



ULTRASTRUCTURE OF OVARIAN GERM CELLS IN THE OSTRICH (*STRUTHIO CAMELUS*) EMBRYO

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Summary

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In this study, the ultrastructural development of germ cells in the ostrich embryo was analysed. The nuclear organisation and morphological characteristics of cytoplasm in the developing germ cells, on embryonic days 20, 26, and 36 and the day of hatching (5 samples from each stage) was analysed using transmission electron microscopy (TEM). Germ cells located in the cortex of left ovaries were identified by their large size and centrally located nucleus, with a conspicuous nucleolus. In these cells, the cytoplasm contained an abundance of mitochondria and free ribosomes. The structure of Balbiani body, a villous-like elevation in wide intercellular space and desmosome junction between two adjacent germ cells was also studied. The germ cells during embryonic development showed structural differences in both the nucleus and cytoplasm.

Key words: development, embryo, germ cell, microscopy, ostrich, ovary

INTRODUCTION

Germ cells are able to carry the parental genome to the next generation and undergo the meiotic division which is fundamental for gametogenesis. There have been several studies on the morphology of germ cells during the embryonic of chicken embryos (Solari, 1977; Ukeshima & Fujimoto, 1991; Tagami & Kagami, 1998; Ukeshima, 2003). These studies have shown that chicken pre-meiotic germ cells (PGCs) originate from the epiblast

and migrate through the developing blood vascular system to the germinal ridges, where they gather as gonadal germ cells. Chicken PGCs at stage 34 (after 8 days of incubation) enter the left ovary and start to divide actively to differentiate to primary oocytes (Schoenmakers *et al.*, 2009). By the 16th day of incubation, the primary oocytes enter the meiotic prophase and cease to develop beyond this stage (Matova & Cooley, 2001).

Although avian PGCs have been extensively studied, their characteristics remain largely unknown. Similarly, the details of germ cell development in other birds are also unknown. There are several reports on the morphology of ostrich ovarian follicles (Bronneberg & Taverne, 2003; Madekurozwa & Kimaro, 2006; Wang *et al.*, 2008; Kimaro, 2011; Suárez-Bonnet *et al.*, 2012) and ultrastructure of the follicular wall in sexually immature ostriches (Bronneberg & Taverne, 2003; Madekurozwa & Kimaro, 2006; Wang *et al.*, 2008; Kimaro, 2011) but no information on the ultrastructure of germ cells in ostrich embryo. Thus, the incentive of this study was to investigate the ultrastructure of pre-meiotic germ cells and the organisation of meiotic prophase in ostrich germ cells. Considering the fact that the period of embryonic development in ostrich is twice longer than the chicken's embryonic development (42 days vs. 21 days), we chose specific days of development in order to register the most important developmental changes (Gefen & Amos, 2001; Kheirabadi *et al.*, 2014). This report has documented the ultrastructural characteristics of ovarian germ cells during development of germ cell in ostrich embryo.

MATERIALS AND METHODS

In this study, 20-day, 26-day, 36-day, and newly-hatched ostrich embryos (5 samples from each stage) were used. The sex of newly-hatched embryos was determined by PCR. For this purpose, DNA isolated from feather follicles was used in the PCR reactions. Specimens of left ovaries (with approximate size of 1 mm³) were routinely fixed in 2.5% glutaraldehyde (0.1 M phosphate buffer, pH 7.4) for 1.5 h at 4 °C, then washed in 0.15% phosphate

buffer, pH 7.4, and post-fixed in 1% osmium tetroxide (0.1 M phosphate buffer, pH 7.4) for 1 h at 4 °C. After washing in the same buffer, tissues were dehydrated in a graded series of ethanol and were embedded in epoxy resin 812. Semithin sections (1 µm thickness) were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate. The latter sections were observed with a Leo 912 transmission electron microscope (AB Germany) (Glauert & Lewis, 2014).

The embryos were sacrificed by cervical dislocation according to the guidelines of The Animal Ethics Committee of Ferdowsi University of Mashhad.

RESULTS

Germ cells were readily recognisable in the cortex on semithin sections. They had a remarkably large size, large spherical nuclei, high nucleus to cytoplasm ratio, and pale cytoplasm. The germ cell nuclei were light-toned by toluidine blue staining in comparison with those in somatic cells. Other cells which were not identified as germ cells were considered to be somatic cells (Fig. 1).

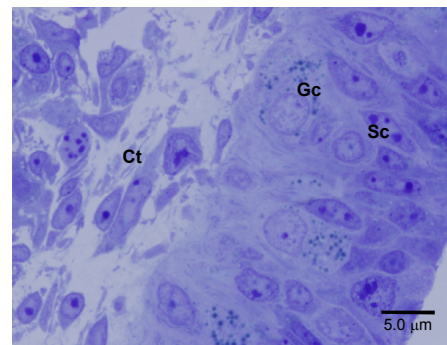


Fig. 1. Semithin section of the left ovary, 20-day-old ostrich embryo. Germ cells (Gc), somatic cells (Sc), connective tissue (Ct).

Nuclear morphology, chromatin organization and cytoplasmic features

In the majority of 20-day old embryos, the nuclei in germ cells contained one nucleolus per nucleus; however, in a few cases two nucleoli per nucleus were also observed. At this stage, germ cells demonstrated condensed chromatin masses in the nuclei. These masses resembled the condensed chromosomes which are commonly seen during mitosis (Fig. 2). Germ cells in 20-day-old embryos contained an abundance of mitochondria and free ribosomes. The Balbiani body, composed of masses of mitochondria and Golgi complex, was observed. Both elongated and spherical mitochondrial profiles were present (Fig. 3).

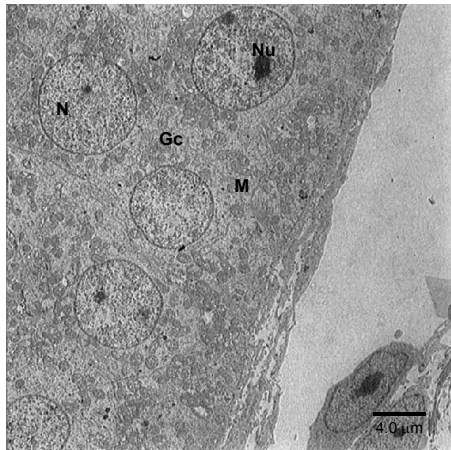


Fig. 2. Electron micrograph of the left ovary. Germ cells (Gc) in the cortex of left ovary have a round, centrally-located nucleus (N), with conspicuous nucleolus (Nu). The cytoplasm of these cells contains numerous mitochondria (M).

At the next developmental time points (26 and 36 days) the nucleoli disappeared from the nucleoli in most germ cells. In 26-day old embryos nuclei were of larger size and nucleoplasm appeared more elec-

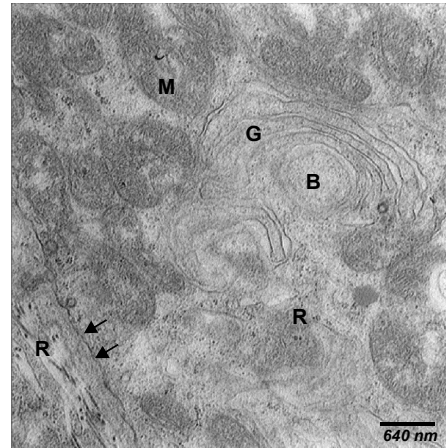


Fig. 3. High magnification of the typical features of the Balbiani body (B), composed by the Golgi complex (G) surrounded by numerous mitochondria (M) and ribosomes (R).

tron-lucent, whereas some electron dense filaments could also be found in the nuclei of many germ cells. At this stage, the number of mitochondria was decreased and some vacuoles were observed in germ cell cytoplasm (Fig. 4, 5, 6). The nucleoli seemed to reappear in the nucleus of germ cells after birth. Some germ cell nuclei in 36-day old embryos showed higher chromatin density indicating the beginning of meiotic prophase. In semithin sections from newly hatched embryos, some germ cells showed dramatic changes of chromatin organisation including condensation and anchorage of chromatin fibres to the inner side of the nuclear membrane, indicating formation of the synaptonemal complex (Fig. 7, 8).

During the first few days after hatching, the chromatin appeared to consist of mostly two levels of fibre density (10 nm and 30 nm).

Germ cell cytoplasm showed endoplasmic reticulum and some dense bodies in 36-day-old embryos. In the newly-hatched embryos, germ cells demonstrated

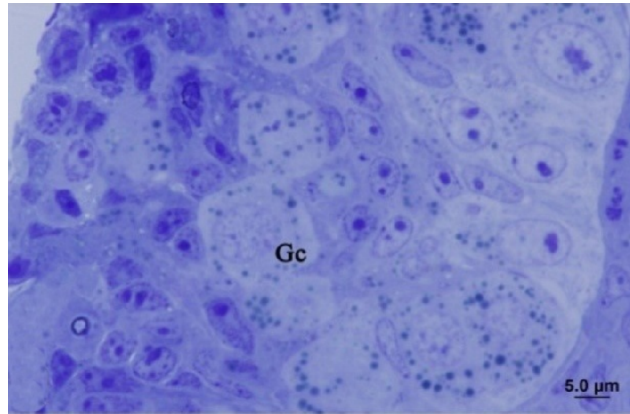


Fig. 4. Semithin section of the left ovary, 26-day-old ostrich embryo showing germ cells (Gc).

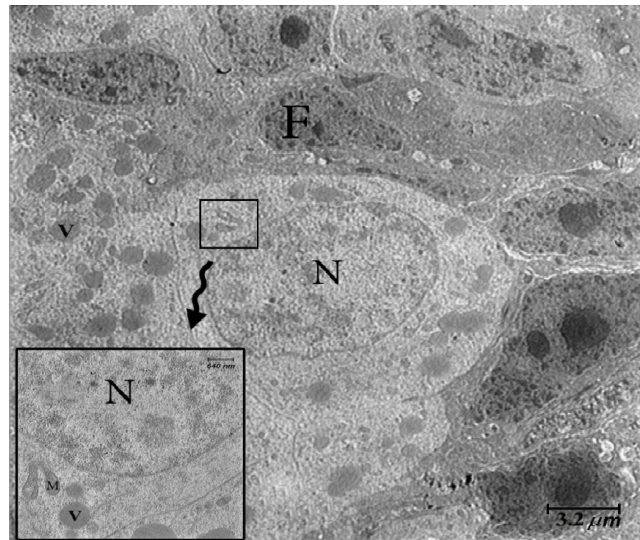


Fig. 5. Electron micrograph, 26-day-old ostrich embryo germ cell in the left ovary cortex. In contrast to the 20-day embryo, the nucleolus disappeared in the nucleus (N) and chromatin organisation has changed. Germ cell in 26-day-old embryo contained several osmophilic vacuoles (V). A few flat cells were arranged around germ cells, suggesting an initial stage in the formation of follicular cells (F); M = mitochondria.

noticeable rough endoplasmic reticulum and both electron-dense and electron-lucent cytoplasmic granules (Fig. 8). In all stages, germ cells were frequently interconnected via desmosome junction, however, in some instances the cell processes

between germ cells were also observed (Fig. 8, 9). Occasionally, wide intercellular space including a limiting membrane was also observed between germ cells and directly beneath the membrane of two neighbouring cells.

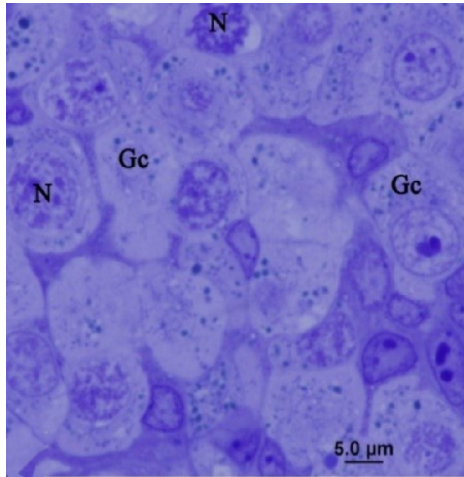


Fig. 6. Semithin section of the left ovary, 36-day-old ostrich embryo. Germ cells (Gc) and their nucleus (N) in the left ovary.

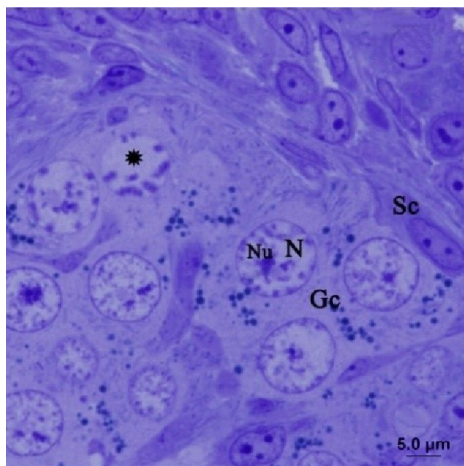


Fig. 7. Semithin section of newly-hatched ostrich germ cell in left ovary cortex. Germ cells (Gc), somatic cell (Sc), nucleus (N), nucleolus (Nu), synaptonemal complex (*).

Medullary germ cells

Some germ cells with a rather diminished cell size were observed in the medullary region of the ovary. These obviously dying germ cells were often found in the medulla throughout the examined stages,

especially in 36-day-old embryos. In these cells, chromatin was strongly condensed while the nuclear matrix was lucent. In these cells, the cytoplasm became sparse, and mitochondria and other membranous structures seemed almost normal in appearance.

DISCUSSION

We studied the morphology of premeiotic and mitotic germ cells by correlative semithin sections and TEM. At day 20, germ cells showed condensed chromatin masses in the nucleus, thus, these germ cells were assumed to be at mitosis phase and active in proliferation. At more advanced stages of embryonic development, the nuclear configuration differed considerably. Nuclear size increased as germ cell development progressed toward the later stage. In addition, important alterations took place in the germ cell's nucleus due to the start of the meiotic process. It has been suggested that around the time of hatch, the germ cell nuclei undergo the early stages of meiotic prophase. We observed that in 36-day-old embryos, some nuclei have started to show meiotic features. In the newly hatched chicks, germ cell nuclei were more electron lucent indicating to be at meiotic arrest. The sequence of morphological and cytological changes associated with the prophase of meiosis has been described in some birds (Ch'in *et al.*, 1978; Galkina *et al.*, 2006; González-Morán, 2007). In chickens, germ cells begin to differentiate into the primary oocytes from the 8th day of incubation. The first meiosis starts at incubation day 13 in the left ovary and stops at the diplotene stage just after hatching (Hughes, 1963; Nakamura *et al.*, 2013). In this investigation the morphological appearance of the chromosomes varied ac-

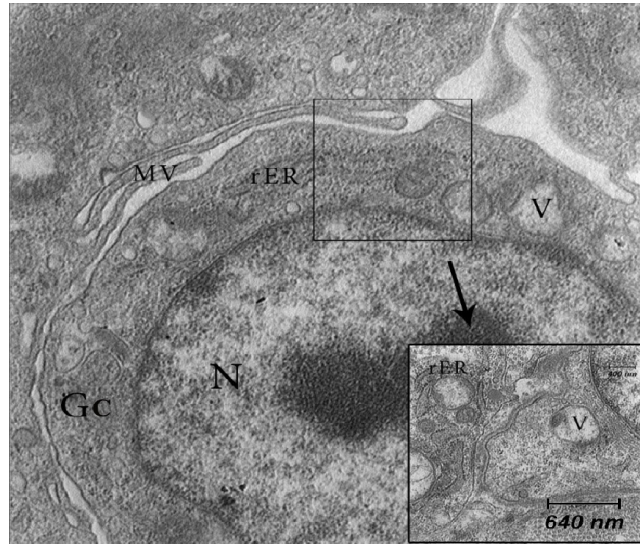


Fig. 8. Micrograph of a single germ cell (Gc). The nucleus (N) was round and contained mostly euchromatin and a conspicuous nucleolus (arrow). Cytoplasm contained endoplasmic reticulum (rER), numerous ribosomes, vacuole (V); MV = microvillus.

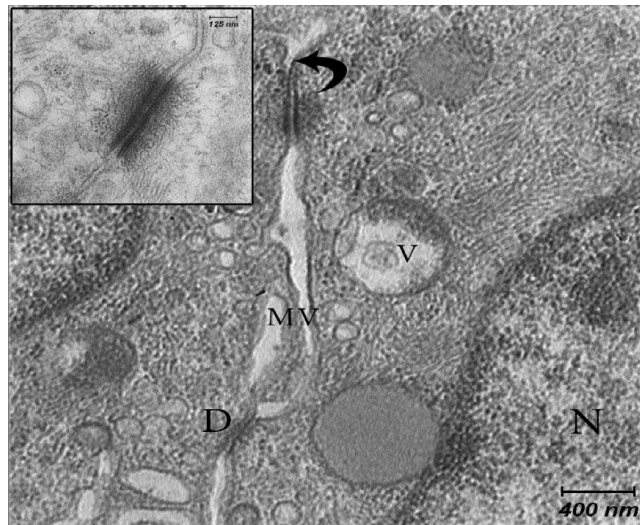


Fig. 9. Electron micrograph of the relationships among germ cells in the newly-hatched ostrich. Germ cells connected via desmosomes (D) and microvillus-like cell processes (MV) are shown between two adjacent germ cells. Nucleus (N) and vacuole (V) are also visible.

According to the age of the embryo. In the newly-hatched ostrich chicks, the germ cells showed a clear nucleoplasm and prominent nuclei.

We observed chromatin fibres that ranged from 10 to 30 nm in diameter. A transition between 10 to 30 nm density has been previously described in other cell

types (Fussner *et al.*, 2010). The morphological organisation of nucleoli reflects the degree of ribosomal synthesis taking place in the cell (Coimbra & Azevedo, 1984). In 20-day-old embryos, germ cells had prominent nucleoli and during development at days 26 and 36 the nucleolus disappeared from the nucleus of most germ cells. However, nucleoli showed a significant reappearance in the nucleus of germ cells of the newly hatched chicks.

Synaptonemal complex has been described as a group of three filaments (two lateral thick and one medial thin) helically twisted and attached by one extremity to the nuclear membrane (Solari, 1977; Oliveira *et al.*, 1995). In this study, these structures were not recognised in the electron microscopic analysis of germ cells. However, in the semithin sections of the gonads of the newly hatched ostrich chicks, the chromatin in some germ cells was condensed and was anchored to the inner side of the nuclear membrane suggesting the formation of synaptonemal complex. Although the meiotic features of the germ cells were frequently found in one-day chicks, some germ cells exhibited these features at day 36. Thus according to our findings, it appears that the meiotic divisions of germ cells in the ostrich begin in the embryonic ovary and cease after hatching.

The isolated chicken PGCs contain a large nucleus and a cluster of large glycogen rich vacuoles in the cytoplasm. PAS (Periodic acid-Schiff) staining of PGCs produced a diffuse staining pattern throughout the cytoplasm indicating a cytoplasm rich in glycogen particles. The lipid rich cytoplasm observed in chicken PGCs is similar to that of migratory human PGCs, but not to the cytoplasm of migratory PGCs in both mouse and pig which do not contain lipid vacuoles

(Macdonald *et al.*, 2010; Naeemipour & Basami, 2013).

The present investigation has found that the Balbiani body disappeared after the embryonic day 20. As described in previous papers (Ukeshima & Fujimoto, 1991) they would be in early oocytes accumulated in limited regions of the cell in chicks, preferentially perinuclear areas, and would spread-out in the cytoplasm during oocyte growth (vitellogenesis). Female germ cells in most of invertebrates grow in clusters of interconnected cells called cysts that demonstrate several distinctive characteristics. Concurrent with the development of vertebrate germ cells from oogonia into the meiotic prophase, intercellular bridges have been described between these cells in a variety of species (Skalko *et al.*, 1972; Pepling & Spradling, 2001). In addition, the finding of some organelles within intercellular bridges has confirmed cytoplasmic flow between connecting dividing germ cells (Motta *et al.*, 2000). The occurrence of germ cell clusters in developing ovaries of ostrich embryo, raises the question of whether early germ cell clusters in embryonic ovaries are cysts or not. That motivated us to study interconnections between cells within the germ cell clusters. According to our observations, in ostrich germ cell clusters, direct cytoplasmic continuity between the germ cells was not detected. In our study in cell cluster, germ cells were joined by desmosomes. Occasionally, some germ cells in wide intercellular space showed a limiting membrane, which suggests that transfer of nutrients and organelles. In fact the significance of such a transfer remains to be determined. Our evidence, however, does not prevent to prove the possibility that there may be intercellular bridges between ostrich germ cells. The insufficient information about

oogenesis proliferation phase and onset of meiosis promoted us to study the embryonic and newly hatched ovaries in ostrich. We present evidence for nuclear re-organisation and cytoplasmic changes during embryonic development of ostrich.

CONCLUSION

Germ cells had a notably large size, and large spherical nuclei. The morphological organisation of nucleoli reflects the degree of ribosomal synthesis taking place in the cell. At day 20, germ cells showed condensed chromatin masses in the nucleus, thus, these germ cells were assumed to be at mitosis phase and active proliferation. Important alterations took place in the germ cell's nucleus due to the start of the meiotic process. In 36-day-old embryos, some nuclei had started to show meiotic features. In the present investigation, it was found that the Balbiani body disappeared after the embryonic day 20. In cell clusters, germ cells were joined by desmosomes. Occasionally, some germ cells in wide intercellular spaces had a limiting membrane, indicating the transfer of nutrients and organelles.

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