In-vitro maturation of camel oocytes using different media and sera

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Why?

- Economic role of dromedary camel
  - Milk
  - Meat
- Therapeutic effect (Knoess, 1984; Abdalla et al., 2015).
- Seasonality of reproduction
  - Male (Allam et al., 2013).
  - Female (Shalash, 1980; Kandil et al., 2014).
- Selection (Abdalla et al., 2015).
- Culture medium (Humblot et al., 2005; Harris and Picton, 2007).
- Very few data (Kandil et al., 2014).
Breeding & non-breeding season

Two maturation media
- TCM-199
- MEM

Three types of serum
- FDCS
- BSA
- ESS

In-vitro maturation of camel oocyte
Material and methods

- Ovaries collection
- Meteorological data
- Laboratory
Ovaries collection

175 non-pregnant she-camel

350 ovaries

110 during breeding season

240 during non-breeding season
Meteorological data

- Ta (°C)
- RH (%)
- THI
- Day light length (hr)
Laboratory

- Harvesting media (PBS)
- Maturation media
- Oocyte collection
- Oocyte categories
- Fixation
- Staining
- Examination for maturation
- Statistical analysis
Oocyte categories

Compact cumulus oocyte
- Oocyte with five or more layers of complete cumulus cells.

Partial denuded oocyte
- Oocyte with cumulus cells present either incompletely surrounding the oocyte.

Denuded oocyte
- Oocyte without cumulus cells and covered by zona pellucida.

Shrunken (degenerated) oocyte
- Ooplasm shrunk away from the zona pellucida or not evenly filling the zona ooplasm looks degenerated with fragment empty zona pellucida.
Photomicrograph of the different oocyte categories.

- Compact cumulus
- Partially denuded
- Denuded oocyte
- Degenerated oocyte
The supplementation included the following media:

- **T1**: TCM-199 + 10% FDCS.
- **T2**: TCM-199 + 6mg/ml BSA.
- **T3**: TCM-199 + 10% ESS.
- **T4**: MEM + 10% FDCS.
- **T5**: MEM + 6mg/ml BSA.
- **T6**: MEM + 10% ESS.
Maturation

- **pH**: 7.2-7.4
- **osmolarity**: 280-300 mOsmol/kg
- **Filter**: 0.22 μm-millipore
- **maturation medium**: 200 μl
- **RH**: 90-95%
- **Temperature**: 38.5 °C
- **Time**: 40 hr
Table 1. Compositions of maturation media and sera

<table>
<thead>
<tr>
<th>Content</th>
<th>Amount Per 100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media:</td>
<td></td>
</tr>
<tr>
<td>TCM-199 or MEM</td>
<td>9 ml</td>
</tr>
<tr>
<td>(liquid)</td>
<td>9 ml</td>
</tr>
<tr>
<td>Sera:</td>
<td></td>
</tr>
<tr>
<td>FDCS</td>
<td>10%</td>
</tr>
<tr>
<td>BSA</td>
<td>6 mg/ml</td>
</tr>
<tr>
<td>ESS</td>
<td>10%</td>
</tr>
<tr>
<td>PMSG</td>
<td>20 µg/ml</td>
</tr>
<tr>
<td>HCG</td>
<td>10 IU/ml</td>
</tr>
<tr>
<td>Oestradiol-17β</td>
<td>1 µg/ml</td>
</tr>
<tr>
<td>Na Pyruvate</td>
<td>20 mMol</td>
</tr>
<tr>
<td>Na Penicillin G</td>
<td>100 IU/ml</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>100 µg/ml</td>
</tr>
</tbody>
</table>
Criteria of maturation

**Germinal vesicle (GV)**
- Inter phase chromosomes enclosed within a nuclear membrane.

**Germinal vesicle breakdown (GVBD)**
- An absence of a visible nuclear membrane and chromatin condensation characterized by a cluster of DNA material without individual chromosomes.

**Metaphase-I (MI)**
- Chromosomes were condensed in pairs and without detected polar body (immature oocytes).

**Metaphase-II (MII)**
- One the larger group of chromosome formed an equatorial plate and the remaining chromosomes are highly condensed or had extruded a polar body (oocytes mature).

**Degenerated**
- Oocytes were vacuolated or had scattered or highly condensed chromatin.
Results
Table 2. Air temperature, relative humidity, temperature-humidity index and length of day light, during breeding and non-breeding seasons.

<table>
<thead>
<tr>
<th>Season</th>
<th>Air temperature (°C)</th>
<th>Relative humidity (%)</th>
<th>Temperature-humidity index (THI)</th>
<th>Length of day light (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Breeding</td>
<td>11.23</td>
<td>21.66</td>
<td>43.02</td>
<td>58.49</td>
</tr>
<tr>
<td>Non-breeding</td>
<td>20.84</td>
<td>34.30</td>
<td>42.67</td>
<td>63.66</td>
</tr>
</tbody>
</table>

- No heat stress $\leftarrow$ 72 - moderate heat stress - 74 - severe heat stress - 78 $\rightarrow$ very severe heat stress
Table 3. Effect of breeding and non-breeding season on in-vitro maturation rate of camel oocytes.

<table>
<thead>
<tr>
<th>Season</th>
<th>Total Oocyte number</th>
<th>Criteria of maturation</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Germinal vesicle</td>
<td>Germinal vesicle breakdown</td>
<td>Metaphase-I</td>
<td>Metaphase-II</td>
<td>Degenerated</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Breeding</td>
<td>710</td>
<td>99</td>
<td>13.9</td>
<td>93</td>
<td>13.1</td>
<td>120</td>
<td>16.9</td>
</tr>
<tr>
<td>Non-breeding</td>
<td>650</td>
<td>86</td>
<td>13.2</td>
<td>90</td>
<td>13.9</td>
<td>111</td>
<td>17.1</td>
</tr>
</tbody>
</table>

a and b: Means denoted within the same column with different superscripts are significantly different (P<0.05).
### Table 4. Effect of maturation media on in-vitro maturation rate of camel oocytes.

<table>
<thead>
<tr>
<th>Type of medium</th>
<th>Total Oocyte number</th>
<th>Criteria of maturation</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GV</td>
<td>N</td>
<td>%</td>
<td>GVB</td>
<td>N</td>
</tr>
<tr>
<td>TCM 199</td>
<td>700</td>
<td>87</td>
<td>12.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>90</td>
<td>12.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MEM</td>
<td>660</td>
<td>98</td>
<td>14.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>93</td>
<td>14.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> and <sup>b</sup>: Means denoted within the same column with different superscripts are significantly different (<i>P</i>&lt;0.05). TCM199 = Tissue Culture Medium; MEM = Minimum Essential Medium.
Table 5. Effect of type of serum on in-vitro maturation rate of camel oocytes.

<table>
<thead>
<tr>
<th>Type of serum</th>
<th>Total oocyte number</th>
<th>Criteria of maturation</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Germinal vesicle</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Germinal vesicle breakdown</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metaphase-I</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metaphase-II</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Degenerated</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>FDCS</td>
<td>465</td>
<td>52 11.2 b</td>
<td>61</td>
<td>13.1 a</td>
<td>70</td>
<td>15.1 b</td>
</tr>
<tr>
<td>BSA</td>
<td>455</td>
<td>64 14.1 a</td>
<td>65</td>
<td>14.3 a</td>
<td>83</td>
<td>18.2 a</td>
</tr>
<tr>
<td>ESS</td>
<td>440</td>
<td>69 15.7 a</td>
<td>57</td>
<td>13.0 a</td>
<td>78</td>
<td>17.7 a</td>
</tr>
</tbody>
</table>

\^a and \^b: Means denoted within the same column with different superscripts are significantly different (\(P<0.05\)). FDCS = Fetal Dromedary Camel Serum; BSA = Bovine Serum Albumin; ESS = Estrus Sheep Serum
Conclusion

use of TCM-199 medium for in vitro maturation of camel oocytes added with fetal dromedary camel serum (FDCS) to promote in vitro camel oocytes maturation for in-vitro fertilization programme to enhance of fertilizing ability of she-camel oocytes, especially during breeding season.
Thank you for your attention