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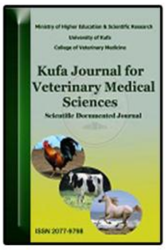

Histological study of ovary through last periods(Oryctolagus cuniculus) of pregnancy in domestic rabbit

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Abstract:

The aim of this work was to study the morphology of the ovaries of rabbit at last peroid pregnancy in order to understand the effect of pregnancy on the development of follicles. Twenty local breed pregnant does in estimated ages, five does prepared for every stage beginning from 22, 24, 26 and 30 days by which gestation occurred. Their ovaries were removed, fixed in 10% of formalin and routinely processed of histotechnique for histological studies. The observations of histological examination shown that the cortex was occupied with different types and various sizes of follicles, Corpora lutea were also observed in ovary at each stage of present study. These results show, and according statistical analyses of the numbers of primordial and primary follicles become decrease with progressive of pregnancy. The diameter of primary follicles can be different according to the average diameter of the oocyte. The results showed that decrease rate of secondary follicles formation through all pregnancy stages of present work compare with the rate of primary follicles. The ratio of follicles growth to atresia folliclea in pregnancy is lower than that of the female at cycle stages. The morphometerical result showed that the increase in rate of the tertiary follicles in last day of gestation.

Key words: Follicle, histology, ovarian, rabbit, pregnance.

دراسة نسيجية للمبيض خلال المراحل الأخيرة من الحمل في الأرانب المحلية

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الخلاصة:

هدف هذا العمل دراسة التطور الشكلي لمبايض الأرانب في المراحل الأخيرة من الحمل ولمعرفه تأثير الحمل علي التطور الجريبي. عشرون من إناث السلالة المحلية، خمسة إناث لكل مرحلة من مراحل الحمل (22يوم ، 24يوم ، 26 يوم ، 30 يوم). استخرجت المبايض وتم تثبيتها بالفورمالين ثم استخدمت طرق التقنية النسيجية المحددة للدراسة النسيجية. اضهرت مشاهدات الفحص النسيجي إن قشرة المبيض تحتوى على جريبات ذات أشكال وإحجام مختلفة، وكذلك وجود الجسم الأصفر في مبيض كل مرحلة من مراحل التجربة. أظهرت النتائج، وبالاعتماد علي التحليلات الإحصائية أن أعداد الجريبات البدائية والجريبات الأولية تبدأ بالتناقص مع تقدم الحمل. إن أقطار الجريبات البدائية يختلف طبقا إلى معدل قطر خلية البيضة. أظهرت النتائج انخفاض في معدل تكوين الجريبات الثانوية مقارنة بمعدل الجريبات الأولية خلال كل المراحل التي تضمنتها هذه الدراسة. إن نسبة نمو الجر يبات إلى الرتق الجريبي خلال فترة الحمل منخفضة مقارنة بمراحل الدورة. أظهرت نتائج العد الشكلي زيادة معدل الجريبات من المرحلة الثالثة في اليوم الأخير من فترة الحمل.

Introduction:

Mammalian species are classified as spontaneous or induced ovulators (1). Spontaneous ovulatos (e.g., cattle, pigs, sheep, humans) display a cyclical increase of estradiol which triggers Gonadotropin releasing hormone (GnRH) release from the hypothalamus, Luteinizing hormone (LH) release from the pituitary, and ultimately ovulation regardless of mating activity (2, 3, 4). Induced ovulators (e.g., rabbits, camelids, cats) however, require stimulation associated with mating to provoke GnRH release and then the surge of LH which is necessary to elicit ovulation (5).

Female rabbits are classified as induced or reflex ovulators because ovulation takes place after mating (6, 7, 8, 9) hence, rabbits do not have a regular estrous cycle (9) as in other domestic species (e.g., cattle). Rabbits display periods of sexual receptivity and non receptivity. Investigators have reported that the period of receptivity lasted about 5 to 6 days (10, 11) or 7 to 10 days (9) in the absence of mating.

Sexual behavior in female rabbits has been studied during pregnancy, pseudopregnancy, and post partum. Some pregnant rabbits accepted mating, but release of gonadotropins and ovulation did not occur (12). Sexual behavior in pregnant rabbits was markedly reduced compared to that in non pregnant rabbits and ended by the final third of pregnancy (13, 14).

The ovaries of rabbits are small, flattened ovoid organs, lying in the right and left lateral pelvic cavity. The surface of rabbit ovary is covered by a single layer of epithelium. A substantial basement membrane (tunica albuginea) separates the surface cells from the underlying ovarian tissue divided into the inner medulla and outer cortex, which consists of follicles and stroma. Studies of the total follicular populations in animals of high and low ovulation rate have shown that there are more follicles at each stage throughout the entire growth phase with a higher ovulation rate (15, 16). However, until this day very few data are available on the study of on the ovarian morphology of the pregnant does has been reported notably the Algerian local rabbit. Recently,(Zoubida et al) studied anatomo-histological changes studies of uteri and ovaries in the post partum phase in rabbits. The scope of the present study was to determine the morphology of the ovaries of rabbit in last pregnancy in order to understand the effect of pregnancy on the development of follicles and to provide the basic knowledge to assist reproductive issues (17).

Materials and Methods:

Twenty local breed pregnant does in estimated ages, five does prepared for every stage beginning from 22, 24, 26 and 30 days by which gestation occurred. The animals were housed individually in cages, fed, watered and received a daily illumination of 16 hours of light. The ovaries were removed from the tract and weighed separately. The ovaries were then fixed in 10% formalin and routinely processed and embedded in paraffin wax for histology, sections are stained by the hematoxylin and eosin (H&E) and periodic acid- Schiff stain (PAS) procedure. The presence of different stages of follicular development, and their relative frequency was assessed by counting them in a series of sections of each ovary(18).

Morphometric Analysis

To counting of ovarian follicles, and according to Pedersen's follicular classification in rodents (19), follicles were divided into 3 main categories; small, (Primordial and Primary) medium (Preantral) and large (Antral or Graffian) follicles respectively.

Results and Discussion:

The ovaries were covered with epithelium, usually consisted of simple cubiodal tissue (figure 1). The cortex was occupied with different types and various sizes of follicles. Corpora lutea were also observed (figure 2). In the central zone of ovarian stroma, the medulla appeared vascularised. Spindle shaped stromal cells were mostly fibroblasts and also included bundles of smooth muscle cells (figure 3). This finding is agree with observation of (Radwan) who described the histological structures in mouse ovary(20).

In each stage of this study, the histological results show at periphery of the cortex, numerous primordial follicles were observed (Figure 4). Each one contained a primary oocyte, surrounded by two or three flattened follicular cells (figure 5). These primordial follicles tend to be evenly distributed throughout the outer cortex. Some intermediary follicles presented both flattened and cuboidal follicular cells were observed. The analogous to this result has been described in rabbit (17) and in the ovary of non pregnant does (21) in herbivores, primordial follicles were regularly distributed through the outer cortex, by comparison, in carnivores the follicles are more clustered.

Primary follicles were also seen. Here, the oocytes were now surrounded with a thin zona pellucida, and a granulosa constituted as a single layer of cuboidal cells (figure 6). The diameter of primary follicles can be different according to the average diameter of the oocyte. These observations indicated that the oocyte enlarged in the largest primary follicles, started the maturation and the growth process. Generally, this result of primary follicular formation is the same in the other lab animals like rat (22), whereas (21) mentioned that the primary oocyte grows large, resulting in multiple Golgi complex and further proliferation of mitochondria, and during this stage of development the oocyte will increase its size by two or more time. These results show, and according statistical analyses (Table 1), the numbers of primordial and primary follicles become decrees with progressive of pregnancy. (23) similarly noted that a progressive decline in the number of follicles in the rabbit ovary that would respond to an LH:FSH combination as pregnancy continued, possibly due to a loss of gonadotropin - receptor activity. However, It is probable that the smaller sized follicles, which accumulate as pregnancy continues, secrete sufficient estrogen to maintain luteal steroidogenic function (24).

Through the histological examination by hematoxylin and eosin staining section of ovary, secondary follicle or preantral follicle was also visible in all stages and these follicles were centered by the oocyte. This oocyte contained a central nucleus. The size of the oocyte was constant whatever the preantral follicle. In the small ones, the granulosa was 2-3 cells thick, the zona pellucida appeared clearly stained with periodic acid-Schiff (figure 7). The preantral follicles were characterized by an enlarged granulosa. The proliferating follicular cells expressed an intense mitotic activity. The granulosa cells appeared as an homogeneous. As the preantral follicle grew, the division of ganulosa cell continued and the number of layers increased to form a stratified epithelium, 2 to 10-12 layer cells thick (figure 8). This histological results which described the formation of secondary follicles though different stage of pregnant does is similar to the finding of (24) in albino rat and (21) in rabbit and canian. According to statistical analysis, which show that decrease rat of secondary follicles formation through all stage of present research (20, 24, 26 and 30) days of pregnancy compare with the primary follicles (Table 1). This observation agree with (25) they described in beef cow and buffaloes. The ratio of follicles growth to atresia folliclea in pregnancy is lower than that of the female at cycle stages. This indicated that the transformation of follicles in pregnancy process is low.

The growing follicles were usually observed in deep cortex of the ovary. The largest preantral follicles developed and its size increased by a proliferation of cells and the accumulation of fluid to form the antrum, a single cavity to form the tertiary follicle or antral follicle (figure 9). Some granulosa cells surrounding the oocyte appeared closely associated between them, constituting the cumulus oophorus, the compact cumulus cell-oocyte complex. The peripheral granulosa layer cells, observed near the basement membrane, formed the mural granulosa cells (Figure 10). This result, histologically is similar to progress of prenatal follicles to tertial follicales through follicular development in most rodent (26). Theses follicles don’t have periodic acid- Schiff (PAS) – positive bodies (Call- Exner bodies). This finding was agree with (21), who mentioned that the granuolsa cells of tertiary follicles can contain Call- Exner bodies in the ewe and cow.

In *Oryctolagus cuniculus*, the large healthy antral follicles developed from the maturing follicles to form preovulatory follicles. These preovulatory follicles were characterized by these oocytes occupied the centre of the follicle but the germinal vesicle became eccentrics in position. The granulosa cells surrounding the oocyte, formed a ring, the corona radiata. The oocyte was embedded in the cumulus area (Figure 11) that became limited by the enlarging antrum.

The morphometerical result show that the increase in the tertial follicles in last day of gestation (3.25 ± 0.62) (Table 1). However, the majority of follicles present in the ovary are small or atretic during periods of elevated progesterone throught luteal phase and pregnancy. (27) likewise reported in rat the reappearance of large follicles apparently associated with progesterone levels dropped in late pregnancy. The rat and rabbit rely on their corpora lutea (CL) as the major source of progesterone throughout gestation. (28). In the rabbit, CL mass increases to about day 16-20 of gestation but then declines gradually to day 28,term is day 32 (29).

In rabbits ovary, the atresia was observed at all stages of follicle development. It seemed that the atresia of primary and secondary follicles progressed very fast, the atretic follicles characterized by hypertrophied granulose cells and the atretic follicule appeare under light microscope with glassy membrane (figure 12). The follicular atresia in does during pregnancy is similar with that reported in by (30), The atresia of different types of follicles in yak might begin at any stage of follicular development and might follow any one of several different patterns.

On other hand, the fact that development of tertiary ovarian follicles is dependent upon primarily the availability of proper concentrations of FSH and LH, and secondly that the estrogen produced by the follicles is well accepted. Failure of growth of the follicle may result from insufficient FSH, LH or estrogen, and atresia may be brought about not only by insufficiency of these hormones, but also by excess LH, estrogen or progesterone. In summary, the pregnancy prevents the development of follicles to Graafian follicles and improves their atresia. Thus, the growth of follicles is restrained and the function of corpura luteum maintained.

Table 1: Follicular number counts in studied groups.

Day of gestation \ Follicles	Primordial follicles	Primary Follicles	Secondary follicles	Tertial follicles
20	65.00 ± 1.77 A	12.25 ± 0.62 A	3.75± 0.47 A	3.25 ± 0.62 A
24	56.50 ± 1.32 B	17.00 ± 0.40 B	3.50± 0.28 A	2.50 ± 0.28 A
26	51.50 ± 1.70 C	14.75 ± 0.62 C	4.00± 0.40 A	3.75 ± 0.47 A
30	32.25 ± 1.31 D	9.50 ± 0.64 D	5.75± 0.47 B	8.00 ± 0.40 B

Values represent mean ±S.E. Different capital letters mean significant differences at ($P<0.05$) results between different periods.

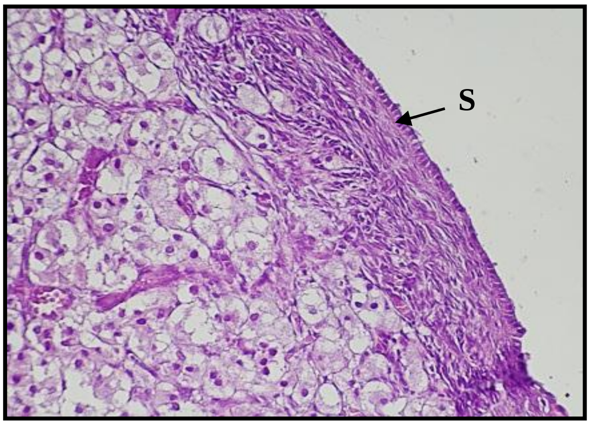


Figure (1): Showing the ovary was covered with simple cubiodal epithelial tissue (S). (H&E stain, X40).

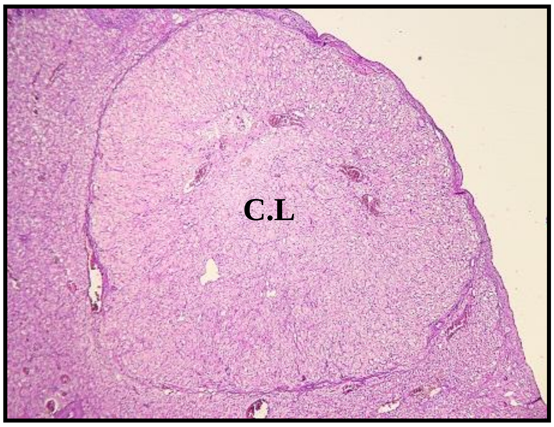
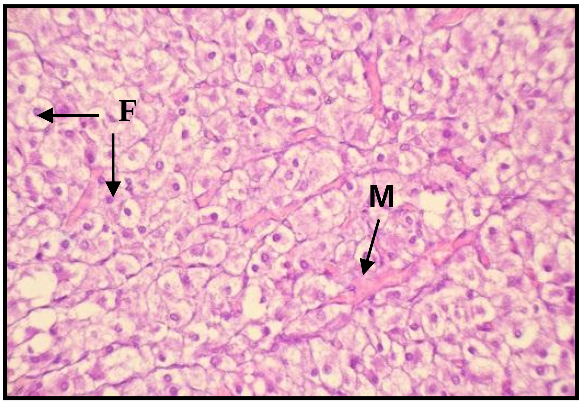


Figure (2): The Corpus luteum(C.L) of the ovary at 20 day of gestation. (H&E stain, X4).



Figure(3): Ovarian section showing stromal fibroblasts (F) and smooth muscle cells (m). (H&E X40).

Figure (4): Ovarian section showing the primordial follicles (P) in the cortex of vary. (H&E X40).

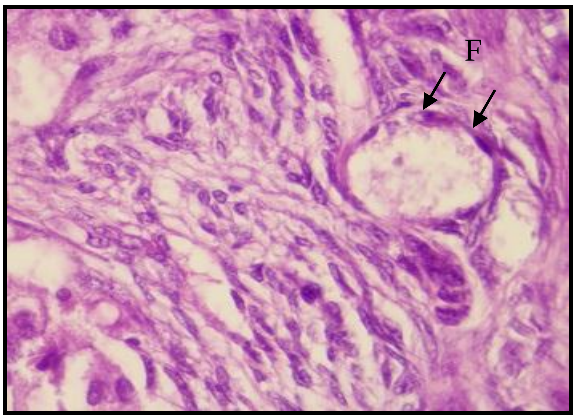


Figure (5): Ovarian section showing the primordial follicles were surrounded by flattened cells (F) (H&E stain, X100).

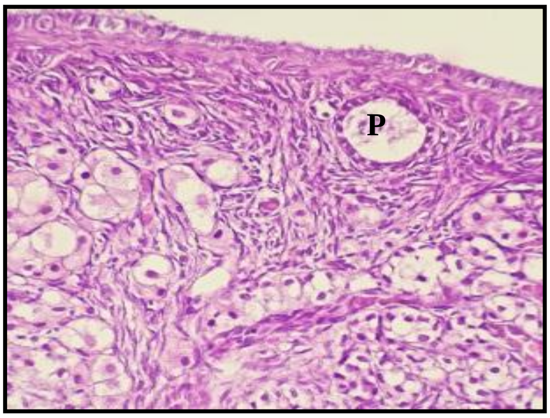


Figure (6): Ovarian section showing primary follicle (P), granulose as a single layer of cuboidal cells. (H&E stain, X40).

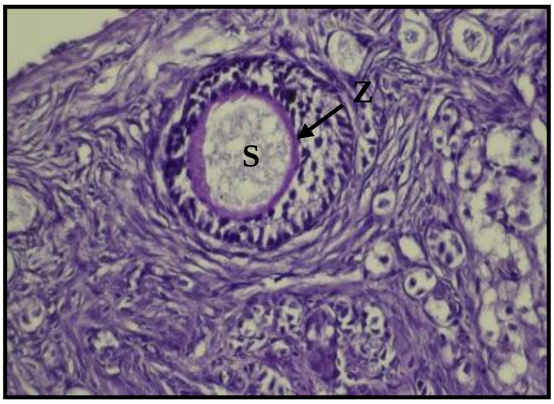


Figure (7): Ovarian section showing secondary follicle(S), the zona pellucida (Z) appeared clearly.(PAS stained with X40).

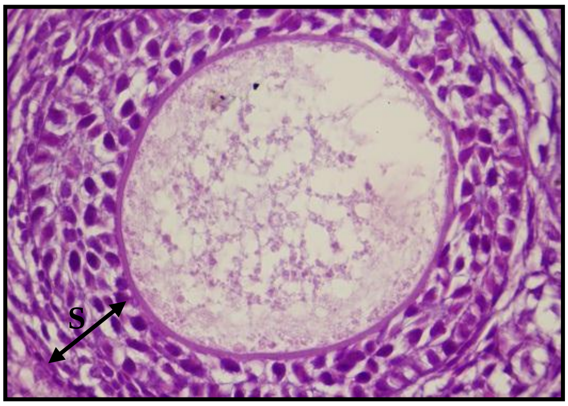


Figure (8): Ovarian section showing preantral follicle, the number of granulosa layers form a stratified epithelium(S), (H&E stain, X40).

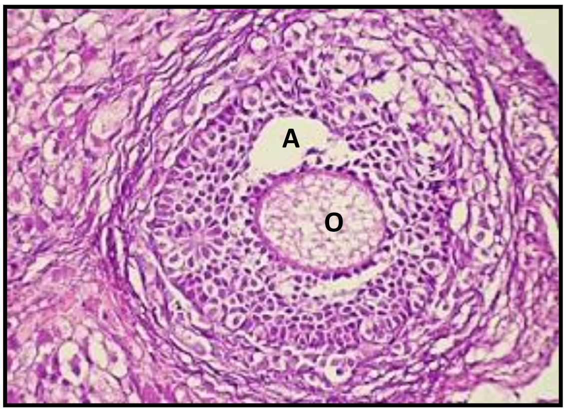


Figure (9): Ovarian section showing the antrum (A),a single cavity and oocyte (O) of the tertiary follicle (H&E stain, X 40).

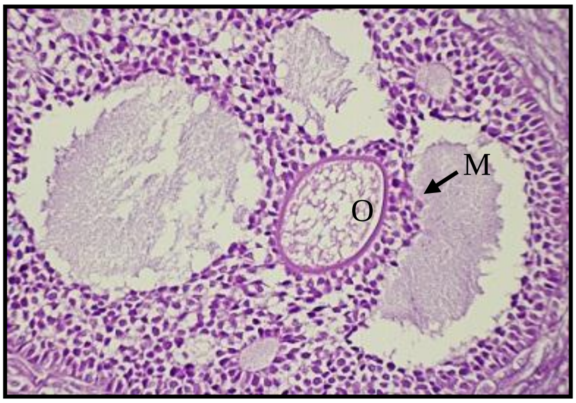


Figure (10): Ovarian section showing mural granulosa cells (M), surrounding the oocyte (O) in the tertiary follicle (H&E stain, X 40).

Figure (11): Ovarian section showing corona radiata surrounding the oocyte, in the tertiary follicle (H&E stain, X 100).

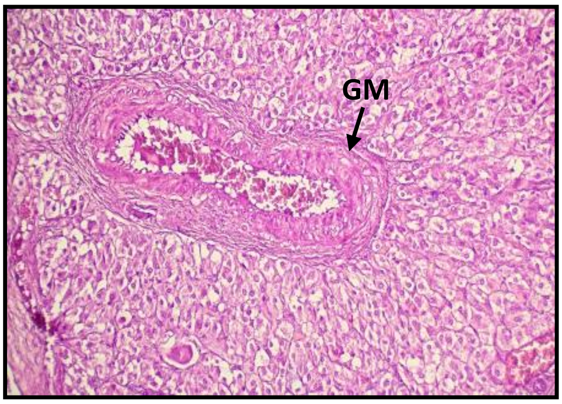


Figure (12): Ovarian section showing atretic follicle with glassy membrane (GM) (H&E stain, X 20).

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