OSMOLARITY AND APOPTOSIS IN IVF BOVINE EMBRYOS

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ABSTRACT

The aim of this research was to establish the optimal conditions for culture of bovine embryos obtained in vitro and the effects of osmolarity on development and apoptosis. The embryos obtained by in vitro fertilization (IVF) procedure were divided into two different groups. The embryos from the control group were cultured for 7 days in B2-Menezo. The embryos from the experimental group were cultured in medium with NaCl, having variation of concentration and osmolarity. Results showed that there are differences in development stage and apoptosis. Increasing the concentration from 90 to 120mM NaCl generated decreasing of the blastocyst stage ($P<0.05$), and also the apoptosis was increased in the blastocyst stage. The optimum concentration of NaCl was 90mM and the optimum osmolarity of the medium was between 260-280 mOsmol for the studied IVF bovine embryos, affecting positively the embryos development.

INTRODUCTION

Since 1967 Potts and Wilson studied the causes of cells dead in mammalian embryos, and in 1972 Kerr and his collaborators described that the cell die by programmed process, called apoptosis – process that assure the regulation of the cell population in the development stages.

Generally, the cell dead can happened by apoptosis and necrosis – which are morphologically different. While the apoptosis affects only single cells, the necrosis acts on a large cells group. Apoptosis is not associated with any inflammatory processes, and entail consecutive distinct stages of morphology. At the beginning, it can be observed the aggregate of chromatin forming a large granular compact mass on the inner nuclear membrane. Then, the cytoplasm is condensed and its membrane and the nuclear membrane become extremely indented.

Also, the embryo culture medium is influenced by osmolarity. As well, the culture medium depends on salt concentration, amino acids content that modifies the osmolarity and cell volume regulation (Liu and Foote, 1996).

These experiments were performed on different animal species (porcine, rabbit, mice, bovine). Liu and Foote (1995) reported their results and they showed that the concentration of sodium chloride solution can not be higher then 93mM for rabbits. As well, the osmolarity of the cultured medium has to be 250-270mOsmol for bovine IVF embryos (Liu and Foote, 1996).

Another experiment presents the cultivation of porcine IVF and NT embryos in medium with various osmolarity. Their results presented that high osmolarity or medium earlier cultured stage had low apoptosis rate compared to the embryos cultured in normal osmolarity (Hwang, 2008).

Preimplantation embryos will develop normally in medium with low salt concentration and low osmolarity. A higher osmolarity of embryo culture medium can allow

embryo development when there are present some organic osmolytes (for example: amino acids) – Baltz, 2012.

MATERIALS AND METHODS

In Vitro Production of Embryo

Cumulus-oocyte complexes (COC) were obtaining by aspirating follicle from 1 to 5 mm. The ovaries bovine were collected of slaughtered cows. The COC only with compact non-atretic cumulus oophorus and graduated cytoplasm were selected for maturation for 24 h at 39 grade C and 5% CO2 in TCM-199 supplemented with 10% fetal bovine serum (FBS).

The oocytes were inseminated with semen sample from a single bull of proven fertility and layered on a discontinuous Percoll gradient (45:90) and centrifuged at 1800 g for 30 min (Suthar and Shah, 2009).

Gametes were coincubated for 18 h under the same conditions as described for oocyte maturation. Presumptive zygotes were cultured in B2-Menezo under mineral oil for 7 day for all experiments on blastocysts at 39 grade C and 5% CO2.

Embryos obtained by in vitro fertilization (IVF) procedures were divided in two groups. Control groups embryos were cultured in B2-Menezo. Other group was cultured in medium with NaCl in concentration of 90 to 120 mM and osmolarity of the medium is 260-280-mOsmol.

Embryo stages after fertilization were selected (Van Soom et al.1997):two-cell, 31h; 4-cell, 38h; 5 to 8 cell, 49 h; 9 to 16-cell, 100h; morula,128h; early blastocyst, day 6; expanded day 7; hatched blastocyst, day 8.

A Tunel assay was used to assess the presence of apoptotic cells. Day 7 embryos were fixed and permeabilized as described for mouse embryos (Brison and Schultz, 1996; 1997) but with an increase in the time of permeabilization in 0.5% Triton X-100 to 180 min. Total number of cells and of apoptotic dead cells were counted.

RESULTS AND DISCUSSIONS

There were some differences founded in the development and apoptosis incidence during the treatment with NaCl solution. Increasing of NaCl concentration from 90mM to 120mM decreased blastocyst formation ($P<0.05$). The apoptosis was observed at 8-16 cells stage of development (stage of genome activating at bovine) decreasing at the morula stage and increasing to blastocyst stage.

Next table presents our results regarding the effect of sodium chloride concentration on different stages of bovine embryos development, and also the apoptotic cell index. Totally we form two different treatments of bovine embryos and one control. After bovine IVF we add to the embryos sodium chloride solution in two different concentrations (90mM to 120mM) for the first three days, and after that cultured in B2-Menezo for the next days. For the control group we cultured the embryos in B2-Menezo for all the cultured period, without any sodium chloride supplementation. Our results of experiment showed that the supplementation with sodium chloride is important for the development of embryos, but the positive effect is depending on the concentration and osmolarity.

Also, we evaluated the apoptotic cell index by TUNEL, and we observed that the apoptosis was smaller in NaCl supplemented embryos compared to the control. Our research presented that there is a decrease of apoptotic cell index depending of concentration of NaCl, meaning that as great is the supplementation with sodium chloride as small is the apoptosis cell death (2.91 for supplementation with NaCl 120mM and 3.47 for NaCl 90mM). But this observation has to be correlated with the blastocyst percent. Blastocyst percent is greater in the supplementation with NaCl 90mM solution (34%) compared to the control (29%) and the other treatment (NaCl 120mM – 20% blastocyst).

This happened even the apoptotic cell index is also greater in supplementation with NaCl 90mM compared to the other treatment (NaCl 120mM).

As well, apoptosis process is depending to the embryos quality (Van Soom et al., 1997). In the presence of fetal bovine serum (FBS) the apoptosis cell death is increased (Byrne et al., 1999). However, the rate of apoptosis is not influenced by heat inactivation of fetal bovine serum – which contains a lot of nutrients for the embryos survival factors, such as: growth factors – peptide nature, and also cytokines or tumor necrosis factor – that can induce apoptosis process (Brison and Schultz, 1996, 1997, 1998; Suthar and Shah, 2009).

Table Effects of NaCl on the development of bovine IVF embryos

Treatment	***No. of oocytes cultured***	***% > 2 cell***	***% blastocyst***	***% Tunel***
Control	*76*	*82*	*29*	*3.88*
NaCl (90mM)	*78*	*88*	*34*	*3.47*
NaCl (120mM)	*75*	*74*	*20*	*2.91*

For a very well development of embryos, after this research, we obtain the optimum concentration of NaCl 90 mM, and the optimal osmolarity of the medium was 260-280mOsmol. Apoptotic dead cell index in day 7 of embryo blastocyst was reversal correlated with the total number of cells.

CONCLUSIONS

Concentration optimal of NaCl is of 90 mM and osmolarity of the medium is 260-280 mOsmol for bovine IVF embryos.

Apoptotic dead cell index in day 7 blastocyst is correlated negatively with the number total of cell. The Tunel percent is greatest for bovine embryos without supplementation of saline solution, and was the lowest for the embryos supplemented with sodium chloride 120mM solution.

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