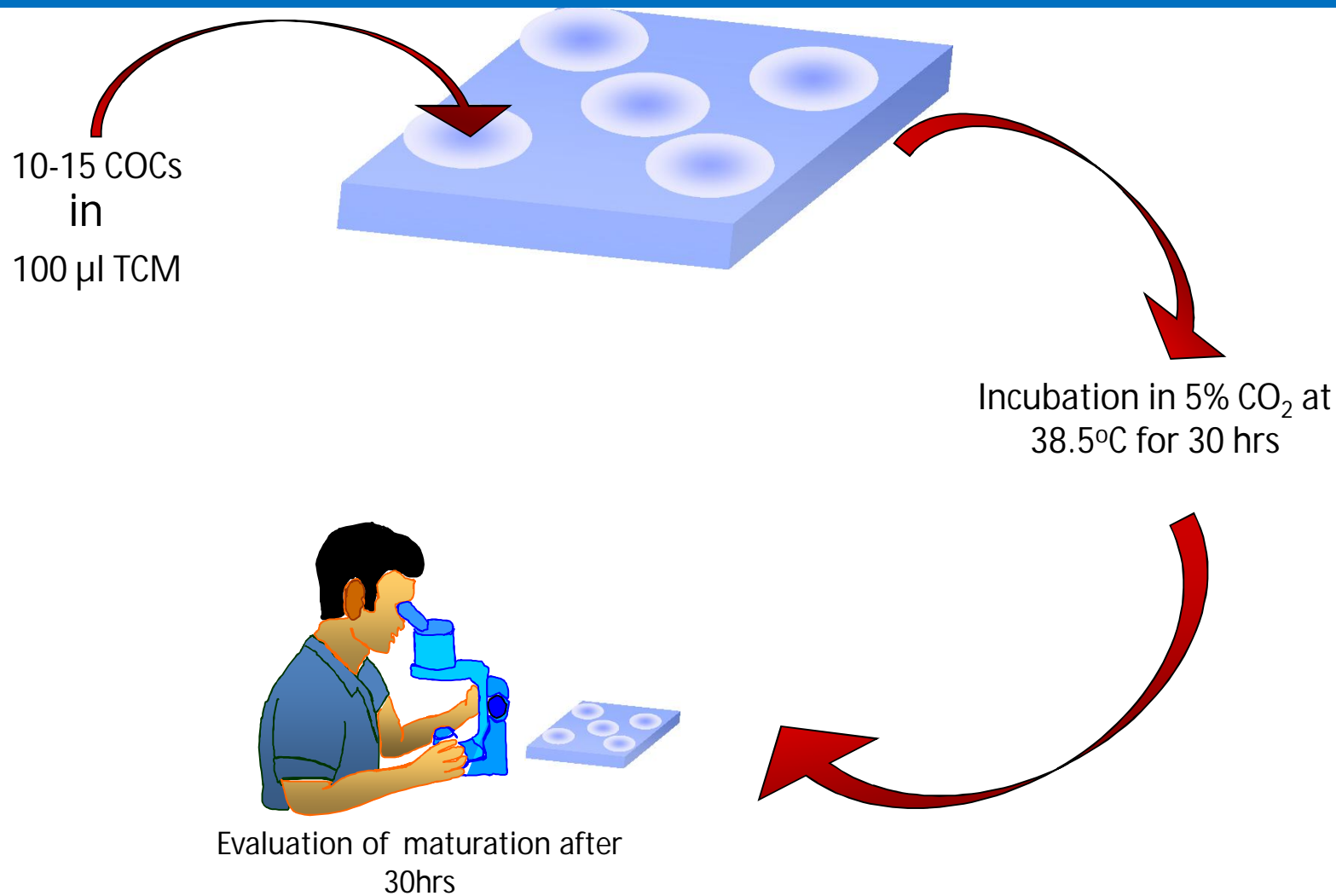
# Material and methods (cont.)



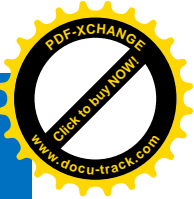
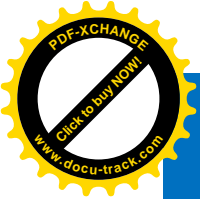
## Oocytes Maturation

- In the day of work, culture medium TCM-199 were supplemented with 10% (v/v) heat-treated (56°C for 30 min) fetal bovine serum (FBS), 1 µg/ml follicle stimulating hormone (FSH), 1 µg/ml estradiol (E2) and 50 µg/ml gentamicin (control group G1)
- The effect of epidermal growth factor (EGF) additive on the potential of oocytes to mature in vitro was study, finally maturation medium TCM-199 was supplemented with 10 ng/ml EGF (treatment group G2)
- The good quality of COCs in two groups (G1 & G2) were washed twice in warm PBS supplemented with 50µg/ml gentamicin and twice in maturation medium

# Material and methods (cont.)







# Material and methods (cont.)



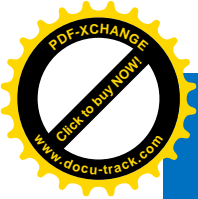
## Effect of Breeding season

- COCs were collected by slicing the ovaries, during breeding season(BS) from January to April and non-breeding season (NBS) from May to September
- The effect of EGF additive on the oocytes to mature *in vitro*, during the BS and NBS, was studied
- The percentage of cumulus expansion was recorded in BS and NBS in every group and maturation rate was calculated.

# Results

Table (1): Mean values of oocytes recovered from ovaries in TCM-199 medium and TCM-199+ Epidermal growth factor (EGF) in dromedary camels

<b>Trait</b>	<b>G1 (TCM-199)</b>	<b>G2 (TCM-199+EGF)</b>
<b>No. of ovaries</b>	55	48
<b>Average of recovered oocytes/ovary</b>	7.45±0.73 (381)	8.14±1.0 (348)
<b>Average of good quality of oocytes/ovary</b>	6.37±0.59 (339)	7.16±0.91 (309)
<b>Average of poor quality oocytes/ovary</b>	0.91±0.18 (42)	0.97±0.21 (39)



## Results (cont.)



Table (2): Effect of EGF on oocytes maturation (%) in dromedary camel after culture in TCM-199 medium measured after 30 h incubation.

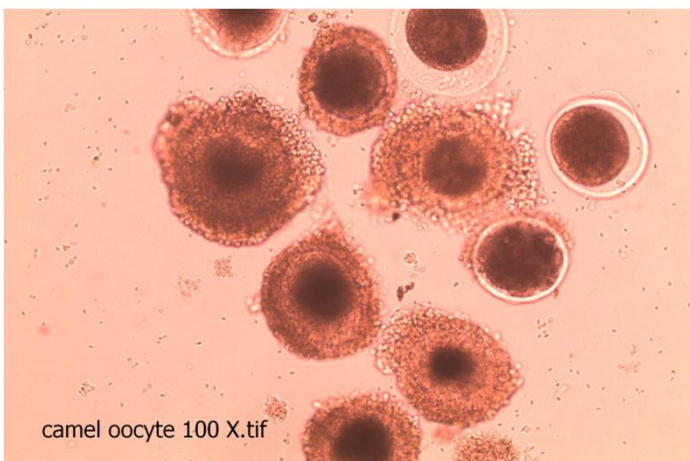
<b>Trait</b>	<b>G1 (TCM-199)</b>	<b>G2 (TCM-199+EGF)</b>
<b>No. of oocytes</b>	339	309
<b>Matured oocytes (%)</b>	62.27±3.82 <sup>b</sup> (205)	76.45±3.86 <sup>a</sup> (243)

# Results (cont.)

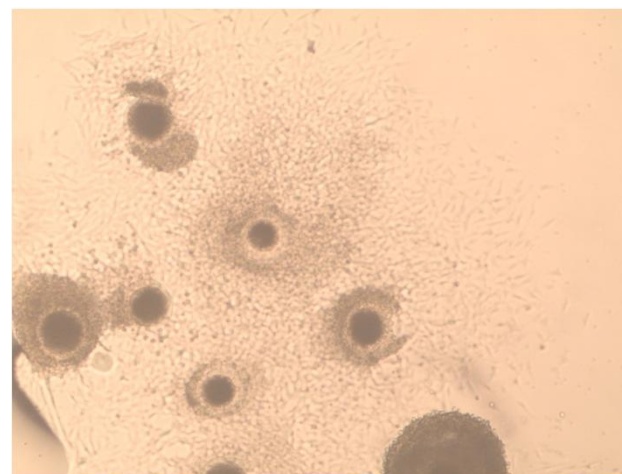
Table (3): effect of EGF and season on maturation rate in camel oocytes after 30h incubation

Trait	Season effect			
	BS		NBS	
	G1 (TCM-199)	G2 (TCM-199+EGF)	G1 (TCM-199)	G2 (TCM-199+EGF)
<b>No. of oocytes</b>	364	383	285	340
<b>Maturation rate (%)</b>	57.88±4.5 (200)	69.82±5.9 (274)	85.53±2.8 (245)	95.74±1.5 (328)

# Selected photos

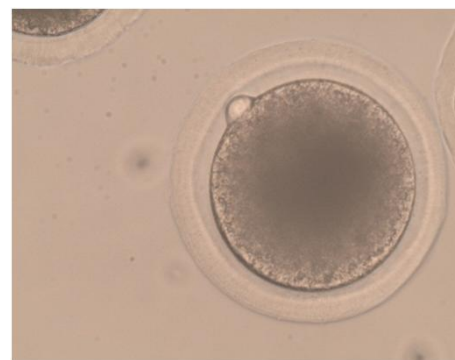


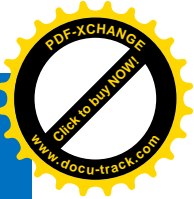
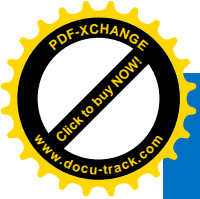
Im-matured oocytes cultured in  
TCm-199 Medium



Matured oocytes cultured in  
TCm-199 Medium

Matured oocyte with the 1st  
polar body

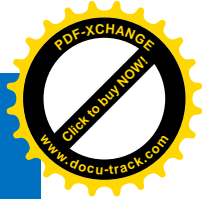
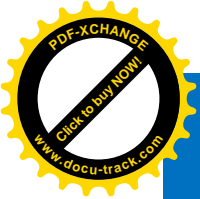




# Conclusion

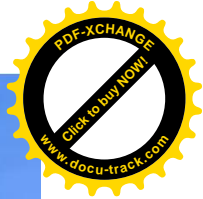
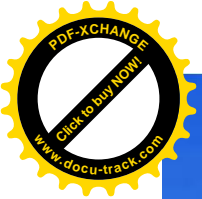


- Maturation of oocytes in TCM-199 medium supplemented with EGF for 30 h resulted in higher percentage of cumulus expansion when compared with oocytes cultured in TCM-199 medium
- There is no significant differences between BS and NBS in maturation rate.



## Selected references

- Skidmore J.A. (2005). Reproduction in dromedary camels: an update. *Animal Reproduction Science*. 2 (3): 161-171. (introduction)
- Khatir H., A. Anouassi, A. Tibary. (2007). Effect of follicular size on *in vitro* developmental competence of oocytes and viability of embryos after transfer in the dromedary (*Camelus dromedarius*). *Animal Reproduction Science*. 99: 413-420.(material)
- Amer H. and A. Moose (2008). Relationship between season of the year, culture medium and *in vitro* oocyte competence in dromedary camels. *Bulgarian Journal of Veterinary Medicine*. 11(3): 195-204.(material)
- Khatir H., A. Anouassi, A. Tibary. (2004). Production of dromedary (*Camelus dromedarius*) embryos by IVM and IVF and co-culture with oviductal or granulosa cells. *Theriogenology*. 62: 1175-1185.(material).



**Thank you for your attention**

