ISSN 2664-4169 (Print) & ISSN 2664-7923 (Online)
South Asian Research Journal of Biology and Applied Biosciences
Abbreviated Key Title: South Asian Res J Bio Appl Biosci

| Volume-7 | Issue-4 | Jul-Aug -2025 | **DOI: https://doi.org/10.36346/sarjbab.2025.v07i04.004**

Original Research Article

Postnatal Histological Development of the Ovary in Awassi Lambs Early and Late Immature Stage

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Article History
Received: 08.06.2025
Accepted: 16.07.2025
Published: 21.07.2025

Abstract: The current study this study investigated the postnatal stages of female sheep were taken the ovary of healthy females, the age of the animals was determined depending on formula of milk teeth and eruption age of teeth. Across two age groups: 2–3 months age and 8–9 months age. In the first group (2–3 months), the ovarian structure lacked clear distinction between cortex and medulla, the germinal epithelium comprised a single layer of cuboidal to low columnar cells, overlying a moderately thick tunica albuginea made of dense connective tissue. The ovarian cortex displayed numerous primordial follicles beneath the tunica albuginea, each containing an oocyte surrounded by flattened granulosa cells. Early primary follicles with an enlarged oocyte and cuboidal granulosa cells were also observed. Occasional secondary follicles appeared with multiple granulosa layers and early thecal differentiation, but no antral cavities, indicating preantral development. The medulla contained loose connective tissue, fibroblasts, and numerous developing blood vessels. In contrast, in the second group (8-9month) which lambs exhibited clear compartmentalization into cortex and medulla. With surface lined by a simple squamous to low cuboidal germinal epithelium underlain by a thick collagen-rich tunica albuginea. The cortex contained follicles at all developmental stages, including primordial, primary, secondary, and Graafian. The medulla featured dense connective tissue, fibroblasts, and vascular networks.

Keywords: Postnatal Development, Ovary, Lamb.

INTRODUCTION

Sheep are important livestock for milk, wool, and meat production. Approximately 65% of animals in Iraq are sheep (FAO, 2010). Iraq has five breeds: Hamadan, Karadi, Arabi, Naeimi, and Awassi, which are considered highly economical. The female reproductive system includes two ovaries and a reproductive tract composed of the oviducts (uterine tubes), uterus, cervix, vagina, and vulva (Samuelson, 2007). Ovaries are the primary reproductive organs with dual functions: the exocrine production of ova and the endocrine secretion of sex hormones (progesterone and estrogen) (Deokar *et al*., 2005). The ovary is covered by a simple squamous serosal epithelium, beneath which lies the tunica albuginea, composed of dense irregular connective tissue. The ovary is divided into two zones: the cortex, which surrounds the centrally located medulla. The cortex contains stromal fibroblast cells, interstitial gland cells, and theca cells involved in follicular development (Dellmann and Brown, 2006). The cortical region houses follicles at various stages: primordial, primary, secondary, and tertiary up to Graafian follicles. Ovulation occurs when a Graafian follicle ruptures, releasing the oocyte. The medulla contains large blood vessels surrounded by loose connective tissue with abundant elastic and reticular fibers (Samuelson, 2007; Junqueira, 2018). Few studies have described fetal ewe ovaries, especially in the Awassi breed. Sheep are considered suitable models for studying follicular development due to their affordability, short gestation, and early puberty. Compared to rodents, sheep have ovarian development more similar to humans (Hejazi *et al*., 2013). This study aims to provide a comprehensive histological description of the postnatal ovary in local Awassi sheep.

MATERIAL AND METHODS

Ethical Approval:

The experimental procedures were approved by the Institutional Animal Ethics Committee and conducted at Veterinary Medicine Collage, Al-Qasim Green University, Iraq.

Experimental Animals:

The purpose of this work was to describe the Histomorphological developmental of ovary in postnatal periods of local awassi sheep's fetuses (Ovis aris) where experimental samples are gathered from September to November2025 16 postnatal stage (8 neonate (2-3 month) and (8 fetus at(8-9 month). The study is conducted on sheep fetuses from slaughtered ewes in Najaf's and Babylon abattoirs for postnatal research. The sheep fetuses at postnatal stages distributed into two groups: (2-3mounth) and (8-9 month)after gestation according to the gestational age which determined depending on the crown rump length (CRL) by using of following formula (Y=2.74X+30.15) (Al-jebori, J.G.A., *et al*., 2020 and Hejazi, *et al*., 2013).

Sample Collection and Light Microscope Technique:

The sheep fetus's ovary was preserved in buffered formalin (10%), dried out using a sequence of increasing alcohol concentrations, cleaned in xylene, and then embedded in paraffin wax. Using a rotary microtome, the blocks were sectioned at 5- 6 µm thickness of slice. According to (banchroft, *et al*., 2018), histological sections were stained with trichrome mason's stain, periodic acid schiff (PAS), and Hematoxyline and Eosin (H&E). Olympus light microscope with USB digital camera attached to computer slides and various magnification settings was used to study the sections.

RESULTS AND DISCUSSION

1. Immature Periods (At 2–3-Month-Old Lamb)

At 3-2 months of gestation, the ovary was distinctly were not fully developed and cortex not distinguish from the medulla). The germinal epithelium formed the outermost layer of the ovarian surface and consisted of a single layer of cuboidal to low columnar epithelial cells. These cells were continuous with the peritoneal mesothelium at the level of the ovarian hilus (Figure1).. And the tunica albuginea appeared moderately thick, forming boundary between the surface germinal epithelium and the underlying ovarian cortex. It was composed of dense connective tissue containing numerous collagen fibers and scattered fibroblasts. The ovarian cortex displayed numerous primordial follicles suite immediately beneath the tunica albuginea compsed of an oocyte surrounded by a single layer of flattened granulosa cells. Additionally, early primary follicles were observed, characterized by a single layer of cuboidal granulosa cells and a slightly enlarged oocyte (Figure2). And secondary follicles, these follicles were characterized by a larger oocyte surrounded by two or more concentric layers of cuboidal granulosa cells, signifying active granulosa cell proliferation (Figure3) and The medullary region contained developing vascular structures and connective tissue components, which contributed to the structural organization and metabolic activity of the ovary. (Figure4)

In sheep, these results were line with those reported by (Sharum *et al*., 2023) who mention that the primordial follicles were mainly located in the peripheral cortex, while the central cortex lacked follicles. Small to medium-sized antral follicles were present, with expanded stroma and a vascular medulla. By two months, antral follicle proportion increased (12.3%), though many showed degeneration. The follicular arrangement remained layered, with growing follicles beneath the primordial zone.

In Azerbaijani river buffalo, these results were agree with those reported (Shahrooz *et al*., 2012), the cortex and medulla were not distinctly separated, and the ovarian parenchyma consisted largely of undifferentiated mesenchymal tissue. Numerous oogonia and clusters of germ cells were scattered throughout the stroma, particularly beneath the tunica albuginea. Only a few primordial follicles were present, characterized by a single oocyte surrounded by flattened follicular cells. Developing follicles beyond the primary stage were absent.

In bovine, these results were support with those reported by (Singh and Adams, 2000). Who mentioned that the ovary was characterized by a high number of primordial follicles located within the cortex, while the medullary region lacked follicular structures. As age progressed, primary and secondary follicles appeared, showing multiple layers of granulosa cells and a distinct theca layer.

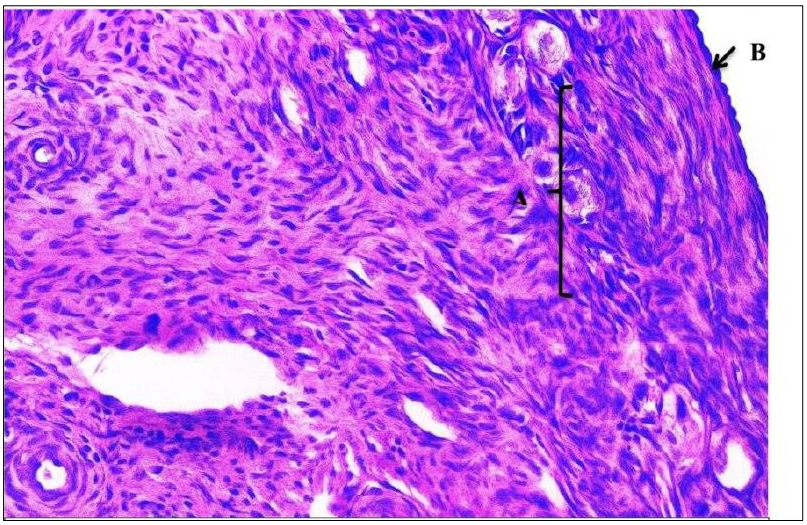

Fig. 1: Histological section explaining the ovary at 2-3 months explaining. A-cortex B-germinal epithelium (H&E,20x)

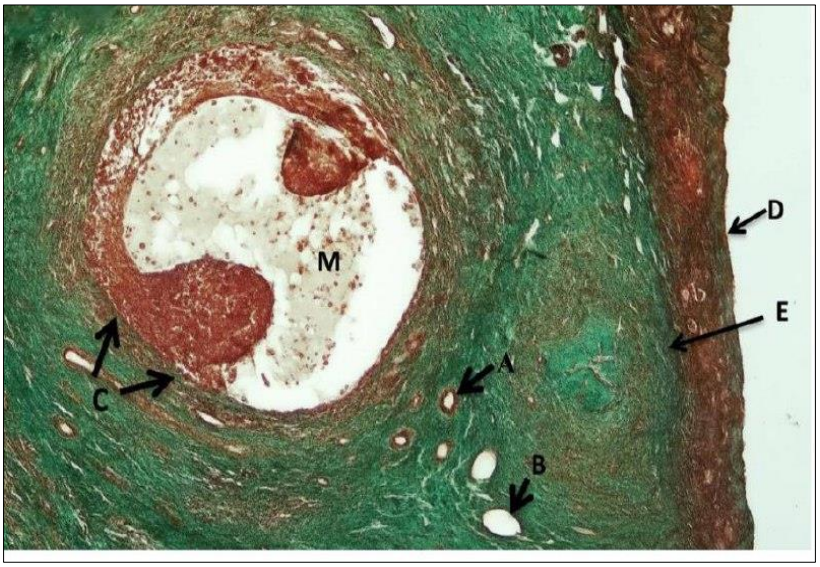

Fig. 2: Histological section explaining the ovary cortex at 2-3 months after gestation in sheep fetuses A: Primordial follicle B: Primary follicle C: Griffin follicle D: Germinal epithelium E: Tunica albuginia M: Antrum (Masson trichrome, 4x)

Fig. 3: Histological section explaining the secondary follicle at 2-3 months. A: Antrum O: Oocyte K: Corona radiata Z: Zona pellucida G: Zona granulosa (H&E, 10x).

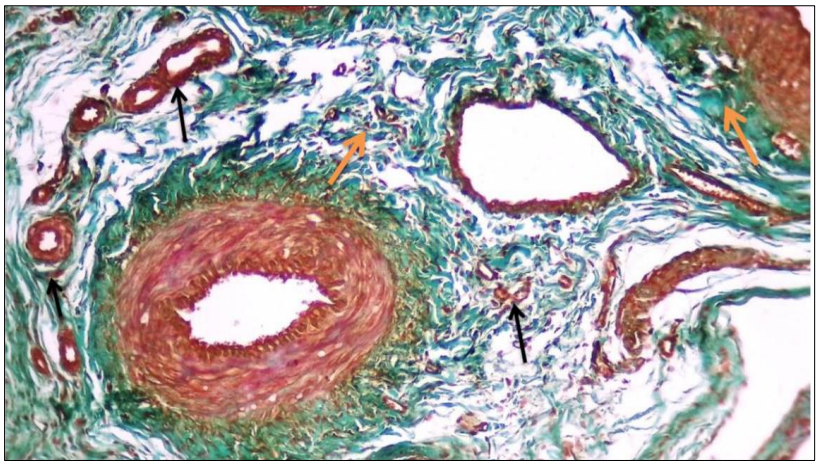

Fig. 4: Histological section explaining the ovary medulla at (2-3month) of showed distribution of the connective tissue stroma in the medulla (orange arrow) and ovarian blood vessels (black arrows),(Masson trichrome, 4x)

2. Mature Periods (At Eight Months Old Lamb Postnatal)

At 8–9 months of gestation, the ovary was distinctly divided into cortex and medulla. The surface was lined by simple squamous to low cuboidal germinal epithelium, underlain by a thick tunica albuginea composed of dense irregular collagenous connective tissue and the cortical region showed advanced folliculogenesis, containing primordial, primary, secondary, and Graafian follicles (Figures 5). Primordial follicles were located just beneath the tunica albuginea, each consisting of a primary oocyte surrounded by flattened follicular cells resting on a basal lamina. primary follicles these follicular were large in size compare with primordial, each primary follicles consist of primary oocyte surrounded by single layer of cuboidal follicles epithelium, while in secondary follicles oocyte surrounded by granulosa cells multiple later based on zona pellucida (Figure6). Secondary follicles exhibited multiple granulosa layers, a distinct zona pellucida, and separated theca interna and theca externa layers (Figure7). Graafian follicles were located near the surface, with a centrally positioned oocyte, surrounded by cumulus oophorus, and a large fluid-filled antrum. A distinct basement membrane separated granulosa cells from a highly vascularized theca interna, surrounded by a fibrous theca externa (Figure8). The medulla was centrally located, composed mainly of fibroblasts and dense collagenous connective tissue. It contained prominent blood vessels, with smaller vessels and capillaries.

In sheep, these results were aligned with (shehan *et al*., 2017). The surface of ovary is covered by a simple squamous epithelium, beneath which lies the tunica albuginea. The cortical region, located beneath the tunica albuginea, contains follicles at various developmental stages. Primordial follicles were most abundant and located near the periphery, consisting of an oocyte surrounded by a single layer of flattened granulosa cells. As follicles matured, they transitioned into primary and secondary follicles, showing cuboidal granulosa cells, zona pellucida formation, more advanced tertiary (vesicular) follicles exhibited a large antrum.

In camels, (Abd-Elrazik *et al*., 2014), the ovarian surface epithelium consists of a single layer of low cuboidal cells over a thick tunica albuginea. The cortex contains primordial and primary follicles peripherally, with secondary, antral, and frequent Graafian follicles deeper within. Oocytes exhibit central nuclei and a distinct zona pellucida. Corpora lutea of varying sizes with luteinized cells and dense vascularization suggest recent ovulation. The medulla comprises loose connective tissue rich in blood vessels, lymphatics, and nerves.

In adult Kano Brown goats, all follicular stages—from primordial to preovulatory—were observed. Early antral (type 4) and Graafian follicles (type 5) were mainly localized in the deeper cortex. Oocytes showed central nuclei with prominent nucleoli and well-defined zona pellucida. Unlike Chima and Ifeoma (2009), corpora lutea were consistently present, indicating ongoing follicular turnover. The medulla was highly vascularized, supporting metabolic and endocrine activity.

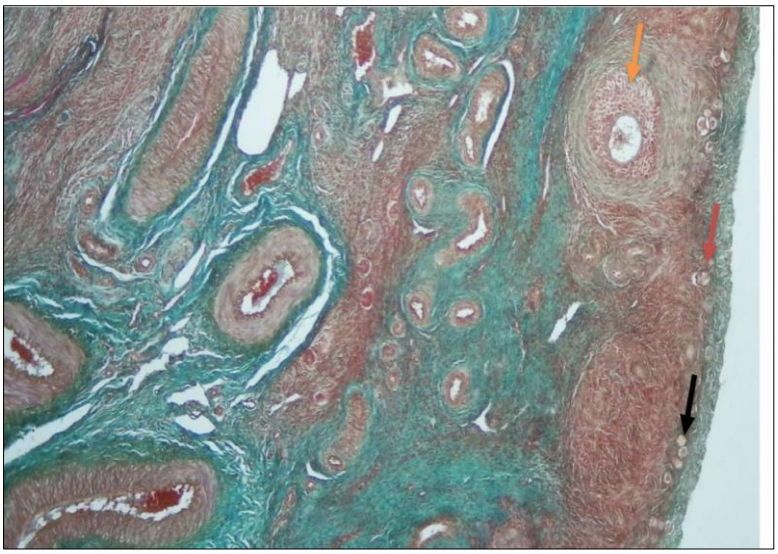

Fig. 5: Histological section explaining the ovary cortex at 8-9 months after gestation in sheep fetuses. A-primordial follicle (black arrow), B-primary follicle (red arrow), C-secondary follicle (orange arrow).(Masson trichrome,4x)

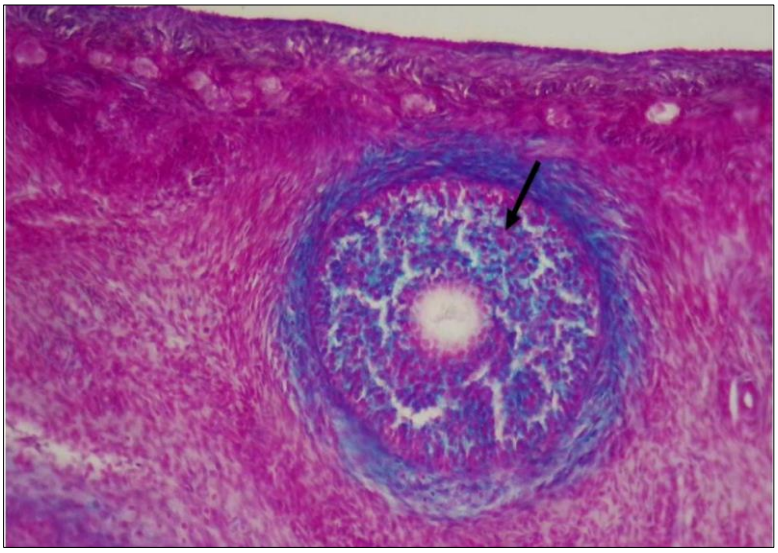

Fig. 6: Histological section explaining the ovary cortex at 8-9 months after gestation in sheep fetuses A- Secondary follicle (black arrow). (H&E,4x)

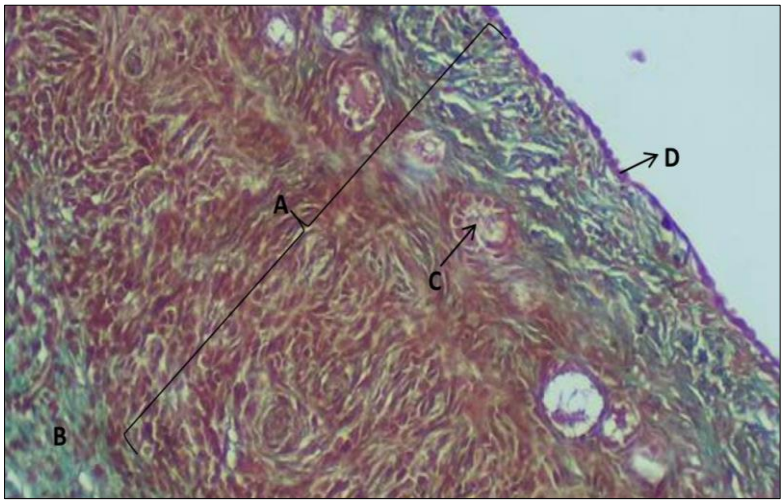

Fig. 7: Histological section the ovary at 8-9 months explaining A-cortex B-medulla C-primary follicles D-Germinal epithelium (Masson trichrome,20x)

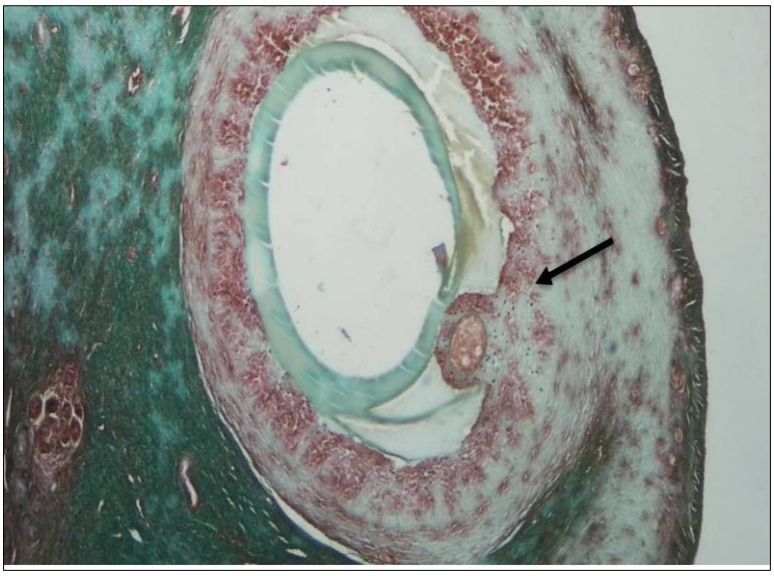

Fig. 8: Histological section explaining the ovary at 8-9 months explaining A- Griffin follicles (black arrow) (Masson trichromr,4x)

Novelty Statement: Present report and finding can advanced several aspects of the ovary development and offer several markers to be considered during effective assess of animal health and production potential.

Funding: No funding was received.

Conflict of Interest: The authors have declared no conflict of interest.

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