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Effect of Culture Media and Epidermal Growth Factor on In Vitro Oocyte Maturation in the One-Humped Camel (Camelus dromedarius)

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Abstract: Culture conditions for in vitro embryo production (IVP) in domestic species have been improved in recent years. Despite the low reproductive performance, camels are probably the last large domestic species to experience the benefit from these in vitro culture techniques. The aim of the present study was to compare the in vitro maturation (IVM) of camel oocytes in different culture media. The effect of epidermal growth factor (EGF) on camel oocyte maturation was also examined.

Oocytes (n=8805) were aspirated from 604 camel ovaries during the breeding season. Selected oocytes were cultured in TCM-199 and CR1aa media at 38.5 ºC, under 5% CO_2 for 40 h. Oocytes were also matured in culture media supplemented with EGF (20 ng/ml).Maturation of camel oocytes was assessed at end of incubation. Assessment of cytoplasmic maturation of camel oocytes was carried out through 4 grades (G0, G1, G2 and G3) of cumulus cell expansion. Nuclear maturation rate (MII) was calculated based on number of oocytes with 1st polar body.

In the present study, retrieval rate of camel oocytes was 14.58%. TCM-199 and CR1aa culture media for camel oocytes showed no significant differences in cytoplasmic maturation expressed as G3 cumulus expansion (66.02%, 63.43% respectively) and nuclear maturation expressed as MII (77.90%, 79.01 % respectively).Addition of EGF (20 ng/ml) to the TCM-199 maturation medium significantly increased the proportion of camel oocytes reaching cytoplasmic maturation rate (G3, 72.46%) and nuclear maturation metaphase II (MII, 87.93 %).When present in CR1aa maturation medium, EGF significantly improved cytoplasmic maturation rate (G3, 68.98%) with 82.00% nuclear maturation rate (MII) of camel oocytes. Hence, our data indicate that TCM-199 and CR1aa media are suitable for in vitro maturation of camel oocytes. EGF supplementation improves camel oocyte maturation especially in TCM-199 culture medium.

Keywords: Camel oocytes, culture media, in vitro maturation, epidermal growth factor.

Introduction

The camel has been a very important animal for centuries in the desert regions and is gaining popularity in many countries of the world because of its ability to survive and perform well under arid and semi-arid climatic conditions. Development of camel racing in the Middle East has led to increased interest in improving reproductive efficiency. The low reproductive performance is one of the most important factors affecting camel productivity [1,2,3,4,5,6,7].

Reproductive technologies have been developed to improve reproductive efficiency and genetics of animals as well as for infertility treatment. Great progress has been made in most farm animals. The camelids are probably the last large domestic species to experience the benefit from these technologies[8,9,10,11,12,13,6,7].

In vitro production of embryos (IVP) is an important reproductive biotechnology for the multiplication of genetically superior animals and the preservation of genetics. Methods for (IVP) are prevalent for cattle; proved by the birth of innumerable calves worldwide[14]. In camelids, relatively limited information is available on development of this technology. The production of embryos by IVM/IVF in camelids was first reported in llamas [15], however, the first offspring's were produced in dromedary camel in 2006 by Khatir and Anouassi[16].

Improvement of in vitro embryo production is mainly dependent on oocyte developmental competence. One of the most important factors regulating the number and quality of oocytes maturing in vitro, is the culture system used for IVM. The components of the culture media and culture conditions can affect and even modulate the meiotic regulation of mammalian oocytes[17,18,19]. Culture conditions for in vitro maturation in other domestic species have been improved in recent years such that nowadays a large percentage of oocytes successfully complete nuclear maturation[20,21,22];Therefore, it is necessary to devise and optimize culture system that takes into account all the factors essential for the completion of oocyte maturation in vitro [23].

TCM-199 and CR1aa media were used successfully for the in vitro maturation of domestic species [19,24,25,26,27]. Moreover, the culture employed in IVM not only affect the proportion of the oocytes to reach metaphase II (MII) and become cable of undergoing in vitro fertilization, but can also influence subsequent embryonic development [28,29,23]. It has also been demonstrated that epidermal growth factor (EGF) participates in the regulation of many ovarian function as a potent mitogen for granulosa cells[30], a biological amplifier of FSH action in the ovary [31] and a better oocyte maturation rate[32].

The present study was carried out to evaluate the:

1. Influence of maturation media (TCM-199 and CR1aa) on in vitro maturation (IVM) of camel oocytes (Experiment 1).

2. Effect of addition of epidermal growth factor (EGF) to the in vitro maturation media on maturation rate of camel oocytes (Experiment 2).

Materials and Methods

The present study was conducted in the Department of Animal Reproduction and Artificial Insemination, Veterinary Research Division, National Research Center, Cairo, in collaboration with the Department of Theriogenology, Faculty of Veterinary medicine, Cairo University. The experimental work was carried out during the breeding season for two successive years from November (2013, 2014) to March (2014, 2015).

1. Materials:

1.1. Ovaries:

Ovaries (n=604) were collected from camels of unknown reproductive history, at El-Bassatin main slaughterhouse in Cairo.

1.2. Chemicals:

All chemicals and media used in the present work were purchased from Sigma-Aldrich (Saint. Louis, MO, USA) unless otherwise mentioned.

1.3. Media:

1.3.1. Aspiration medium

Phosphate buffer saline (PBS) supplemented with 3 mg/ml bovine serum albumin (BSA) and 50 µg/ml gentamycin was used as aspiration medium.

1.3.2. Basic maturation media

9 m1 TCM-199 or CR1aa media were supplemented with10% fetal calf serum (FCS, Sigma), 10µg/m1 FSH (Foltrobin-V) and 50 µg/m1 gentamycin sulphate with or without addition of 20 ng/m1 epidermal growth factor (EGF, Sigma). All media were sterilized before use by passing through Millipore filter 0.22 µm in diameter fitted on 10 ml syringe to remove bacteria particulates[32].

2. Methods

2.1. Ovaries Collection

Camel ovaries were selected from main slaughterhouse in Cairo and placed into a thermos containing warm normal saline solution (NSS, 0.9% NaCl) at 37ºC to be 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 normal saline (NSS) supplemented with 100 IU/m1 penicillin and 100 µg/m1 streptomycin[33].

2.2. Retrieval Rate of Camel Oocytes

Camel ovaries (604) were selected for aspiration of oocytes. Retrieval rate was calculated by dividing total number of aspirated oocytes by total number of ovaries X100.

2.3. Cumulus Oocytes Complex (COCs)

The cumulus oocytes complex (COCs) were aspirated from 2-8mm diameter follicles using sterile disposable 10 ml syringe with 18 gauge needle containing 1 m1 of aspiration medium (PBS).

The contents of each syringe were placed into 15 ml sterile Falcon tube (Falcon, USA) and kept in water bath at 37ºC for 15 minutes, allowing oocytes to settle down. The sediment at the bottom of the falcon tube was aspirated using pasture pipette and placed into sterile Petri dish (Nunclon, Denmark) containing 5ml aspiration medium (5ml PBS plus 3mg/ml bovine serum albumin BSA, Sigma, USA) for searching oocytes under stereomicroscope (Olympus, Japan) at 90x.

2.4. Oocytes Quality

Camel oocytes quality (COCs) was determined according to Kandil et al. (2014) and classified under stereomicroscope (90X) into four categories (Figure 1) based on their cumulus investment and evenly granulated ooplasm as follows:

Excellent quality (EX.): oocytes with five or more layers of complete cumulus-cells and evenly granulated dark ooplasm.

Good quality (G.): oocytes with 1-4 layers of cumulus-cells and evenly granulated dark ooplasm.

Fair quality (F.): oocytes with cumulus-cells incompletely surrounding the oocytes and little granulation in ooplasm.

Denuded (D.): oocytes without cumulus cells and covered by zona pellucida.

2.5. Experiments

2.5.1. Experiment1: Effect of Culture Media (TCM-199 and CR1aa) on In Vitro Maturation (IVM) Rate of Camel Oocytes

Excellent and good oocytes with more than 3 layers of cumulus-cells were selected for in vitro maturation. Selected oocytes were cultured in two culture media (IVM): TCM-199 and CR1aa[34,24]. Selected oocytes were matured in 9m1 TCM-199 (Sigma) or 9m1 $CR1_{aa}$supplemented with10% fetal calf serum (Sigma), 10µg/ml FSH (Sigma) and 50 mg/ml gentamycin. Cultured oocytes were incubated in 5% CO_2 at 38.5ºC for 40 h.

2.5.2. Experiment II: Effect of Addition of Epidermal Growth Factor (EGF) to the Culture Media on Maturation Rate of Camel Oocytes

Excellent and good oocytes were cultured in TCM-199 and CR1aa media with addition of 20 ng/ml epidermal growth factor (EGF, Sigma) at 38.5 ºC, under 5% CO2 for 40 h in humidified air [34.24].

2.5.3. Assessment of Maturation of Camel Oocytes

Assessment of cytoplasmic and nuclear maturation of camel oocytes was carried out according to Abd El-Kader [35]. Cumulus oocytes complexes (COCs) maturation was judged into 4 grades (G0, G1, G2, G3), according to degree of cumulus-cell expansion (Cytoplasmic maturation).

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.

At the end of maturation period, oocytes were recovered from the culture medium. Cumulus-cells were removed to count the number of oocytes with first polar body in the perivetteline space as matured oocytes (Nuclear maturation).Nuclear maturation (MII) rate was calculated (number of oocytes with 1st polar body divided on the total number of cultured oocytes X100).

2.3. Statistical Analyses

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. The percentage differences were carried out by using decision analyst STATS 2.0.

Results

1. Retrieval Rate of Camel Oocytes

The camel oocytes (n=8805), aspirated from 604camel ovaries during the breeding season, characterized by high number of excellent and good quality oocytes (n= 8161) compared to low number of fair and denuded oocytes (n=644) (Figure 1, Table 1). The retrieval rate was 14.58% (8805/604%).

Table 1: Retrieval rate of aspirated camel oocytes

Items	Total No.
Number of ovaries	604
Total number of oocytes	8805
Excellent and good oocytes	8161
Fair and denuded oocytes	644
Retrieval rate	14.58%

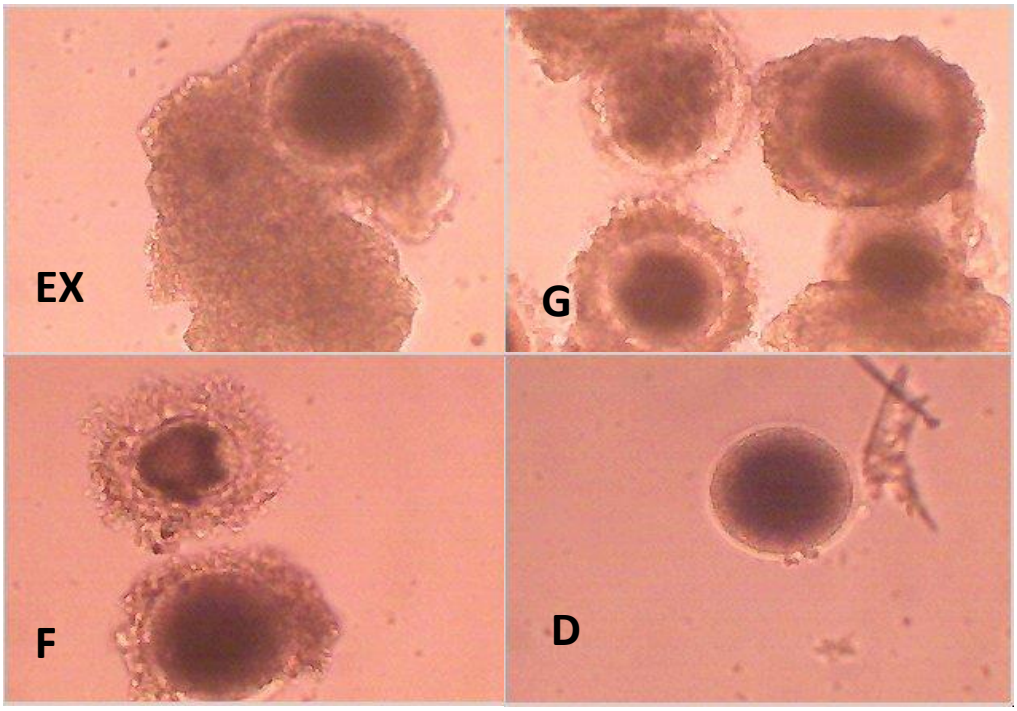

Figure 1: Different quality of immature camel oocytes: Excellent (EX), Good (G), Fair (F) and Denuded (D) under inverted microscope 200X

2. Effect of Culture Media on In Vitro Maturation Rate of Camel Oocytes

2.1. Cytoplasmic Maturation

Table 2 shows the number (M±SE and sum) of excellent and good oocytes cultured in the TCM-199 and CR1aa media. The cytoplasmic maturation (Figure 2) expressed as cumulus expansion G3and G2was not significantly higher in TCM-199 (66.02% and 24.22% respectively) when compared with CR1aa (63.43% and 22.86% respectively). The G1 and G0 expansion showed significance difference ($P<0.05$) between TCM-199 (7.28% and 3.21% respectively) and CR1aa (6.69% and 7.02% respectively).

Table 2. Effect of TCM-199 and CR1aa maturation media with or without EGF on the in vitro maturation (Cumulus expansion rate) of camel oocytes

Group	No. of oocytes		G3			G2			G1			G0		
	mean ± SE	Sum	mean ± SE	%	Sum	mean ± SE	%	Sum	mean ± SE	%	Sum	mean ± SE	%	Sum
TCM-199	144.64 ± 5.13^{a}	2170	94.57 ± 2.61^{b}	66.02 ± 1.89^{c}	1419	34.86 ± 2.64^{a}	24.22 ± 1.68^{a}	523	10.64 ± 0.88^{a}	7.28 ± 0.49^{bc}	160	4.57 ± 0.96^{b}	3.21 ± 0.71^{b}	69
TCM-199 +EGF	136.21 ± 5.68^{a}b	2043	98.5 ± 3.88^{b}	72.46 ± 0.66^{a}	1477	28.71 ± 1.01^{b}	21.41 ± 0.88^{ab}	431	6.57 ± 0.92^{b}	4.56 ± 0.54^{c}	99	3.07 ± 0.59^{b}	2.19 ± 0.41^{b}	46
CR1-aa	131.20 ± 2.33^{b}	1968	83.20 ± 1.42^{b}	63.43 ± 0.06^{c}	1248	30.00 ± 0.53^{b}	22.86 ± 0.01^{ab}	450	8.80 ± 0.35^{a}	6.69 ± 0.20^{b}	132	9.20 ± 0.31^{a}	7.02 ± 0.21^{a}	138
CR1-aa +EGF	132.00 ± 3.08^{ab}	1980	91.00 ± 1.97^{b}	68.98 ± 0.24^{b}	1365	27.00 ± 0.72^{b}	20.45 ± 0.21^{b}	405	10.60 ± 0.50^{a}	7.99 ± 0.25^{a}	159	3.40 ± 0.27^{b}	2.58 ± 0.20^{b}	51

a-b, b-c,a-c between rows of the same column, values with different superscripts indicate a significant difference (P<0.05).

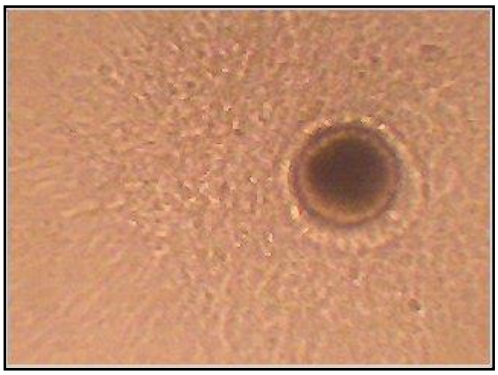

Figure 2: Cumulus expansion (G3, Cytoplasmic maturation) of in vitro matured camel oocytes after 40 h

2.2. Nuclear Maturation

As showed in Table 3, maturation rate (MII) (Figure3) was slightly higher in the CR1aa medium when compared with the TCM-199 medium (79.01% vs. 77.90%, respectively). The rate of cultured oocytes without 1st Pb was higher (P<0.05) in CR1aa (15.68±0.17 %) thanTCM-199 (10.93±0.44 %).

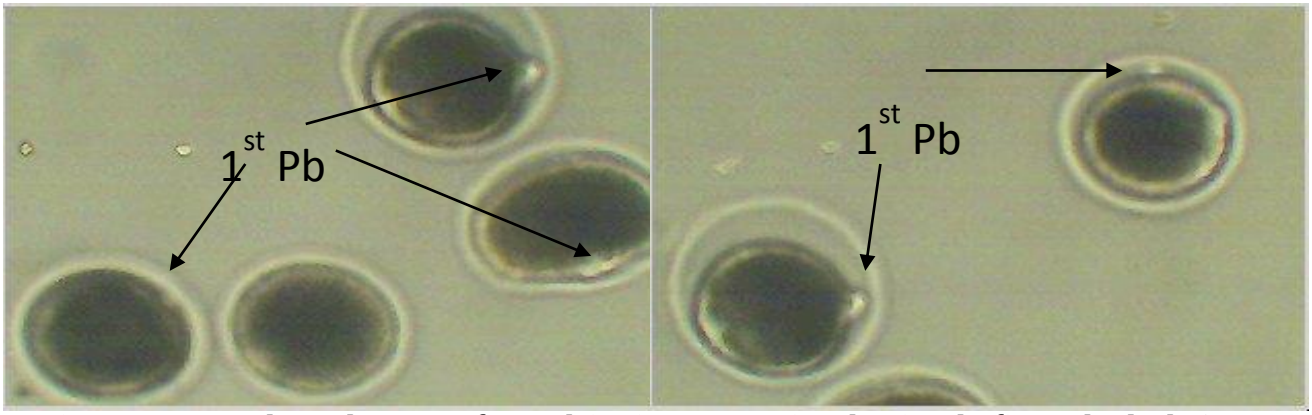

Figure 3: Matured camel oocytes after 40 h in vitro maturation showing the first polar body (MII, nuclear maturation)

Table 3. Effect of TCM-199 and CR1aa maturation media with or without EGF on the in vitro maturation (Nuclear maturation) of camel oocytes

Group	No. of oocytes		1st pb			Without 1st pb			Degenerated		
	mean ± SE	Sum	mean ± SE	%	Sum	mean ± SE	%	Sum	mean ± SE	%	Sum
TCM-199	144.64 ±5.13^{a}	2170	112.29 ±3.27^{ab}	77.90 ±0.59^{c}	1684	15.79 ±0.83^{b}	10.93 ±0.44^{b}	236	16.57 ±1.56^{a}	11.17 ±0.75^{a}	248
TCM-199 +EGF	136.21 ±5.68^{ab}	2043	119.14 ±4.08^{a}	87.93 ±1.06^{a}	1787	11.57 ±0.75^{c}	8.47 ±0.38^{c}	173	5.50 ±1.59^{b}	3.60 ±0.93^{c}	82
CR1aa	131.20 ±2.33^{b}	1968	103.6 ±1.79^{b}	79.01 ±0.61^{c,b}	1554	20.60 ±0.50^{a}	15.68 ±0.17^{a}	309	7.00 ±0.61^{b}	5.31 ±0.44^{bc}	105
CR1aa+ EGF	132.00 ±3.08^{ab}	1980	108.2 ±2.46^{b}	82.00 ±0.37^{b}	1623	15.80 ±1.51^{b}	11.72 ±0.86^{b}	237	8.00 ±1.03^{b}	6.29 ±0.88^{b}	120

a-b, b-c,a-c between rows of the same column, values with different superscripts indicate a significant difference (P<0.05).

3. Effect of Epidermal Growth Factor (EGF) on Maturation of Camel Oocytes Cultured in TCM-199 and CR1aa Media

3.1. Cytoplasmic Maturation

In vitro matured camel oocytes showed higher (P<0.05) cumulus expansion rate G3 (Table 2, figure4) in culture media (TCM-199 and CR1aa) supplemented with EGF when compared with media without EGF (72.46% and 68.28%,vs. 66.02% and 63.43%, respectively). The cumulus expansion rate of G2 and G0 was higher (P<0.05) in CR1aa without EGF. GI expansion rate was also higher (P<0.05) in TCM-199 medium without EGF.

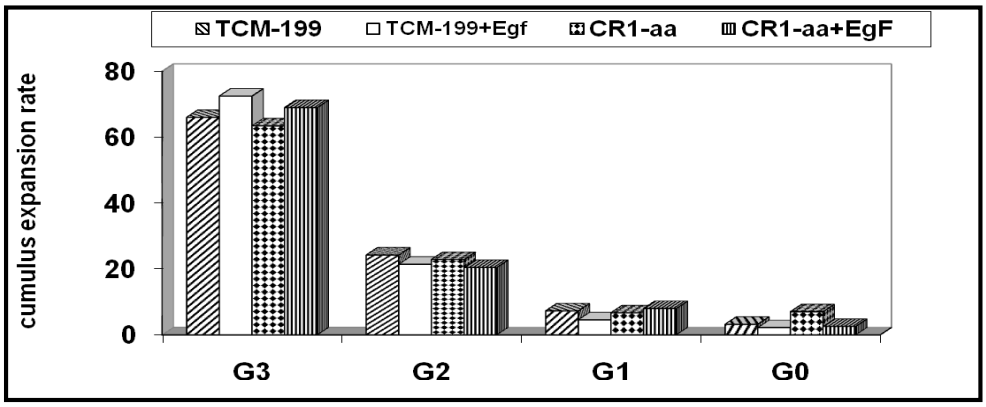

Figure 4: Effect of in vitro maturation media with or without EGF on cumulus expansion rate of camel oocytes

3.2. Nuclear Maturation

Epidermal growth factor (EGF) increased (P<0.05) the maturation rate (Table 3,Figure 5) of oocytes (with 1st Pb) cultured in TCM199+EGF (87.93±1.06%) versus TCM-199 (77.90±0.59 %).Addition of EGF also, decreased (P<0.05) the oocytes without Pb as well as the degenerated oocytes.

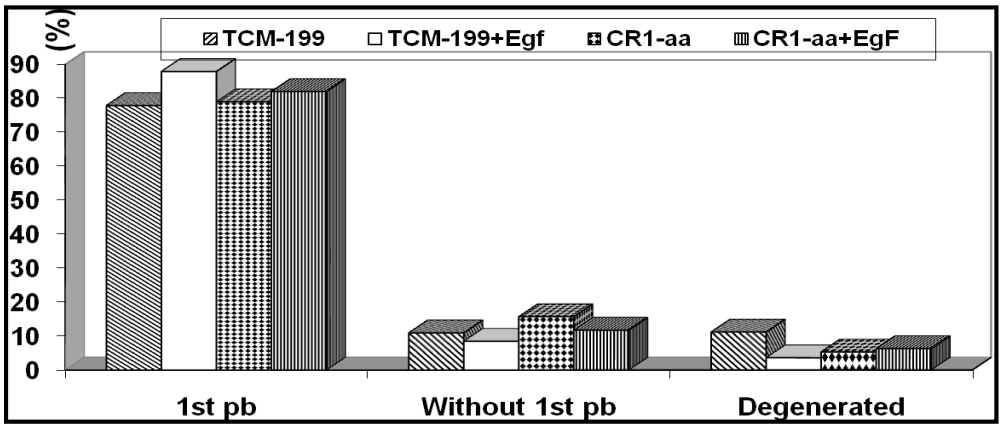

Figure 5: Effect of in vitro maturation media with or without EGF on nuclear maturation rate of camel oocytes

Supplementation of EGF to the CR1aa medium improved the maturation rate of camel oocytes, as indicated by higher oocytes with 1st Pb and lower (P<0.05) oocytes without 1st PB and degenerated oocytes (Table 3,Figure 5).TCM-199+EGF significantly increase (P<0.05) the maturation rate (MII) when compared with CR1aa+EGF in camel oocytes.

Discussion

1. Retrieval Rate of Camel Oocytes

A wide range in number of oocytes per camel ovary was observed, being 3.5 [36], 3.99[37], 4.1 [38], 4.23 [39], 5.3 [40], 6.67[41], 7.64 [42], 9.4% [29], 10.85 [23] and 12.4[43]. In the present study, the number of aspirated oocytes/ ovary (Retrieval rate) of dromedary camels during the breeding season was 14.58%. This wide variation in number of oocytes / ovary may be attributed to differences in animal age and season [44,45,42], reproductive status[42,46] , site of the ovary [37,47], method of oocytes collection [48,49,50,51] and periods of ovary preservation[43].

2. Influence of Culture Media on In Vitro Maturation of Camel Oocytes

The current results showed that cumulus expansion rate of camel oocytes was significantly higher using tissue culture medium TCM – 199 compared with Charles Rosenkrans 1 (CR1aa). In accordance, Brackett and Zuelk [52], Nowshari [53] and Kandil et al. [23] concluded that for IVM and IVF of bovine and camel oocytes, TCM – 199 was superior to oocytes cultured in CR1aa.The expansion of cumulus cells depends largely on the culture media used for maturation of the oocytes, differences in maturation percentage may be attributed to the composition of the media itself as well as supplements added to these media[54].

The finding of no significant differences in nuclear maturation of camel oocytes cultured in TCM-199 and CR1aa disagrees with Barakat et al.[55], who revealed significantly increased nuclear maturation and greater cumulus expansion of buffalo oocytes during IVM using TCM-199 medium in comparison with CR1aa medium. A variety of media have been used for in vitro maturation of follicular oocytes, TCM-199 medium was the basic maturation medium in many studies[56,57,58] .

3. Effect of Epidermal Growth Factor on Maturation Rate of Camel Oocytes

A significant improvement in the maturation rate of camel oocytes cultured in TCM-199 and CR1aa media supplemented with EGF as indicated by a marked increase in GIII cumulus expansion percentage and nuclear maturation (MII). This finding agrees with reports of Wani and Wernery [59],El-Sayed et al. [29] and Kandil et al [23] on same species. According to former authors, maturation rates in media supplemented with 20 ng/ml were superior to rates of oocytes cultured in media with 0 ng/ml (controls), 10 ng/ml or 50 ng/ml of EGF. Therefore, in agreement with Wani and Wernery[59], 20 ng/ml of EGF in the maturation medium is optimal and improves the maturation rate of dromedary oocytes. However, El-Sayed et al.[29] found that10 ng/ml EGF improved

camel oocytes maturation and cleavage rates. EGF has been reported to promote oocytes nuclear maturation in several species, such as pigs [60,61], cattle[62,63,64], sheep [65], rat [66] and buffalo [67]. The mechanism of its action could be due to disruption of oocyte communication with cumulus cells [68], creation of a positive maturational signal [69]and ⁄or mediation of effect via a tyrosine kinase dependent intracellular mechanism [70].

The action of EGF, is likely to be on the oocytes itself, as was suggested by Lonergan et al., [71] in bovine oocytes. The zona pellucida allows the passage of molecules as large as 150 kDa in the mouse [72], the camel zona may be somewhat similar in this regard. Hence, the relatively small EGF molecules (6k Da) 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 [73]. Thus, EGF may have exerted its positive effects directly on the oocyte after binding to its receptor and activating the tyrosine kinase [74].

In accordance with Lonergan et al.[71],supplementation of TCM-199 with EGF alone during IVM at physiological concentrations stimulates cumulus cell expansion and improves the percentage of oocytes undergoing nuclear maturation. EGF also increases the proportion of embryos attaining the blastocyst stage[71]. These authors suggested a physiological role for EGF/EGF-like molecules in the intra-follicular regulation of oocyte maturation. EGF acts by binding with high affinity to epidermal growth factor receptors (EGFR) on the cell surface and stimulating the intrinsic protein – tyrosine kinase activity of the receptor[69] . The tyrosine kinase activity, in turn initiates a signal transduction cascade that results in a variety of biochemical changes within the cell – a rise in intracellular calcium levels, increased glycolysis and protein synthesis and increases in the expression of certain genes including the gene for EGFR – that ultimately lead to DNA synthesis and cell proliferation [69]. The EGFR is expressed in both cumulus cell and oocytes [75,76], and its expression in oocytes increases with acquisition of meiotic competence [77].

In conclusion, the retrieval rate of camel oocytes during the breeding season was 14.58%. The TCM-199 and CR1aa media were suitable for in vitro maturation of camel oocytes. Supplementation of EGF to TCM-199 culture medium improved in vitro maturation rate of camel oocytes.

Acknowledgement

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References

1. Wilson R T. Reproductive performance of the one-humped camel. The empirical base. Revue Elev Med Vet Pays Trop., 1989, 42:117-125.
2. Musa BE, Siema H, Merkt H, Hago B, Cooper M, Allen W , Jochle W. Manipulation of reproductive functions in male and female camels. Anim Reprod Sci., 1993, 33:289–306.
3. Al Eknah MM. Reproduction in old world camels. Anim Reprod Sci., 2000, 60–61:583–592.
4. Tibary A, Anouassi A, Sghiri S. “Factors affecting reproductive performance of camels at the herd and individual level”. In: Faye B, Esemov P, editors. Desertification combat and food safety: the added value of camel producers NATO Science Series Series I: Life and Behavioural Sciences, 2005, vol. 362. Amsterdam: IOS Press., p. 97–114.
5. Skidmore JA, Billah M. The main challenges of artificial insemination and embryo transfer in the dromedary camel. ICAR 2012 Satellite Meeting on Camelid Reproduction, August 3-5, 2012, Vancouver, Canada, pp 43-48.
6. Hemeida NA. Development and application of the reproductive technologies to camel breeding. International Conference on Sustainability of Camel Production and Population. College of Agriculture & Food Sciences, 2013, King Faisal University, February 17-20, Al-Ahsa, Saudi Arabia.
7. Hemeida NA. Reproductive Technologies in Camelids. Review Article. Veterinary Medical Journal -Giza (VMJG), 2014, 60:1-15.
8. Skidmore JA. The main challenges facing camel reproduction research in the 21st century. Reprod Suppl., 2003, 61:37-47.

9. Tibary A, Anouassi A, Sghiri A, Khatir H. Current knowledge and future challenges in camelid reproduction. Soc Reprod Fertil Suppl., 2007, 64:297-313.
10. Anouassi A, Tibary A. Development of a large commercial camel embryo transfer program: 20 years of scientific research. Anim Reprod Sci., 2013, 136:211-221.
11. Nagy P, Skidmore JA, Juhasz J 2013. Use of assisted reproduction for the improvement of milk production in dairy camels (Camelus dromedarius). Anim Reprod Sci., 2013, 136:205-210.
12. Skidmore JA, Morton KM, Billah M. Unique strategies to control reproduction in camels. Soc Reprod Fertil Suppl., 2010, 67:467-474.
13. Skidmore JA, Morton KM, Billah M. Artificial insemination in dromedary camels. Anim Reprod Sci., 2013, 136:178-186.
14. Galli C, Duchi R, Crotti G, Turini P, Ponderato N et al. Bovine embryo technologies. Theriogenology, 2003, 59:599-616.
15. Del Campo MR, Del Campo CH, Donoso MX, Berland M, Mapletoft RJ. In vitro fertilization and development of llama (lama glama) oocytes using epididymal spermatozoa and oviductal cell co-culture. Theriogenology, 1994, 41:1219-1229.
16. Khatir H, Anouassi A. The First Dromedary (Camelus dromedarius) Offspring obtained from in vitro matured, in vitro fertilized and in vitro cultured abattoir-derived oocytes. Theriogenology, 2006, 65:1727-1736.
17. Downs SM, Mastropolo AM . Culture conditions affect meiotic regulation in cumulus-cell enclosed mouse oocytes. Mol Reprod Dev., 1997, 46:551-566.
18. Kito S, Bavister BD. Gonadotropins, serum and amino acids alter nuclear maturation, cumulus expansion and oocyte morphology in hamster cumulus-oocyte complexes in vitro. Biol. Reprod., 1997, 56:1281-1289.
19. Kandil OM, Abdoon ASS, Murakami M, Otoi T, Suzuki T. New technique, using a portable CO incubator, for the production of in vitro fertilized Egyptian buffalo embryos. J. Reprod Develop., 1999, 45:315-320.
20. Eppig JJ. Intercommunication between mammalian oocytes and companion somatic cells. Bio Essays, 1991, 13:569–574.
21. Krisher RL, Bavister BD. Responses of oocytes and embryos to the culture environment. Theriogenology, 1998, 49:103–114.
22. Songsasen N, Wildt DE. Oocyte biology and challenges in developing in vitro maturation systems in the domestic dog. Anim Reprod Sci., 2007, 98:2–22.
23. Kandil OM, El-Nahla AM, Siam AA, Abdoon ASS, Al-Monifi FA, Mermillod P. Effect of Media on In vitro Maturation Rate of Dromedary Camel Oocytes. Global Veterinaria, 2014, 13:159-165.
24. Abdoon ASS, Kandil OM.Factors affecting number of surface ovarian follicles and oocytes yield and quality in Egyptian buffaloes. Reprod Nutr Dev., 2001, 41:71-77.
25. Matsukawa K, Akagi S, Adachi N, Sato F, Hasegawa T, Takahashi S. In vitro development of equine oocytes from preserved ovaries after intracytoplasmic sperm injection. J Reprod Dev., 2007, 53:877–885.
26. Wani PC, Hao ZD, Zhou P, Wu Y, Yang L, Cui MS, et al. Effects of SOF and CR1 media on developmental competence and cell apoptosis of ovine in vitro fertilization embryos. Anim Reprod Sci.,2009, 114:279–288.
27. Somfai T, Inaba Y, Aikawa Y, Ohtake M, Kobayashi S, Konishi K, et al. Development of bovine embryos cultured in CR1aa and IVD101 media using different oxygen tensions and culture systems. Acta Vet Hung, 2010, 58:465–474.
28. Bavister BD, Rose-Hellekant TA, Pinyopummintr T. Development of in vitro matured/in vitro fertilized bovine embryos into morulae and blastocysts in defined culture media. Theriogenology, 1992, 37:127-145.
29. El-Sayed A, Sayed HA, El-Hassanein EE, Murad H, Barkawi AH.Effect of epidermal growth factor on in vitro production of camel (camelus dromedarius) embryos by using frozen semen.Egyptian J Anim. Prod., 2012, 49, Suppl. Issue:39-45
30. Hernandez ER, Resnick CE, Svoboda ME, Van Wyk JJ, Payne DW, Adashi EY. Somatomedin-C/insulin-like growth factor-I as an enhancer of androgen biosynthesis by cultured rat ovarian cells. Endocrinology, 1988, 122:1603-1612.
31. Hsu CJ, Holmes SD, Hammond JM. Ovarian epidermal growth factor-like activity: concentrations in porcine follicular fluid during follicular enlargement. Biochem Biophys Res Commun., 1987, 147:242-247.

32. Leibfried ML, First NL. Characterization of bovine follicular oocytes and their ability to mature in vitro. J Anim Sci., 1979, 48:76-86.
33. Leibfried Rutledge ML, Critster ES, Eyestone WH, Northey DL, First NL. Development potential of bovine oocytes matured in vitro. Biol Reprod., 1987, 36: 376-383.
34. Del Campo MR, Donoso MX, Delcampo CH, Rojo R, Barros C, Parrish JJ, Mapletoft RJ. In vitro maturation of llama (Lama glama) oocytes. In: Proceedings of the 12th International Congress on Anim.Reprod., 1992, Vol. 1, pp. 324–326.
35. Abd El-Kader HM.In vitro maturation, fertilization and culture to blastocyst of buffalo oocytes in different culture media. Ph D Thesis, 2005, Faculty of Science, Cairo University, Giza, Egypt.
36. Khatir H, Anouassi A, Tibary A. Production of dromedary (Camelus dromedarius) embryos by IVM and IVF and co-culture with oviductal or granulosa cells. Theriogenology, 2004, 62:1174–1185.
37. El-Harairy MA, Shamia SHM, Abdel-Khalek AE, Khalil WA. Effect of pregnancy status and harvesting technique on recovery rate and categories of dromedary camel oocyte. Egypt J Basic Appl Physiol., 2007, 2:255–268.
38. Nowshari MA, Syed AA. Effect of season and gonadotropins on the superovulatory response in camel (Camelus dromedarius). Theriogenology, 2005, 64: 1526-1535.
39. Tajik P, Puyan HR, Ghasemzadeh-Nava H. Dromedary camel cool stored oocyte maturation in different concentrations of fetal calf serum. FAVA-OIE Joint Symposium on Emerging Diseases, 2012, pp: 295-296.
40. Mahmoud KGH, El-Shahat MKH, El-Nattat WS. Chromosome configuration during in vitro maturation of dromedary camel oocytes. Vet Med J Giza, 2003, 51: 411-420.
41. Ali A, Abdel-Razek AKH. Comparison of number and quality of oocytes in the Egyptian buffaloes (Bubalus bubalis), cows (Bos taurus) and camels (Camelus dromedarius). Assiut Vet Med J., 2001, 45:317–325.
42. Abdoon ASS. Factors affecting follicular population, oocyte yield and quality in camels (Camelus dromedarius) ovary with special reference to maturation time in vitro. Anim Reprod Sci., 2001, 66:71–79.
43. Abdel-Khalek EA, El-Harairy MA, Shamiah ShM, Khalil WA.Effect of ovary preservation period on recovery rate and categories of dromedary camel oocytes.Saudi J Biol Sci., 2010, 17:231–235.
44. Musa BE, Abausineina ME. The estrous cycle of the camel (Camelus dromedarius). Vet. Rec., 1978, 103:556-557.
45. Sghiri A, Driancourt MA. Seasonal effects on fertility and ovarian follicular growth and maturation in camels (Camelus dromedarius). Anim Reprod Sci., 1999, 55:223-237.
46. Torner H, Heleil B, Alm H, Ghoneim IM, Srsen V, Kanitz W, Tuchscherer A, Fattoh EM. Changes in cumulus-oocyte complexes of pregnant and non-pregnant camels (camelus dromedaries) during maturation in vitro. Theriogenology, 2003, 60:977-987.
47. Amer H, Moosa A. Effect of season and culture media on the competence of dromedary camel oocyte to mature in vitro. Global veterinaria, 2009, 3:01-08.
48. Yoo HJ, Choi SC, Lee SH. Maximization of the numbers of follicular oocytes recovered from the bovine ovaries. Korean J Anim Reprod., 1993, 17:149-157.
49. Alm H, Torner H, Kantiz W, Becker F, Hinrichs K. Comparison of different methods for recovery of horse oocytes. Equine Vet J., 1997, Suppl 25:47–50.
50. Heleil B, Torner H, Alm H, Greising T. Effect of follicle size on cumulus morphology, chromatin configuration and meiotic competence of bovine cumulus-oocyte complexes. Reprod Dom Anim., 1998, Suppl 5:11.
51. Sansinena MJ, Taylor SA, Taylor PJ, Schmidt EE, Denniston RS, Godke RA. In vitro production of llama (Lama glama) embryos by intracytoplasmic sperm injection: effect of chemical activation treatments and culture conditions. Anim Reprod Sci., 2007, 99:342–353.
52. Brackett BG, Zuelke KA. Analysis of factors involved in the in vitro production of bovine embryos. Theriogenology, 1993, 39:43-64.
53. Nowshari MA. The effect of harvesting technique on efficiency of oocyte collection and different maturation media on the nuclear maturation of oocytes in camels (Camelus dromedarius). Theriogenology, 2005, 63:2471-2481.
54. Nandi S, Ravindranatha BM, Gupta PS, Sarma PV. Timing of sequential changes in cumulus cells and first polar body extrusion during in vitro maturation of buffalo oocytes. Theriogenology, 2002, 57:1151-1159.
55. Barakat IA, Hassan AM, Alam SS, Khalil WK. Genetic and biochemical effects of natural extracts on in vitro maturation of Egyptian buffalo oocytes. Cytologia, 2010, 75:243–253.

56. Li Q, Niwa K, Hunter MG. Effects of 17B-Estradiol on in vitro maturation of pig oocytes in protein-free medium. J Rep Dev., 2004, 50: 305–313.
57. Jamil H, Samad HA, Rehman NU, Qureshi ZI, Lodhi LA. In vitro maturation and fertilization of riverine buffalo follicular oocytes in media supplemented with oestrus buffalo serum and hormones. Acta Vet Brno., 2007, 76:399-404.
58. Amer H, Moosa A.Relationship between season of the year, culture medium and in vitro oocyte competence in dromedary camels. Bulg J Vet Med., 2008, 11:195- 204.
59. Wani NA, Wernery U. Effect of different protein supplementations and epidermal growth factor on in vitro maturation of dromedary camel (Camelus dromedarius) oocytes. Reprod Dom Anim., 2010, 45:e189–e193.
60. Reed ML, Estrada JL, Illera MJ, Apetters RM. Effect of epidermal growth factors, insulin-like growth factor-1 dialyzed porcine follicular fl uid on porcine oocyte nuclear maturation in vitro. J Exp Zoology, 1993, 266:74-78.
61. Wang W, Niwa K. Synergistic effect of epidermal growth factor and gonadotropins on the cytoplasmic maturation of pig oocytes in a serum free medium. Zygote, 1995, 3:345-350.
62. Harper MJ, Brackett BJ. Bovine blastocyst development after in vitro maturation in a defi ned medium with epidermal growth factor and low concentrations of gonodotropins. Biol Reprod., 1993, 48:409-416.
63. Lorenzo PL, Lorenzo MJ, Illera JC, Illera M. Enhancement of cumulus expansion and nuclear maturation during bovine oocyte maturation in vitro by the addition of epidermal growth factors and insulin-like growth factors. J Reprod Fertil., 1994, 101:697-701.
64. Nandi S, Ravindranatha BM, Gupta PS, Raghu HM, Sarma PV. Development competence and post-thaw survivability of buffalo embryos produced in vitro: effect of growth factors in oocytes maturation medium and embryo culture system. Theriogenology, 2003, 60:1621-1631.
65. Guler A, Poulin N, Mermilod P, Terqui M, Cognie V. Effect of growth factors, EGF and IGF-I and estradiol on in vitro maturation of sheep oocytes. Theriogenology, 2000, 54:209-228.
66. Feng P, Catt KJ, Knecht M. Transforming growth factor b stimulates meiotic maturation of the rat oocytes. Endocrinology, 1989, 122:181-186.
67. Kumar D, Purohit GN. Effect of epidermal and insulin-like growth factor-1 on cumulus expansion, nuclear maturation and fertilization of buffalo cumulus oocyte complexes in simple serum free media DMEM and Ham's F-10. Vet Arhiv., 2004, 74:13-25.
68. Deckel, Scherizly I. Epidermal growth factor induces maturation of rat follicle enclosed oocytes. Endocrinology, 1985, 116:512-516.
69. Downs SM. Specificity of epidermal growth factor action on maturation of the murine oocyte and the cumulus oophorus in vitro. Biol Reprod., 1989, 41:371-379.
70. Lorenzo PL, Liu IK, Illera JC, Picazo RA, Carneior GF, Illera MJ, Conley AJ, Enders AC, Illera M. Influence of epidermal growth factor on mammalian oocyte maturation via tyrosine kinase pathway. J Physiol Biochem., 2001, 57:15-22.
71. Lonergan P, Carolan C, Van Langendouck FA, Donnay I, Khalir H, Mermillod P. Role of epidermal growth factor in bovine oocyte maturation and pre-implantation., embryo development in vitro. Biol Reprod., 1996, 54:1420-1429.
72. Legge M. Oocyte and zygote zona pellucida permeability to macromolecules. J Exp Zool., 1995, 271:145–150.
73. Maruo T, Ladines-Llave CA, Samoto T et al. Expression of epidermal growth factor and its receptor in the human ovary during follicular growth and regression. Endocrinology, 1993, 132:924–931.
74. Gill GN. Regulation of EGF receptor expression and function. Mol Reprod Dev., 1990, 27:46–53.
75. Uhm SJ, Chung HM, Kim SW, Kim NH, Lee HT, Chung KS. Expression of epidermal growth factor receptor in porcine oocytes and preimplantation embryos. Theriogenology, 1999, 5:258.
76. Gall L, Boulesteix C, Ruffini S, Germain G. EGF-induced EGF receptor and MAP kinase phosphorylation in goat cumulus cells during in vitro maturation. Mol Reprod Dev., 2005, 71:489–494.
77. Gall L, Chene N, Dahirel M, Ruffini S, Boulesteix C. Expression of epidermal growth factor receptor in the goat cumulus–oocyte complex. Mol Reprod Dev., 2004, 67, 439–445.

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