Histological changes of Uterine Horn in the pregnant and non- pregnant Iraqi ewes (*Ovis aries*).

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Abstract:

The present study was designed to describe the histological changes in the uterine horn of pregnant and non- pregnant adult Iraqi ewes. uterine horn samples of Twenty slaughtered ewes were collected from Kerbala abattoir and divided into four groups, group 1 represented non pregnant ewes, group 2 represented pregnant ewes in the early stage of gestation period (20 – 30) days, group 3 represented pregnant ewes in the middle stage of gestation period (60 – 90) days, group 4 represented pregnant ewes in the late stage of gestation period (100 – 130) days, age of the fetus was determined by measuring crown-rump length. The samples were fixed in 10 % formalin, routine histological technique was done and stained with hematoxylin and eosin. Thickness of endometrium of the uterine horn was increased significantly ($p \leq 0.05$) in pregnant ewes especially in the middle stage of pregnancy, myometrium significantly decreased ($p \geq 0.05$) in the late stage of pregnancy while there were no significant differences in the perimetrium between pregnant and non- pregnant ewes. The number and the diameter of endometrial glands also showed a significant increase ($p \leq 0.05$) in pregnant ewes rather that in non-pregnant one and height of luminal epithelium was significantly decreased ($p \geq 0.05$) with the pregnancy advanced, also the glandular epithelium significantly decreased ($p \geq 0.05$) in the middle stage of pregnancy.

التغيرات النسجية لقرن الرحم في النعاج العراقية الحامل والغير حامل

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المستخلص:

صممت الدراسة الحالية لوصف التغيرات النسجية في قرن الرحم للنعاج العراقية البالغة عند عدم وجود حمل وخلال فترة الحمل. جمعت عينات قرن الرحم لعشرين نعجة بعد الذبح من مجزرة كربلاء وقسمت الى اربع مجاميع. المجموعة الاولى تمثل النعاج الغير حوامل، المجموعة الثانية تمثل النعاج في المراحل الاولى من الحمل (20–30) يوم، المجموعة الثالثة تمثل النعاج في المرحلة الوسطى من الحمل (60– 90) يوم، المجموعة الرابعة تمثل النعاج في المراحل المتأخرة من الحمل (100– 130) يوم، ثبتت العينات بمحلول الفورمالين بتركيز 10% واجريت عليها التحضيرات النسجية الروتينية وصبغت بصبغة الهيماتوكسَيلين والايوسين. اظهرت النتائج وجود زيادة معنوية ($p \geq 0.05$) في سمك بطانة الرحم وخصوصا في المرحلة الوسطى من الحمل بينما سجلت الطبقة العضلية للرحم انخفاض معنوي في سمكها ($p \leq 0.05$) في المرحلة

الاخيرة من الحمل. بالمقابل لم يكن هناك اي فروقات معنوية في سمك الطبقة المصلية للرحم بين النعاج الحوامل والغير حامل، كما لوحظ وجود زيادة معنوية ($p \geq 0.05$) في عدد وقطر الغدد الرحمية بالنعاج الحوامل مقارنة بالغير حوامل و لوحظ وجود انخفاض معنوي ($p \leq 0.05$) في ارتفاع ظهارة التجويف مع تقدم فترة الحمل بينما سجلت الظهارة الغدية اقل انخفاض معنوي ($p \leq 0.05$) في ارتفاعها في الفترة الوسطى من الحمل.

Introduction

Sheep plays an important role in agriculture economy, most of this importance represented by the number of lambing per year that depending on the reproductive activity of sheep, As well as the importance of sheep in producing meat, milk and wool [25]. Reproductive qualification is one of the important factors that determine the ability of sheep flock production especially in countries in which sheep industry is important [11]. The uterus in all ruminants, is bicornuate. The cornua or the uterine horns enclosed by a layer of serosa and muscular coat. The cranial part of the two uterine horns twisted to form comma shape, and suspended by the broad ligament within the abdomen[1].

Number of off spring per year increase by the presence of healthy reproductive organs especially the uterine horns in small ruminant in which the implantation of the zygote occurs [10].

Gestation, is the duration of embryo and fetus development which start by the fusion of sperm and oocyte and ended by birth [5]. In sheep, the ovoid conceptus of about 1 mm in length on day 11 begins to elongate on day 12 and forms a filamentous conceptus of 15 to 19 cm or more in length by day 15 that occupies the entire length of the uterine horn ipsilateral to the corpus luteum with extraembryonic membranes extending into the contralateral uterine horn [6].

The information on histology of uterine horn in pregnant and non- pregnant Iraqi ewes are rare. This present study aims to documenting information on the progressive histological changes in the uterine horn in pregnant and non- pregnant ewes.

Materials and Methods

The tissue samples were collected from middle part of uterine horns immediately after slaughter of twenty healthy and mature ewes in kerbala abattoirs and divided into four groups, group 1 represented non pregnant ewes, group 2 represented pregnant ewes in the early stage of pregnancy (20 – 30) days, group 3 represented pregnant ewes in the middle stage of pregnancy (60 – 90) days, group 4 represented pregnant ewes in the late stage of pregnancy (100 – 130) days, age of the fetus was determined by measuring crown-rump length [22].

The samples were fixed in 10 % formalin, Routine histological technique was done and stained with hematoxylin and eosin [15]. The prepared slides were examined in light microscope (Motic, Malaysia). Thickness of endometrium, myometrium and perimetrium, also the height of luminal epithelium, height of glandular epithelium,

number and diameter of uterine gland was calculated using ocular micrometer in different stages of pregnancy and non-pregnant ewes. The number of uterine glands were counted per microscopic field selected randomly under low power (X10) objective lens and repeated for 5 animals [18]. The slides were imaged by using digital camera (14.1 megapixel power resolution camera).

Statistical analysis:

One way ANOVA was used to estimate mean ± SE of the data and the variance was analyzed, Post hoc test was used LSD to specify the significant difference among means the software package IBM SPSS Program version 20 was used for the analysis of data [24].

Results

Histologically the uterine horn of pregnant and non- pregnant ewes was consisted of three layers, endometrium, myometrium and perimetrium that was similar to those of other mammals (Figure 1).

Endometrium in pregnant ewes was significantly thicker ($p \leq 0.05$) than that in non – pregnant ewes (Table 1). Endometrium of the uterine horn in non- pregnant ewes represented by the presence of small uterine glands with narrow lumen and surrounded by large amount of connective tissue. The epithelium of uterine gland in non-pregnant ewes was simple columnar with rare evidence of secretory activity (Figure 1).

In pregnant ewes, the endometrium of middle stage of pregnancy showed a significant increase ($p \leq 0.05$) in thickness as compared to the other stages of pregnancy (Figure 2, 3 and 4). Increase in the endometrial thickness in the middle stage of pregnancy was as a result to the hyperplasia in the uterine glands on account of the connective tissue that represented by a thin strands around the uterine glands, while the hypertrophy in the uterine glands was very clear in the later stage of pregnancy (Figure 3 and 4). So that the significant increase ($p \leq 0.05$) in the number of uterine glands were appeared in middle stage of pregnancy while the significant increase ($p \leq 0.05$) in the diameter of uterine glands were in the late stage of gestation period (Table 2). Most of the uterine glands in early and middle stage of pregnancy were round to oval in shape (Figure 2, 3) and in some sections of middle stage of pregnancy were appeared elongated (Figure 5). While in late stage of pregnancy, most of the uterine glands were appeared saculated (Figure 6).

The present study revealed there were a significant differences ($p \leq 0.05$) in the thickness of glandular epithelium, the uterine gland in the early and late stage of pregnancy was lined with tall simple columnar epithelium (Figure 6 and 7) while the most glandular epithelium in the middle stage of pregnancy was low columnar to cuboidal epithelium (Figure 8). So that the highest of glandular epithelium was significantly declined ($p \geq 0.05$) declined in middle stage of pregnancy as compared to other stages of pregnancy and non- pregnant ewes (Table 2).

Secretory activity of glandular epithelium was very clear especially in early and middle stage represented by the accumulation of products in the lumen of the uterine glands (Figure 7 and 8). Some of the glandular epithelium in the middle stage of

pregnancy was large and had light stain cytoplasm with spherical nuclei and some cells also were in mitotic division (Figure 8).

The present study revealed that there were a significant differences ($p \leq 0.05$) in the thickness of luminal epithelium, The luminal epithelium was simple columnar cells with oval nuclei near the basement membrane in all sections of the non- pregnant and pregnant ewes in early stage of gestation (Figure 7 and 9), while the luminal epithelium was simple columnar to cuboidal in the sections of middle and late stage of gestation, the luminal epithelium in early stage of pregnancy was significantly higher ($p \leq 0.05$) than that other groups (Table 2). In the late stage of gestation, the luminal epithelium was ragged and worn in many sections (Figure 4).

Thickness of myometrium of the uterine horn was significantly decreased with the progress of pregnancy (Table 1), myometrium in all sections of non- pregnant, early and middle stage of pregnancy was composed of two layers, inner circular and outer longitudinal (Figure 1 and 2), the two layers were very distinguished except in late stage of pregnancy, the two layers were difficult distinguished and were appeared as one layer (Figure 4).

The differences in thickness of Perimeterium was unclear in pregnant and non-pregnant ewes and also in all sections of pregnant stages.(Table 1). Perimetrium was composed of single layer of mesothelium supported by a thin layer of connective tissue (Figure 1, 2 and 4).

Table (1) showing Averages thickness of three layers of uterine horn in ewe in non- pregnant and with different stages of pregnancy.

parameter / stage of pregnancy	Average Thickness of Endometrium (**µm**) Under X 10 magnification	Average Thickness of Myometrium (**µm**) Under X 10 magnification	Average Thickness of Perimetrium (**µm**) Under X 10 magnification
Non pregnant	58.9	63.4	8.4
Early	113.2	71.6	8.2
middle	163	64.2	8.6
Late	78	49.2	7.2
L. S. D	14.66	9.64	1.77

Table (2) showing averages of number, diameter, height of epithelium of the uterine gland and height of luminal epithelium of uterine horn in ewe in non- pregnant and with different stages of pregnancy.

parameter / stage of pregnancy	Average number of uterine gland / microscopic field Under X 10 magnification	Average uterine gland diameter (**µm**) Under X 40 magnification	Average height of glandular epithelium (**µm**) Under X 40 magnification	Average height of luminal epithelium (**µm**) Under X 40 magnification
Non pregnant	24.2	5.46	11.18	11.92
Early	76	16.98	12.26	13.52
Middle	99.2	28.62	9.82	8.5
Late	36.8	42.42	14.72	7.7
L. S. D	7.73	9.22	1.98	0.79

Discussion

Luminal epithelium was significantly higher ($p \leq 0.05$) in the early stage of pregnancy than that in non- pregnant and pregnant ewes in mid and late stage, in this stage the luminal epithelium was appeared tall columnar, where's in the advanced of pregnancy, epithelium was tend to be cuboidal, this result similar to result of [8] when revealed that the embryo, chorio vetelline membrane and embryonic fluids apparently compressed the endometrium with its luminal epithelium. [21] reported the same result and revealed that the blastocyst expansion in early stage of pregnancy requires a substances found in the secretion of the luminal epithelium. [20] also explained that the period of greatest thickness of the luminal epithelium correlated with the period of blastocyst expansion. [17] reported that increase in the cellular organelles during the secretory state of cells lead to increase in the cellular volume during pregnancy.

Thickness of endometrium and number of uterine glands were significantly increased from non- pregnant to pregnant ewes except in late stage of pregnancy in which thickness of endometrium and uterine glands index were significantly declined ($p \geq 0.05$), these results was similar to the results of [9 and17] when they observed that the physiology hyperplasia in uterine gland during pregnancy was necessary as a result to the increase the demand for the nutrients by the fetus. Also similar to the result of [12] when he reported that the histiotrophe represent an important source of nutrient from the uterine glands during pregnancy for the developing embryo also uterine gland secrets product as well as to histiotrophe, such as leukemia inhibitory factors and calcitonin which are require for implantation. In sheep, as in other mammalian the uterine glands undergo a program of hyperplasia followed by hypertrophy that appears to be dependent on temporal and spatial actions of hormones from the

ovary (progesterone) and placenta (placental lactogen and growth hormone) [2, 6and 9]. This result also agree with the result of [4, 16 and 19] when they observed that There were close relationships between the endocrine changes and histological modification, thickness of endometrium and number of uterine glands corresponding positively with increase of progesterone in pregnancy and leuteal phase of estrus cycle, Progesterone not only important to prepare the uterus for implantation of the embryo, but also aids in maintaining pregnancy by providing nourishment to the cnceptus.

The decrease in the thickness of endometrium in late stage of pregnancy may explained as a result to decrease in the number of uterine glands as described by [13] when mention that the connection of maternal and embryonic tissues might facilitate transport during late stage of pregnancy when metabolic processes are highest, this result were in accord with those of [23] who referred that the number of uterine glands decreased after mid stage of pregnancy where's the total uterine diameter continued to increase, suggestion that the nutritive role of uterine gland epithelium decreased during late stage of gestation.

The secretory activity of uterine glands were very clear in mid stage of pregnancy due to the increasing in the number of uterine glands and accumulation of its products in the lumen, in spite of the highest of glandular epithelium in this stage was reported least values as compared to the other stages of pregnancy. the present study suggest that the glandular epithelium was secreted there products to the lumen so that it was cuboidal in shape. This result agreed with the result of [14] in secretory cells of mammary gland in buffalo when revealed that the most of secretory cells were cuboidal in shape while the lumen of alveoli were filled with secretion. Also there were many large and light stained glandular epithelium of mid stage of pregnancy, similar result was done by [7] when described glandular cells resemble goblet cells in that they each contained a large secretory vesicle. Also same result was reported by [19] when observed that the epithelial cytoplasmic vacuolation increased with the pregnancy advanced as a result to increase in the level of progesterone which reflecting increased secretory activity when Approximately half cells had a sub nuclear glycogen vacuole. Mitotic division was clear in many glandular cells of middle stage of pregnancy in the present study, mitosis correlated with the increase in the concentration of progesterone during pregnancy and leuteal phase [19]

Myometrium thickness decreased with the advanced of gestation, this result agreement with the result of [7] who reported that the myometrium was significantly decrease in thickness after second half of gestation, the two layers of myometrium (inner and outer) where undistinguished as compared to other stages of pregnancy. The present study suggested that the increasing in the fetus weight, fetus fluids and fetal membranes caused the excessive pressure on the myometrium.

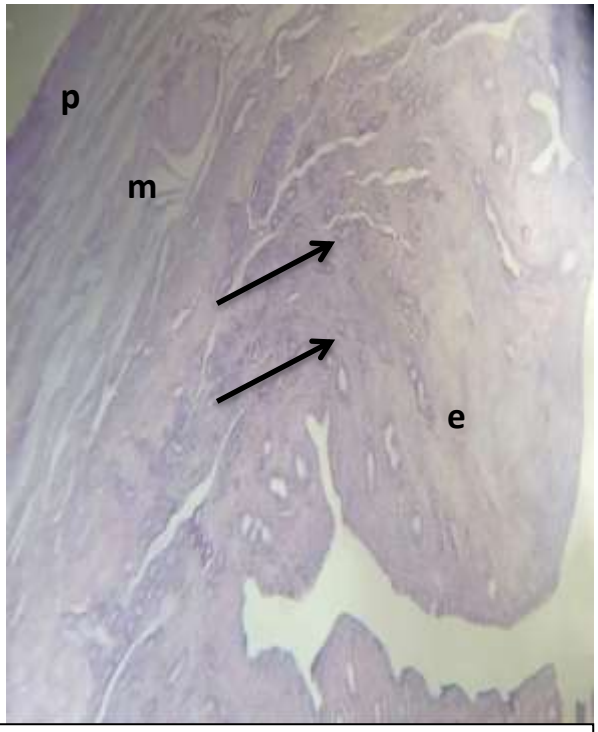

Figure 1: Histological section of uterine horn in non-pregnant ewe showing the endometrium(e) with little and small uterine glands (arrows), myometrium(m) and perimetrium(p). (X 4) H & E stain.

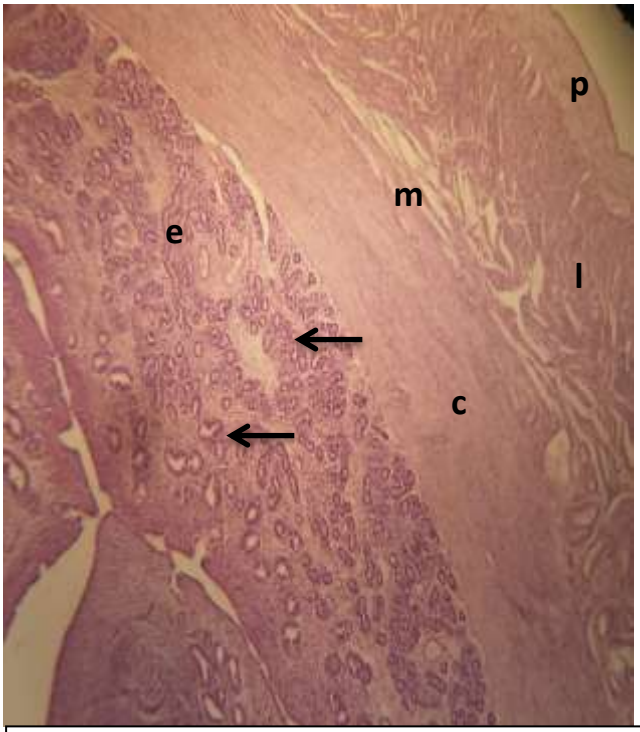

Figure 2: Histological section of uterine horn in early stage of pregnancy showing the endometrium (e) with increase in uterine glands (arrows). myometrium (m) consist of inner circular (c) and outer longitudinal (l). perimetrium (p). (X 4). H&E stain.

Figure 3: Histological section of uterine horn in middle- stage of pregnancy showing the hyperplasia in the uterine glands (thin arrows) filled with secretion within endometrium(thick arrows). (X 4) H&E stain.

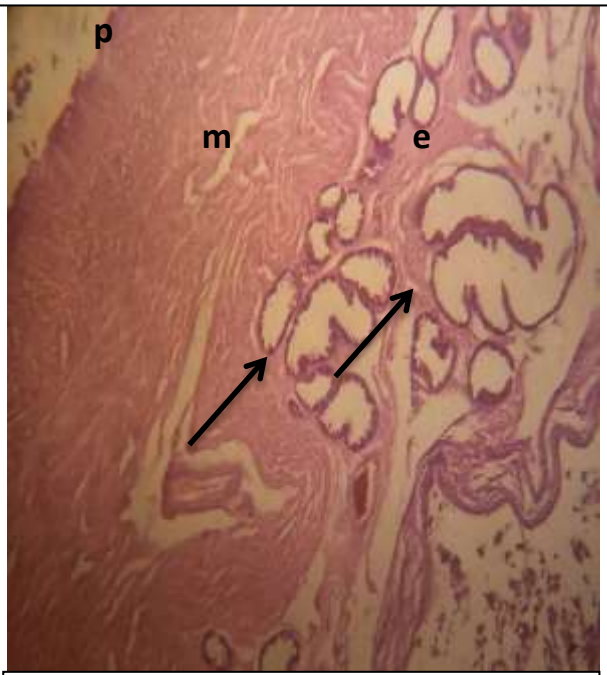

Figure 4: Histological section of uterine horn in late- stage of pregnancy showing the hypertrophy in the saculated uterine glands (arrows) within endometrium (e), myometrium (m) and perimetrium (p). (X 4) H&E stain.

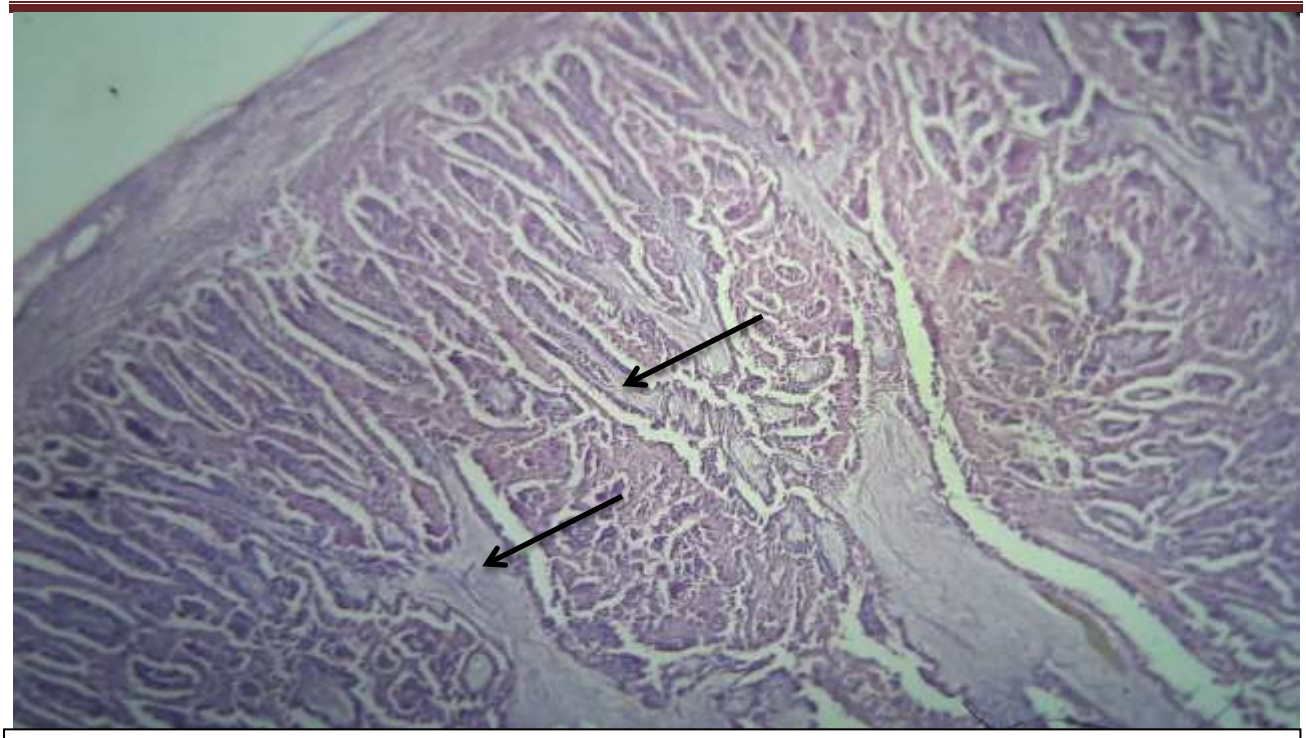

Figure 5: Histological section of uterine horn in middle - stage of pregnancy showing the uterine glands within endometrium filled with secretion (arrows) and most of the glands were straight. (X 10) H& E stain.

Figure 6: Histological section of uterine horn in late- stage of pregnancy showing the uterine glands (u) within endometrium (e), lined with simple columnar epithelium(arrows) with few secretion in their lumen. (X 40). H&E stain.

Figure 7: Histological section of uterine horn in early- stage of pregnancy showing the luminal epithelium lined with simple columnar (L) and the uterine glands (u) also lined with simple columnar epithelium with the presence of secretion in their lumen (arrow). (X 40) H&E stain.

Figure 8: Histological section of uterine horn in mid- stage of pregnancy showing the uterine glands (u) was lined with simple cuboidal epithelium with the presence of secretion in their lumen as well as the presence of some large and light stain glandular cells (thin arrows) and mitotic division (thick arrows). (X 40) H&E stain.

Figure 9: Histological section of uterine horn in non- pregnant ewe showing the luminal epithelium lined with simple columnar epithelium (arrows). (X 10). H&E stain.

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