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Microscopical Structure of The Corpus Luteum of Rabbit During Postpartum

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Abstract

In rabbit, The corpora lutea of pregnancy persist for some time during the postpartum period. The morphological characteristics of growth and regression of rabbit corpora lutea were investigated by light and scanning electron microscopy during the postpartum. The corpus luteum (CL) of rabbit ovary was greatly decreased in size at 3-10 days postpartum. This change was accompanied by a decrease in the size of the lutein cells. As these cells shrink in size, a gradual invasion of collagen fibers and fibroblasts appear in the central cavity of the CL. Also during structural regression the mean number of large lutein cells was markedly and significantly decreased, while the mean number of the interstitial gland cells was significantly increased. Macrophages and other connective tissue cells were not observed during regression indicating that these cells in the rabbit, unlike other species, may not play an important role in luteolysis as apoptosis do. The survival of the corpora lutea being apparently related to the duration of lactation and the frequency of suckling as it was suggested by some authors.

Keywords: Doe, Ovary, Corpus luteum, Pregnancy, Post-partum, Luteolysis, Luteinization

Introduction

Ovarian components play an important role in the regulation of the reproductive process. In some primates, the presence of the active corpus luteum (CL) inhibits folliculogenesis **(Koering, 1974a; Baird et al., 1975)**, while in the rabbit, estradiol secreted by the follicles is essential for CL maintenance **(Keyes and Nalbandov, 1967; Holt et al., 1975)**. When progesterone levels decrease during luteolysis in rabbit, ovulation can occur **(Hammond and Marshall, 1925)**. Although degeneration of the rabbit CL takes place following parturition and can be induced experimentally by either estrogen deprivation **(Miller and Keyes, 1975)** or by prostaglandin **$pGF2_\alpha$** administration **(Koering and Kirton, 1973; Keyes and Bullock, 1974)**. The morphological persistence of the CL beyond functional activity may be relevant to a better understanding of the relationship of the CL to the development of both follicles and interstitial gland tissue which is abundant in the mature rabbit ovary **(Koering and Kirton, 1973 and Mori and Matsumoto, 1973)**. Another aspect to be considered in the luteolytic process is the role of the macrophages and vasculature. This can be examined by use of a morphological tracer such as colloidal carbon. This method had previously been employed to locate phagocytic cells in various organs **(Wislocki, 1924)**. **Moreover,** it help the interpretation of vascular morphology as it appears normally **(Morris and Sass, 1966)**.

This study aimed to describe the micro-morphological characters of the CL of the rabbit ovary during the postpartum period.

Materials and Methods

Seven New Zealand White adult healthy female rabbits (6-9/phase) were the source of CL. They aged 14 to 20 weeks and weighing 2.2 to 3.5 Kg. and were obtained from a local farm (Physiology lab) of Faculty of Veterinary Medicine - Cairo University. Rabbits were individually housed in separate cages with daily illumination of 16 hours of light and allowed free access to water and rations.

The day of mating of the does with the fertile bucks was called 0 Day of gestation. Animals were sacrificed by an over dose of pentobarbital sodium (100 mg/ Kg body weight). The ovaries were removed from 3 does at the 3rd and 10th days postpartum and were prepared for:

Light microscopy (LM): Ovaries were fixed in Bouin's fluid, dehydrated in ethanol, embedded in paraffin, sectioned and stained with H&E, Toluidine blue,

Osmium tetroxide as outlined by Bancroft and Stevens (1990).

Scanning Electron Microscopy (SEM): Ovarian samples were fixed in 1.25% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.2), dehydrated in ethanol. Then critical point dried from carbon dioxide, attached to stubs with colloidal carbon, and coated with gold palladium in a sputtering device. Specimens were examined and photographed with Joel SEM operating at 25 KV at Al-Azhar University.

Results

At the end of pregnancy, the corpus luteum remained slightly active. Following parturition, pronounced sings of retrogression of the corpora lutea were recognized. The lutein cells, in lactating animals, were smaller than before parturition and had fewer lipid inclusions.

At **the 3rd day** postpartum clear signs of regression could be seen in the corpus luteum represented by its marked decrease in size. It was surrounded by thin fairly distinct highly vascularized fibrous capsule (Fig.1). Many lutein cells were healthy and appeared large polyhedral in shape (Fig.2). Their nuclei were spherical, eccentric with obvious nucleolus. Numerous vacuoles and relatively few small osmiophilic lipid droplets were recognized in the cytoplasm (Fig. 3). Some of these vacuoles, in a few examined sections, were joined to form large empty ones of different sizes (Fig.3). The coalescence of these vacuoles resulted in the appearance of large irregular vacuoles in the cytoplasm of the lutein cells (Fig.4). However, most of the remaining lutein cells were markedly decrease in the size (Fig.5). They exhibited signs of degeneration indicated by pyknotic deeply stained nuclei and ill-distinct cell boundaries. Their cytoplasm appeared condensed and more vacuolated. The pericapillary spaces in between the lutein cells were less obvious and appeared narrow Their diameter was significantly decreased. Signs of apoptosis were demonstrated in the regressing CL. In addition, the density of the connective tissue between the lutein cells was markedly increased, particularly in the central area of the CL. This CT was represented by newly formed fibrous tissue with fine vascular spaces, particularly lymphatics (Fig.6).

By the **10th day** postpartum, signs of structural regression or luteolysis of the CL were noticed. The size of the CL was significantly decreased than before. The regressing CL was observed as a small faint cellular mass embedded in the ovarian cortex (Fig.7). It appeared as irregular, ill-demarcated pale staining area. Many parts of the CL, particularly at the periphery, were completely disappeared and replaced by the interstitial gland cells and large blood vessels. The cytoplasm of the regressed lutein cells appeared faint, more vacuolated than before due to the increase of lipid droplets (Fig.8). Some of these lipid droplets were coalesced with each other to form large lipid droplets or vacuoles (Fig.9). At higher magnification the cytoplasm of the lutein cells possessed relatively few faintly stained osmiophilic lipid droplets (Fig. 10). Most of the nuclei showed deformities and signs of karyolysis. The nuclear membrane was lost in most of the cells.

The pericapillary spaces, permeating the lutein cells were enlarged and became more prominent than before (Fig.11).

The interstitial gland cells, lying adjacent to the CL, were easily distinguished from the luteal cells of the regressing CL by their smaller size (Fig.12) and uniform-sized lipid droplets. They were usually separated from the CL by a less distinct connective tissue boundary. As the CL regressed, this fibrous boundary usually disappeared, allowing the luteal cells to intermingle with the interstitial gland cells. Most of the apoptotic lutein cells disappeared and were replaced by newly formed network of collagen infiltrated with fibroblasts particularly in the central region of the CL (Fig.13). A gradual invasion of the fibrous tissue occurred with masses of collagen fibres and fibroblasts. As regression advances the intercellular collagen increases (Fig.14,15&16). No macrophage cells could be found. Some parts of the same CL presented a more fibrosed stroma indicating an advanced stage of involution. This indicated that the involution of the

CL and its transformation into a hyalinized corpus albican was a gradual process that begun at the center of the CL from which it extended to the other parts. However, in the examined ovaries the corpora lutea disappear without leaving any scars.

Discussion

In many animals (e.g. primate, cow, ewe, hamster, mouse) the corpora lutea of pregnancy persist for some time during the postpartum period. According to **Wagner and Hansel, (1969)** the survival of the corpora lutea being apparently related to the duration of lactation and the frequency of suckling.

In the lactating rhesus monkey the corpus luteum of pregnancy may still be present postpartum **(Weiss et al., 1973)**. In lactating animals the lutein cells are smaller than before parturition and had fewer lipid inclusions.

The corpora lutea of pregnancy survive for a variable length of time during the postpartum period in mice and rats **(Baird et al., 1975)** and in cow and sheep ovaries **(Wagner and Hansel, 1969)**.

In the non-lactating primate, ewe and cow **Wagner and Hansel, (1969)** described that the CL of pregnancy has regressed by the 30th postpartum day.

They suggested that the suckling stimulus is an important factor in maintaining the corpus luteum after parturition.

The main cell in luteal tissue, the granulosa lutein cell, is a large cell with acidophilic cytoplasm, a vesicular nucleus and a prominent nucleolus. In this study only the granulosa derived lutein cell can be distinguished in the rabbit. Similar finding was described in rat **(Enders and Lyons, 1964)**.

The transformation of the granulosa cells into lutein cells, in the rabbit, is accomplished by hypertrophy of the cell, by the accumulation of lipids and by changes of the cytoplasmatic organelles. The cell size increases, but an increase in the cell number does not usually take place.

In other species, a second smaller lutein cell type arising from the theca (theca lutein cells) becomes part of the CL **(Koering, 1969; Mossman and Duke, 1973)**. In the primate the theca lutein cells become a prominent part of the corpus luteum **(Corner Jr., 1956; Baird et al., 1975)**.

The lutein cells develop abundant sER **(Bjersing, 1967; Koering, 1969)**. pleomorphic mitochondria with tubular cristae, well-developed Golgi complex and abundant lipid droplets **(Adams and Hertig, 1969a,b; Koering et al., 1973a,b)**. These findings lead to the suggestion that the lutein cells may act as storage sites for cholesterol and cholesterol esters as it was mentioned by **Fienberg and Cohen (1966)** and for progesterone **(Koering et al., 1973a,b)**.

In the present study, cells similar to the interstitial gland cells are scattered within the regressing CL. In some areas a distinction between the regressing luteal cells and the interstitial gland cells is practically impossible to detect under the light microscope. This suggests that either: Luteal cells are converting to interstitial gland cells or certain cells are differentiating into interstitial gland cells, or the interstitial gland cells are migrating into the regressing CL. Further support can be given to each of these 3 possibilities **(Saunders, 1966)** or because they may be capable of reversible differentiation **(Gurdon and Woodland, 1970)**.

In many species the CL is composed of cells derived not only from granulosa cells but also from cells located in the theca interna **(Mossman and Duke, 1973)**. A finding has not been observed in the rabbit. However, the theca cells could be the ones that now resemble the interstitial gland cells but were not seen previously as they were in an undifferentiated state. This is further supported by data which revealed that much of the interstitial gland tissue in the rabbit is of theca interna origin **(Davies and Broadus, 1968; Mori and Matsumoto, 1973)**. Another possible explanation for the presence of interstitial gland-like cells is that these cells move into the regressing CL as the connective tissue boundary disappears and will eventually replace the original CL **(Mossman and Duke, 1973)**. **Koering (1974b)** described that the luteolytic process in the rabbit is more gradual and complex process.

Signs of regression in the cycling animal start to appear post-ovulation soon after

the corpus luteum has formed in rhesus monkey (**Koering, 1969**), in human (**Corner Jr., 1956**) and in sheep (**Deane et al., 1966**). The lutein cells shrink in size show clumping of chromatin, karyorhexis and vacuolization of the cytoplasm becomes marked. Moreover, large lipid filled vacuoles, which eventually occupy most of the cytoplasm (**Lennep and Madden, 1965**).

In agreement with **Cohen et al., (1977)** a gradual invasion of connective tissue occurs with masses of collagen fibres and fibroblasts. As regression advances the intercellular collagen increases causing the corpora lutea to disappear without leaving any scars as in rat, mice and hamster.

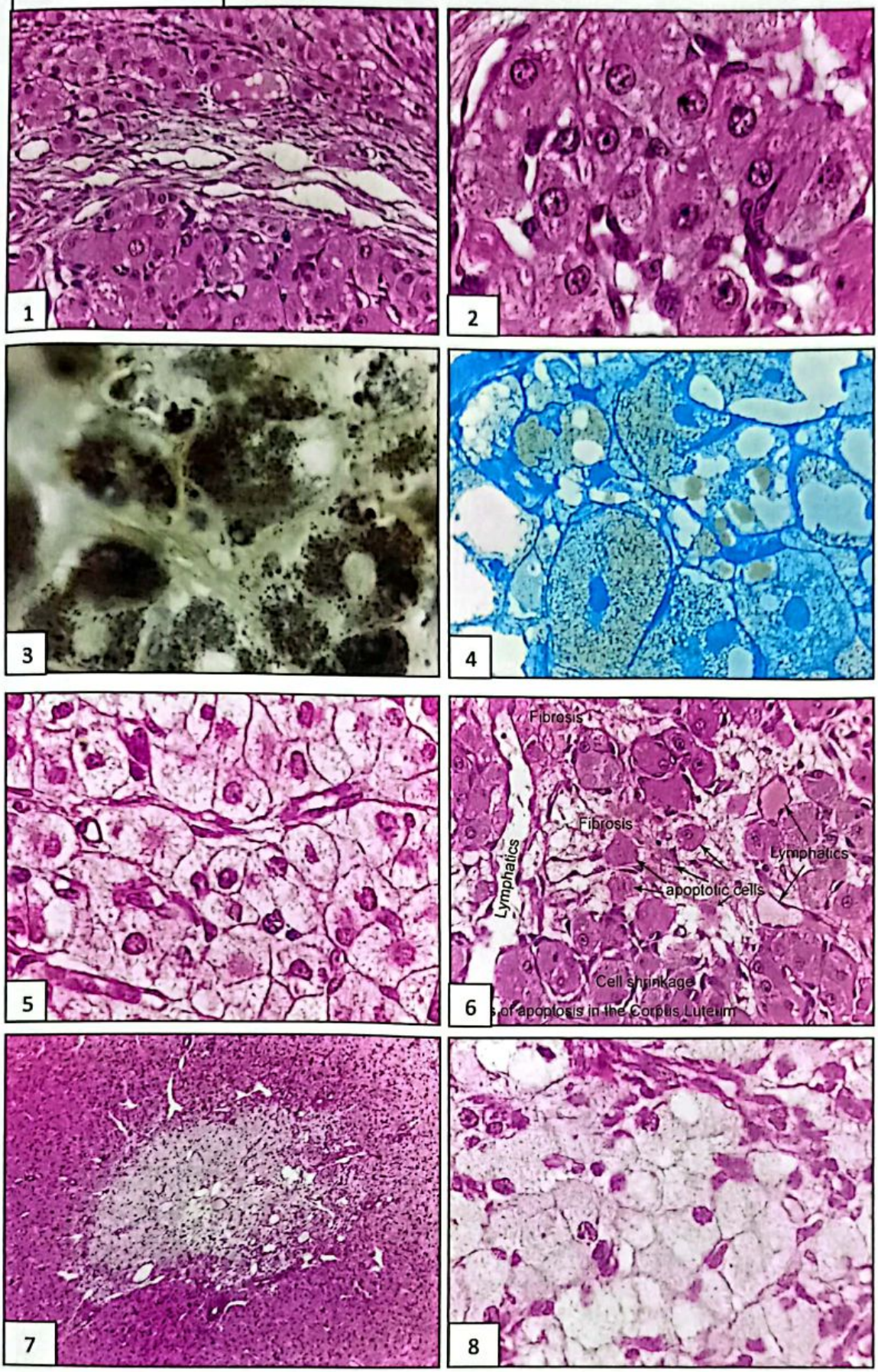
Some CL maintains their separation from the adjacent interstitial gland tissue by a connective tissue band and large blood vessels. The breakdown of this band is likely due to collagenase which can be formed by the mesenchymal cells located in the area (**Gross, 1974**).

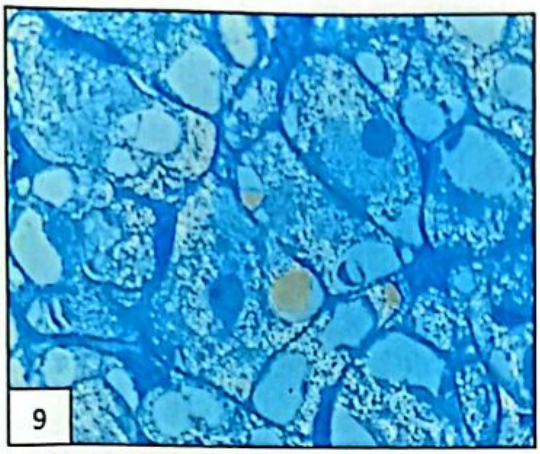
Fibroblasts appear in the central cavity of the CL as the large lutein cells begin to shrink and become increasingly vacuolated. Similar finding was described by (**Han et al., 1977**). Invasion of connective tissue increases during the lactation phase and elimination of the cavity by fibroblasts and connective tissue is usually accomplished by the end of lactation.

Macrophages are known to take an active part in the atrophy of various tissues (**Helm inen, 1975**) including such involuting organs as the mammary gland (**Helminen and Ericsson, 1968**) and uterine endometrium (**Padykula and Campbell, 1976**). Macrophages engulfed extracellular materials as regression progressed **Padykula and Taylor, 1976**). Macrophages have also been seen during luteolysis in the rat (**Dott, 1969**), but were not identified during the stages of regression in the rabbit as also mentioned by (**Koering, 1974b**). In order to investigate the possible involvement of macrophages in luteolysis, **Koering and Thor (1978)** used carbon particles as a tracer to assist in the location of these cells. In the present study neither macrophages nor other cells resembling macrophages were identified in rabbit. This is contrary to what was previously reported in guinea pig where macrophages were abundant during luteolysis suggesting that a species difference may exist (**Paavola, 1977**). However, during CL degeneration in the rabbit, cellular activity has been observed. This activity is attributed to the presence of fibroblast-like cells and endothelial cells (**Han et al., 1977**). It is quite possible that some of that activity is due to the presence of the interstitial gland-like cells seen in the present study.

The intercellular gaps which had been described in the vasculature of the rat CL by **Morris and Sass (1966) were** not seen in the regressing CL of rabbit.

Plates of Figures





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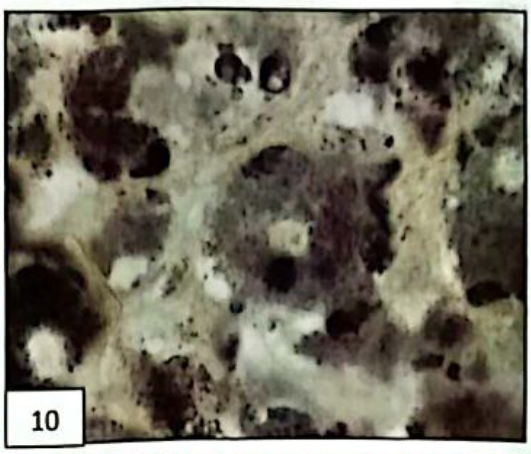

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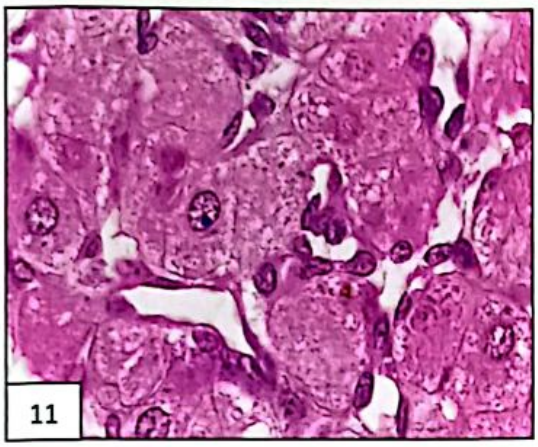

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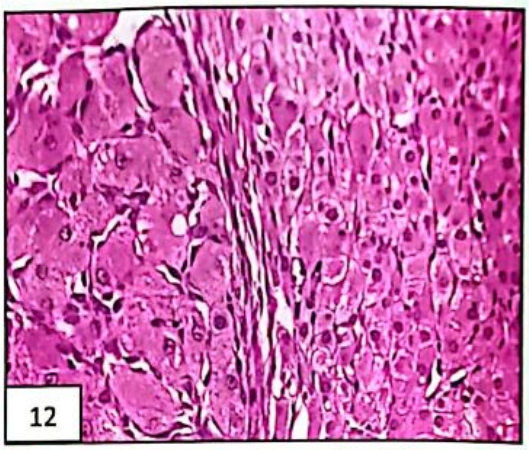

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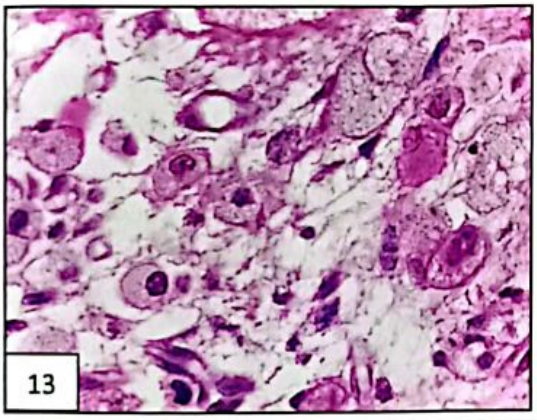

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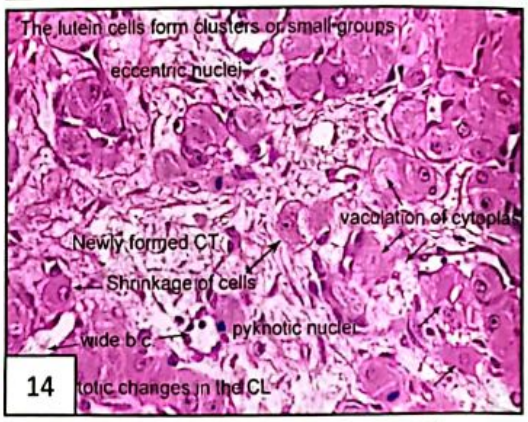

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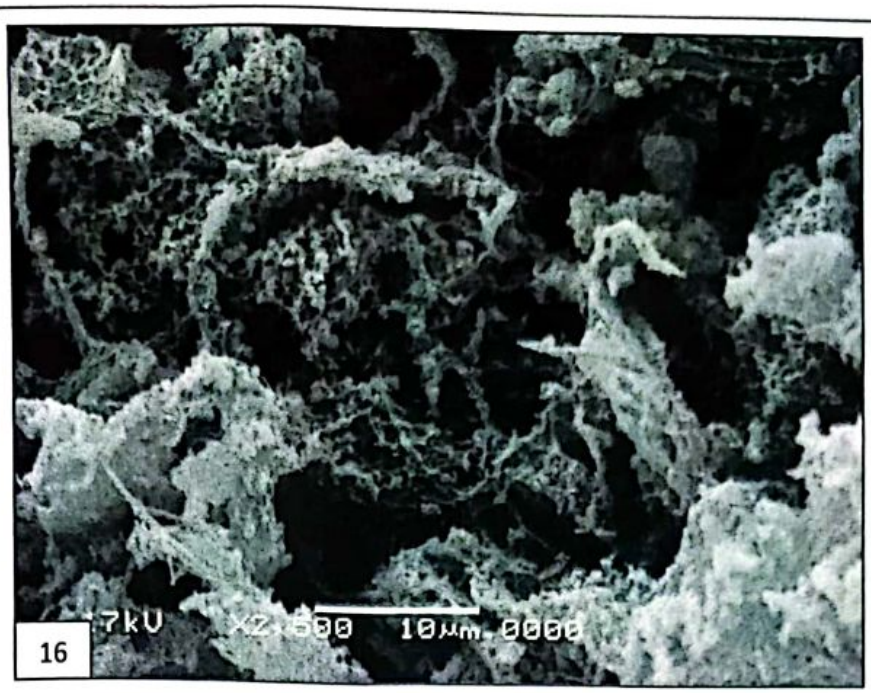

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الملخص العربي

دراسة ميكرومورفولوجية للجسم الأصفر فى الأرانب أثناء ما بعد الولادة

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في الأرانب، تستمر الأجسام الصفراء للحمل لبعض الوقت خلال فترة ما بعد الولادة .وقد تم دراسة الخصائص الميكرومورفولوجية لنمو وتراجع الجسم الأصفر بالمجهر الضوئي والمجهر الإلكتروني الماسح أثناء فترة مابعد الولادة . لوحظ انخفاض حجم الجسم الأصفر في مبيض الأرانب إلى حد كبير في اليوم الثالث إلى اليوم العاشر بعد الولادة .ورافق هذا التغيير انخفاضاً ملحوظاً في حجم الخلايا اللوتينية. وقد صاحب هذا التقلص في حجم هذه الخلايا غزو تدريجي لألياف الكولاجين والخلايا الليفية إلي التجويف المركزي للجسم الأصفر. أيضا خلال الانحدار الهيكلي للجسم الأصفر إنخفض متوسط عدد الخلايا اللوتينيه بشكل ملحوظ ، في حين ارتفع متوسط عدد الخلايا الخلالية الغدية بشكل كبير .لم يلاحظ وجود خلايا بلعمية كبيرة أو غيرها من خلايا النسيج الضام أثناء الانحدار الهيكلي للجسم الأصفر مشيرا إلى أن هذه الخلايا في الأرنب، على عكس الأنواع الأخرى، قد لا تلعب دورا هاما في إنحلال الجسم الأصفر مثل الدور الذي يقوم به موت الخلايا المبرمج . واعتقد البعض أنه من المحتمل أن بقاء الجسم الأصفر لبعض الوقت خلال فترة ما بعد الولادة في الأرانب يتعلق بمدة وتواتر الرضاعة.