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APCBEE Procedia 8 (2014) 272 – 276

Procedia APCBEE

www.elsevier.com/locate/procedia

2013 4th International Conference on Agriculture and Animal Science (CAAS 2013)
2013 3rd International Conference on Asia Agriculture and Animal (ICAAA 2013)

# 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.


Selection and peer review under responsibility of Asia-Pacific Chemical, Biological & Environmental Engineering Society

*Keywords:* Early Apoptosis, Bovine Oocyte, *In Vitro* Maturation

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doi:10.1016/j.apcbee.2014.03.039

## 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 apoptosis 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° - 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.