In vitro Maturation of Camel (Camelus dromedarius) Cumulus-Denuded Oocytes

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Abstract: The present study was designed to investigate the effect of different culture media, adding gonadotropin hormones and length of culture period on in vitro maturation (IVM) rate of camel denuded oocytes (DOs). Camel ovaries were collected from local slaughterhouse. Oocytes were aspirated from follicles 3 to 10mm in diameter with a 20 gauge needle attached to a 5ml syringe. The oocytes in a denuded (DOs) form were selected and recovered. The DOs were cultured either in TCM-199, Ham's F-10 or RPMI-1640. These media supplemented with or without gonadotropin hormones (GHs). The GHs were in form PMSG + hCG. The culture periods involved three times, 28, 32 and 42hrs. The DOs were matured at 39°C under 5% CO₂ and 95% humidity.2 The results showed that the proportions of DOs reaching metaphase II (MII) were significantly increased (P<0.05 or P<0.01) in groups cultured with TCM-199 compared to Ham's F-10 and RPMI-1640 (23.97% vs. 18.44% and 12.52%, respectively). The supplementation with gonadotropins to culture media significantly (P<0.05) improved meiotic maturation rate of DOs than those cultured in gonadotropin-free media (20.58% vs. 16.04%). The IVM rate significantly elevated (P<0.05 or P<0.01) after 42hrs of culture time in comparison with other maturation times 28 or 32hrs (24.59% vs. 11.96% and 18.38%, respectively). In conclusion, the present study adds evidence that, it is possible to restore and success the IVM of camel DOs without adding cumulus cells, when oocytes were matured in simple culture system consisting of TCM-199 plus gonadotropins in form PMSG + hCG and use of maturation time of 42hrs. The successful of IVM DOs that usually discarded in the standard IVM-IVF system could be remunerated the inefficient of IVM of cumulus –oocyte complexes (COCs) especially prepubertal and poor quality oocytes.

Key words: Camel • Denuded oocytes • IVM • Maturation medium • Hormone additives • Maturation time

INTRODUCTION

One serious problem associated with in vitro maturation and fertilization in camel is poor recovery rate of immature oocytes in comparison with other large domestic farm animals [1,2]. In order to increase the number of oocytes available for in vitro maturation and fertilization it may be possible to use the denuded oocytes with normal cytoplasm which are recovered in substantial numbers from buffalo [3], bovine [4] and sheep [5] ovaries. It is generally accepted that cumulus cells during the maturation period support maturation of oocytes to metaphase II stage and are involved in the cytoplasmic maturation that needed for developmental competence and postfertilization [6,7]. However, there are several reports showed that cumulus-denuded oocytes (DOs) could complete meiotic maturation in mice [8], rats [9], sheep [10] and cattle [11] in vitro. Das et al. [12] observed that addition of cumulus cells to the artificially denuded buffalo oocytes (AD+C) reversed the decrease in the nuclear maturation, the nuclear maturation rate in AD was 51%, while, it was 64% in AD + C, however, the addition of cumulus cells had no effect on oocytes which had been recovered in a denuded form (D), the IVM rate was 42% in DOs oocytes, while, it was 46% in denuded oocytes with adding cumulus cells (D + C). Also, in many species (mouse [13] rabbit [14] bovine [15] human [16], immature oocytes denuded of cumulus cells have been reported to complete meiosis, fertilize and initiate early cleavage. Schroeder and Eppig [17] and Yamazaki et al. [18] noted that the in vitro maturation of denuded murine oocytes can display the same developmental competence as

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in vivo matured oocytes. Chang et al. [19] indicated that 95% (B6SILFl) and 94% (B6D2F1) of the denuded mouse strains oocytes reached MII when they matured in vitro, 39% and 40% of them were fertilized and 29% and 30% developed to blastocyst.

Moreover, several reports suggested that the presence of cumulus cells in oocytes are not always necessary for IVM processes and the in vitro competence of the oocytes could be controlled by in vitro culture conditions [7,18,19]. Culture medium, hormone supplements and the length of culture period have been considered the main factors that affect efficient in vitro maturation system for animal oocytes. These factors play an important role for maximizing success not only in the subsequent maturation rate but also for embryonic development following IVF[20]. TCM-199 is the most widely used culture medium for IVM of different animal oocytes [21]. Moreover, Ham's F-10 [22,23] and RPMI [5] have also ability for promoting the developmental competence of oocytes in different mammalian species to mature in vitro. Farag et al. [24] showed in camel that the use of TCM-199 for culturing of cumulus-oocytes complexes (COCs) led to a significant increases (P<0.05 or P<0.01) of in vitro maturation rate in comparison to other culture media such Ham's F-10 and RPMI-1640. However, the comparison of efficiency of using the different culture media (TCM-199, Ham's F-10 and RPMI-1640) on improving IVM of camel denuded oocytes were not discussed previously.

On the other hand, gonadotropins were found to exert advantageous effects on an oocyte's competence to mature, fertilize and support normal preimplantation development [19]. PMSG or PMSG plus hCG used to reach the rate of IVM of denuded mouse oocytes for 85% [19]. In camel, Farag et al.[ 24] observed that the supplementation of maturation media with PMSG plus hCG resulted in significant improvement of meiotic maturation rate of cumulus-oocytes complexes (COCs) than in hormone-free media. However, the comparison of efficiency of using the different culture media (TCM-199, Ham's F-10 and RPMI-1640) on improving IVM of camel denuded oocytes were not discussed previously.

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Furthermore, the culture period is also considered to be an effective factor for IVM of camel oocytes. Several studies reported only different optimal maturation times for IVM of COCs type. Abdoon [25] and Wani and Nowshari[1] found that the optimal durations for IVM of camel COCs were 36 and 44h, respectively. Whereas, Mahmoud et al. [26] and Kafi et al. [27] observed that camel COCs could be matured in vitro in an optimal culture time of 30h and prolonged of maturation length result in the degeneration of the oocytes. Farag et al. [24] revealed that the proportions of camel COC, oocytes reaching MII were significantly elevated (P < 0.05 or P< 0.01) after 42hrs of culture time than other maturation times (28 or 32hrs). However, the optimal maturation time for IVM of camel denuded oocytes has not been discussed previously. So, the present study was designed to investigate the effect of use of different culture media (TCM-199; Ham's F-10 and RPMI-1640), gonadotropin hormones in form PMSG-hCG and three different maturation times (28, 32 and 42hrs) on IVM rate of camel denuded oocytes.

**MATERIALS AND METHODS**

**Chemicals and Plastics:** TCM-199 (M-4530), Ham's F-10 (N 6013), RPMI-1640 (R5886) media and mineral oil (M-8410) were purchased from Sigma chemicals CO. (St. Louis, MO, USA). hCG Pregnyl®, was provided from Nile Co. for Pharmaceutical & Chemical Industries A.R.E. PMSG folligon®, Intervet International B.V. Boxmeer, Holland. D-PBS (Cat. No: 21300-017) was obtained from GIBCO/BRL (Grand Island, N.Y. USA). Polystyrene plastic culture dishes (35 x 10mm, 60 x 10 mm) and 0.22um millipore membrane filters were purchased from Nuncelon, Nalge Nunc International, Roskide, Denmark.

**Preparation of Pregnant Dromedary Camel Serum (PDCS):** Pregnant dromedary camel serum (PDCS) was used in this study as supplementary factor for three different media (TCM-199; Ham's F-10 and RPMI-1640) and prepared according to the method of Mahmoud et al. [26] as follows: Twenty ml of blood were obtained from pregnant she-camels slaughtered at a local slaughterhouse. The pregnancy was proved by presence of conceptus in different stages in uterus after slaughter according to Mahmoud et al.[26]. The fetal age ranged from 6-7 months. The blood was centrifuged at 500g for 10 min. The serum was separated and stored at -20°C until required for the culture of the oocytes.

**Preparation of Gonadotropin Hormones:** PMSG (pregnant mare serum gonadotropin) and hCG (human chronic gonadotropin) were separately solved in 0.9% NaCl, aliquoted and stored at -20°C until use. Stock solutions of PMSG and hCG were freshly prepared every two months [1].
Table 1: Types of culture media and additives

<table>
<thead>
<tr>
<th>Medium</th>
<th>Additives</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCM-199</td>
<td>10 % PDCS + 50 µg /ml Gentamycin (used as control)</td>
</tr>
<tr>
<td></td>
<td>10 % PDCS + 50 µg /ml Gentamycin + 10 µg /ml PMSG + 10 µg /ml hCG</td>
</tr>
<tr>
<td>Ham's F-10</td>
<td>10 % PDCS + 50 µg /ml Gentamycin (used as control)</td>
</tr>
<tr>
<td></td>
<td>10 % PDCS + 50 µg /ml Gentamycin + 10 µg /ml PMSG + 10 µg /ml hCG</td>
</tr>
<tr>
<td>RPMI-1640</td>
<td>10 % PDCS + 50 µg /ml Gentamycin (used as control)</td>
</tr>
<tr>
<td></td>
<td>10 % PDCS + 50 µg /ml Gentamycin + 10 µg /ml PMSG + 10 µg /ml hCG</td>
</tr>
</tbody>
</table>

Preparation of Maturation Media: Each type of medium (TCM – 1999; Ham's F-10 and RPMI–1640) was supplemented with 10% PDCS + 10 µg/ml PMSG(Folligon) + 10ug/ml hCG(Pregnyl) + 50ug/ml gentamycin sulfate (antibiotic). However, the control of medium was without adding of gonadotropin hormones. The types of culture media and additives were showed in Table 1. Each maturation medium was adjusted to pH 7.2 – 7.4 and filtered by 0.22 um – Millipore filter.

Collection of Ovaries: Camel ovaries were collected at a local abattoir within 15 min after slaughter. The ovaries were transported to the laboratory in 0.9% saline supplemented with 50 µg/ml gentamycin sulfate at 37°C within 2-3hrs.

Collection of Denuded Oocytes (DOS) and in vitro Maturation: Oocytes from all visible antral follicles (3-10 mm in a diameter) in collected ovaries were aspirated with a 22 gauge hypodermic needle attached to a 5ml disposable syringe containing 1ml of aspiration medium. The aspiration medium consisted of Dulbecco's phosphate buffer saline (D-PBS) supplemented with 50 µg/ml gentamycin sulfate [24,28,29]. Cumulus-denuded oocytes (DOS) with homogeneous cytoplasm were recovered under stereomicroscope. The DOS were washed three times with aspiration media (TCM-199 or Ham's F-10 or RPMI-1640), that enriched with 10% PDCS and 50 µg/ml gentamycin sulfate and without any gonadotropin hormones supplementation. The same non-supplemented media (TCM-199 or Ham's F-10 or RPMI-1640) were used as controls for three different culture media supplemented with gonadotropin hormones as showed in table 1. The maturation times in each of controls and medium plus gonadotropins were 28,32 and 42hrs. Each treatment was consisted of three replicates. 15-20 of DOS were transferred into a 100ul drop of each type of culture media (control medium or medium plus gonadotropin hormones) and covered with sterile mineral oil in a polystyrene culture dish (3.5 mm x 10 mm) which had been previously kept for about 2hrs in a CO₂ incubator before oocytes were added. Oocytes (DOS) were cultured for different three times 28, 32 and 42hrs at 39°C in an atmosphere of 5% CO₂ in air with 95% humidity.

Assessment of the Nuclear Maturation by Cytogenetic Analysis: For examining the rate of nuclear maturation (the proportion of oocytes which their nuclei reached metaphase II), DOS were fixed in acetic: ethanol (1:3 v/v) in culture dishes (35 x 10mm) for at least 45hrs at 4°C. After that, the oocytes (DOS) were stained for 30 min with 1% (w/v) orcein in 45% (v/v) acetic acid[30]. Oocytes were examined under a light microscope (1000 x magnification) and classified as being at one of the following stages: germinal vesicle stage (GV), germinal vesicle breakdown (GVBD), metaphase I (MI), anaphase I (AI), telophase I (TI) and metaphase II (MII). Oocytes with no visible or abnormal chromatin configuration were classified as degenerated ones[31].

Statistical Analysis: Data of the obtained results were statistically analyzed by ANOVA using SAS program[32]. Fisher's least significant difference test (LSD) at 5% significant level (P < 0.05) was used to test the differences between means of treatments.

RESULTS

In vitro Meiotic Maturation Rate of Camel Denuded Oocytes as Affected by Types of Culture Media: The meiotic maturation rate of camel denuded oocytes in vitro matured in culture media (TCM-199, Ham's F-10 and RPMI) are presented in Table (2). The results showed that the proportion of oocytes reaching MII was increased in group treated with TCM-199 medium than those found in Ham's F-10 and RPMI groups (23.97 vs 18.44 and 12.52%, respectively). This increase was significant (P<0.05) than those found in Ham's F-10 group and highly significant (P<0.01) than those observed in RPMI group. Also, meiotic maturation rate of camel denuded oocytes significantly (P<0.05) increased in group treated with Ham's F-10 compared to RPMI group (18.44 vs 12.52%).

In vitro Meiotic Maturation Rate of Camel Denuded Oocytes as Affected by Gonadotropins Treatment: Proportions of camel denuded oocytes which their nuclei reach MII in the control (culture media supplemented with camel serum) and hormone supplemented (culture media plus camel serum and gonadotropin hormones) groups are given in Table (3). The results revealed that
Table 2: *In vitro* meiotic maturation rate of DOs of camel as affected by type of culture media

<table>
<thead>
<tr>
<th>Maturation media</th>
<th>No. Of cultured Dos</th>
<th>No. Of fixed oocytes (Homogeneous)</th>
<th>Meiotic maturation of fixed oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GV (M %)</td>
<td>GVBD (M %)</td>
</tr>
<tr>
<td>TCM-199</td>
<td>629</td>
<td>314</td>
<td>29 (12.62)</td>
</tr>
<tr>
<td>Ham’s F-10</td>
<td>711</td>
<td>329</td>
<td>74 (20.61)</td>
</tr>
<tr>
<td>RPMI</td>
<td>708</td>
<td>350</td>
<td>81 (21.08)</td>
</tr>
</tbody>
</table>

Table 3: *In vitro* meiotic maturation rate of DOs of camel as affected by gonadotropins (PMSG +hCG) treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Of cultured Dos</th>
<th>No. Of fixed oocytes (Homogeneous)</th>
<th>Meiotic maturation of fixed oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1012</td>
<td>464</td>
<td>88 (20.4)</td>
</tr>
<tr>
<td>Treatment with gonadotropins</td>
<td>1036</td>
<td>529</td>
<td>105 (15.54)</td>
</tr>
</tbody>
</table>

Table 4: *In vitro* meiotic maturation rate of DOs of camel as affected by length of culture time

<table>
<thead>
<tr>
<th>Maturation times</th>
<th>No. Of cultured Dos</th>
<th>No. Of fixed oocytes (Homogeneous)</th>
<th>Meiotic maturation of fixed oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 hours</td>
<td>717</td>
<td>262</td>
<td>48 (18.74)</td>
</tr>
<tr>
<td>32 hours</td>
<td>649</td>
<td>310</td>
<td>49 (14.75)</td>
</tr>
<tr>
<td>42 hours</td>
<td>682</td>
<td>421</td>
<td>96 (20.47)</td>
</tr>
</tbody>
</table>

In above tables (2, 3 & 4):
- Values in the same column with different superscripts differ significantly ($P<0.05$).
- Table represents mean numbers of oocytes (%) in each stage of maturation (Mean %).
- GV = Germinal vesicle, GVBD = Germinal vesicle breakdown, MI = Metaphase I, AI= Anaphase I, TI = Telophase I, M II = Metaphase II and Deg. = Degenerated.

Table 5: Interaction effect among the maturation media, length of culture times and gonadotropins supplementation on *in vitro* meiotic maturation rate of denuded camel oocytes

<table>
<thead>
<tr>
<th>Medium</th>
<th>Maturation Time</th>
<th>Additives</th>
<th>No. of cultured DOs</th>
<th>No. Of fixed oocytes (Homogeneous)</th>
<th>Meiotic maturation of fixed oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCM-199</td>
<td>28 h</td>
<td>-H</td>
<td>121</td>
<td>43</td>
<td>8 (19.37)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+H</td>
<td>122</td>
<td>65</td>
<td>4 (18.71)*</td>
</tr>
<tr>
<td></td>
<td>32 h</td>
<td>-H</td>
<td>86</td>
<td>42</td>
<td>7 (15.97)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+H</td>
<td>90</td>
<td>47</td>
<td>4 (7.28)*</td>
</tr>
<tr>
<td></td>
<td>42 h</td>
<td>-H</td>
<td>107</td>
<td>50</td>
<td>6 (10.99)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+H</td>
<td>103</td>
<td>67</td>
<td>1 (1.25)</td>
</tr>
<tr>
<td>Ham’s F-10</td>
<td>28 h</td>
<td>-H</td>
<td>127</td>
<td>40</td>
<td>9 (23.24)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+H</td>
<td>110</td>
<td>39</td>
<td>6 (15.28)*</td>
</tr>
<tr>
<td></td>
<td>32 h</td>
<td>-H</td>
<td>109</td>
<td>47</td>
<td>9 (16.94)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+H</td>
<td>135</td>
<td>66</td>
<td>12 (12.84)*</td>
</tr>
<tr>
<td></td>
<td>42 h</td>
<td>-H</td>
<td>121</td>
<td>74</td>
<td>27 (33.42)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+H</td>
<td>109</td>
<td>63</td>
<td>14 (19.97)*</td>
</tr>
<tr>
<td>RPMI</td>
<td>28 h</td>
<td>-H</td>
<td>110</td>
<td>33</td>
<td>6 (17.57)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+H</td>
<td>127</td>
<td>42</td>
<td>17 (38.30)</td>
</tr>
<tr>
<td></td>
<td>32 h</td>
<td>-H</td>
<td>117</td>
<td>53</td>
<td>10 (18.38)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+H</td>
<td>112</td>
<td>55</td>
<td>10 (17.08)*</td>
</tr>
<tr>
<td></td>
<td>42 h</td>
<td>-H</td>
<td>114</td>
<td>82</td>
<td>23 (25.98)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+H</td>
<td>128</td>
<td>85</td>
<td>25 (29.19)*</td>
</tr>
</tbody>
</table>

- Values in the same column with different superscripts differ significantly ($P<0.05$).
- Table represents mean numbers of oocytes (%) in each stage of maturation (Mean %).
- GV = Germinal vesicle, GVBD = Germinal vesicle breakdown, MI = Metaphase I, AI = Anaphase I, TI = Telophase I, M II = Metaphase II and Deg. = Degenerated.
- +H = treated with gonadotropins. -H = without gonadotropins

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supplementing maturation media with gonadotropin hormones significantly \((P<0.05)\) improved oocyte maturation rate compared to the control group \((20.58\ Vs\ 16.04\%)\).

**In vitro Meiotic Maturation Rate of Camel Denuded Oocytes as Affected by Length of Culture Time:** Effect of maturation times on meiotic maturation rate of camel denuded oocytes is listed in Table (4). Present results showed that the proportion of oocytes reaching MII was increased after 42hrs of culture time compared to other maturation times; 28h and 32h \((24.59\ Vs\ 11.96\ and\ 18.38\%\ respectively)\). This increase was significant \((P<0.05)\) than those observed after 32h and highly significant \((P<0.01)\) than those found after 28h maturation time. Also, meiotic maturation rate in camel oocytes significantly elevated \((P<0.05)\) after 32h than those found after 28h of maturation time \((18.38\ Vs\ 11.96)\).

**Effect of Interaction among Types of Media, Adding of Hormones and Different Maturation Times on in vitro Meiotic Maturation of Camel Denuded Oocytes:** Present results (Table, 5) observed that the effect of interaction among types of media, gonadotropins supplementation and different maturation times on the proportion of denuded oocytes reaching MII was not significant, although the highest proportion of oocytes reaching MII was found in TCM-199 medium group supplemented with gonadotropins after 42h of maturation time \((32.08\%)\).

**DISCUSSION**

The present study we aimed to design an improved system of IVM for camel denuded GV-stage oocytes, that are usually discarded in the standard IVM-IVF system. So, we investigated the effect of different culture media, adding gonadotropin hormones and use different culture periods on IVM rate of camel DOs.

**Effect of Culture Media on IVM of Camel DOS:** The present findings showed that the proportions of DOs oocytes reaching MII significantly \((P<0.05\ or\ P<0.01)\) elevated in groups cultured in TCM-199 than those of cultured in Ham's F-10 or RPMI-1640 medium. These results were supported by several reports on buffalo[12], Pig [7] and bovine [33], these reports found a good quality of meiotic stages especially metaphase II in denuded oocytes that cultured in TCM-199.

The effect of different culture media on IVM of animal oocytes was only discussed previously on COCs. So, the present findings were similar with that observed by Farag et al. [24] on camel COCs, the observations found that TCM-199 was the most efficacious medium for IVM of oocytes compared to the other types of media (Ham's F-10 and RPMI-1640). In sheep, Attia [24] found a high maturation rate \((80\%)\) of ovine COCs oocytes when cultured in TCM-199 compared to 70\% maturation rate achieved when the oocytes cultured in Ham's F-10. Also, Farag et al.[5] showed that the proportion of sheep COCs oocytes reaching MII significantly increased \((P<0.05)\) when they matured in TCM-199 compared to those matured in RPMI-1640 \((19.84\ Vs\ 3.98)\). The higher maturation rates in goats[35] and buffalo [36] COCs oocytes were achieved in TCM-199 than in Ham's F-10 medium. In addition, Gliedt et al. [37] showed that TCM-199 medium is superior to RPMI-1640 medium in promoting IVM of bovine COCs oocytes.

The differences among different culture media in oocyte IVM may be due to the composition of the media: Rexroad and Powell [38] concluded that the improved maturation index could be related to additional factors in TCM-199 such as insulin which stimulates DNA and RNA synthesis and enhances cell division. Also, the higher IVM rate of camel oocytes which achieved with TCM-199 than other media (Ham's F-10 and RPMI-1640) in the present study may be attributed to the differences in their ionic concentration and in the concentration of energy sources[39].

**Effect of Gonadotropins on IVM of Camel DOS:** The results in the present study, showed that the supplementation of gonadotropins (PMSG-hCG) to culture media significantly \((P<0.05)\) improved meiotic maturation rate of camel denuded oocytes than that cultured in hormone-free media. Our findings were similar with that reported by Dey et al. [33] who found that the proportion of bovine denuded oocytes reaching MII significantly increased when they cultured in TCM-199 supplemented with gonadotropin (FSH) in comparison to those cultured in gonadotropin-free medium. Also, Feng et al. [40] cultured groups of cat denuded oocytes in Ham's F-10 of cat denuded oocytes in Ham's F-10 with or without gonadotropins (FSH/LH) and subjected to IVM. Their results showed that the groups of DOs cultured in the presence of gonadotropins reached more advanced meiotic stages \((P<0.05)\) than in the absence of
gonadotropins. Previous study by Pawshe et al. [35] showed that the gonadotropins are primary regulators of nuclear maturation in mammalian oocytes in vitro and one from the functions of its preovulatory surge is to suppress the granulosa cell factor that inhibits meiosis. The effect of such hormones on oocyte maturation could be explained by the presence of receptors for gonadotropins on the cell membrane of oocytes that, in turn, triggered meiotic resumption, as it has been demonstrated in human and pig [41] and cat [40] oocytes. In this respect, Vanhoutte et al. [42] reported that the oocyte is not just a passive recipient of developmental signals from its associated somatic cells (cumulus cells), but also plays a principal regulatory role of its own meiotic and developmental competence.

Effect of Length of Culture Period on IVM of Camel DOS: The present results showed that increasing the culture period from 28 to 42h has improved the meiotic maturation rate of camel denuded oocytes. The proportions of DOs reaching MII were significantly elevated after 42h of culture time compared to other maturation times 28 or 32h. To our knowledge the comparison of effects of length of culture period on improvement the IVF rate of DOs of camel or of other mammalian species was not discussed previously. However, there were several studies used one time only for IVM of DOs oocytes: For example, the proportions of DOs reaching MII were 42%, 38.5-62.9% and 60-70% in buffalo [12]; bovine [33] and pig [7], respectively, after 24h of in vitro culture. Also, the rate of IVM of mouse DOs was 85% after 14-15h of culture period [19] while, it was 16% of cat DOS after 24h of culture time [40]. The DOs in previous different species were cultured without adding cumulus cells, but the different IVM rate might be due to different of culture condition. On the other hand, our findings were similar with that reported on camel COCs oocytes by Farag et al.[24], who found that the proportions of COCs reaching MII at 42h was higher ($P < 0.05$ or $P <0.01$) than those observed at other maturation times 28 and 32h ($P < 0.05$ or $P <0.01$) than those observed at other maturation times 28 and 32h (57.39 vs. 24.30 and 36.02%, respectively).

In conclusion the present study adds evidence that, it is possible to restore the in vitro meiotic maturation of camel DOs by utilization of appropriate the in vitro maturation conditions without presence of or adding cumulus cells. This in vitro culture condition was achieved by employment TCM-199 medium supplemented with gonadotropin hormones in form PMSG+hCG and using maturation time of 42h. Such simple culture system could be a solution to success of IVM of DOs oocytes and consequently lead to remuneration the inefficient of IVM of COCs especially of prepubertal oocytes [43] and poor quality oocytes [44,45]. It might also be a technical solution for the in vitro culture of immature denuded oocytes for micromanipulation or cryopreservation [46-48].

REFERENCES


