Influence of Early Apoptosis Incidence on In Vitro Maturation of Bovine Oocytes

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Abstract

Apoptosis in oocyte could be a good marker for oocyte quality and development competency. The study aims to investigate the relation between early apoptosis occurrence in different morphological groups of oocytes, i.e. Group A, B and C, and their developmental potential in terms of meiotic resumption to metaphase II. Annexin-V staining was used to detect early apoptosis in oocytes and Giemsa staining for meiotic resumption. Immature oocytes in Group B and C showed significantly high incidence of early apoptosis compared to Group A oocytes (A: 10.20%, B: 19.00% and C: 20.60%). After maturation, no differences were observed in the incidence of early apoptosis among oocytes from different groups (A: 28.40%, B: 18.20% and C: 23.00%). However, the mean percentage of early apoptosis increased among Group A oocytes after maturation. The progression to metaphase II were similar among the different groups of oocytes (A: 34.09%, B: 31.54% and C: 33.45%). In conclusion, early apoptosis occurrence in bovine oocytes is related to developmental competence.

Keywords: Early Apoptosis, Bovine Oocyte, In Vitro Maturation
1. Introduction

The importance of the study on apoptosis and its influence in oocyte and embryo quality, especially in in vitro technologies lays on the fact that a very heterogeneous oocyte population, at different stages of growth and atresia, is used to produce embryos. In the ovary, apoptosis is responsible for follicular atresia, whereby most follicles present at birth are lost during further development [1]-[3]. Therefore, when oocytes from slaughtered animals are recovered by slicing, most of these oocytes will have arisen from atretic follicles and blastocyst production could be consequently impaired. Apoptosis in oocytes could be a good marker of oocyte quality and its capacity to develop into a viable embryo. Previous studies have reported that apoptosis in the oocyte may affect embryo quality, because of the presence of molecules that regulate the apoptotic mechanism in the maternal mRNA stored in the oocyte [4]-[6].

However, information on apoptosis in bovine oocytes is limited and studies on the incidence of apoptosis produced conflicting results. A study [7] demonstrated that apoptosis occurs both in mature and immature oocytes by using TUNEL staining to detect late apoptosis, while another, detected no apoptotic oocytes before or after maturation [8]. A previous study reported that early apoptosis is related to improved developmental potential in bovine oocyte. Many immature oocytes showed early apoptosis i.e. positive Annexin-V staining compared to late apoptotic using TUNEL assay [9]. While another reported that apoptosis assessed by both Annexin-V and TUNEL assay was detected in both mature and immature oocytes [10].

The present study aims to investigate the occurrence of early apoptosis in immature and matured bovine oocytes and its relation with the developmental competence evaluated by nuclear maturation. First, the incidence rates of early apoptosis in different groups of immature bovine oocytes were determined. Next, immature oocytes were matured for 24 h and the incidence of early apoptosis and their progression to metaphase II were evaluated.

2. Materials and Methods

2.1. Collection and Classification of Oocytes

Bovine ovaries were obtained from local slaughterhouse and transported to the laboratory in Dulbecco’s phosphate buffered saline (D-PBS, Sigma) maintained at 30°C - 38°C within 2 hours of slaughter. The cumulus-oocyte complexes (COCs) were recovered by slicing in the collecting medium i.e. D-PBS supplemented with 10% steer serum and transferred into 35 X 10 mm culture dishes containing 3 ml of TCM 199 supplemented with 25% steer serum, gentamycin, 20 mM sodium pyruvate, 100 mM L-glutamine and 1 μg/ml estradiol-17β. Oocytes showing heterogenous ooplasm were selected and classified into three groups, i.e. A, B and C according to the layer of cumulus cells [11]. Oocytes with compact and dense cumulus cell layers were classified as Group A. Group B consists of oocytes with compact but not dense cumulus cell layers (1 to 5 layers) and Grade C, oocytes with thin or little remnants of cumulus cell layers, and expanded.

2.2. Annexin-V Staining

Oocytes were denuded and then stained using Annexin-V kit according to the manufacturer’s instructions (Sigma). The procedure consists of the binding of Annexin-V-FITC to phosphatidylserine in the membrane of cells, which are the beginning of the apoptotic process. The samples were also stained with propidium iodide (PI) to differentiate live cells from dead cells. Briefly, oocytes were washed twice in PBS and incubated in 100 μl of binding buffer containing Annexin-V and PI for 10 minutes at room temperature in the dark. Oocytes were then mounted on slides and observed under a fluorescent microscope.
2.3. **In vitro Maturation**

For maturation, groups of 15 to 20 COCs were transferred into droplets of 100 μl of IVM medium i.e. TCM 199 under silicone oil (Sigma) pre-equilibrated in a CO2 incubator. They were incubated in 5% CO2 humidified incubator for 24 hours to achieve complete maturation. For nuclear maturation assessment, matured oocytes were denuded, fixed and stained using Giemsa to determine the oocyte progression to metaphase II.

2.4. **Statistical Analysis**

The statistical analysis was carried out using SPSS 21 for Windows. The rates of Annexin-V positive oocytes and nuclear maturation (metaphase II) were analyzed by one-way ANOVA and Duncan post-hoc test.

3. **Results and Discussion**

3.1. **Early Apoptosis in Immature Oocytes**

It is generally accepted that cumulus-oocyte complexes (COCs) that come from non-atretic follicles show compact cumulus cell layers and an homogeneous oocyte cytoplasm [12] as well as showing higher developmental competence [13]-[15], [8]. Nevertheless, some studies provided conflicting results concluding that COCs showing mild signs of atresia lead to higher blastocyst rates in cows [12], [16]. Apoptosis incidence in oocytes has been used as one of the approaches to find out the characteristics that affect oocyte quality. In this study, early apoptosis were detected both in mature and immature oocytes by using Annexin-V staining which are similar to the findings by [9] and [10]. The Annexin-V staining detected early apoptosis in both immature and mature oocytes from all morphological groups based on the layers of cumulus cells surrounding the oocytes. The mean percentage of the rates of early apoptosis in different groups of immature oocytes is shown in Table 1. Among immature oocytes, the incidences of early apoptosis in Groups B (19.00%) and C (20.60%) were found to be significantly high (p<0.05) compared to Group A oocytes (10.20%).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of oocytes</th>
<th>Early apoptosis (Mean % ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>63</td>
<td>10.20 ± 2.82a</td>
</tr>
<tr>
<td>B</td>
<td>73</td>
<td>19.00 ± 2.28b</td>
</tr>
<tr>
<td>C</td>
<td>89</td>
<td>20.60 ± 2.36b</td>
</tr>
</tbody>
</table>

*a,b Values with different superscript differ significantly (p<0.05)

Immature oocytes with compact and not dense cumulus cells (Group B), and oocytes with thin or little remnants of cumulus cell layers and expanded (Group C) showed a significantly high rate of early apoptosis compared to oocytes with compact and dense cumulus cells (Group A). The occurrences of early apoptosis in these oocytes were twice as high as in Group A. All groups of oocytes analyzed showed heterogenous ooplasm i.e. one of the characteristics of early signs of atresia. These oocytes may be at earlier stages of apoptosis before DNA breakdown since they are the last compartment to be affected by apoptosis during atresia of antral follicle [12]. However, it is still not known whether oocytes with compact and more layers of cumulus cells surrounding the oocytes such as Group A come from late atretic follicles.
3.2. Early Apoptosis in Mature Oocytes

After 24 h maturation, it was found that the incidence of early apoptosis did not differ between Group A, B and C. While the rate of early apoptosis incidence in Group A increased after maturation, the rates for Group B and C remained as they were before maturation. Table 2 represents the early apoptosis and nuclear maturation rates observed in bovine oocytes after 24 h maturation. No significant difference was found in the incidence of early apoptosis between all groups of oocytes. Group A showed an increase in the rate of early apoptosis incidence after maturation. The mean percentage of the rate of early apoptosis for Group A, B and C oocytes were 28.40, 18.20 and 23.00% respectively.

Table 2. Early apoptosis and metaphase II in mature oocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>Total oocytes</th>
<th>Used</th>
<th>Early apoptosis (Mean % ± S.E)</th>
<th>Metaphase II (Mean % ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>136</td>
<td>136</td>
<td>28.40 ± 3.59a</td>
<td>34.09 ± 6.32a</td>
</tr>
<tr>
<td>B</td>
<td>185</td>
<td>185</td>
<td>18.20 ± 3.09a</td>
<td>31.54 ± 4.08a</td>
</tr>
<tr>
<td>C</td>
<td>179</td>
<td>179</td>
<td>23.00 ± 3.49a</td>
<td>33.45 ± 6.47a</td>
</tr>
</tbody>
</table>

Values with different superscript differ significantly (p<0.05)

For nuclear maturation, the study demonstrated that the progression of oocytes to metaphase II after 24 h maturation do not differ between all groups of oocytes. The mean percentage of oocytes reaching metaphase II in Group A is 34.09%, Group B, 31.54% and Group C, 33.45%. This may indicate that in vitro maturation increased the occurrence of early apoptosis in Group A oocytes. Moreover, no difference was observed in the rates of oocytes reaching metaphase II after 24 h in vitro maturation between all groups of oocytes. It is possible to suggest that although Group A oocytes showed heterogenous ooplasm they may not derived from late atretic follicles since the early apoptosis incidence in immature oocytes were low. Grade B and C immature oocytes showed a relatively high occurrence of early apoptosis compared to Group A, nevertheless their meiotic resumption to meiosis II were similar to Grade A oocytes. This may indicate that in vitro maturation had no effect on the rate of early apoptosis incidence in these oocytes. These findings are consistent with previous reports by [9] and [17], indicating that oocytes with early signs of atresia have good developmental potential. Thus this study supports the notion that the occurrence of early apoptosis in oocytes does not necessarily develop into late apoptosis, which decreases their developmental competence [10], [18]. A low level of atresia in follicle tends to improve the in vitro competence of oocytes [11], [16], [19], [20].

4. Conclusion

The occurrence of early apoptosis in bovine oocytes, mainly among oocytes with early signs of atresia i.e. having less dense or thin cumulus cell layers and showing heterogenous ooplasm may be associated with the developmental competence.

Acknowledgements

This research was supported financially by the University Malaya Research Grant (Grant No. RG128-10AFR).
References