ABSTRACTS

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Viability of domestic cat morulae cells according to ovarian status during breeding season and non-breeding season

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OBJECTIVES AND METHODS: This experiment was designed to determine the mean number of viable cells with normal nuclei (VN) and non-viable cells with fragmented nuclei (NVF) of domestic cat morulae produced from oocytes recovered from queen ovaries of three distinct status – follicular, luteal or inactive (1) – during two different reproductive seasons experienced by cats in southeast of Brazil (22º53'09" S and 48º26'42" W) – non breeding season (NBS), from January to March; and breeding season (BS), August to October. Twenty eight queens were neutered. Ovaries were classified according to their status and were sliced in PBS for cumulus oocyte complex (COC) releasing. Grade I COC were washed three times in H-MEM supplemented with 3 mg/mL BSA, 2.0 mM glutamine, 1.0 mM sodium pyruvate, 0.13 mM cystein, 100mg/mL streptomycin and 100 UI/mL penicillin. Oocytes were incubated in groups of 20-30 in 400 µL of DMEM supplemented with 10 µg/mL FSH, 1 µg/mL LH, 1 µg/mL estradiol, 20 ng/mL IGF-I and 10 ng/mL basic fibroblast growth factor under mineral oil for 30 or 36 hours at 38°C in humidified environment of 5% de O₂, 5% CO₂ and 90% N₂. COC were fertilized in Ham’s F-10 medium supplemented with 3 mg/mL BSA, 0.13 mM cystein, 1 mM pyruvate and 100 mg/mL streptomycin and 100 UI/mL penicillin (culture medium) with fresh semen selected through swim up technique (2). Eighteen hours later, the presumptive zygotes were denuded, the percentage of cleavage was determined and every 10 zygotes were transferred to 100 µL drops of culture medium for culture during two days. After 48 hours of culture, morulae were stained using acridine orange and propidium iodide (3), examined under fluorescent microscope and morulae cells were classified as VN or NVF. For statistical analysis SAS was used. The procedures were in accordance with the ethical standards of the committee on animal experimentation of Unesp.

RESULTS: Results can be visualized on tables 1 and 2, respectively and on figure 1.

Table 1- Viability of morulae produced from oocytes recovered from domestic cat ovaries of three distinct status during NBS

<table>
<thead>
<tr>
<th>Morulae/ovarian status (n)</th>
<th>Cell number/morulae ± SD</th>
<th>VN ± SD (%)</th>
<th>VNF ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive (20)</td>
<td>10.70 ± 2.27</td>
<td>9.9 ± 2.12</td>
<td>(92.52)</td>
</tr>
<tr>
<td>Luteal (20)</td>
<td>10.30 ± 1.56</td>
<td>9.2 ± 1.55</td>
<td>(89.81)</td>
</tr>
<tr>
<td>Follicular (20)</td>
<td>10.35 ± 2.36</td>
<td>9.8 ± 2.14</td>
<td>(94.69)</td>
</tr>
</tbody>
</table>

p=0.505 p<0.1 p<0.05

VN: viable cells with normal nuclei; NVF: non-viable cells with fragmented nuclei. Same letters in the same column indicate no statistical difference (Tukey test).

Table 2- Viability of morulae produced from oocytes recovered from domestic cat ovaries of three distinct status during BS

<table>
<thead>
<tr>
<th>Morulae/ovarian status (n)</th>
<th>Cell number/morulae ± SD</th>
<th>VN ± SD (%)</th>
<th>VNF ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive (20)</td>
<td>10.26 ± 2.25</td>
<td>9.65 ± 1.75</td>
<td>(91.04)</td>
</tr>
<tr>
<td>Luteal (20)</td>
<td>10.50 ± 1.43</td>
<td>9.40 ± 1.5</td>
<td>(89.52)</td>
</tr>
<tr>
<td>Follicular (20)</td>
<td>10.55 ± 2.11</td>
<td>9.55 ± 1.53</td>
<td>(90.52)</td>
</tr>
</tbody>
</table>

p=0.505 p<0.1 p<0.05

VN: viable cells with normal nuclei; NVF: non-viable cells with fragmented nuclei. Same letters in the same column indicate no statistical difference (Tukey test).

CONCLUSION: In the conditions of our study, no statistic difference was verified in the mean number of viable and non-viable morulae cells when comparing the three ovarian conditions in both seasons. Although this technique is suitable is based on detection of the DNA fragmentation that occurs in early apoptotic program, apoptosis should be confirmed independently by electron micrograph analysis (3).
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Figure 1 – Eight-cell embryo produced from a luteal status ovary during the breeding season stained with acridine orange (highlighting an NVF cell at upper right edge) and propidium iodide (highlighting a VN cell just right and above the center).