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A Histomorphological and Histochemical Changes of Postnatal Developmental in the Ovaries and Uterus of Local Rabbits (*Oryctolagus cuniculus*) in Duhok Province

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Abstract

THIS study aimed to detect histomorphology and histochemical changes of the ovaries and uterus in local rabbits (*Oryctolagus cuniculus*) during postnatal development from 7 to 150 days. Eighty rabbits were used from local breeders and divided into 8 groups, with 10 rabbits selected for each age group. The rabbits were euthanized and subsequently dissected. The ovaries and uterus were obtained and stored in a solution of 10% neutral buffered formalin for preservation. Subsequently, the sample was subjected to standard histology procedures. Ultimately, 5 µm slices were utilized and subjected to hematoxylin-eosin Masson Trichrome staining, as well as alcian blue and periodic acid shift staining. Results of the microscopic investigation indicated clear morphological changes in the ovaries and uterus during the postnatal maturation of the young. The study indicated that the rabbit ovary, which was between one week and six weeks old, consisted of follicles of type 1-5b. Nevertheless, G7 and G8 exhibited the presence of sizable follicles categorized as type 6 and 7. This study detected a growth in all layers of the uterus, especially in the tunica mascularis, throughout postnatal development. These findings indicated that young female rabbits undergo significant developmental progress and changes after being weaned. This study determined that pre-ovulatory follicles and Corpus luteum were observed in mature female rabbits at the age of 4 months, suggesting that these rabbits reached puberty at that age. The study concluded that the maturation of the follicles and uterus in this breed based on the age of the individual.

Keywords: Folliculogensis, endometrial glands, Histomorphomerty, Histochemistry, Rabbit.

Introduction

In the previous several decades, rabbits were increasingly utilized as experimental animals after mice and rats. Fischer [1]. Rabbits reproductive organs contains two ovaries, two oviducts, two uterine tubes, vagina, and vestibule. Insufficient and non-restorable oocyte reserves in neonatal ovaries affect rabbit fertility and reproductive lifetime. In sheep and rabbits, the primordial follicle assemblage forms during fetal development or early postnatal life [2]. A tiny percentage of primordial follicles in the ovary are recruited into the developing pool and/or ovulated or atretic degenerated immediately after creation [2]. As is known, primordial germ cells differ from ovarian somatic cells in shape and marker gene expression. Vascular-associated cell families, generic somatic cell ancestry, and pre-granulosa cells are somatic cell lineages [3]. Ovarian development and function depend on somatic cell-oocyte contact, which is essential for follicular integrity [3]. After birth, the ovary has two pre-granulosa cell populations. First class become follicular granulosa cells in the medulla, second class in the cortical area of the fetal ovary [3,4]. These cells will differentiate into cortical follicle granulosa cells that are gradually activated and produce the eventual pool of primordial follicles during the animal's reproductive lifetime [4]. It has been documented that postnatal uterine development in rabbit is marked by some

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morphogenetic events which include differentiation of endometrial stroma; endometrial gland development, and myometrium differentiation. Even though the uterus is critical for female fertility, the cell, hormonal and molecular pathways that regulate uterine development are less understood [5]. Post-natal uterine gland morphogenesis is predominant. Specifically, prenatal and postnatal uterine tissue "programming" establishes adult uterine functional capability [6]. The reproductive tract organogenesis and differentiation in mammals occur during fetal development [6]. In lab animals, humans, and domestic animals, uterine histoarchitecture is not completely established and differentiated at birth [7].Organized, fragile fluctuations in uterine gene expression enhance cellular connections throughout fetal uterus development and cytodifferentiation. These create uterine tissue developmental trajectory [8]. Uterine gland development is critical for pregnancy because its secretions affect implantation, stromal cell decidualization, and placental development [9]. Ovarian, oviduct, and uterine epithelial homeostasis processes are poorly understood. Stem cells in many adult epithelia are dangerous tissue regenerators [10]. Studies have shown epithelia on ovulatory follicles and fimbrial fringes with stem cell-like structure [11]. For that reason, this study was designed to understand the morphological, morphometrical and histochemical characteristics of the ovary and uterus during their postnatal growth.

Material and Methods

Animals and study design

This research studied the uterus and ovaries of clinically healthy inbred indigenous rabbits (*Oryctolagus cuniculus*) from birth to 150 days after birth for histological, Morphological, and histochemistry (HC) characteristics. Designed study allocated randomly into 80 rabbits were divided into 8 groups with 10 kits each. Group 1 (one-week-old rabbits) were newborn kits. The 2-week-old Group 2 kits compromised suckling kits. Group 3 (3-week-old kits) received food and mother's milk. Group 4 (4-week-old kits) was weaned and given greens only. After weaning, Group 5 (35 day old kits), Group 6 (42 day old kits), Group 7 (70 day old kits), and Group 8 (150 day old kits) ate basic solid and greenish food. Animals were kept with free food and water under optimal conditions. All kits followed a standardized euthanasia technique, and ovaries and uteri were collected and stored in 10% neutral buffer formalin and Boun's solution for histo-morphometrical analysis [12].

Sampling

Firstly, the rabbits were anaesthetized intramuscularly with ketamine at dose 35-50 mg/kg and xylazine at dose 5 mg/kg of body weight; then animals were euthanized intravenously with ketamine at dose of 600 mg as describe by [13]. The subsequently, the ovaries and uterus were collected and washed thoroughly with 1% phosphate buffer saline (PBS), then stored and fixed in 10% neutral buffer formalin and/or Bouni's solution.

Histological analysis

Routine histological processing was performed on 5µm uterine and ovary sections using a rotatory microtome (Leica RM 2245, Germany). Following fixation, the tissue specimens were rinsed with flowing tap water. The sample was dehydrated using ascending concentrations of ethyl alcohol, then treated with xylene for clarification. After that, it was impregnated and enclosed in paraffin wax (58-60 ^{o} C). Tissue slices were stained by following stains: Harris hematoxylin and eosin stain (H&E) (to demonstrate the general tissue architecture): Masson's trichrome stain (used for the identified the presence of collagen and muscle fibers) and Periodic acid-Schiff' (used for demonstration neutral polysaccharides and different type of glycoproteins). A Swiftcam 18 Megapixel, USB 3 digital microscopic camera (SC 1803R, China) and picture analysis software (Swift Imaging 3.0) were used to analyze these micromorphometric data for the ovaries and uterus [15]. For classification of the ovarian follicles see Table 1[14].

Statistical analyses

Statistical analyses were performed in Genstat 19.0 (VSN International, Hemel Hempstead, UK). All data were checked for normality using residual plots and homogeneity of variance by Bartlett's test. The micro-morphometric parameters of uterus and ovaries were presented as mean and standard error of mean. One way ANOVA (Tukey test) was used for comparisons of the variants among the age groups. Differences were considered significant at $P<0.05$.

Results

Classification of ovarian follicles

The study found that in kittens aged one and two weeks, the majority of ovarian follicles were type 1, with percentages of 100% and 76%, respectively (Table 2, $P<0.05$). In other groups, the distribution varied. In G3, type 1 follicles were highest at 35.4%, while in G4, type 3a follicles were most prevalent at 38.5%. G5 showed a mix of follicle types, with type 1 at around 33%, type 2 at 30.4%, and type 3a at 27.8%. G6 had all types of follicles, with type 2 being the highest at 40.8%. G7 had various follicle types present, with type 4 being the most prevalent at 34%. Finally, G8 had all types of follicles, with type 3a being the highest at 24.1%.

Micromorphometric measurements of the uterus

The micro morphometric investigation showed that epithelial height, lamina propria thickness, tunica muscularis thickness, and serosal thickness

were increased with age. Table 3 indicated significant differences (P<005) in all age groups for all measurements except serosal thickness, which did not vary across G3, G4, and G5 (Table 3).

General histological description characteristic of the ovary

Ovaries contain a simple cuboidal epithelial surface layer throughout development except at the hilum. There was a thin layer of loose collagenous connective tissue (tunica albuginea) underlying this epithelium with a rounded central nucleus on the ovary surface from 1 week to 150 days old (Fig. 1A). Parenchyma had a cortex and medulla (Fig.1B). Cortex had large, spherical germ cells called oogonia dominate the cortex (Fig.1C). Spindle-shaped stroma cells supported these oogonia. In contrast, the medulla containd numerous blood vessels and connective tissue (Fig.1D). The ovarian cortex and medulla developed at two weeks. Oogonia (immature germ cells) in the cortex were surrounded by connective tissue (Fig. 2A). Oogonia form primordial follicles in the cortex's inferior portion, according to this study. The cortex produced Type 1, 2, and 3a follicles (Fig. 2B). Types 1, 2, and 3a small follicles increased, whereas 3b and 4 decreased. Type 3b oocytes were surrounded by around 20 cuboidal follicular cells. Type 4 primary follicles have two cell layers around the oocyte. Three-week-old kittens have primordial (Type 1 and 2) and primary (Type 3a, 3b) peripheral cortical follicles. Type 4 follicles penetrated the cortex (Fig. 2C). Like three weeks, four weeks has an excess of microscopic follicles (Type 1, Type 2, Type 3a) and an increase in medium-sized ones (Type 3b and Type 4, Fig. 2D). This study discovered unique 5-week-old kitten cortical follicles (Type 4). Type 4 follicles predominated and Type 5a appeared. Fig2E shows type 5a follicles with one oocyte and pre-granulosa cells. Medium-sized Type 5a follicles increased at six weeks, making the cortex and medulla difficult to detect (Fig. 2F). Cortical thickness increased whereas medulla thickness decreased with age. Age groups had different cortex-to-medulla thickness ratios (Fig. 2G). Immature rabbit ovaries contained cuboidal germinal epithelium and a thick tunica albuginea at 10 weeks (Fig.3A). Many small follicles (Type 1, 2, and 3a) and medium-sized (Type 3b, 4 and 5a) were in the peripheral cortex beneath the tunica albuginea, whereas large follicles (Type 5b, 6 and 7) were in the core and recognized for the first time (Fig.3A As follicles grew from cortex to core, the medulla extended (Fig.3A). Type 5b follicle oocytes had a zona pellucida, granulosa cells, a central vesicular nucleus, and theca-like cells (Fig.3B). Small follicular fluid space between granulosa cells and enhanced oocyte-circle cell development was detected in Type 6 follicles (Fig.3C). Graafian follicles type7 had large liquid-filled chambers. The zona pellucida and corona radiata embrace this space. Cumulus oophorous cells linked the ovum to stratified mural granulosa cells that surrounded the follicular cavity. Theca interna had larger glandular spindle-shaped cells with weakly stained cytoplasm and conspicuous nucleoli, whereas theca externa contains circular collagen fiber bundles (Fig.3D). Immature ovaries showed well-developed interstitial glands with clusters of polyhedral round cells, whereas the medulla contained thick bundles of irregular connective tissue and many blood vessels (Fig.4A). The connective tissue-scattered cells had transparent cytoplasm and black nuclei (Fig.4B). The cortical periphery and primordial and primary follicles were thinner in 4-month-olds than immature ones (Fig. 5A). This period produced corpus luteum. Mature (5-month-old) does had more corpora lutea and fewer follicles. Corpora lutea were huge, microscopic luteal cells with clear cytoplasm and black nuclei. Granulosa cells produced large luteal cells, whereas theca interna produced little ones (Fig. 5B).

General histological description of the uterus

One-week-old kittens had endometrium, myometrium, and perimetrium. Endometrium was lined with pseudostratified columnar epithelium resting basement membrane, lacked uterine glands. The myometrium had few circular smooth muscle fibers. Endometrium base included lumina epithelialis, lamina propria, undifferentiated mesenchymal cells, lymphocytes, and blood vessels. The myometrium was a thin layer of smooth muscle fibers with blood vessels, whereas the outer perimetrium was loose connective tissue covered with simple squamous epithelium (mesothelium) and blood vessels (Fig.6A&B). Oval-nucleated simple columnar endometrial epithelium appeared at two weeks. In the uterine gland primordium (bud development), shallow epithelial invaginations began lamina epithelialis adenogenesis (Fig.7A). Myometrium had plenty of blood vessels. In the outer perimetrium, simple squamous epithelium (mesothelium) covered loose connective tissue, blood vessels, and nerves (Fig.7A). Histologically, the uterus formed at 3-4 weeks. Nascent glands from lamina epithelial growth penetrated the lamina propria and began tubulogenesis (second-stage adenogenesis) (Fig. 7B), undulating the mucosa. The sixth week noticeable the end of adenogenesis. From the lamina propria to the myometrium's border, branching and coiling (third stage) formed simple uterine glands with discrete lumens (Fig.7C). Also, the myometrium was divided into inner circular and outer longitudinal muscle fibers (Fig. 7 D). Sparse deep invaginations formed crypts in the endometrium at 10 weeks, which evolved into uterine glands with simple cuboidal epithelium. Myometrium grew. A narrow, slit-like lumen results from nearly closed endometrial folds (Fig.8A &B). Folded uterine mucosa at 4 months. Thick collagenous stroma contains simple tubular uterine glands with low

connective tissue-to-glandular tissue ratio. Well-organized uterine glands with lumens and simple cuboidal epithelium were observed in the mucosal folds. Myometrium thickened gradually. Uterine maturity was indicated by presence of loose connective tissue between myometrial layers and uterine arteries and veins (Fig. 9A). Epithelial proliferation, crypt development, luminal folding complexity, and endometrial gland densification started at 5 months. Uterine glands with simple cuboidal epithelium remained tubular (Fig.9B). Uterine wall muscle reached its final structure. Well-developed blood vessels and nerve plexuses were noticed. In myometrial muscle fiber connective tissues, ganglionic cells had large pale nuclei and conspicuous nucleoli. A few had two nuclei. These cells were numerous in 4- to 5-month-old rabbits (Fig.9C).

Histochemical analysis

Masson trichrome

Ovarian sections of one-week-old kittens showed the tunica albuginea, a thin layer of loose connective tissue below the epithelium with sparse collagen fibres within oogonia clusters (Fig. 10A). Collagen fibers differentiated in primordial follicles after 2 weeks (Fig. 10B). G3 and G4 showed thicker collagen fiber bundles between growing follicles from cortex to medulla (Fig. 10C). At 5 months, older does had less connective tissue density, especially in the medullary zone around large blood vessels, and many collagen fiber branches pierced the cortex around large ovarian follicles (Fig. 10D). In female kittens, immature does, and mature does, Masson's trichrome stain identified collagen fibers in the uterus by color and structure. Results demonstrated collagenous fibers in different uterine layers, depending with rabbit age: wider lamina propria and thinner myometrium in female kittens, well-developed lamina propria in immature does, and scarce, mature lamina propria in mature does due to well-developed uterine gland Mature myometrium thickened whereas connective tissue diminished (Fig. 10E&F).

Carbohydrate Histochemical results of the uterus

The study found age-related kitten and doe staining differences: The endometrium of 2-week-old kittens reacted strongly with alcian blue (AB) indicating acidic mucopolysaccharides but negatively with PAS stain (Fig.11A). The endometrium reacted strongly to AB at 3 weeks, but the lining epithelial cells reacted weakly to PAS and AB (Fig.11C). AB reaction was moderate in the lamina propria stroma and strong in the lining surface epithelium at 6 weeks (Fig.11D). The ground substance in the lamina propria reacted favourably to AB at 10 weeks, and newly developed uterine glands had weak PAS and moderate AB reactions (Fig.12). In mature does at 4 and 5 months old, endometrial cells displayed positive AB reactions and uterine wall constituents mild positive to negative PAS reactions (Fig.13A-D). These studies showed uterine carbohydrate content changes which related to age.

Discussion

This study found that female rabbit ovaries aged 1-6 weeks contained various follicle types (1, 2, 3a, 3b, 4, with some 5a and 5b). Only types 5a and 5b developed into large follicles, indicating incomplete maturity. Mature does (G7 and G8) exhibited significant numbers of type 6 and 7 follicles, suggesting pituitary gland development, particularly in the pars distalis. Type 7 graafian follicles persist in the ovarian cortex and enlarge during estrus. These results were parallel with [15] they found that large follicles type 7 and 8 were only seen in mature rabbit. Microscopically, the ovary had germinal epithelium, but the hilum, where blood vessels entered and departed, did not. Under such epithelium is tunica albuginea. This investigation pointed with the outer cortex and inner medulla of the ovary core were discovered in rabbits [16] and golden hamster [17]. This research demonstrated that primordial follicle development during folliculogensis requires the large oogonia pool in the one-week-old cat ovarian cortex. Oogonia make vibrant nests. According to previous study [18], newborn rabbits' ovarian cortex includes mainly germ cells. Not all oogonia reach prophase in the first meiotic division and become primordial follicle precursors [18]. At 14 days, kittens developed a one-cell layer of flattened pregranulosa cells that formed Primordial follicles and started occupying the inner cortex. Folliculogensis begins at this stage, according to these studies. This investigation confirmed by another workers [18] Discovered that the New Zealand rabbits with high ovarian FSHR and LHR gene expression develop folliculogensis at two weeks of age. Folliculogensis begin at two weeks of life and increase FSHR and LHR gene expression in the procine ovary [19]. Large numbers of tiny and medium-sized primary and secondary follicles reached the inferior cortex at 4-6 weeks [18]. These data confirm [20] that primary and secondary follicle development enhances FSHR gene expression. Granulosa cells grew, forming theca externa. The zona pellucida, a thick glycoprotein around the oocyte, becomes apparent throughout growth. This research found antrum pockets between granulosa cells in developing secondary follicles at 10 weeks. These morphologies resemble immature doe ovaries [21]. Similar to [18] pituitary gland response affects early infantile rat ovarian antrum development. Immature ovaries have 2-4 kinds of large graafian follicles that develop to the ovarian surface for maturity and ovulation. A liquor-filled C-shaped antrum created by follicles with a corona radiate layer surrounding the secondary oocyte. According to [15], 8-10 week-old local rabbit ovaries contained

3-4 large immature follicles. In the ovarian medulla, interstitial glands steroid-secreting cells were initially detected within collagen fibers at 10 weeks of age. Clear cytoplasm and a dark nucleus characterized these polyhedral spherical cells. According to [22], 8-week-old rabbit ovaries contained steroidogenic cells. Actually, these glands developed from atretic antral follicle theca interna cells. Golden hamsters mature at 16 weeks [16] and have these glands [20]. Mature rabbits (4-5 months) had fewer tiny and medium-sized follicles than pre-puberty ovaries. Multiple follicular phases and several active corpora lutea indicated complete ovarian development, presumably owing to ovulation. This implies a favorable postnatal link between pituitary, hypothalamus, and ovary. These results agree with [15], who consider this period mature in does. Increased progesterone may restrict follicle development, reducing follicle counts. The dominant follicle secretes growth hormones including IGF1 and estradiol and increases LH receptors in granulosa cells during advanced follicular development. IGF1 stimulates oestrogen production, which is necessary for granulosa cell proliferation, antrum formation, and gap junction building [23]. Through the estrous cycle, the hypothalamic-pituitary-ovary axis governs this process. The hypothalamus releases GnRH due to high estradiol levels from the follicle before ovulation, which encourages the pituitary gland to produce LH and FSH. After ovulation, estradiol levels drop, transforming theca and granulosa cells into luteal cells [23]. Postnatal epithelium height, lamina propria thickness, tunica muscularis thickness, and serosal thickness increased significantly on uterine wall microscopy. In kittens, measurements were 10-12 µm, 60-202 µm, 16-114 µm, and 14-59 µm. Immature does had measures of 18 µm, 352 µm, 225 µm, and 92 µm, whereas mature does had readings of 22 µm, 597 µm, 281 µm, and 167 µm. These results match [17], who reported comparable measurements. The current study shows that postnatal uterine histological changes are comparable to those in New Zealand White Rabbits [20] and indigenous rabbits [18]. This includes uterine gland production, mucosal fold growth, and myometrium thickness and specialization. The uterine histology of one-week-old kittens was studied in this research. The initial uterine stages have pseudostratified columnar epithelium-lined endometrium. This endometrium had no endometrial glands and was surrounded by undifferentiated mesenchyme. The uterus' three layers were not yet differentiated. [25]found similar rodent developmental changes. This study that rabbits started adenogenesis, the formation of endometrial glands, at two weeks. This procedure developed shallow epithelial invaginations that become glandular buds. These results parallel to rabbit research [20]. According to [25]rodent buds developed at 5 PND. Reproductive tract development varies by species, habitat, and gestation length. Before birth, the vagina, cervix, and oviduct develop, whereas the uterus develops after birth [25]. Nascent glandular epithelial buds formed tubular structures in the stroma at 3 weeks. [20] discovered nascent buds in 4 week rabbits and 7 and 9 PND rats [25]. Rabbit and rat adenogenesis time may anatomically, histologically, and histochemically [27-30]. Myometrium was completely developed, thickened, and arranged into two inner circular and outer longitudinal layers by 4 weeks postnatally, unlike [20] histological data. Smooth muscle and extracellular matrix hypertrophy may cause myometrium growth [20]. Postnatal myometrium development and differentiation may affect functions [25]. Adenogenesis ends with endometrial glands coiling and branching to the inner myometrium after 5 weeks, whereas in rodents it occurs at 9–15PND [25]. Different gestational lengths may explain this. Rabbits have tubular endometrial glands like rats and mice [25], but large mammals have coiled glands [31]. In the present study at age 10th week postnatally, simple tubular endometrial glands invade the lamina propria, longitudinal mucosal folds, and deep epithelial invaginations. Myometrium mature. [18] found similar results with 8-10-week rabbits. Structural and functional maturity not occur here. According to [32] prenatal and postnatal uterine tissue training may affect mature function.4-month-old doe uterine walls alter drastically. This includes bigger myometrium and deeper endometrial glands. In connective tissues, ganglionic cells surround myometrium muscle fibers. This supports [20] conclusion that 4-month-old New Zealand White Rabbits have histologically developed uterine walls. [18] reported 5-month-old local breed rabbits with perfect uterine maturity. At 4 months, endometrial glands imitate mammogenesis, lactogenesis, and involution. Myometrial ganglionic cells may induce CNS ovulation during sexual activity. Numerous autonomic ganglionic cells in the female reproductive tract govern smooth muscle contraction, uterine gland secretion, and central nervous system linkage [33]. Functional and morphological maturity vary among mammals. Uterine maturation occurs at 15 PND in rats [33], 120 PND in pigs [35]. After delivery, endometrial glands, folds, caruncles, and myometrium proliferate. Early FSHR and LHR mRNA and protein expression and uterine development from birth to maturity (4months) were investigated [18]. Results reveal endometrial gonadotropin receptors' importance in uterine growth. According to [36] and later experiments using HCG to induce pseudopregnancy, gonadotropins are essential for uterine health during pregnancy. Postpartum uterine development required stroma-derived fibroblast-like, hepatocyte, and insulin-like growth factors. Steroid hormone receptors ER, PR, and PRL promote uterine gland adenogenesis and branching [36]. Masson's

trichrome staining of newborn kitten ovarian sections revealed delicate tunica albuginea collagen fibers. At 5–6 weeks, connective tissue extended throughout the cortex, collagen fiber thickness increased, and dense collagenous bundles developed amid growing follicles. However, connective tissue stroma density decreased in immature and mature ovarian sections, notably in the medullary area around big ovarian blood arteries, and collagen fiber density decreased in the cortex between large ovarian follicles. These results parallel [37]in adult rabbits, and [38] in adult Guinea Pigs. Steroid hormones like oestrogen and progesterone may affect connective tissue density at different ages. Research shows that oestrogen and progesterone increase collagen fiber production. Oestrogen increases collagen III in the ovarian stroma, whereas progesterone increases collagen I [39]. Masson's Trichrome staining revealed age-related changes in female rabbit connective tissue. A large, underdeveloped, highly vascularized lamina propria and circular smooth muscle fiber in the myometrium characterize kittens' uterine walls. Similar results were found in rabbit [20]. The lamina propria of juvenile and adult female deer was primarily thick collagen bundles and less dense. Collagen fiber in the lamina propria increased with progesterone activity, which is necessary for their formation [40]. Immature and adult myometrium was thick and layered longitudinally and circularly. This study detected connective tissue surrounding major blood veins in the vascular layer between myometrial layers. Perimetrium with serosa. In [31], comparable results were reported. Myometrium outnumbers connective tissue in immature and mature does, indicating uterus preparation for pregnancy. [40] discovered that uterine connective tissue and smooth muscle fiber proportions impact female horse fertility.In contrast, [18] on indigenous rabbit kitten uterus exhibited negative AB and PAS stain in the columnar epithelium and endometrium, perhaps due to the lack of secretory cells. This research found minimal PAS stain reactivity and neutral mucopolysaccharaids in newly produced uterine gland endometrial cells. The juvenile uterine luminal epithelium contains a thin sulfated acidic mucopolysaccharid coating. In rabbit uteri, [41-42] found similar results. Mature uteri with weak positive to negative PAS stain on columnar cells had no secretory mucosa-lined uterus. Adult guinea pigs' lining mucosa had two types of secretory cells and a positive PAS stain [18,24]. Newborn kittens' guts contain acidic mucin to combat pathogens [25].

Conclusion

This study concluded that the histological and morphological maturation of the reproductive organs (ovaries and uterus) in local rabbit was mainly age related. This study found that rabbit reach puberty at age of 4 months with the presence of large follicles type 7 (Graafian follicles). Rabbits needed to be stimulated by mating with males in order to cause the rapid maturation and rupture of ovarian preovulatory Graafian follicle.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The research methodology and animal experiments received approval from the Animal Welfare and experiments Ethics Committee at Duhok University, College of Veterinary Medicine (VM2020/0212ud authorized on December 2nd 2020), in accordance with their requirements

TABLE 1. Classification of ovarian follicles at different ages during postnatal development in local.

Type	Morphological appearance of follicles
1	Follicle only consist of a small oocyte with no follicular cells attached to its surface
2	Follicle consist of a small oocyte with a few follicle cells attached to its cell surface, but not a complete ring of cells
3a	Follicles consist of oocyte with a complete ring of follicle cells surrounds it, which is typically small or might have in progress to grow
3b	Follicle characterized by one complete ring of follicle cells surrounds a growing oocyte.
4	Growing oocytes surround by two layers of follicular cells
5a	Growing oocytes surround by three layer of follicle cells. It is a transitory stage between medium and large sized follicles
5b	Follicle has a large oocyte surround by many layers of cells. The follicle cells are separated by scattered areas of fluid.
6	This type of follicle characterized by a single cavity containing follicle fluid. The cumulus oophorus (but not the stalk) has formed.
7	Large follicle with a single cavity filed with fluid and characterized by well-formed cumulus stalk. This sort of follicle called preovulatory follicle

TABLE 2. Classification of ovarian follicles in local rabbit at different ages during postnatal development

Type of Follicles	Group of Animals							
	1	2	3	4	5	6	7	8
Type 1	9(100%)^{a}	30 (62.5%)^{a}	17 (35.4%)^{a}	17 (32.7%)^{a}	26 (32.9%)^{a}	17 (12.4%)^{a}	1(1.9%)^{ag}	46(16.8%)^{a}
Type 2	0^{b}	9 (18.8%)^{b}	16(33.3%)a	8 (15.4%)^{b}	24 (30.4%)^{a}	56 (40.8%)^{b}	4(7.5%)^{be}	47(17.2%)^{a}
Type 3a	0^{b}	5 (10.4%)^{c}	5(10.4%)^{c}	20 (38.5%)^{c}	22 (27.8%)^{b}	4(2.9%)^{c}	7(13.2%)^{c}	66(24.1%)^{e}
Type 3b	0^{b}	2(4.2%)^{d}	5(10.4%)^{c}	5(9.5%)^{d}	4(5.1%)^{c}	31(22.6%)^{d}	10(18.9%)^{c}	42(15.3%)^{ac}
Type 4	0^{b}	2(4.2%)^{d}	5(10.4%)^{c}	2(3.8%)^{e}	2(2.5%)^{d}	8(5.8%)^{e}	18(34.0%)^{d}	21(7.7%)^{c}
Type 5a	0^{b}	0^{e}	0^{b}	0^{f}	1 (1.3%)^{d}	8(5.8%)^{e}	5(9.4%)^{ef}	19(6.9%)^{f}
Type 5b	0^{b}	0^{e}	0^{b}	0^{f}	0^{b}	13(9.5%)^{f}	4(7.5%)^{ef}	10(3.6%)^{g}
Type 6	0^{b}	0^{e}	0^{b}	0^{f}	0^{b}	0^{g}	3(5.7%)^{f}	20(7.3%)^{c}
Type 7	0^{b}	0^{e}	0^{b}	0^{f}	0^{b}	0^{g}	1(1.9%)^{g}	2(0.7%)^{d}
Total number	**9**	**48**	**48**	**52**	**79**	**137**	**53**	**273**

Letters a,b,c,d,e,f,g are significant al level (P<0.05) at the same column

TABLE 3. Comparisons of the histo-morphometrical features of the uterine walls among different postnatal age groups of rabbit

Variables	Groups of Animals (Mean±SEM)							
	1	2	3	4	5	6	7	8
Epithlium height (µm)	10.18±1.03^{a}	11.53±1.03^{b}	8.7±1.03^{c}	9.65±0.73^{d}	12.8±1.03^{e}	12.32±1.03^{f}	18.12±1.03^{g}	21.86±0.73^{h}
Lamina Properia (µm)	60.0±64.95^{a}	102±64.95^{b}	160.0±64.95^{c}	172.8±64.95^{d}	181.3±64.95^{e}	202.6±64.95^{f}	351.8±64.95^{g}	596.5±45.93^{h}
Tunica Mascularis (µm)	16.00±30.77^{a}	51.70±30.77.1^{b}	62±30.77.7^{c}	64.2±21.76^{cd}	74.3±30.77^{e}	114.7±30.77^{f}	225.00±30.7^{g}	280.8±21.76^{h}
Serosa(µm)	14.33±23.93^{a}	73.92±23.93^{b}	43.07±23.93^{c}	43.26±16.92^{c}	44.23±23.93^{c}	59.16±23.92^{d}	91.82±16.92^{e}	167.34±23.93^{f}

Letters a,b,c,d,e,f,g are significant al level (P<0.05) at the same row.

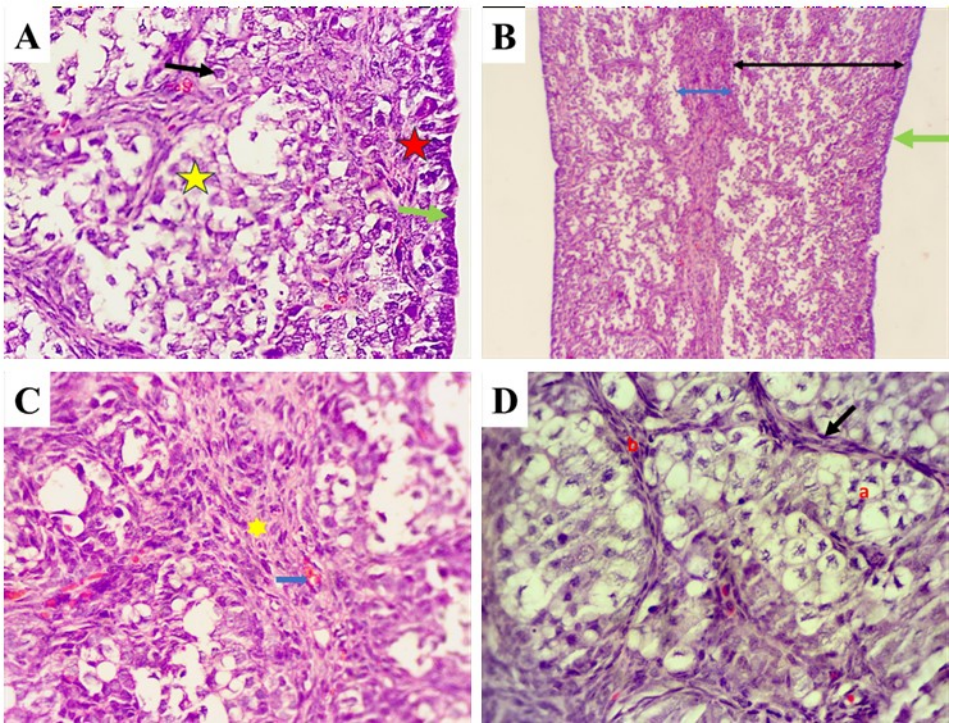

Fig.1. Histological section of ovary of one week aged kitten. A) Shows the germinal epithelium (green arrow), tunica albuginea (red star), cortex (yellow star) (oogonium (black arrows) H&E, 400x. B) Shows the cortex (black double heads arrow), medulla (blue double heads arrow), and germinal epithelium (green arrow). H&E, 100x. C): Shows the blood vessels (blue arrow) formed within the medulla (yellow star) H&E, 400x. D) Shows the oogonium (letter a), connective tissue (letter b) and stromal cell (black arrow), H&E, 400x.

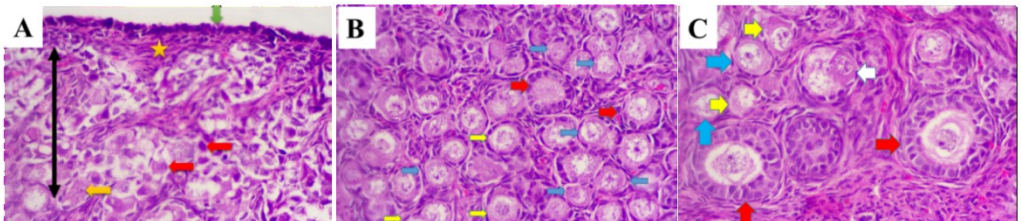

Fig. 2. A) Histological section of ovary of two week aged kitten show the germinal epithelium (green arrow), tunica albuginea (yellow star), cortex (black double head arrow) type1 primordial follicle (red arrows), type 2 Primordial Follicle (yellow arrows), irregular dense connective tissue (green star) H&E, 400x. B) Shows the type 1 follicle (blue arrows) type 2 follicle (yellow arrows) type 3a follicle (red arrows) .100x H&E. C) Histological section of ovary of three week aged kitten showed the type 1 (yellow arrows), type 2(blue arrows), type 3a (white arrow), type 4 (red arrows), 400x H&E. D) Histological section of ovary of four weeks aged kitten shows type 2 follicle (blue arrows), 3b (green arrow), type 4 (red arrow). 400 X H&E. E) Histological section of ovary of five week aged kitten shows the type 2 (red arrows), type 3a (yellow arrows), type 3b (black arrows), type 4 (green arrow).400X H&E. F) Histological section of ovary of six week aged kitten shows the type 1 and type2 follicles (yellow star), type 3a (yellow arrows), 5b (green arrows) 100x H&E. G): Histological section of ovary of six week aged kitten shows the type 5b (green arrows) 400x H&E.

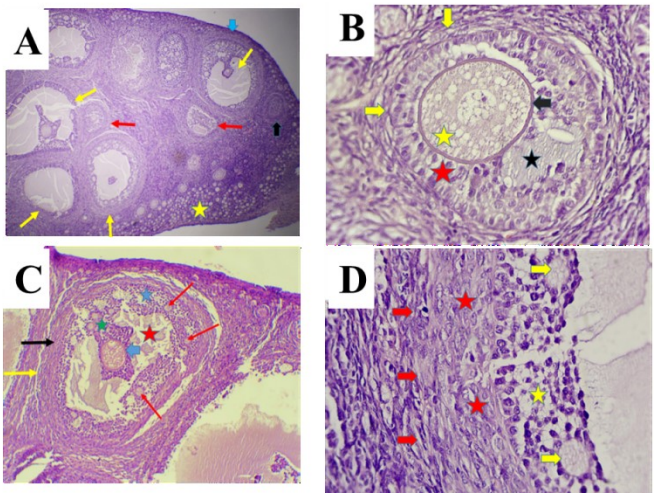

Fig.3. Histological section of ovary of 10 weeks aged immature does. A) Shows small follicles (yellow star), medium follicles (red arrows), large follicles (yellow arrows), germinal epithelium (blue arrow), atretic follicle (black arrow), 100X H&E. B) Shows type 6 follicle contain oocyte (yellow star) centrally, zona pelucida (black arrow), granulosa cell (red star), theca cell (yellow arrow) newly formed antrum (black star).400X H&E. C) Type 7 follicle (red arrows), corona radiata (blue arrow), cumulus oophorus (green star), antrum (red star), granulosa cells (blue star), theca interna (black arrow),theca externa (yellow arrow) 100x H&E. D) Shows type 7 follicle (red arrow): antrum pocket (yellow arrows), granulosa cells (yellow star), theca interna (red star),theca externa (red arrow), antrum pocket (red arrows) 400X , H&E.

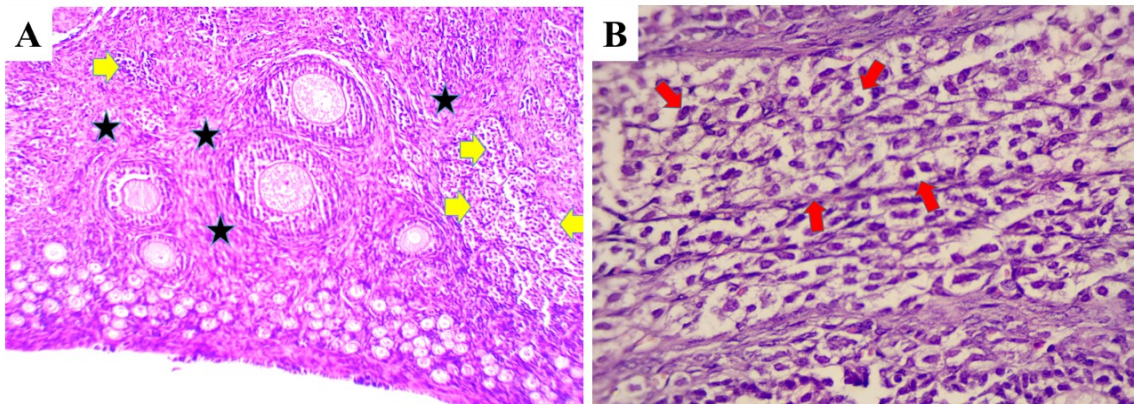

Fig.4. Histological section of ovary of 10 weeks aged immature does. A) Shows medulla formed of dense connective tissue (black stars) interstitial glands (yellow arrows).40XH&E. B) Shows interstitial gland cells (Res arrows) are seen in the ovary of immature does characterize by polyhedral round cells with clear cytoplasm and prominent dark nucleus, dispersed between connective tissue 400XH&E.

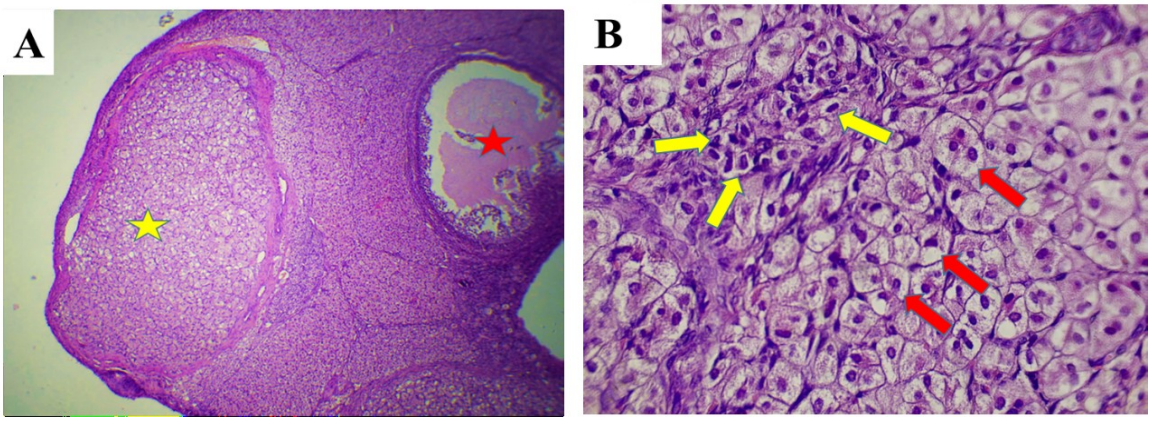

Fig.5. Histological section of ovary in 5month aged in mature does. A: Shows formation of corpus luteum (yellow star) and mature follicle (red star) 100X. B: Shows histological section of corpus luteum (yellow star) composed of large (red arrows) and small lutein cells (yellow arrows), 400X, H&E.

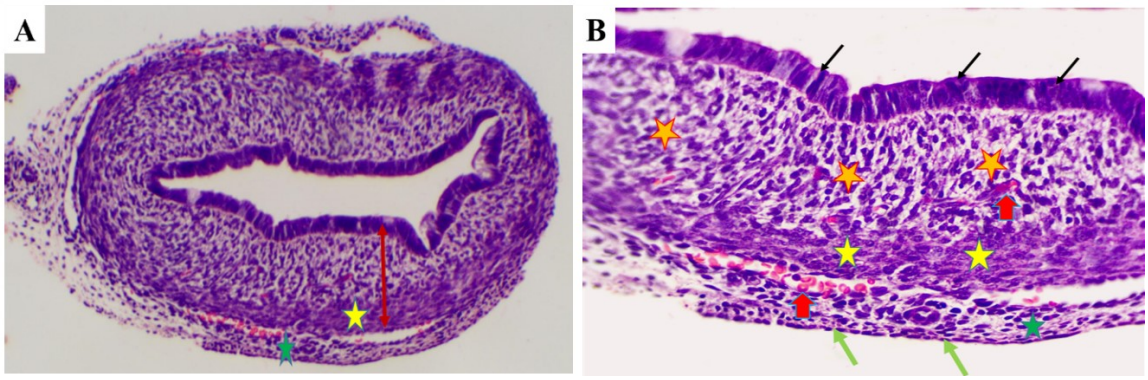

Fig. 6. Histological section of uterus in one-week aged kitten. A) Shows the three layers endometrium (double heads red arrow), myometrium (yellow stars), perimetrium (green stars), 100 X. B): Show epithelium characterized by pseudo stratified columnar epithelium (black thin arrows) rest on basement membrane, lamina propria (orang stars), myometrium (yellow stars), perimetrium (green stars), mesothelium (green arrows) and blood vessels (red arrows), 400. H&E.

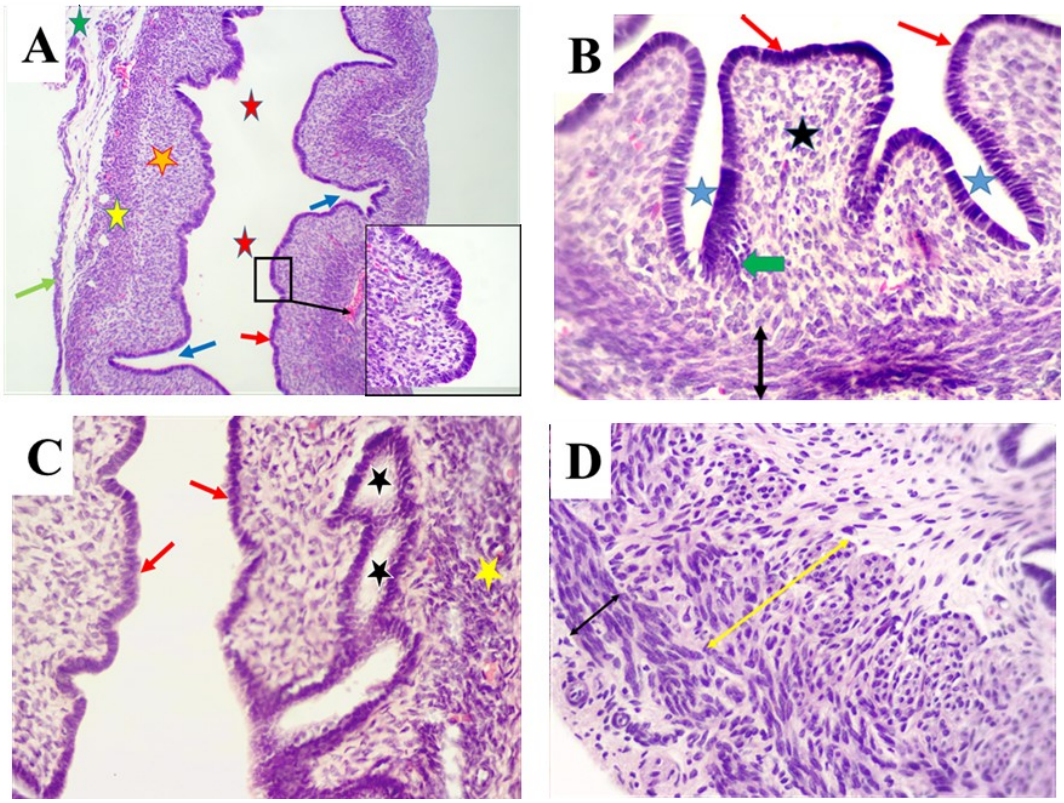

Fig. 7. A) Histological section of uterus in two weeks aged kitten shows uterine lumen (red stars), endometrium (orange star), simple columnar epithelium (red arrow), myometrium (yellow stars), perimetrium (green stars) bud formation (blue arrows), mesothelium (green arrow).100X, H &E. B) Histological section of uterus of four week aged kitten shows simple columnar epithelium of the uterus (red arrows), lamina properia (black star) , muscular layer (double head black arrow) tubular structure (second stage of adenogenesis , tubulogenesis) (blue stars), nascent gland (green arrows).400X, H&E. C) Histological section of uterus of five week aged kitten shows simple columnar epithelium (red arrow), last stage of adenogenesis branching and coiling (black stars) and myometrium (yellow star). 100X, H&E. D) Histological section of uterus of five weeks aged kitten showed inner muscular layer (yellow arrows) and outer muscular layer (black arrow) of myometrium.400X. H&E.

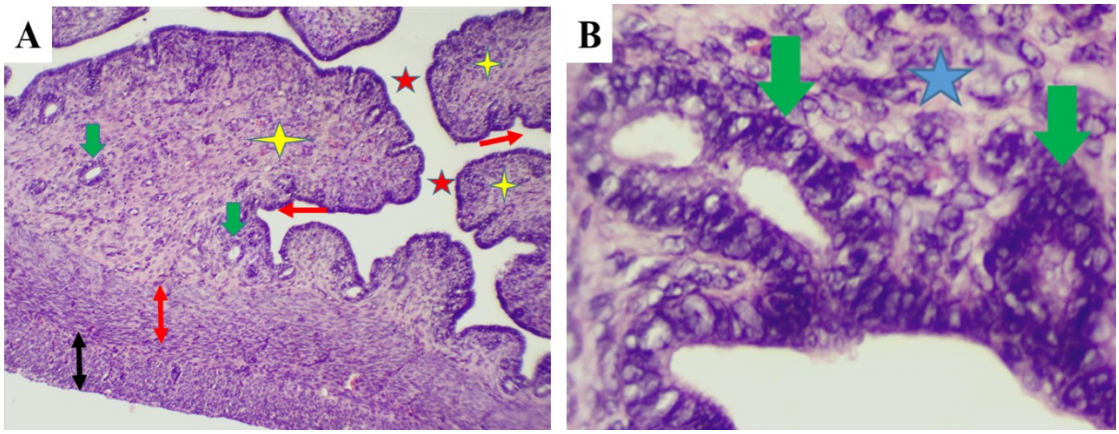

Fig.8. Histological section of uterus of ten weeks aged immature does. A) Shows narrow lumen (red stars), crypt (red arrows), lamina properia (yellow stars), developing uterine glands (green arrows) and inner myometrium (red double head arrow) and outer myometrium (black double head arrow) 100X, H&E. B) Shows uterine gland (green arrows) lined with simple cuboidal epithelium, lamina propria (blue star) 400X H&E.

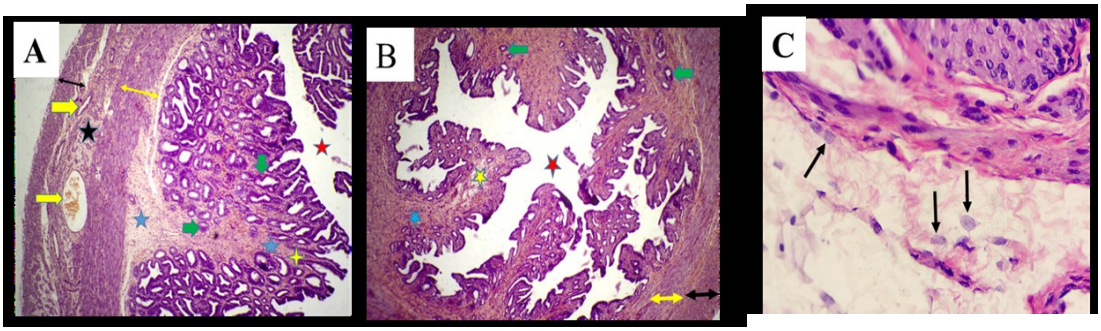

Fig.9. A) Histological section of uterus 4 month mature does shows lumen (red star), increase endometrial folds (yellow star), uterine glands (green arrows), lamina propria (blue stars), internal layer of myometrium (double head yellow arrow) and external layer of myometrium (double head black arrow), connective tissue between two layers of endometrium (black star) containing blood vessels (yellow arrows) 100X, H&E. B) Histological section of uterus 5month mature does showed lumen (red star), endometrial folds (yellow stars), uterine glands (green arrow), lamina propria (blue stars), internal layer of myometrium (double head yellow arrow) and external layer of myometrium (double head black arrow), connective tissue between two layers of endometrium (black star) containing blood vessels (yellow arrows) 100 X H&E. C) Ganglionic cells dispersed within the connective tissues among muscle fibers of myometrium, have large pale nucleus with prominent nucleus, some ganglionic cells have two nuclei in 4 and 5 months of age (black arrows) 400 X H&E.

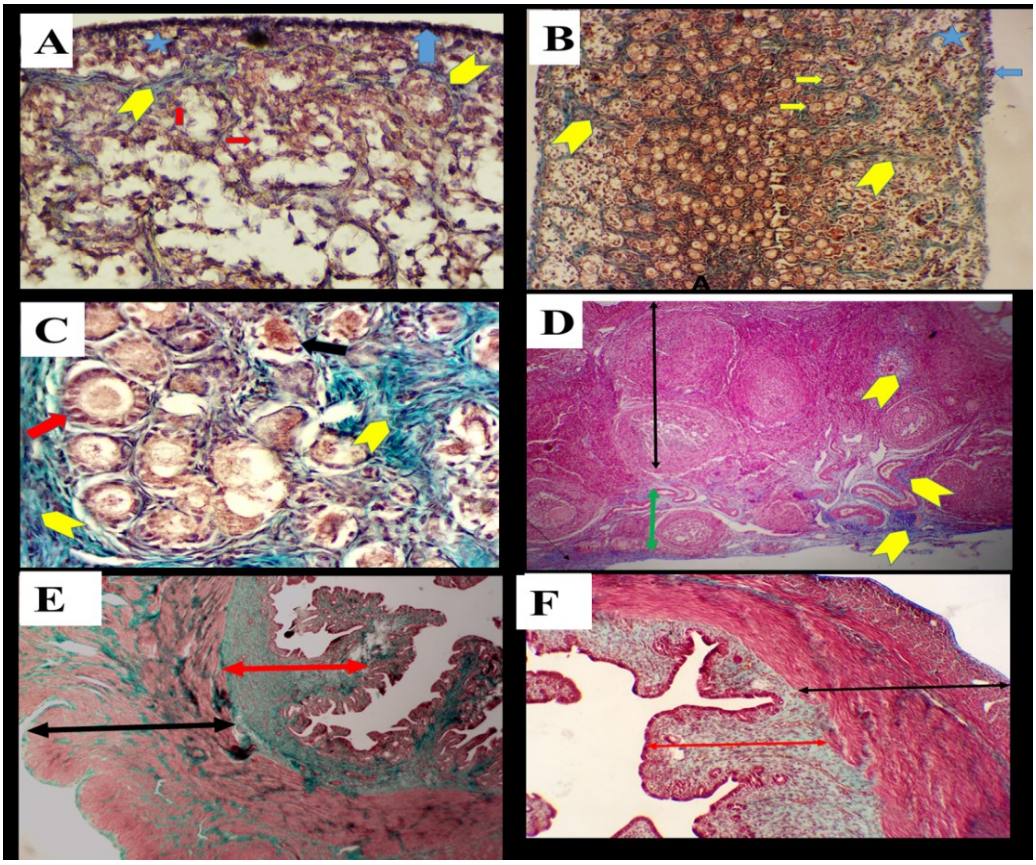

Fig.10. Histochemical staining of the ovary. A) One week of aged kitten shows thin collagen fiber (yellow chevron) intervening among the oogonia (red arrows), germinal epithelium (blue arrow), tunica albuginea (blue star). B) Two weeks of aged kitten shows more distinct collagen fibers (yellow chevron) became more distinct distributed among the primordial follicles (yellow arrows) germinal epithelium (blue arrow), tunica albuginea (blue star). C) Three to four weeks aged shows thick, long bundles of collagen fiber (yellow chevron) distributed among the growing follicles primary follicle (red arrow). D) Four months does shows distribution of the collagen fibers (yellow chevron) in the cortex (double head black arrow) and in the medulla (double head green arrow). E) Ten weeks aged kitten. F: 5 months aged rabbits this figure showed that the development of connective tissue (double head red arrow) present in the endometrium to the myometrium (double head black arrow) B; C&E100, A; D&F: 400X. Masson's trichrome stain.

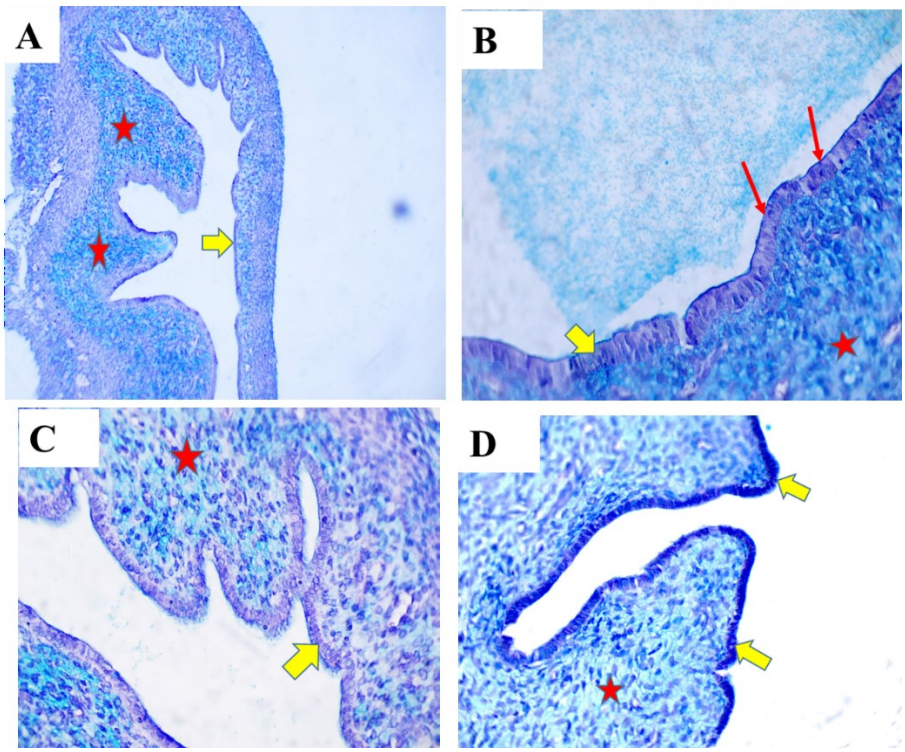

Fig.11. The microphotograph of the uterus section in different ages. A&B) Two weeks aged kitten show positive reaction of ground substance (red stars) toward alcian blue (PH2.5) stain, the luminal surface of epithelium shows strong positive reaction with alcian blue (red arrow) and negative reaction with PAS stain (yellow arrow). C) Three weeks aged kitten shows the stroma exhibits a positive reaction toward AB (pH 2.5)(red star), epithelial cells had weak reaction toward both PAS - AB (pH 2.5) (yellow arrow). D) Six weeks old kitten, the ground substance in endometrium shows moderate positive reaction toward AB (pH 2.5) (red star), whereas the lining surface epithelium shows strong reaction (yellow arrow). A: X100, B, C, D: X400. PAS - AB (PH2.5).

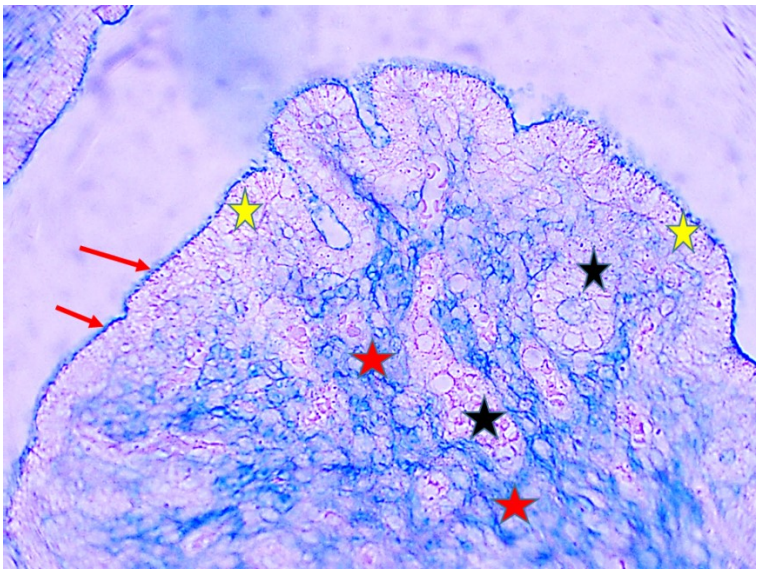

Fig.12. The microphotograph of the uterus section in 10 weeks old kitten showing positive reaction of ground substance (red stars) and in uterine epithelium (red arrows) toward combined PAS AB (pH2.5) stain contain acidic mucin, weak PAS reaction in the luminal epithelium (yellow stars)and in glandular epithelium of uterine gland (black stars),100x PAS-AB (pH 2.5) .

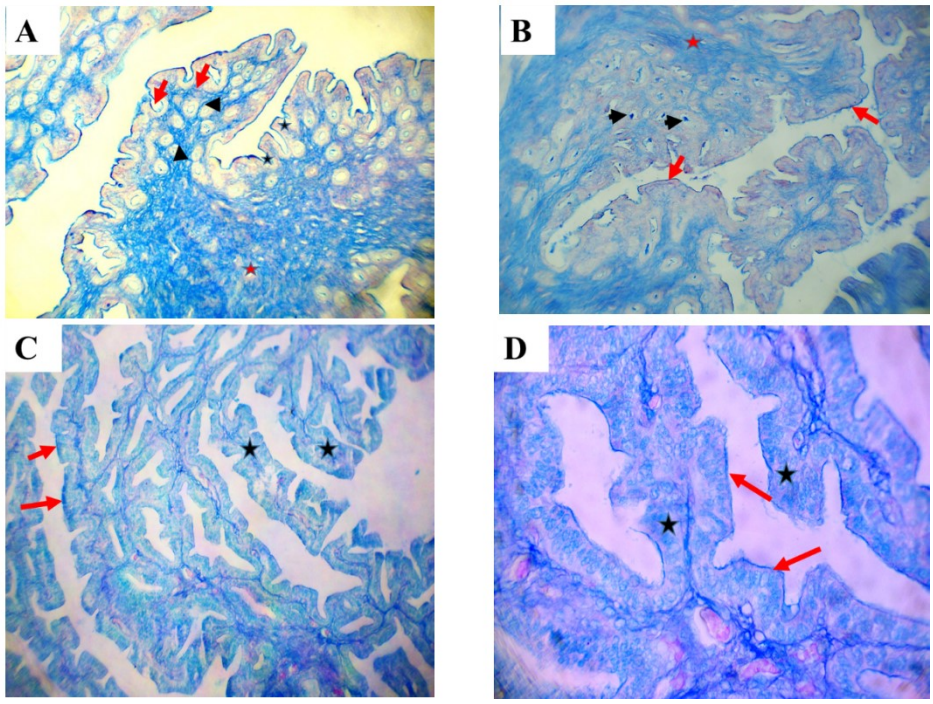

Fig.13. The microphotograph of the uterus sections in different age groups in adult does 5 months of age A, B, C&D: In both ages the ground substance of endometrium show positive reaction (blue color) toward AB stain (red star).Whereas, the epithelial cells of the endometrium and uterine gland exhibit mild positive to negative reaction against PAS (black stars) and AB stain (red arrows). A-C100X, D: 400X PAS-AB (PH 2.5).

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التغيرات التطورية بعد الولادة في المبيض والرحم للأرانب المحلية (*Oryctolagus cuniculus*) في محافظة دهوك: "دراسة نسجية وكيميائية نسجية

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الملخص

هدفت هذه الدراسة الى الكشف عن التغيرات في التركيب والشكل والتركيب الكيميائي النسيجي للمبيض والرحم في إناث الأرانب المحلية (*Oryctolagus cuniculus*) خلال فترة النمو بعد الولادة من 7 إلى 150 يوما. لإجراء هذه الدراسة تم اقتناء 80 أرنباً من المربين المحليين وتم تقسيمهم إلى 8 مجاميع حسب الفئات العمرية المختلفة وبواقع 10 أرانب لكل فئة عمرية. تم اجراء القتل الرحيم للأرانب ثم تشريحها للحصول على المبيضين والرحم وتم حفظهم في محلول 10٪ من الفورمالين الدارئ المتعادل. وبعد ذلك، خضعت العينات للتمريرات النسيجية الروتينية للحصول على شرائح بسمك 5 ميكرومتر وصبغت بملون الهيماتوكسلين والايوسين وملون ماسون ثلاثي الصبغ، بالإضافة إلى ملون الاليشان الازرق وملون حامض شف الدوري. كشف الفحص المجهري عن تغيرات شكلية ملحوظة في المبيضين والرحم أثناء نضوج الصغار بعد الولادة. وبينت الدراسة أن مبيض الأرنب، الذي كان عمره بين أسبوع وستة أسابيع، يتكون من جريبات مبيضية من النوع 1-5ب. ومع ذلك، اظهرت المجموعتين السابعة والثامنة وجود جريبات مبيضية كبيرة مصنفة على أنها من النوع 6 و7. كشفت هذه الدراسة عن نمو في جميع طبقات الرحم، وخاصة في الغلالة العضلية طوال فترة ما بعد الولادة. اشارت هذه النتائج إلى أن إناث الأرانب الصغيرة تخضع لتغييرات تنموية كبيرة بعد الفطام مع تقدم العمر. بينت هذه الدراسة أن جريبات ما قبل الإباضة والجسم الأصفر كانت موجودة في إناث الأرانب الناضجة في عمر 4 أشهر، مما يشير إلى أن هذه الأرانب وصلت إلى سن البلوغ في هذا العمر. وخلصت الدراسة إلى أن نضوج الجريبات المبيضية والرحم في هذه السلالة يعتمد على عمر الفرد.

الكلمات الدالة: نشوء الجريبات ، غدة بطانة الرحم ، قياسات النسيجية ، الكيمياء النسيجية ، الارنب.