ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). <a href="https://doi.org/10.21070/ijhsm.v2i2.287">https://doi.org/10.21070/ijhsm.v2i2.287</a>

# The Effect of Physiological Status and Pregnancy on Histological Structure and Hormonal Levels of Ovary and Uterus in Mice *Mus Musculus*

Shaimaa Hameed Hassan

Department of the anatomy/ Colla. of medicine, University of Sumer / Iraq Email; shaima.hamid@uos.edu.iq Phone number: +9647818626622

**Abstract.** The reproductive system in female mammals is highly dynamic, with significant structural and hormonal changes occurring during the estrous cycle and pregnancy to support fertilization, implantation, and fetal development. In mice (Mus musculus), understanding the histological and hormonal variations in the ovary and uterus is crucial for advancing reproductive biology, disease control, and experimental models in biomedical research. Despite extensive studies on reproductive physiology, few have integrated hormonal profiling with detailed histomorphological changes across different reproductive phases in mice. This study aimed to investigate the effects of physiological status and pregnancy on ovarian follicle development, uterine histology, and hormonal regulation in adult female mice. Sixty ovaries and uteri were examined across six reproductive stages, revealing significant changes in follicular growth, corpora lutea formation, and endometrial structure. Estrogen peaked during estrus, stimulating Graafian follicle development and a thin endometrium, while progesterone dominated pregnancy, maintaining uterine quiescence and promoting decidualization. Pregnancy induced marked endometrial vascularization, increased myometrial thickness, and glandular proliferation, supporting implantation and fetal nourishment. This study is among the first to comprehensively correlate histological remodeling with hormonal dynamics across reproductive stages in mice. The findings enhance understanding of the endocrine-morphological interplay underlying reproductive success and provide valuable baseline data for future research in reproductive biology, fertility regulation, and comparative anatomy.

#### **Highlights:**

- 1. Significant hormonal fluctuations were observed across reproductive stages, with estrogen peaking during estrus and progesterone dominating pregnancy.
- Pregnancy induced major uterine changes, including increased vascularization, decidualization, and myometrial hypertrophy to support implantation and fetal development.
- 3. Ovarian histology revealed dynamic follicular development, luteal formation, and atresia patterns influenced by hormonal regulation.

**Keywords:** Estrus, Pregnancy, Mice, Hormones, Ovary, Uterus.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). https://doi.org/10.21070/ijhsm.v2i2.287

#### **Introduction**

As natural carriers of many zoonotic illnesses, especially plaque, rodents play a crucial role in preserving and spreading infections within endemic areas (1). Researchers have also looked into the suppression of animal fertility by the use of environmental endocrine-disrupting substances and synthetic hormones. Hormonal agents, such as levonorgestrel and nongestural, have been used extensively to control reproduction in wildlife, livestock, and captive animals (1-4). The mammalian reproductive cycle's most sensitive stage is pregnancy, which makes it particularly susceptible to external disturbances. Immune tolerance, redox homeostasis, and the precise control of uterine hormone transmission are all necessary for a successful pregnancy (5,6). It is possible to measure hormones from a wide range of biological sources, including blood, saliva, feces, urine, hair, cerebral fluid, and more (7,8). The availability of the source material, the type of details that is wanted, the range of species' steroid hormone elimination and metabolism pathways, the analytical techniques available, and other complex aspects all play a role in choosing the optimal material to sample (9-11). According to studies comparing the overall amount of follicles in rodents with different ovulation rates, those with higher ovulation frequencies had more follicles during the entire growth period (12,13). A possible ecological strategy for managing rodent populations and controlling illness is to implement reproductive interventions that target the pregnant stage, as wild rats are important hosts of plagues and various other infectious diseases (14,15). Nevertheless, prior to the productive capability being utilized, the reproductive potential must be improved (16). The goal of the current study was to determine the histological features and hormonal condition of ovaries and uteri of mice at different stages in order to comprehend the effects of estrus on the ovary and pregnancy on follicle development. In addition to highlighting the need for more research, this thesis may help with disease identification and, consequently, the most effective treatment. and this study has enhanced our understanding of the reproductive biology of the animals.

#### **Materials and Method**

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). https://doi.org/10.21070/ijhsm.v2i2.287

**Ethical approval:** Every procedure was followed in compliance with the requirements for animal care. The animals were killed in compliance via ethical and animal care standards, and their organs were subsequently used for research at the University of Sumer, College of Medicine from March to August 2025.

Animals: 60 uteri and ovaries from mature female mice Mus musculus was split up into six groups, each representing a distinct sexual phase. The study's subjects were 10 mice, each of which was 13 weeks old. The animals were raised in the Department of Anatomy's Experimental Laboratory at the University of Sumer's College of Medicine. The animals were kept in bedded in wood cages (81 cm  $\times$  70 cm  $\times$  55 cm) which complied with all requirements in a 12 x 25 m enclosed hall with ventilated air and water. The farm's mean temperature varied between ten to fifteen degrees Celsius, and the animals were kept in a 14 L:10 D system with Sixty lux of light intensity, 14 hours of light, and ten hours of darkness. Between fifty and sixty percent humidity prevailed (5). The animals were given access to clean water and a complete granular diet, and a veterinarian kept a close eye on them. The cages and feeder were regularly cleaned and sanitized according with the needs of the animals. Give every one of the animals an overdose of xylazine and ketamine. After blood was drawn, the samples were stored at 4 °C for two hours and centrifuged for ten minutes at 3500 rpm to separate the serum. ELISA tests (Elabscience, Wu Han, China) were used to test the levels of follicle-stimulating hormone (FSH), estrogen, progesterone, as well as luteinizing hormone (LH). These mice's ovaries and uterus inherited their histological characteristics. Seven 1 cm<sup>3</sup> specimens were obtained for histological examination from various ovarian and uterine regions (horns, body, and cervix) (17), Using analysis of variance (ANOVA), the data collected at the end of the experiment was assembled and statistically assessed. The mean spacing was ascertained where required using Duncan's novel multi range test (DMRT) (3).

#### **Results and Discussion**

**Estrogen hormone**: During the early and mid-stage of pregnancy, estrogen levels are frequently lower than those during estrus (Histogram 1). This demonstrates the pre-estrus

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). https://doi.org/10.21070/ijhsm.v2i2.287

phase's capacity for estradiol production by the Graafian follicle. Estrogen is the primary hormone responsible for regulating the estrus period. Estrogen may therefore be impacted by the substances secreted in bodily exudates during estrous. Elevated levels of estradiol were found prior to and after the preovulatory spike in gonadotropins. Throughout the course of the oestrous cycle, concentrations fluctuate somewhat before falling to their initial levels in the hours immediately follow. Following a peak of 35 milligrams every milliliter during the onset of estrus, the concentration of LH declined and stayed low throughout luteal phase (3 ng/ml). For the majority of mammals, one of the most important hormones during pregnancy and delivery is estrogen. Being a hormone-producing organ, the placenta is an organ that actively aids in the detection of pregnancy and generates progesterone and estrogen while a woman is pregnant. Just before igniting, on day 28, the E2 level soared after increasing significantly during the second half of pregnancy (2,17,18).

**Progesterone**: According to Histogram 1, progesterone levels are frequently higher during pregnancy than during estrus. This outcome is consistent with (19). The corpus luteum generates progesterone during the first half of pregnancy, and the placenta takes over approximately 10 days after estrus. After the luteal stage, the levels of progesterone in periphery plasma start to rise gradually. They peak about 12 days after estrus and keep rising for the whole 21-day pregnancy. Keeping the uterus quiescent during gestation is another way to prevent an early start to labor. It also helps with embryonic implantation and keeps the process going (4,20).

**LH** Compared to the pregnancy animals; the non-pregnant animals had a considerably greater LH level (P<0.05) (Histogram 1). By adversely influencing the anterior part of the pituitary and hypothalamus, progesterone suppresses the release of LH through the corpus luteum. Because ovulation is not required, there is no LH surge during pregnancy. LH helps to sustain pregnancy by halting the reproductive cycle and increasing at baseline levels during the oestrous cycle. The presence of an estrogen peak prior to the preovulatory rise in LH and an upward relationship between LH as well as estrogen within the 24 hours prior to the LH surge indicate that oestradiol may aid in the release of LH, despite the lack of evidence that it actually boosts LH secretion (3,21).

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). <a href="https://doi.org/10.21070/ijhsm.v2i2.287">https://doi.org/10.21070/ijhsm.v2i2.287</a>

**Follicle-stimulating hormone (FSH):** Although the levels of this hormone fluctuate during the period of estrous, they are typically highest in the early stages of estrous (Histogram 1). FSH is necessary for the formation and maturation of follicles in females, but its levels are influenced by progesterone and estrogen (22).

**Prolactin hormone:** Pregnancy frequently results in higher prolactin levels than estrus (Histogram 1). The hormone called prolactin had to rise sharply to reach its peak height. That on every day of pregnancy was far less than this. Compared to the other hormones, the levels of prolactin were lower on the actual day of delivery than they were during the balance of the pregnancy. Furthermore, they attributed these results to the increased prolactin levels in pregnant and lactating animals, which increase feed consumption and restrict prolactin production. Pregnancy-related changes in prolactin levels affect the subsequent phase of mammary gland maturation. The beginning of this phase occurs around parturition to prepare the mammary gland to emit colostrum and then milk (23).

The four phases of the estrus cycle which constitute the uterine cycle modifications brought on by the pituitary hormones activating on the ovary are proestrus, the period of estrus, metestrus, and diestrus. The epithelium lining the ovaries was composed of simple cubiodal tissue. The cortex included follicles of varying diameters. A granulosa made up of a single layer of cuboidal cells, a thin zona pellucida that now surrounded the oocytes, and several primordial follicles have all been seen in the cortex. Several flattened follicular cells surrounded a main oocyte in each. Corpora lutea were also observed. In the central zone of the ovarian stroma, the medulla which houses the main blood vessels, lymphatic vessels, and nerves appeared vascularized. These spindle-shaped stromal cells, which were primarily fibroblasts, also had smooth muscle cell strands. Usually, primordial follicles are distributed uniformly over the outer cortex. In a small number of intermediate follicles, many flattened cells of the follicle have been seen. (Fig. 1-5). This result is consistent with the findings of (10) that identified the histological structures in hamster and mouse ovaries, and it supports the theory that the epithelium plays an essential role in protection and ovarian surface renewal following ovulation, as well as the histological features of follicles at different phases of growth in ovine.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). <a href="https://doi.org/10.21070/ijhsm.v2i2.287">https://doi.org/10.21070/ijhsm.v2i2.287</a>

The ovaries' cortex was crammed with small follicles which were both common and plentiful. Larger follicles than medium-sized ones were observed in the ovary, which had Graafian follicles. Since the organism is polyestrous, the development of multiple Graafian follicles in the diestrous stage indicates the need for them in subsequent stages, when they comprised a substantial amount of ovarian cortex. Graafian follicles also had the potential to produce additional oocytes (Fig. 6-12). They were similar to the results published by (23). The other ovarian follicles experience atresia at different phases of development, but only a small portion are chosen to ovulation throughout ovarian follicular growth (11,24).

The diameter of primary follicles can change to match the diameter of the oocyte. The oocyte growing within its largest primary follicles started during the growth and maturation phase (Table 1); based on these data, the follicular development result typically looks the same in other experimental animals, like mice (2). But (25) pointed out that the main oocyte gets big, which causes several Golgi complexes and more mitochondria to form. The oocyte will also get bigger by multiple times at this stage of development. The amount of ovarian follicles which would respond to the FSH: LH combination gradually declined over the course of pregnancy, possibly due to a decline in gonadotropin-receptor function (12,27). To maintain luteal steroidogenic activity, the smaller follicles, which proliferate during pregnancy, probably release adequate estrogen (28).

Secondary follicles were seen in every stage, located within the egg, according to histological investigation utilizing an ovarian sample stained with several stains. It was an oocyte with a core nucleus. The oocyte was the same size regardless of preantral follicle. In the minuscule ones, granulosa was two to three cells thick, and the zona pellucida was plainly stained with periodic acid-Schiff (Fig 6,8,11). There have been reports of a similar result in rabbits (12). The appearance of those follicles, which seemed prolate due to the gathering of granulosal cells on both ends in along the long axis, specifically did not match the primary follicle's morphology. Between each pole were only slender handles of granulosal cell that stretched toward the oocyte. The differences in both primary and secondary follicle sizes that our data revealed were supported by (23,24), who postulated that oocyte expansion initiates follicular growth, which is completed by the time the antrum occurs.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). <a href="https://doi.org/10.21070/ijhsm.v2i2.287">https://doi.org/10.21070/ijhsm.v2i2.287</a>

In order to form a stratified epithelium, layers multiplied and the granulosa cells kept dividing as the initial follicle matured (Fig. 8,9). Similar to those observed in rabbits, these histology results described the growth of additional follicles at different phases of pregnancy (24). During pregnancy, the ratio of atresia folliclea to follicular development is smaller than in other cycle stages (Table 1). In light of the ovary's surface bulges, that may indicate yellow bodies, which would indicate altered reproductive activity, this revealed that there is minimal follicular alteration during pregnancy. The ovaries seemed more unequal and lobulated as a result of the ovarian activity (25).

Usually, the deep cortex of the ovary was where the growing follicles were visible. In Graafian follicle which governed the ovaries, the cells of the granulosa were positioned in a distinct area that is situated within the granulosa cell and the ovum on one side along with granulosa cell and the stroma, theca externa, and theca interna on the other. However, the granulosa cells that were found in the treated ovary were spread randomly and had lost their typical structure, with the possible exception of a few that were not perfectly aligned round the fertilized egg site (Fig. 8,10). This is similar to what was shown in white Swiss mice with (29). Fig. 9 shows that these follicles do not react with periodic acid-Schiff (PAS). The large, robust antral follicles that developed from the growing follicles to produce preovulatory follicles supported this observation (2,30). These preovulatory follicles had oocytes in the middle, but the germinal vesicle began to travel eccentrically. Encircling the oocyte is a ring of granulosa cells called the corona radiata. The oocyte was stuck in the granulation area (Fig. 9) that was bordered by the growing antrum. On the last day of pregnancy, the results show an increase in larger follicles (Table 1). Nonetheless, most ovarian follicles are small or atretic during periods of elevated progesterone throughout the luteal phase and pregnancy.

Primary and secondary follicular atresia seemed to be progressing rapidly; atretic follicles and follicules containing hypertrophied granular cells are apparent under a light microscope (Fig.10). Follicle enlargement in does during pregnancy is similar to that reported by (24). Rats also showed a resurgence of large follicles related to a drop in levels of progesterone in late pregnancy. Progesterone is mostly obtained from the corpora lutea (CL) of Angora rabbits during pregnancy (26). In mice ovaries, atresia was observed throughout the whole follicle

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). <a href="https://doi.org/10.21070/ijhsm.v2i2.287">https://doi.org/10.21070/ijhsm.v2i2.287</a>

development process (Fig.10). The atresia of different follicle types in yak can appear in a number of ways and can begin at any stage of follicular maturity. On the other hand, the ovary's ability to develop tertiary follicles is contingent upon the availability of suitable amounts of FSH and LH, followed by the follicles' ability to take the estrogen they make. Both an overabundance of levels of LH, estrogen, or progesterone whereas a deficiency of these hormones can result in atresia, while insufficient hormones such as FSH, LH, or estrogen can prevent the follicle from developing. Pregnancy prevents follicles from maturing into Graafian follicles and improves their atresia (8).

A basic cuboidal epithelium covered the germinal epithelium, limiting follicle growth and maintaining the function of the corpura luteum. The epithelial layer was absent from the ovary's hilus, which is wherever blood vessels traverse the organ. Beneath the germinal epithelium was an outermost layer of dense, disorganized connective tissues fiber, which was the tunica albuginea (Fig.9). The medulla of ovaries consisted of thick, irregular bundle of connective tissue that occupied the heart of the ovary and passed across the hilus to the medullarystroma, the area where large follicles were obviously surrounded by a lot of blood vessel branching and connective tissue running between follicles. These findings contradicted those in rabbit (24). In order to fertilize and ensure the successful continuation of the species, the female gonad known as the ovary must differentiate and release the fully developed oocyte. Progesterone and estrogen, the two main female sex hormones, are produced in the ovaries (9). (27, 28) found that corpus luteum significantly affects ovarian growth and that the size of the ovaries depends on the age, diet, and number of births of the animal. (29) suggested that a thick theca layer of androgen substrate may be required within the developing premenstrual follicle to maintain follicular oestradiol synthesis.

In the current investigation, there was an obvious difference between the images of a pre-ovulatory follicle, displayed a blood region around the antral cavity, and an atretic follicle, that lacked visible blood flow and gradually shrunk in diameter (Fig.6,7,9,10). This result is in line with (27) who suggested that follicular vascularization and blood flow velocity might be used to identify healthy follicles and predict the time of ovulation. The anovulatory follicle has a less vascularized membrane and atresia than the preovulatory follicle (Fig. 6,8,9). The findings

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). <a href="https://doi.org/10.21070/ijhsm.v2i2.287">https://doi.org/10.21070/ijhsm.v2i2.287</a>

of the most recent investigation supported those of previous studies (30, 31). (9) which had proposed that poor vascularity might be associated with low amounts of trophic hormones, such as LH. The most recent results from (2) completely support the earlier discovery from (32) indicating the largest and recent preovulatory follicles situated on the other side of the corpus luteum. (11) suggested that the short lifespan of the corpus luteum and the rapid growth of follicles could account for these findings, while (22) suggested that ovarian activity alternated between the ovaries in future cycles. (28) suggested that the follicular microenvironment had a crucial role in the identification of the dominant follicle. But as demonstrated by (33) the corpus luteum became one of most vascularized structures during ovulation and absorbed the highest rate of blood flow per unit of tissue due to the high amount of LH. (10) found that the ovary produces survival factors in addition to steroids FSH and LH, which are essential for the proper growth of follicles. Sexual receptivity affects the chemicals prolactin, FSH, and LH. As a result, T3 and T4 may have had the highest levels of prolactin, FSH, and LH as well as the highest receptivity. Treatments 3 and 4 demonstrated 15% of rhizolith absorption rates, respectively, and maximal prolactin production, which may be related to T4's greatest birth weight.

In all study animals, the uterine wall is composed of three fundamental histological components: endometrium, myometrium, and perimetrium. The endometrium is made up of the visceral layer with connective tissue that has many blood arteries and dense fibrous cells. An examination of female's uterus at nine days gestation for various dosages revealed a noticeable deficiency in the amount of uterine glands. The simple columnar epithelium lining the uterus cavity displayed several divisions and tubular endometrial glands that reached into the connective tissue. The tubular, branching uterine glands were coiled glands. The perimetrium is composed of a longitudinal band of smooth muscle at the outside end and a thick, circular layer of myometrium inside. The peritoneal mesothelium's simple squamous cells line its outside. It is composed of loose connective tissue which is home to numerous tiny lymphatics, blood, and nerve vessels. In addition to simple tubular uterine glands, blood arteries, and immune cells, the stroma is further divided as stratified partitions that comprise the basal endometrium, a more loosely structured layer inside the stratum spongiosum, and a highly organized fibroblasts zone (Fig. 1-5), as results of (1) It was noted that the uterine cavity's mucous lining is made up

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). <a href="https://doi.org/10.21070/ijhsm.v2i2.287">https://doi.org/10.21070/ijhsm.v2i2.287</a>

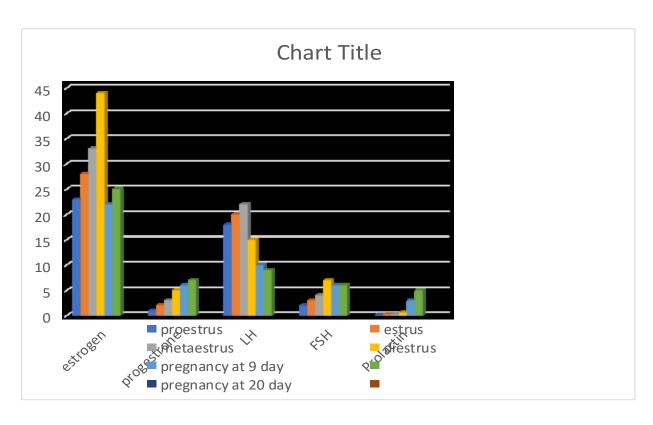
of epithelial cells, and that the endocrine glands are what give this layer its richness. This validates the findings of their study, which demonstrated the significance of glandular endometrium for pregnancy success. Throughout gestation, the mouse uterus alters dynamically to aid in the conceptus's growth (6). To generate a pregnancy, the embryo must develop through the blastocyst phase, implant into uterus endometrium, and form a viable placenta. Ensuring appropriate trophoblast development and controlling invasive into the endometrium for establishing blood supply to the conceptus are ultimately critical to the success of implantation (9).

Uterus is influenced by reproductive state. Endometrial fluid and the endometrium play a crucial role in reproduction. In order to accommodate and promote the fetus's healthy growth, the uterus undergoes considerable changes in shape, form, and placement throughout pregnancy. After mating, females can transition from a state of reproductive responsiveness to nonreceptivity and eventually establish pregnancy thanks to the estrus cycle. The luteal stage (eighteen days) as well as the phase of follicles (six days) are the two distinct phases that make up the cycle. In the luteal phase, corpus luteum forms after ovulation, but in the follicular phase, it persists until ovulation. In order to facilitate conception, the ovulatory follicle grows and lets out an egg into the oviduct during the follicle stage (24). During the estrous phase, the endometrial fibrous tissue was shown to exhibit a typical structure. Decidualization was seen to move towards the anti-mesometrial edge that comprises uterine tissue, wherein implanted would take place, on the ninth day of pregnancy. Maternal blood vessels were dilated, the decidual area's vascularization increased, and the endometrial stroma's glands were dispersed and significantly smaller on the eleventh day of pregnancy. Collagen tissue, which is denser in myometrium, seen around the deciduous cells of the endometrium on the sixteenth day of the pregnancy, after primary decidual zone receded and secondary decidual barrier emerged. The myometrium also showed more thick elastic fibers. The myometrium, which envelops the endometrium, grows during pregnancy as a result of increased hyperplasia and smooth muscle enlargement (hypertrophy). In order to strengthen the uterus throughout pregnancy, myometrium comprises collagen and elastic fibers, particularly in loose connective tissues runs between the muscle bundles. The amount of collagen fiber and elastic fiber that was highly

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). https://doi.org/10.21070/ijhsm.v2i2.287

stained dropped on the eleventh day of gestation, whereas the density of the fibers grew once more on the seventh day of pregnancy. This led to a uterus to receive a 10th-day embryo showing both more elastic fibers and collagen for its insertion to an elastic solid uterine wall and more carbs for its nutrient demands and immunological privilege. As decidualization accelerated on the seventh, the myometrium's elastic and collagen fibers grew, whereas connective tissue elements decreased. The effect of the hormones produced during estrous, that modify the mechanical barrier affecting sperm movement and accelerate the pace of conception, was reflected in changes to cervical mucus characteristics. Morphogenetic events include the differentiation and proliferation of the myometrium and coordinated growth of the female reproductive organs are characteristics of postnatal uterine morphogenesis (19,20). During gestation, the stromal cells that surround the embryo undergo a process called decidualization, which is essential for implantation. During pregnancy, the uterus, an essential organ, alters morphologically and physiologically to promote the growth and development of the fetus and embryo A procedure of decidualization and a healthy uterus that can accept and nourish a fertilized embryo are essential for a successful pregnancy.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). <a href="https://doi.org/10.21070/ijhsm.v2i2.287">https://doi.org/10.21070/ijhsm.v2i2.287</a>



**Diagram (1):** level of the reproductive hormones, in mice, nmol/liter

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). <a href="https://doi.org/10.21070/ijhsm.v2i2.287">https://doi.org/10.21070/ijhsm.v2i2.287</a>

**Table, (1):** histological measurements of ovary and uterus, at different stages, μm

Measure Phase	Cortex of ovary	Medulla of ovary	Primary follicle	Secondar y follicle	Mature follicles	Myometri um of uterus
proestrus	620.5±1.5	25.7±0. 1 <sup>b</sup>	46.2±0. 2 <sup>c</sup>	75.7±0.2	674.3±2.4	302.6±0.5
Estrus	630.8±1.6 a	28.3±0. 8 <sup>b</sup>	50.5±0. 2 <sup>c</sup>	82.2±0.1	711.2±0.1	322.5±1.2
metestrus	634.7±0.6	32.3±0. 6 <sup>b</sup>	38.7±0. 6 <sup>c</sup>	79.4±0.6	873.7±0.5	332.4±3.5
diestrus	640.6±1.8	36.2±0. 3 <sup>b</sup>	35.2±0. 5 <sup>c</sup>	83.7±0.2	987.7±0.2	344.1±0.5
Pregnancy at 9 days	733.2±0.5	42.2±0. 5 <sup>b</sup>	28.2±0. 5°	33.2±0.5	Absent	684.3±1.3
Pregnancy at 20 days	753.2±0.5	45.2±0. 5 <sup>b</sup>	22.2±0. 5°	32.2±0.1	Absent	774.6±2.1

Values with the small letters denote to significant differences (P>0.01)

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY).

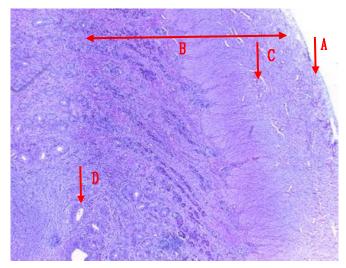


Fig. 1. section of the body of uterus in proestrus phase; A. endometrium, B. Vascular zone, C. blood vessels, D. Gland, H&E stain X100.

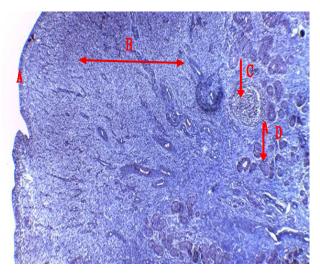


Fig.2. section of the horn of uterus in diestrus phase; A. endometrium, B. Vascular zone, C. blood vessels, D. Gland, PAS stain X100

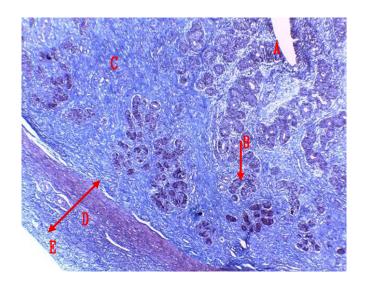


Fig.3. section of the body of uterus in Estrus cycle; A. epithelium, B. Gland, C. connective tissue, D. Myometrium, E. perimetrium, Masson trichrome stain X100.

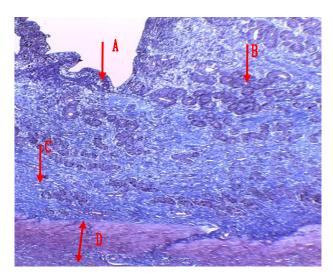


Fig.4. section of the body of uterus in pregnant at 9-day, A. endometrium, B. show increase in the uterine gland, C. Connective tissue, D. myometrium, Masson Trichrome X100

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY).

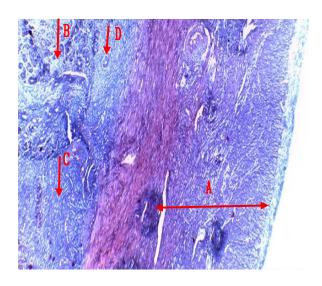


Fig.5. section of the body of uterus in pregnant at 20-day: A. show increase in the thickness of myometrium, B. glands, C. Connective tissue, D. blood vessels, Masson Trichrome X200.

Fig.6. section of ovary in pro-estrus phase: A. Epithelium, B. secondary follicle, C. cortex of ovary, D. medulla of ovary, AB stain X200.

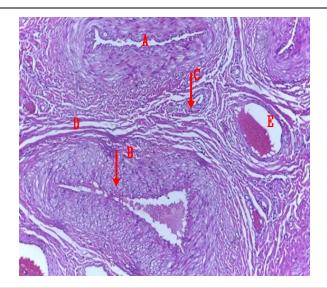


Fig.7. section of the ovary in estrus phase: A. secondary follicle, B. territory follicle, C. blood vessels, D. connective tissue, E. mature follicle, H&E stain X40.

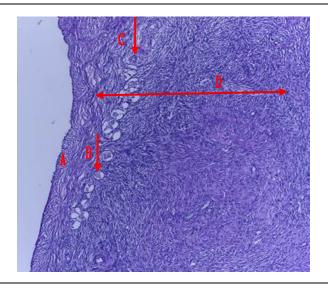


Fig.8. section of the ovary in pregnancy phase: A. Epithelium, B. secondary follicle, C. blood vessels, D. cortex of ovary, H&E stain X20.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY).

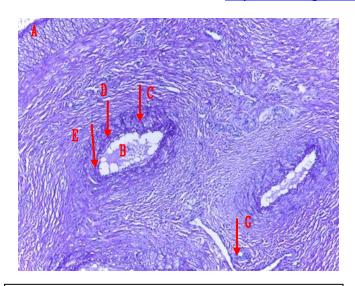


Fig.9. section of the ovary in metestrus phase, 1. A. Epithelium, B. Graffin follicle, C. Theca layer, D. granulosa layer, E. Zona pelucida, F. Antrum, G. Blood vessels, PAS stain X400.

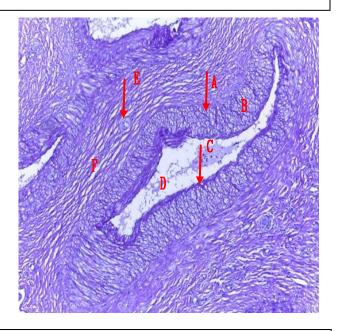


Fig.11. section of the ovary in diestrus phase, A. Theca layer, B. granulosa layer, C. Zona pelucida, D. Antrum, E. blood vessels, F. connective tissue, PAS stain X400.

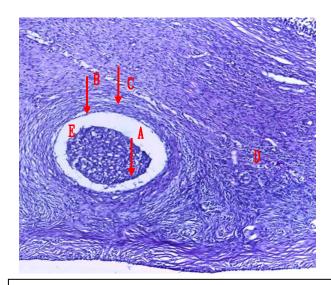


Fig.10. section of the ovary in estrus phase, A. Graffin follicle with oocyte, B. Zona pelucida, C. granulosa layer, D. connective tissue, E. Antrum, H&E stain X400.

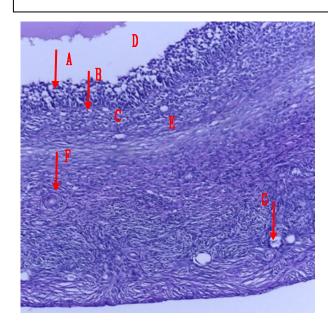


Fig.12. section of the ovary in estrus phase, A. Zona pellucida, B. granulosa layer, C. Theca layer, D. Antrum, E. Corna radita, F. blood vessels, G. Secondary follicle, AB stain X400.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). https://doi.org/10.21070/ijhsm.v2i2.287

#### **Conclusion**

Their methods of reproduction are demonstrated by the uterine and ovarian histology, which shows signs of elevated follicular activity. To aid in the formation of the conceptus, the uterus of a pregnant mouse changes dynamically throughout the gestational period. These findings could be useful for studying animal care and offer valuable new insights into the reproductive systems of common lab and domestic animals. Reproductive hormone profiles of the lab animals reveal a well-tuned system that is appropriate for induced ovulation. Research on rabbit hormone regulation is very beneficial to reproductive endocrinology, animal health care, and reproduction initiatives. The hormone patterns, including the roles of FSH, LH, as well as estrogen, and progesterone, among others, demonstrate how tightly controlled endocrine systems control fertilization in these animals. The findings also reveal distinct morphological and functional alterations associated with reproductive status. Large, fully grown Graafian follicles with a thin endometrium in the ovaries exhibit the highest levels of estrogen activity and fertilization readiness during estrus. In contrast, the ovaries have a strong corpora lutea, which is responsible for the continuous production of progesterone, and the uterus experiences considerable endometrial advancement, vascularization, and decidual changes to facilitate embryo implantation and fetal development during pregnancy. These results show how hormone regulation and tissue remodeling interact in a complex way to support mice's successful reproduction.

**Acknowledgment:** All of the volunteers who helped us complete and publish this research are acknowledged. We are grateful for their efforts.

**Conflict of interest**: According to the authors of the article, no conflicts related to interests arose during the drafting phase.

#### References

[1] S. Rahelinirina, K. Scobie, B. Ramasindrazana, V. Andrianaivoarimanana, F. Rasoamalala, L. N. Randriantseheno, J. S. Rakotoniaina, O. Gorge, X. Lambin, and E. Valade, "Rodent

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY).

- control to fight plague: Field assessment of methods based on rat density reduction," *Integrative Zoology*, vol. 16, pp. 868–885, 2021.
- [2] R. Patel, J. D. Moffatt, E. Mourmoura, L. Demaison, P. T. Seed, L. Poston, and R. M. Tribe, "Effect of reproductive ageing on pregnant mouse uterus and cervix," \*Journal of Physiology\*, vol. 595, no. 6, pp. 2065–2084, Mar. 2017, doi: 10.1113/JP273350.
- [3] J. L. Hornick, C. Van Eeneame, O. Van Gèrard, I. Dufrasne, and L. Isasse, "Mechanisms of reduced and compensatory growth," *Domestic Animal Endocrinology*, vol. 19, pp. 121– 132, 2000.
- [4] S. T. Hoy and M. Verga, "Welfare criteria in housing of rabbits," \*World Rabbit Science\*, vol. 16, pp. 111–120, 2008.
- [5] H. A. Khalil, W. H. Kishk, O. Essa, and M. M. Awad, "Evaluation of productive and physiological performance of Baladi Red compared to New Zealand White rabbits under the same managerial conditions," \*Egyptian Journal of Animal Production\*, vol. 51, pp. 200–209, 2014.
- [6] E. Sajadian Jaghargh, M. T. Vajed Ebrahimi, A. Attaranzadeh, A. Parham, and A. R. Mohammadnia, "Histopathological evaluation of the viability of auto-transplanted ovary following vitrification-thawing process in rabbit as an animal model," \*Veterinary Research Forum\*, vol. 15, no. 10, pp. 545–550, 2024.
- [7] D. F. Avilés, A. M. Martínez, V. Landi, and J. V. Delgado, "The guinea pig (Cavia porcellus): an Andean resource of interest as an agricultural food source," \*Animal Genetic Resources\*, vol. 55, pp. 87–91, 2014, doi: 10.1017/S2078633614000368.
- [8] F. Al-Saffar and H. Al-Ebbadi, "Histomorphological and histochemical study of the ovary and the uterine tubes of the adult guinea pigs (Cavia porcellus)," \*Egyptian Academic Journal of Biological Sciences, D. Histology & Histochemistry\*, vol. 11, no. 2, pp. 1–22, 2019, doi: 10.21608/eajbsd.2019.56024.
- [9] Y. Cui, Y. Yong, and Y. Sijiu, "Follicular morphology in yak in early pregnancy," in \*Proceedings of the International Congress on Yak (Session IV: Reproduction and Physiology)\*, Chengdu, Sichuan, P.R. China, 2004.
- [10] J. Griffin, R. E. Benjamin, I. Huang, C. M. Peterson, and T. C. Douglas, "Comparative analysis of follicle morphology and oocyte diameter in four mammalian species (mouse, hamster, pig, and human)," \*Journal of Experimental & Clinical Assisted Reproduction\*, vol. 3, no. 2, pp. 1–9, 2006.
- [11] H. D. Dellmann and J. A. Eurell, \*Textbook of Veterinary Histology\*, 5th ed. Philadelphia, PA: Awoters Kluwer Company, 1998, pp. 287–391.
- [12] B. Zoubida, M. Theau-Clément, G. Bolet, P. Brown, and R. Kaidi, "Behavior at birth and anatomo-histological changes studies of uteri and ovaries in the post-partum phase in rabbits," \*European Journal of Scientific Research\*, vol. 34, no. 4, pp. 474–484, 2009.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). https://doi.org/10.21070/ijhsm.v2i2.287

- [13] J. A. C. Eurell, \*Veterinary Histology\*. U.S.A.: Teton New Media, 2004, pp. 64–67.
- [14] M. R. Islam, M. A. M. Y. Khandoker, S. Afroz, M. G. M. Rahman, and R. I. Khan, "Qualitative and quantitative analysis of goat ovaries, follicles and oocytes in view of in vitro production of embryos," \*Journal of Zhejiang University Science B\*, vol. 8, no. 7, pp. 465–469, 2007.
- [15] G. Brecchia, A. Bonanno, G. Galeati, C. Federici, M. Maranesi, A. Godetti, M. Gerani, and C. Boiti, "Hormonal and metabolic adaptation to fasting: effects on the hypothalamic-pituitary-ovarian axis and reproductive performance in rabbit does," \*Domestic Animal Endocrinology\*, vol. 31, pp. 105–122, 2005.
- [16] R. Cardinali, A. Dal Bosco, C. Castellini, C. Boiti, and G. Brecchia, "Serum level of hormones and metabolites in pregnant rabbit does," \*Italian Journal of Animal Science\*, vol. 8, suppl. 2, pp. 778–780, 2009.
- [17] S. K. Suvarna, C. Layton, and J. D. Bancroft, \*Bancroft's Theory and Practice of Histological Techniques\*, 8th ed. Philadelphia, PA: Churchill Livingstone Elsevier, 2018, pp. 176–725.
- [18] I. Maraček, M. Krajnicakova, M. Kostecky, G. Greserova, and I. Valocky, "Tertiary follicular growth wave dynamics after oestrus synchronization and superovulation in ewes and goats," \*Acta Veterinaria Brno\*, vol. 71, pp. 481–486, 2002.
- [19] B. M. Jaśkowski, H. Bostedt, M. Gehrke, and J. M. Jaśkowski, "Ultrasound characteristics of the cavitary corpus luteum after oestrus synchronization in heifers in relation to the results of embryo transfer," \*Animals (Basel)\*, vol. 11, no. 6, p. 1706, 2021.
- [20] A. Kürüm, A. Özen, S. Karahan, and Z. Özcan, "Investigation of mast cell distribution in the ovine oviduct during oestral and luteal phases of the oestrous cycle," \*Kafkas Üniversitesi Veteriner Fakültesi Dergisi\*, vol. 20, pp. 9–15, 2014.
- [21] T. Krzymowski and S. Stefanczyk-Krzymowska, "Local retrograde and destination transfer of physiological regulators as an important regulatory system and its role: facts and hypothesis," \*Journal of Physiology and Pharmacology\*, vol. 63, no. 1, pp. 3–16, 2012.
- [22] A. V. Makarevich, A. V. Sirotkin, P. Chrenek, and J. Bulla, "Effect of epidermal growth factor (EGF) on steroid and cyclic nucleotide secretion, proliferation and ERK-related MAP-kinase in cultured rabbit granulosa cells," \*Experimental and Clinical Endocrinology & Diabetes\*, vol. 110, pp. 124–129, 2002.
- [23] P. Massanyi, J. Slamečka, N. Lukáč, and R. Jurčík, "Seasonal variations in the morphometric analysis of the ovary and uterus and in progesterone and 17β-oestradiol production in the brown hare (Lepus europaeus)," \*Journal of Animal and Feed Sciences\*, vol. 9, pp. 697–708, 2000.
- [24] Z. Szendrő and L. Maertens, "Maternal effects during pregnancy and lactation in rabbits," \*Acta Agraria Kaposváriensis\*, vol. 5, no. 2, pp. 1–21, 2001.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY). https://doi.org/10.21070/ijhsm.v2i2.287

- [25] H. A. Awojobi, G. O. Opiah, and G. Sotiminu, "The effect of physiological status on digestibility and some nutrient blood parameters of the New Zealand White doe rabbit," \*Journal of Agriculture, Forestry and the Social Sciences\*, vol. 2, no. 2, pp. 95–103, 2004.
- [26] N. Çetin, T. Bekyürek, and E. Çetin, "Effects of sex, pregnancy and season on some haematological and biochemical blood values in Angora rabbits," \*Scandinavian Journal of Laboratory Animal Science\*, vol. 36, no. 2, pp. 155–162, 2009.
- [27] U. G. Atila, H. Uslu, and Y. Adali, "Hepatoprotective and nephroprotective effects of \*Trigonella foenum-graecum\* L. (fenugreek) seed extract against sodium nitrite toxicity in rats," \*Biomedical Research and Therapy\*, vol. 6, no. 5, pp. 3142–3150, 2019.
- [28] A. K. Allaw, A. K. Belqees, Z. Yousof, I. Al-Ani, M. Bracamonte, and A. Sadeeq, "Is there any effect of fenugreek seeds aqueous extract (FSA) on the quantity of ovarian follicles and estrus cycle of female rats," \*Journal of Medicinal Plants Research (JMPR)\*, vol. 1, no. 1, pp. 55–63, 2016.
- [29] A. J. Al-Yasery, I. M. Khaleel, and M. H. Al-Aameli, "Histological and hematobiochemical study of fenugreek (\*Trigonella foenum\*) and ginger (\*Zingiber officinale\*) aqueous extracts on ovary structure in white Swiss mice (\*Mus musculus\*)," \*Life Science Archives (LSA)\*, vol. 3, no. 2, pp. 962–973, 2017.
- [30] A. A. Khaleel and K. R. Hamad, "Effects of fenugreek (\*Trigonella foenum-graecum\*) seeds aqueous extract on the tissue structure of the anterior pituitary gland and some organs of the reproductive system in male albino rats," \*Journal of Pharmaceutical and Allied Sciences (JOPAS)\*, vol. 3, no. 3, pp. 102–115, 2011.
- [31] S. Esson, L. Ric, O. Ekin, and I. Krush, "Effect of fenugreek (\*Trigonella foenum\*) on gonad development and body vital values in mice," \*Indian Journal of Comparative Histology (IJCH)\*, vol. 64, no. 4, pp. 387–395, 2005.
- [32] K. A. Suad, J. S. Al-Shamire, and A. A. Dhyaa, "Histological and biochemical evaluation of supplementing broiler diet with β-hydroxy-methyl butyrate calcium (β-HMB-Ca)," \*Iranian Journal of Veterinary Research (IJVR)\*, vol. 19, no. 1, p. 27, 2018.
- [33] T. Solomon, Z. Largesse, A. Mekbeb, M. Eyasu, and D. Asfaw, "Effect of \*Rumex steudelii\* methanolic root extract on ovarian folliculogenesis and uterine histology in female albino rats," \*African Journal of Laboratory Medicine (AJOL)\*, vol. 10, no. 4, pp. 353–361, 2010.