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STRUCTURAL AND ULTRASTRUCTURAL ANALYSIS OF GERM CELLS FROM OVARIES OF FETUSES OF BUFFALOES

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ABSTRACT

The objective of this research was the structural and ultra structural characterization of the oogonia, primary oocytes and preantral follicles of buffalo foetuses at different gestational stages. For this study twenty-nine female foetuses of buffaloes where gathered in a slaughterhouse for the analysis, and were divided into 3 experimental groups: foetuses of the first (G1, n = 10), second (G2, n = 9) and third (G3, n = 10) trimester of pregnancy. After obtaining the foetus from the pregnancy uterus, both ovaries were removed from each foetus, one of which was processed by the analysis of classical histology and the other by analysis of transmission electron microscopy. The formation of different germinal cell line was observed in the structural analysis of the ovaries during the three stages of gestation. In G1, foetuses already had oogonia in mitotic division grouped in cords and often associated with primary oocytes which, in some cases, were accompanied by surrounding somatic cells. In a more developed stage, the foetuses of G2 presented in the ovary many pre-enthral follicles completely formed, being mostly primary and secondary primordial follicles. In G3, the ovary was full of pre-enthral follicles at different stages of development, as well as enthral follicles. The ultra-structural analysis of oogonia, primary oocytes and oocytes from preantral follicles showed that such cells contained few organelles as mitochondria, endoplasmic reticulum and Golgi complex, been that the mitochondria were the most frequent observed in all stages. There was an increase in number of organelles, according to the development of the gem cells. These results indicate that the structure and ultra-structure of cells from strain germination of buffaloes are similar to those seen in cattle, as described in the literature for this species.

Keywords: buffaloes, germ cells, ovaries.

INTRODUCTION

Females of domestic species have a finite stock of germinal cells established during fetal life. There are thousands of primordial follicles in mammalian ovaries, but almost all are eliminated *in vivo* by follicular atresia (Santos et al., 2006). The structural and ultrastructural studies of fetal germinative cells are very important for the development of techniques for culture of fetal ovarian follicles with applications in trangenesis, conservation of extinct species and formation of genetic banks.

The aim of the present study was to characterize ovogones, primary oocytes and preantral follicles of buffalo fetus in different ages of gestation.

MATERIALS AND METHODS

For this experiment, 29 fetuses were collected from a slaughterhouse (Frigol – Lençóis Paulista – SP – Brazil) and crown-rump lengths (Figure 1a) were measured to estimate the fetal age according to the method described by Bhavsar et al. (1993) divided into 3 experimental groups: foetuses of the first (G1, n = 10), second (G2, n = 9) and third (G3, n = 10) trimester of pregnancy (Table I). Within 20 to 30 minutes after slaughter, the ovaries were removed (Figure 1b) and washed in 70% ethanol for 10 minutes and in 0.9% saline solution. The ovarian tissue was processed for classic histology and transmission eletron microscopy examination.

The ovogones and primary oocytes were classified according to Rüsse (1983). The preantral follicles were classified according to their stage of development in primordial, primary or secondary.

RESULTS

There were different cells of the germinal line in training in structural analysis of the ovaries of fetuses in the three stages of pregnancy. In G1, fetuses of 9 cm had already ovogones 1 in mitotic division. Juxtaposed Ovogônias were present in all fetal ovaries of this group, forming groups or cords, often with somatic cells located at the periphery of them. During ovarian development, it is observed that the cells more deeply embedded in ovarian tissue are the first to become primary oocytes due

to the increased size of the gonad growth. Thus, the cords of germ cells in different stages of maturation, but not all germ cells, were in the same stage of development.

Somatic cells were located near the walls of germ cells, or between ovogônias and primary oocytes. There is only a small number of somatic cells between germ cells.

Primary oocytes were also shown, in the first semester of pregnancy. They are characterized by their larger size, shiny, and often by the presence of figures of meiosis in the nucleus. In the first semester of pregnancy was identified preantral follicles in early formation.

The analysis of transmission electron microscopy showed that the ultrastructure of ovogones changes with the stage of development and division. The ovogônia contains few organelles, among them, mitochondria, most often round, endoplasmic reticulum and Golgi complex. Electro-dense particles were also commonly observed in all ovogônias evaluated. The primary oocytes are typically larger than the ovogones and have a greater number of organelles, especially mitochondria. You can note accompanying somatic cells (light and dark) around the primary oocyte. The primordial follicle in the early stages of formation, indicated by the approximation of accompanying somatic cells that begin to acquire the form decks, had greater number of organelles in the cytoplasm of the oocyte and often the number of mitochondria.

Table I: Determination of the fetal age according to crown rump length (cm).

Gestation stage (months)	Mean of Crown rump length (cm)
1-2	2.5
2-3	7.3
3-4	14.0
4-5	21.8
5-6	29.1
6-7	37.3

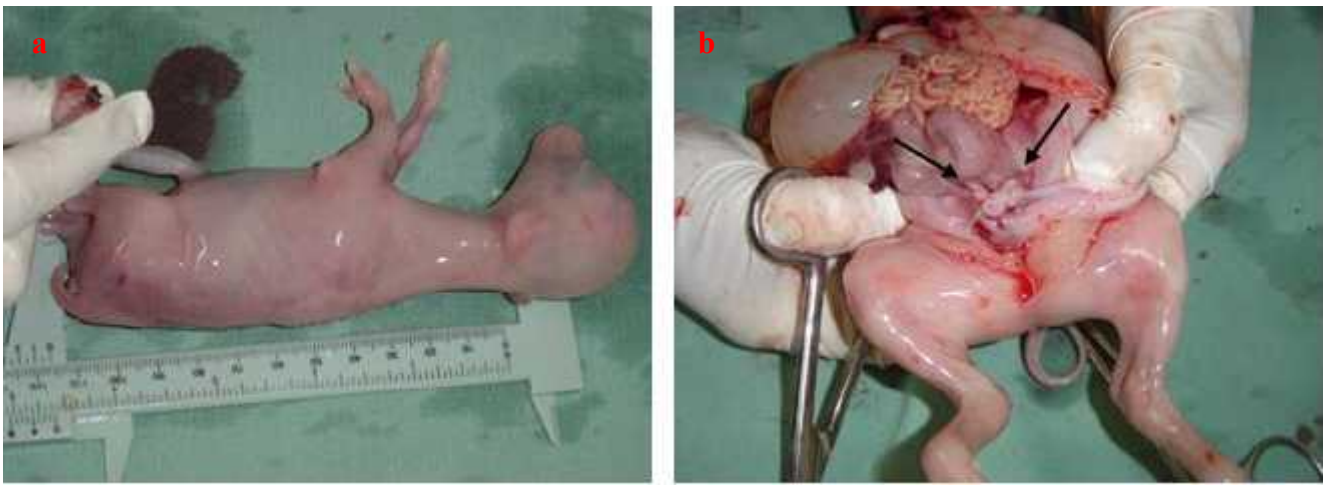

Figure 1: a) The buffalo fetus was measured (crown-rump length) with a paquimeter; b) methodology for collection of fetal ovaries (arrow) after opening the abdominal cavity.

DISCUSSION

This study showed a morphological analysis of germ cells from foetuses of buffaloes in different stages of development. Fetuses of nine centimeters already presented in ovogones in division. Other authors, in cattle (Rüsse, 1983, Tanaka et al., 2001) and sheep (McNatty et al., 1995) have shown consistent results, although this study has not been possible to estimate the fetal age in days of gestation, but in months.

The total number of oocytes in the ovary are derived from a set number of primordial germ cells, which in turn derived from the inner cell mass of blastocyst in development. These cells were characterized by being large, by having a round nucleus with one or more nucleoli, few organelles, small mitochondria, endoplasmic reticulum, polirribossomos, Golgi complex and a variable number of particles of glycogen and lipid in the cytoplasm (Gosden 1995, Motta et al., 1997). After formed, the primordial germ cells migrate to the epithelium of the vitelline sac, through the mesentery into the gonadal ridge of the embryo mesonephro starting to populate the primitive ovary. The particles of lipid and glycogen, that was stored by primordial germ cells, can

be used as energy reserves during their migration to the genital ridge (Pincton, 2001). Initially, the transport of the primordial germ cells toward the ovary depends on a mass transfer, occurring as a consequence of changes in the organization of the embryo growth. This study could not assess the primordial germ cells due to the fact that they are present in the ovary just in the early stages of pregnancy and lower fetus used in this study had nine centimeters in length (Crow-rump length).

Once the proliferation of germ cells in the developing ovary, the primordial germ cells begin to differentiate into ovogones. The primordial germ cells lose their ability to ameboid movement, become more spherical and with few cytoplasmic organelles. In this stage, the germ cells form groups of several cells in division, which exhibit similar chromosomal configurations (Pincton, 2001). These cells may represent often joined together by bridges intracellular, which may represent incomplete divisions of cell bodies during rapid mitotic divisions (Motta et al., 1997). The population of ovogones spreads through a pre-determined number of species-specific mitotic divisions until the cells enter into meiosis and become oocytes. The results of this experiment showed that the formation of strings of ovogones occurred in a very initial stage of development of pregnancy and that in the second semester of pregnancy, they were still present in the ovaries of the fetus, although at that stage was already possible to observe the presence of different germinal cell line, like ovogones, primary oocytes and preantral and primary follicles

The first ovogones to undergo meiotic division are located in more internal areas of the ovarian cortex. In cattle, the half until the end of pregnancy, many stages of developing germ cells are present in the ovary simultaneously. In this study, we can observe the presence of ovogones in animals at the first trimester of pregnancy (group 1). In animals of group 2 could distinguish the presence of ovogones and primary oocytes, which often were found near the somatic cells present in the ovarian stroma.

After the meiosis begins in the germ cells, now called primary oocytes, continues with the stages of leptotene, zygotene and pachytene of the first meiotic prophase, before stopping at the stage of diplotene.

The size of the germ cell increases with the development of the oocyte. Having suffered a genetic recombination of paternal and maternal DNA, other important changes occur in the oocyte. In the cytoplasm, the mitochondria become more numerous, are arranged along the inner surface of the nuclear membrane and often are associated with microtubules (Motta et al., 1997). The Golgi complex is also located near of the nucleus and surrounding the centriole. It is likely that the nuclear polarization of organelles, essential for the metabolism of oocytes, depends on the microtubules activity. After this reorganization, primary oocytes remain in their quiescent stage until puberty, when the follicle is selected to ovulate (Picton, 2001).

The initiation of meiosis in oocytes coincides with the beginning of folliculogenesis. Before follicle formation, there is a massive colonization of the fetal ovary by mesonephrics cells that can become precursor sources of follicular cells. Somatic cells of the medullary region of the fetal ovarian branch is staying and surrounding ovogones and oocytes. During this process, dictyate oocytes lose their intercellular crests and become surrounded by a single layer of pre-granulosa cells from pre floors or polyhedral shape, forming the primordial follicle. The pre-granulosa cells rest on a delicate basement membrane in apposition to the stromal cells, some which can differentiate into a layer tecal after the initiation of follicular growth begins (Gougeon, 1996). Once established, the follicular unit helps to keep the oocyte in a controlled environment and helps to isolate the cell of potentially harmful substances that circulating in the blood. The oocytes that are not incorporated by somatic cells to form the primordial follicles degenerate. Although it was possible to observe ovogones and primary oocytes with signs of degeneration, the results show that in fetuses evaluated there were low rates of degeneration in these cells, because most of the cells evaluated showed normal morphological aspect.

Groups of primordial follicles are clearly recognizable in the ovaries of fetal sheep around day 74 of gestation. Rüsse (1983) reported that primordial, primary and secondary follicles of cattle appear on days 90, 140 and 210 of gestation, respectively. In this study, fetuses in group 1, with 11.5 cm in crown-rump length, already showed the early formation of the follicular unit, as demonstrated by the presence of somatic cells surrounding the primary oocytes. Although the size of the primordial follicle and the number of precursor cells of the granulosa cells varies between species, the origin of the pre-granulosa cells in primordial follicle remains obscure (Picton, 2001). As the germ cells are known originated from the vitelline sac, the somatic cells originate from the gonadal ridge and are derived from the *rete ovari* or epithelial surface (Byskov, 1986). The primordial follicle is the stock of germ cells in the postnatal ovary and their number varies with the species and age (Gosden and Telfer, 1987). Shortly after the stock of primordial follicles is established, the follicular recruitment is restarted and continues uninterrupted until the ovary is completely depleted. The follicular growth is a continuous process, finalized at the time of ovulation or during the degeneration of the follicle and the oocyte (Picton, 2001).

These results indicate that the structure and ultra-structure of cells from strain germination of buffaloes are similar to those seen in cattle, as described in the literature for this species.

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