—Atlas—

Atlas of Human Oogonia and Folliculogenesis

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Abstract: *The fine structure of human oogonia in foetal ovaries and the stages of folliculogenesis in fertile ovaries are documented with digital images. The human oogonia show progressive changes in fine structure during folliculogenesis and oogenesis until they are ovulated and ready for fertilization. Parallel changes also occur in their relationship with the surrounding follicle cells, which support and nurse the growing and maturing oocytes untill ovulation. The inactivation of the maternal centrosome is the most significant event during human oogenesis*

Key words: *Ovary, Oogonia, Follicles, Graafian follicle, Oocyte, Centrosome, TEM*

Introduction

The ovary, the female gonad, is a highly complex organ both structurally and physiologically, with a variety of somatic cells and, of course, follicles containing oocytes in various stages of development, ranging from primordial, growing to mature antral follicles. It is amply supplied with connective tissue, blood vessels, nerves and smooth muscle and is surrounded by a surface, coelomic epithelium. Its stroma is differentiated into a cortex (with developing follicles) and a medullar (with blood vessels). The ovary is a major endocrine organ interacting with the pituitary gland and the uterus during each menstrual cycle and is regulated by gonadotrophins (FSH, LH and hCG). It also secretes steroid hormones-estrogen and progesterone, which interact with the endometrium. The various stages of folliculogenesis and oogenesis are presented in these published texts and in numerous publications by Prof. P. M. Motta and his colleagues in Rome [1–7].

The human oocyte, the female germ cell, shows progressive changes in fine structure during folliculogenesis and oogenesis until it is ovulated and ready for fertilization. Parallel changes also occur in the relationship between the oocyte and the surrounding follicle cells, which support and nurse the growing and maturing oocytes till ovulation. The vast majority of oocytes (~7 million) are lost, undergoing atresia, during the lifetime of a woman from foetus to sexual maturity and only about 400–500 oocytes are normally ovulated from puberty to menopause. Apoptosis, programmed cell death, is believed to be a cause of this oocyte loss [8]. Hence the majority of oocytes and follicles are destined to undergo atresia. Ovarian follicles are now been frozen and cultured [9–11] whilst ovarian tissue banking is now a reality. *In vitro* maturation (IVM) and vitrification of oocytes are currently being used in assisted reproduction with increasing success rates. Atlases portraying the structure of oocytes have been published [12,13].

Methodology

Three foetal ovaries were obtained after termination of pregnancy, cultured or frozen, to generate oocytes for assisted reproduction, an emerging technology. We examined the ultrastructure of oogonia at 13–15 weeks of gestation, since ultrastructural evaluation could be used as a control for culture and freezing of foetal ovaries for ART [9, 14].

Three fertile adult ovaries of women who conceived (ages 27–32) were also examined, as described previously to study folliculogenesis [14]. Each ovary was held with a nontooth forceps and cortical biopsies were taken with a rounded scissor from the ovary.

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Haemostasis was secured using bipolar cautery. All biopsies were immediately placed in fixative and stored for TEM.

The foetal ovaries and ovarian biopsies were routinely fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3), postfixed in 1% aqueous osmium tetroxide and rapidly processed in Epon or Araldite. Thick survey sections were stained with 1% toluidine blue in borax and thins were stained in alcoholic uranyl acetate/ Reynold's lead citrate and examined by Leitz Digital and Joel electron microscopes, respectively. Images were edited and some were coloured with Adobe Photoshop 6 and Paint Shop Pro 5 for publication.

Observations and Discussion

Oogonia are largely located in the ovarian cortex, whilst primordial germ cells (PGC) and somatic follicle cells compose the surface epithelium. Oogonia are derived from PGC after they migrate to the ovary from the wall of the yolk sac in the 3rd week of development via the genital ridges. Oogonia and PGC have large vesicular nuclei with clear cytoplasm, compared to denser follicle cells which have polymorphic nuclei (Figs. 1–18). Follicle cells intermingle with oogonia and establish close contacts at the beginning of folliculogenesis. Nuclei of oogonia contain 1–3 highly reticulated nucleoli, reflecting high levels of RNA synthesis at the onset of growth. Rough endoplasmic reticulum form stacks of cisternae associated with numerous ribosomes. Prominent organelles in the ooplasm are elongated mitochondria with dense matrices and tubular cristae presenting a multilocular appearance. Typical Golgi complexes, dense bodies and clear vacuoles are present and microfilaments are located beneath the plasma membrane.The origins of Balbiani bodies (see follicles) can be traced to oogonial centrosomes that nucleate cellular organelles close to nuclei.

The most remarkable feature of oogonia is that they have two typical juxtanuclear centrioles (diplosomes) with dense pericentriolar material which nucleate microtubules, characteristic of functional centrosomes. This is quite unlike mature oocytes and resemble centrosomes organizing the cytoskeleton in somatic cells. The mature oocyte has no centrioles, since the maternal centrosome is inactivated or reduced during oogenesis, while the paternal centrosome is dominant. This reduction of the maternal centrosome is important, since only one centrosome need be functional in either gamete to ensure normal fertilization [15, 16]. Centrioles are involved in mitosis of oogonia, before they mature into oocytes at the onset of meiosis. Oogonia are also important in the context of PGC for stem cells, like embryonic stem cells.

The various stages of folliculogenesis were traced in adult ovaries from oogonia in foetal ovaries, as shown in Figs 19–28. The human oocyte shows progressive changes in fine structure during folliculogenesis and oogenesis until it is ovulated and ready for fertilization. Parallel changes also occur between the oocyte and in its relationship with the surrounding follicle cells, which support and nurse the growing and maturing oocytes until ovulation. In IVF, ovaries are stimulated to recruit more follicles and produce more oocytes in each cycle, to increase the chances of fertlization and multiple embryo production for embryo transfer.

At the onset of follicle development, the primordial follicle has a single layer of cells surrounding a diplotene oocyte. Next the primary follicle develops 2 to 3 layers of follicle cells now resting on a basal lamina. The early Graafian follicle shows a developing antrum and a multilayered granulosa of follicle cells. The antrum expands with fluid and separates the cumulus cells adjacent to the oocyte, from granulosa cells in its wall. The follicular oocyte has a reticulated nucleolus, rough endoplasmic reticulum, Golgi membranes and a layer of cortical granules The mature Graafian follicle has a large antrum and a secondary, nucleated (germinal vesicle) oocyte about to intiate the first meiotic maturation division, just prior to ovulation. The oocyte is now clearly surrounded by the gelatinous zona pellucida and a crown of cells, the corona radiata. The zona pellucida is believed to be secreted by both the oocyte and follicle cells.

Preovulatory oocytes are those retrieved for IVF and ICSI, just prior to ovulation. The final stages of oocyte maturation are usually completed *in vitro* after induction of ovarian stimulation, particularly with hCG. Germinal vesicle breakdown is a critical stage when follicle cells retract from the oocyte surface, uncoupling cell junctions. The second maturation division is completed only at fertilization, when the sperm fuses with the mature oocyte and introduces the paternal centrosome. All stages from germinal vesicle oocyte to the mature oocyte (Metaphase II) have been well documented [15,17,18].

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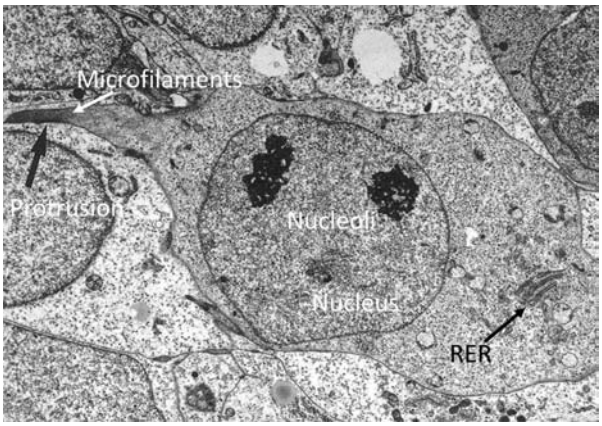

Fig. 1. Migrating primordial germ cell in embryo.
Six week embryo showing fusiform PGC, which arise from the dorsal wall of the yolk sack in week 3 (× 5,800, from Motta, 1995).

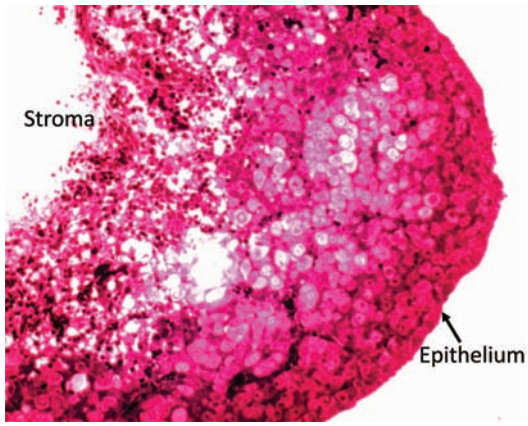

Fig. 2. Foetal ovary (15 weeks)—oogonia (lm).
Several oogonia (purple) are nesting within the ovary. The epithelium and stroma are evident (× 100).

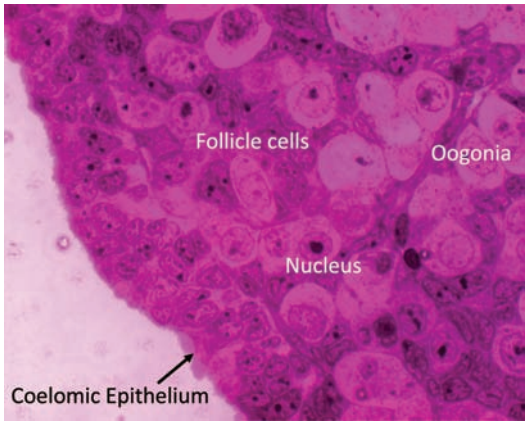

Fig. 3. Foetal ovary—epithelium & oogonia (lm).
The epithelium is multilayered (stratified). The oogonia arise from PGC in week 9 and have large nuclei with nucleoli (× 1,000).

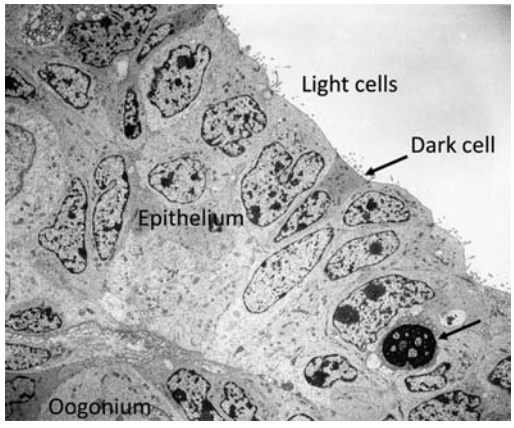

Fig. 4. Foetal ovary—coelomic epithelium (tem).
The epithelium is stratified and consists of light and dark cells. Note degenerating cell (arrow) (× 2,000).

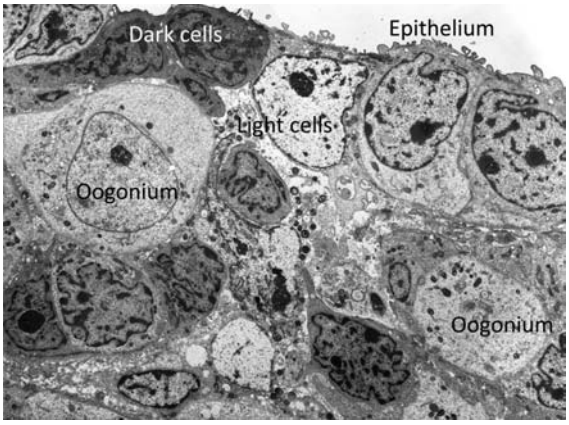

Fig. 5. Foetal ovary—epithelium & oogonium (tem).
Epithelium consists of light and dark cells. Note superficial oogonium surrounded by potential follicle cells (× 2,500).

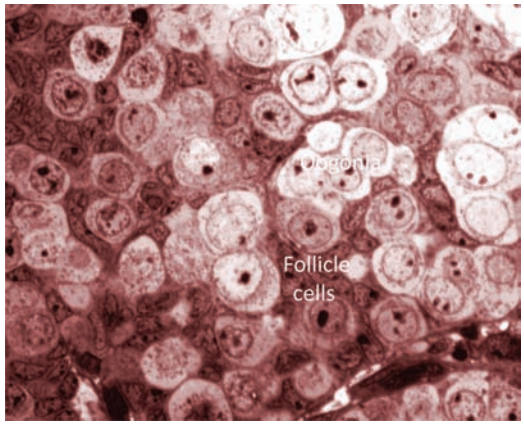

Fig. 6. Foetal ovary—nest of oogonia (lm).
Lighter oogonia are separated by darker, potential follicle cells. Oogonia have large nuclei and few cytoplasmic organelles (× 1,000).

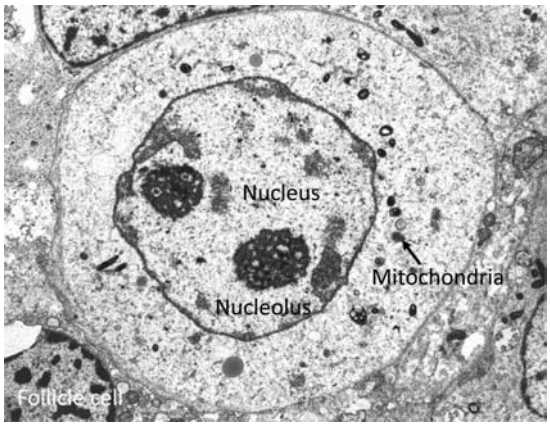

Fig. 7. Foetal ovary—single oogonium (tem).
The oogonium is surrounded by potential follicle cells. Note large nucleus with reticulated nucleoli (× 5,000).

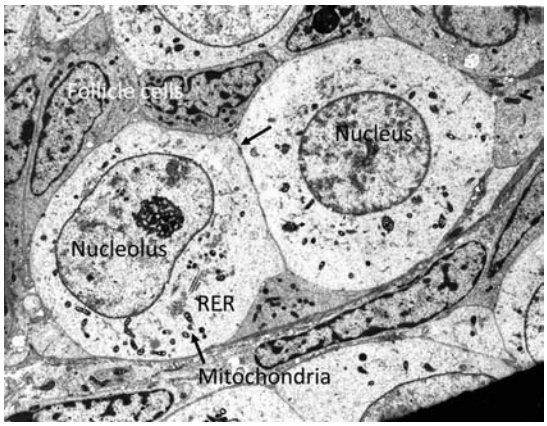

Fig. 8. Foetal ovary(15 weeks)—two oogonia (tem).
The two oogonia have an intervening cell junction consisting of primitive desmosomes (arrow) (× 4,000).

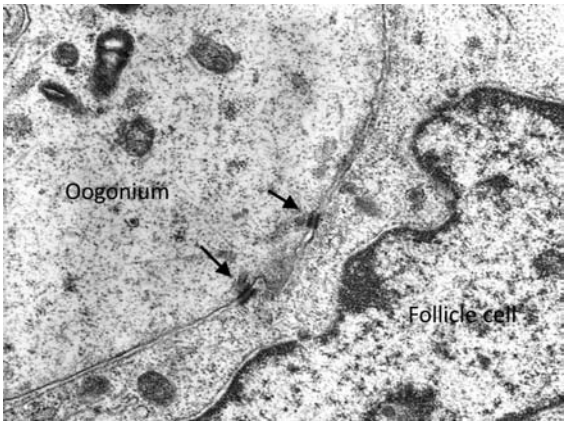

Fig. 9. Desmosome between follicle cell & oogonium.
Two desmosomes (arrows) are seen in the primitive cell junction between the 2 cells (× 20,000).

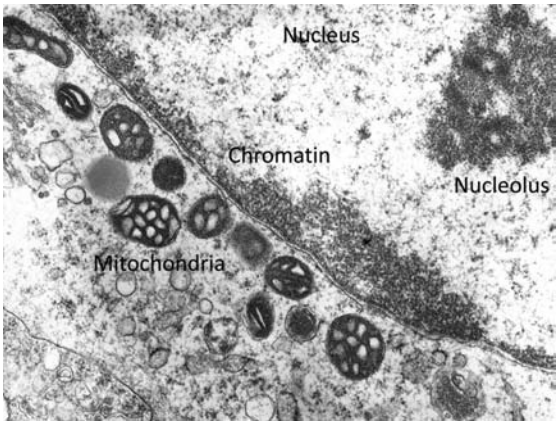

Fig. 10. Oogonium—nucleus & mitochondria (tem).
The large nucleus has a reticulated nucleolus and peripheral chromatin. Mitochondria have tubular cristae (× 20,000).

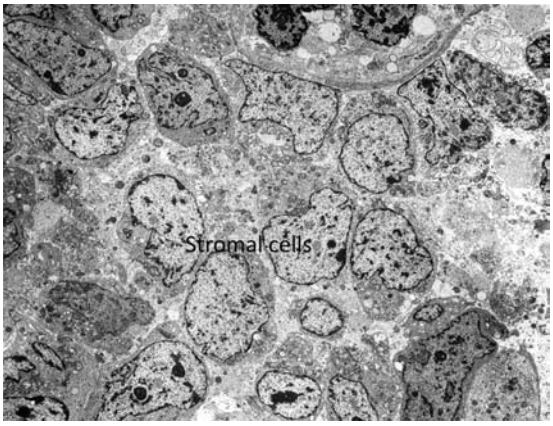

Fig. 11. Stroma in foetal ovary (15 weeks).
Stroma consists of connective tissue cells with few collagen fibrils and blood capillaries (not shown) (× 2000).

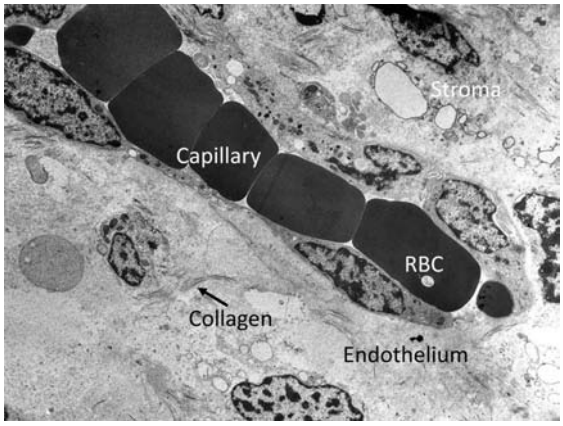

Fig. 12. Blood capillary in stroma—foetal ovary.
The capillary has red blood cells surrounded by an endothelium. Note few collagen microfibrils (× 2500).

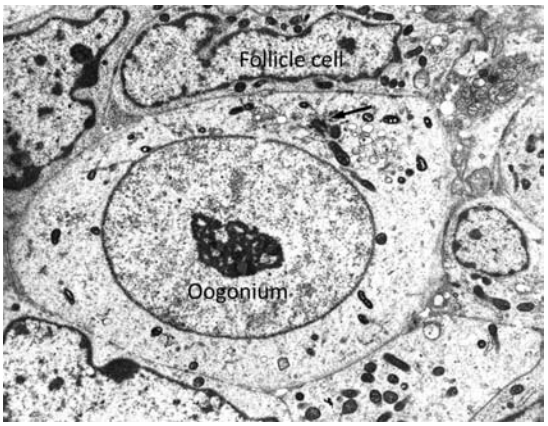

Fig. 13. Centrosome with two centrioles in oogonium.
Oogonia have typical centrosomes with 2 centrioles (arrow), unlike mature oocytes, and resemble somatic cells (× 6,000).

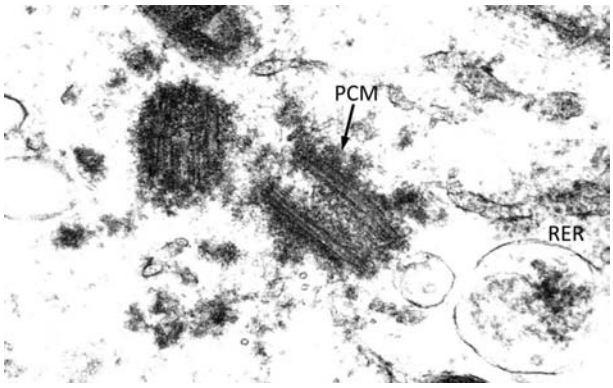

Fig. 14. Two centrioles in centrosome—oogonium.
Dense pericentriolar material (PCM) surround centrioles which are barrel shaped and show triplets of microtubules (× 100,000).

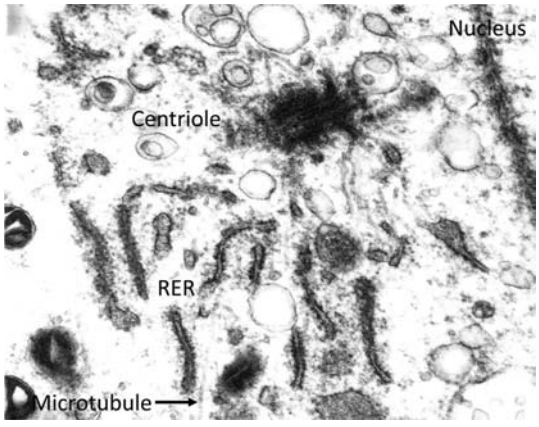

Fig. 15. Centrosome organizing cell organelles.
Rough endoplasmic reticulum (RER) and fine microtubules radiate from the centrosome in an oogonium (× 70,000).

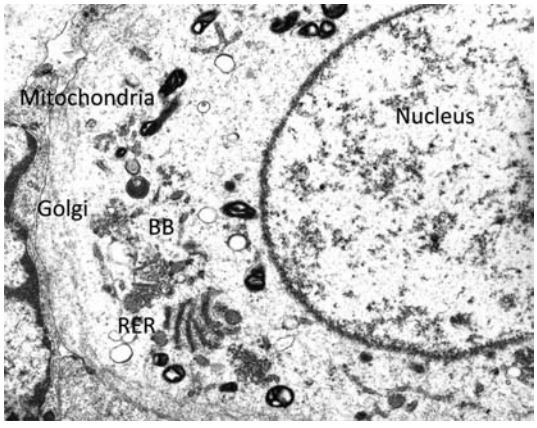

Fig. 16. Oogonium showing origins of balbiani body.
The Balbiani body (BB) is an aggregation of cell organelles close to the nucleus (× 10,000).

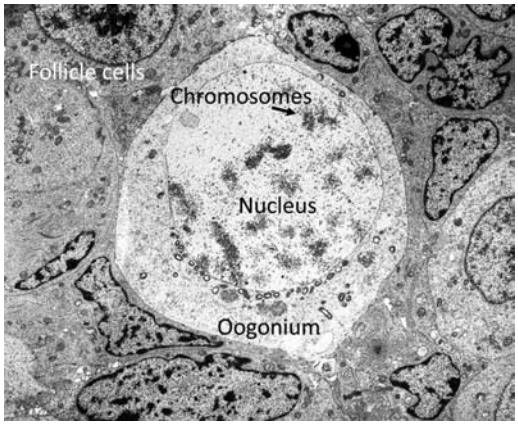

Fig. 17. Oogonium at prophase of mitosis.
The chromosomes are decondensing within the nucleus. Oogonia initiate meiosis at about 13 weeks and arrest at prophase (× 3,000).

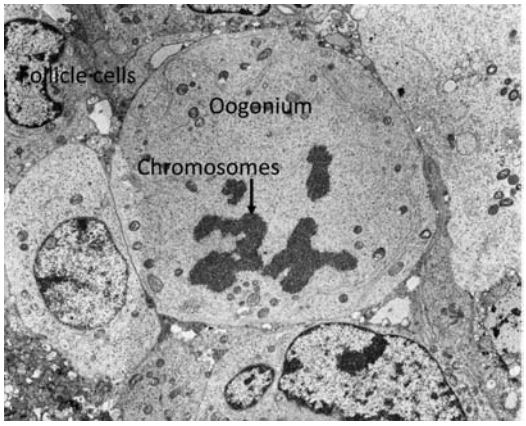

Fig. 18. Oogonium at metaphase of mitosis.
Chromosomes have condensed on a spindle, which is organized by centrioles (not seen). Nucleus has disorganized (× 4,000).

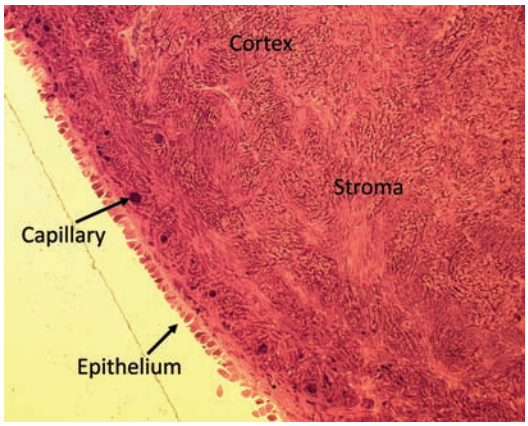

Fig. 19. Fertile ovary (27 years)—epithelium & stroma. Epithelium is cuboidal and forms a single layer. No follicles are present in the cortex (× 400).

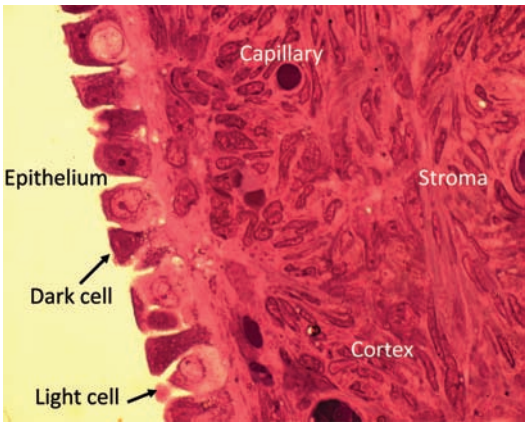

Fig. 20. Fertile ovary (27 years)—epithelium & stroma. The cuboidal epithelium has two types of cells—light and dark. Stroma consists of fibrous connective tissue (× 1,000).

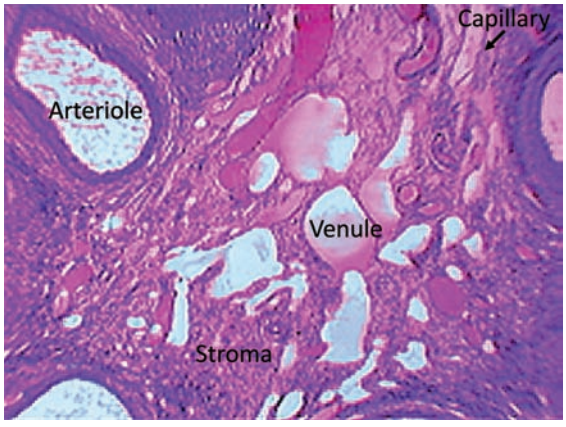

Fig. 21. Fertile ovary (30 years)—medulla. Blood vessels consist of arterioles, venules and capillaries in the central stroma (× 400).

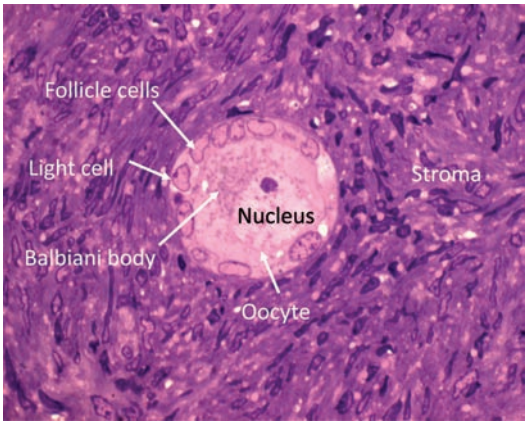

Fig. 22. Fertile ovary (27 years)—primordial follicle. The follicle has a single layer of cells surrounding this diplotene oocyte. Note stroma surrounding follicle (× 1,000).

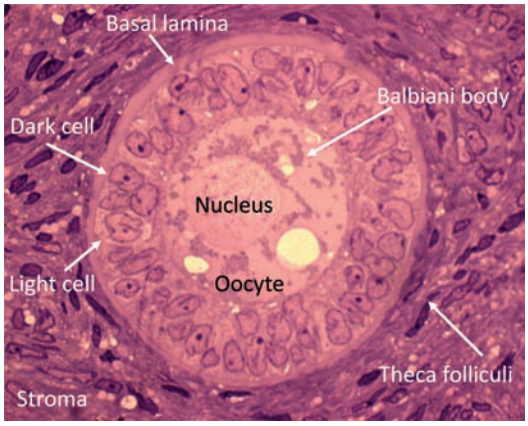

Fig. 23. Fertile ovary (27 years)—primary follicle. The follicle has 2 to 3 layers of cells resting on a basal lamina. The oocyte shows vacuoles-early atresia (× 1,000).

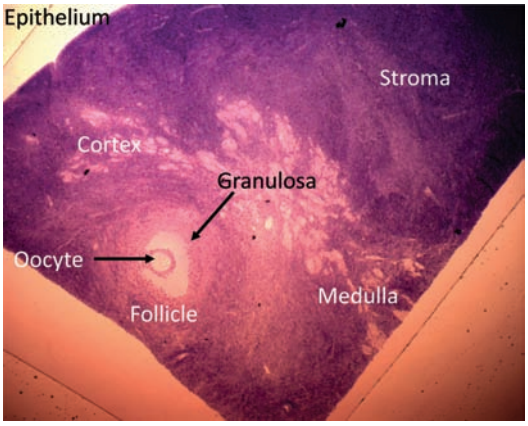

Fig. 24. Fertile ovary (27 years)—graafian follicle. Early Graafian follicle shows developing antrum and a multilayered granulosa. Note oocyte in cumulus (× 50).

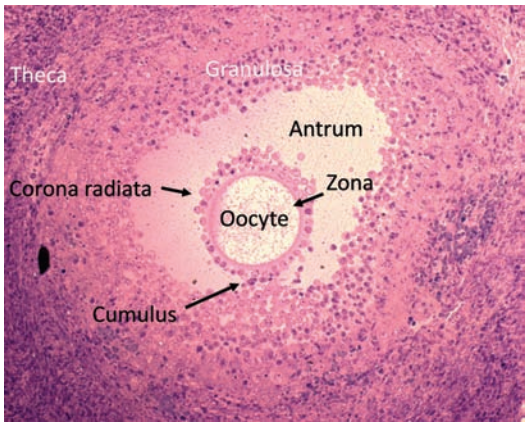

Fig. 25. Fertile ovary (27 years)—graafian follicle.
Early Graafian follicle has an antrum and a secondary oocyte about to intiate 1st meiotic maturation prior to ovulation (× 200).

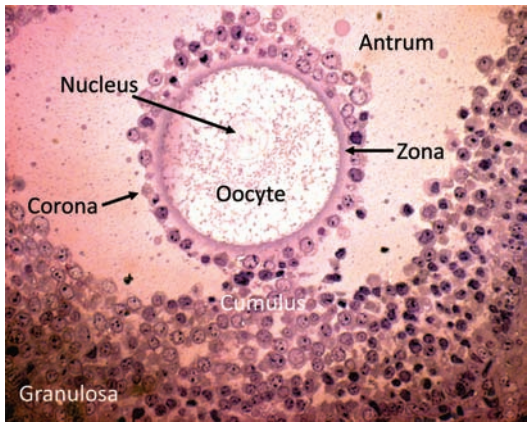

Fig. 26. Graafian follicle—secondary oocyte.
The oocyte is clearly surrounded by the zona pellucida and corona cells. Note nucleus in oocyte and fluid filled antrum (× 400).

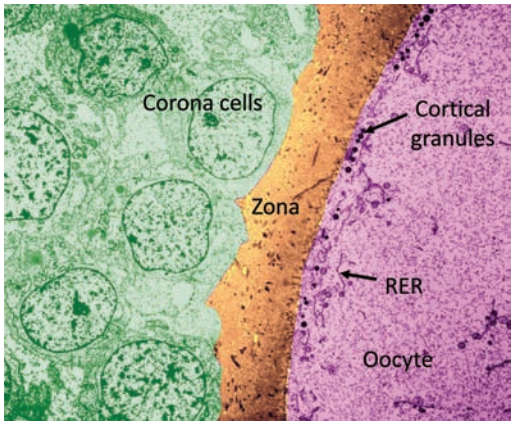

Fig. 27. Graafian follicle—secondary oocyte (tem).
The oocyte has a single layer of cortical granules beneath the surface, surrounded by the zona and corona cells (× 2,000).

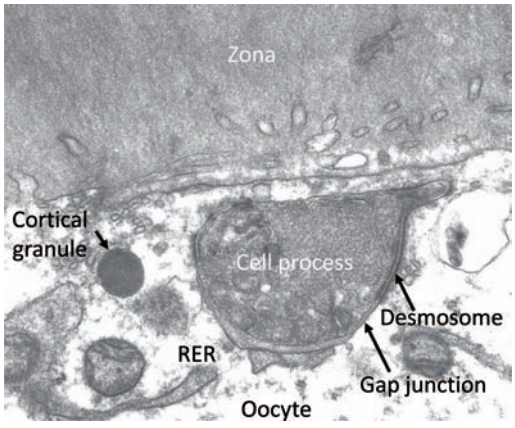

Fig. 28. Graafian follicle—corona cell junction.
The corona cell process ends in a junction in oocyte—shows 2 desmosomes and possibly 2 gap junctions? (arrows) (× 20,000).