



A study of pathological abnormalities of genitalia in ewes in Duhok, Iraq

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Abstract

Congenital or acquired abnormalities in genital tracts of ewes leading to sterility, which cannot easily be identified by routine clinical examination. Reproductive tract abnormalities regarded as a major cause lead to loss of sheep production. This study aimed to examine reproductive tracts in slaughtered ewes grossly and microscopically. This study was conducted on 270 genital tracts of non-pregnant ewes at Khanky slaughterhouse in Duhok province. Samples were collected within the period from September 2019 to March 2020. The results showed that 24.1% exhibited acquired abnormalities and 75.9% were exhibited normal. The rate of uterine abnormalities was about 3.3%, ovarian abnormalities comprised 17.8% and oviduct abnormalities were 3.0%. The most common pathological conditions of the uterus were endometritis at rate 2.2%, hydrometra (pseudopregnancy) and mummified foetus at rate 0.4% and 0.4%, respectively. In the ovary, the common abnormalities were ovarobursal adhesion 14.4%, ovarian cysts 1.1% and Para ovarian cysts 0.4%. The abnormalities of the oviduct were uterine tubes obstruction 2.6%, followed by hydrosalpinx 0.7%. It is concluded that pathological lesions, such as endometritis, ovarian cysts and hydrosalpinx in ewes could result in infertility or sterility of the ewes leading to economic losses to farmers.

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Introduction

Genital tracts abnormality in ewes regarded as an important factor causing subfertility or infertility cannot simply be determined by routine clinical examination. Local small ruminants breed (ewes) at Duhok province is fertile. Local sheep are used to provide a wide range of products such as meat, milk, skin for leather making (1). Production of sheep plays an important role in the economy. Ewes regarded as fertile small ruminants deliver twin in birth is rare. Knowledge of genital tract abnormalities in ewes acts as an essential tool for minimizing the incidence of infertility in ewes and increased reproductive efficiency (1). There are limited studies carried out on the abnormalities in the genital tract in ewes (2). The common abnormalities in the genital tract are ewes have been reported in the previous studies are Para-ovarian cyst, follicular cysts, hydrosalpinx, salpingitis and ovarobursal adhesion (3). It has been reported that abattoirs regarded as an important way to obtain the sort of

genital tract lesions and the incidence of abnormalities of genital tracts (1). There is a variation in the incidence of genital tract abnormalities with different places, in Baghdad is about 20.22% (4), in Basra is 35.0% (3), in Iran is about 16.6% (5). In Sulaimani (6) found the incidence of the ovarian lesion in goats is 21.176%. It should be noted that, there are not enough information available on the cause of infertility and female genital tracts abnormality in small ruminants in Duhok province. For that reason, this study aimed to investigate macroscopic and microscopic lesions of the genital tract abnormalities in ewes slaughtered at local abattoir of Duhok province, Iraq.

Materials and methods

Tissue collection

Non-pregnant reproductive tracts from both nulliparous and pluriparous ewes were collected from Khanky abattoir at Duhok Province. Samples (N=270) were collected at least

twice times in a week started from September 2019 to March 2020. The samples were collected into a container and immediately transferred to Fertility laboratory at College of Veterinary Medicine, The University of Duhok University. Information regarding animals identifies and history was not obtained for this study. After samples were arrived to the laboratory an excessive tissue such as broad ligaments, loose connective tissues or fat tissue that surrounding the vulva and retroperitoneal part of the vagina were removed in order to examine the samples easily and better examination. Reproductive status of the individual ewe was assisted through the ovarian condition. All samples were examined started from the cervix, uterine body, both uterine horns, uterine tubes and both ovaries. Reproductive tracts were evaluated by visual examination, palpation and incision.

The first part of the genital tracts was examined are both ovaries. Ovaries from non-pregnant ewes were examined externally and internally for detection and any gross pathological conditions such as fibrosis, tumours, follicular cysts, luteal cysts and /or para-ovarian cysts, inflammatory conditions, congestion, and other abnormal lesions. Ovarian cysts were confirmed when the diameter was > 1 cm were. Ovarian cysts were classified in to two types including follicular cyst characterised by thin-walled and luteal cysts characterised by firm luteal tissue (7). In addition, ovarian bursa for each side was examined to check for adhesions; adhesions were ranged from mild with a thin thread of connective tissues to severe adhesion between the ovarian bursa and the ovary or ovaries were completely surrounded by fibrous tissues. The uterus was examined visually and by palpation to check any abnormalities. The uteri were opened from the cervix to both horns to check for the presence of pus in the uterus, hydrometra; mummified foetus or intramural fibrosis. At the same time cervix were examined for abnormalities such tumour, fibrosis or congestion. From the tip of the uterine horn both fallopian tubes were injected with nigrosen stain for checking both uterine tubes status whether they were blocked. Any lesions bearing from genital tracts on any segment were selected for histopathological study.

Section preparation

A piece of samples about 5cm in diameter were taken from the lesion or abnormal part of the female genital tracts were place in a container with 10% neutral buffered formalin for fixation (8). The fixed tissue specimens were put in to cassettes and dehydrated then embedded with molten paraffin. The embedded tissue section was sectioned at 5 µm using a rotary microtome (HM355, Germany) then sections were floated onto water 40°C and placed onto slides. Sections were stained with haematoxylin and eosin to check histological appearance using light microscope (9). All recorded data transferred into Excel sheet, and presented in percentage.

Results

The overall prevalence of abnormal reproductive tracts in ewes in this study was 24.16% (65/270) and of 12 different abnormalities was observed by the gross examination. Results showed that the highest abnormalities were related to ovary about 18%, (Table 1, Figure 1 and 2), followed 3.3% related to the uterine tubes and then nearly 3.0%, was associated to the uterus (Table 1; Figure 3).

Ovaro-bursal adhesion

The results found that ovarian bursa adhered to the ovary and recorded the highest incidence about 14.4% (39/270) of abnormal reproductive tracts. The incidence of mild and sever ovaro-bursal adhesion were 10.37% and 4.0%, respectively (Table 1). Bilateral adhesion was limited in number and rest were unilateral, the higher number of adhesion was seen on the right ovary compared to left ovary (Table 1). Adhesions were varied from mild to severe (Figure 1).

Parovarian cysts

The incidence of parovarian cyst in this study was 0.4% (1/270). The cyst was small in size as grape-sized, which measured about 2-10 mm in diameter. These cysts were similar to the bright pearl white in shape and it was filled clear fluid. This cyst was attached to the fimbriae of the oviducts (Figure 2).

Paraoviductal cysts

The results show that 0.7% (2/270) of paraoviductal cyst was found (Table 1). Grossly paraovarian cyst characterised by thin-walled and varied size 3-9 mm (Figure 2).

Follicular and luteal cysts

The results found 4 follicular cysts 0.7% (Table 1) and 1 luteal cyst 0.4% (Table 1) among the total number of reproductive tracts. Anatomically, these cysts were measured about 1.5-2.0cm in diameter and seen unilaterally (Figure 3 and 4). The cyst wall was thin in follicular cyst compared to that of luteal cyst. Histologically, the follicular cysts were lined with a thin layer of granulosa cells and lined with swollen theca interna and congested blood vessels were seen in the theca externa. No ovum was seen in the lumen of cyst (Figure 5). In terms of luteal cyst, the cavity of the cyst was lined with a thin layer fibrous connective tissue layer and surrounded by a thick layer of luteinised tissue, infiltration of the inflammatory cell into the cystic lumen (Figure 6).

Ovarian atrophy

Two cases of ovarian atrophy 0.7% were found in this study, Table 1. Anatomically, ovaries characterised by small in size and smooth external surface which free from form mature follicles and corpora lutea (Figure 7).

Microscopically, the stroma of the ovary was fibroses and the follicles were low in number in a field. The majority of

follicles were primordial and primary follicles were seen (Figure 7).

Table1: Frequency distribution of gross abnormalities of genital tracts

Abnormalities	Right side	Left side No.	Total	%
Abnormalities of ovary				
Para-ovarian cyst	1	0	1	0.4
A- Mild ovaro-bursal adhesion	19	9	28	10.37
B- Sever ovaro-bursal adhesion	6	5	11	4.07
Total Ovarso-bursal Adhesion	25	14	39	14.44
Ovarian atrophy	0	2	2	0.7
Follicular cyst	2		2	0.7
Luteal cyst	0	1	1	0.4
Para oviductal cyst	2	0	2	0.7
Adhesion between two ovaries			1	0.4
Total			48	17.8%
Abnormalities of uterine tubes				
Hydrosalpinx	1	1	2	0.7
Uterine tube obstruction	4	3	7	2.6
Total			9	3.3%
Abnormalities in the uterus				
Hydrometra (pseudopregnancy)			1	0.4
Endometritis			6	2.2
Mummified fetus			1	0.4
Total			8	3.0%
Total number of abnormal reproductive tracts			65	24.1%
Total number of normal reproductive tracts			205	75.9%
Total number of reproductive tract			270	

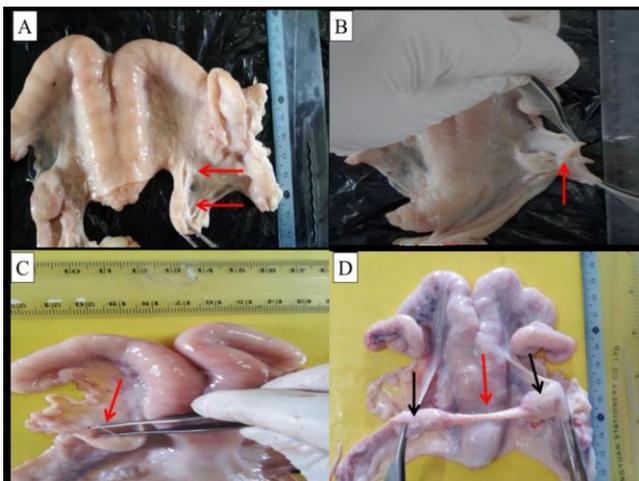


Figure 1: A photograph shows adhesion on the ovary; A) sever unilateral ovarobursal adhesion (red arrows); B) Severe unilateral adhesion between ovary and uterine tube (red arrow); C) Mild unilateral lateral ovarobursal adhesion (red arrow); D) sever adhesion (red arrow) between two ovaries (black arrows).

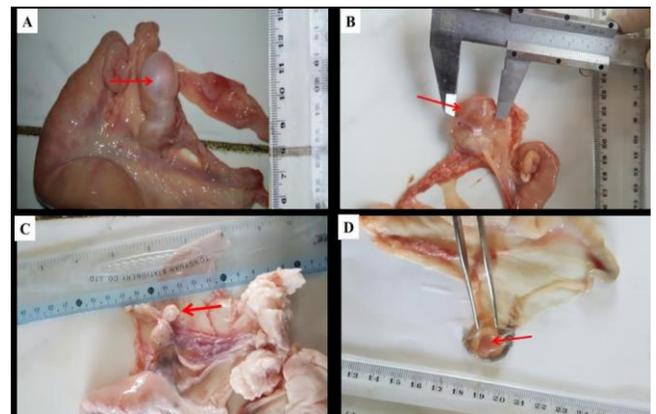


Figure 2: A photograph shows abnormalities on the ovary; A) Follicular cyst (red arrows); B) Luteal cyst (red arrow); C) Para-ovarian cyst (Red arrow); D) Para oviduct cyst (red arrow).

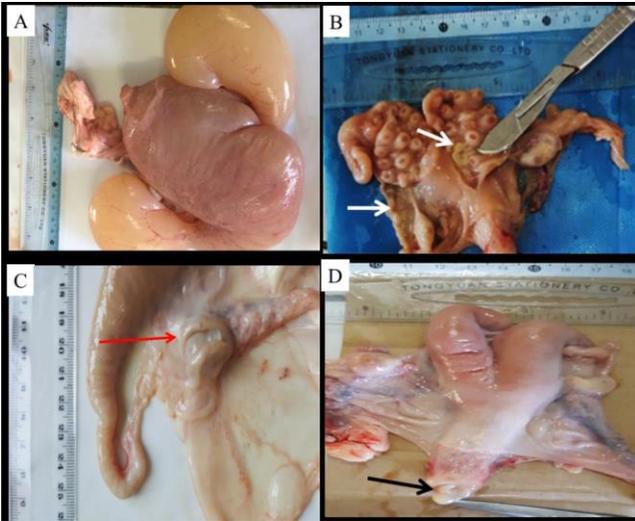


Figure 3: A photograph shows abnormalities in the ovary and the uterus; A) Hydrometra (pseudopregnancy); B) Endometritis, pus in the uterus (white arrows); C) Severe ovaro-bursal adhesion (red arrow); D) Endometritis, drainage of pus from the cervix (black arrow).

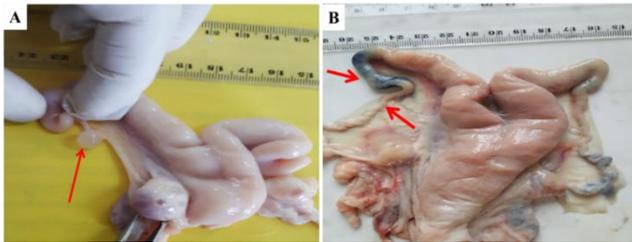


Figure 4: A photograph shows abnormalities in the oviduct; A) Unilateral hydrosalpinx (red arrow); B) Unilateral obstruction of oviduct (red arrow) checked with an injection of nigrosine stain (red arrow).

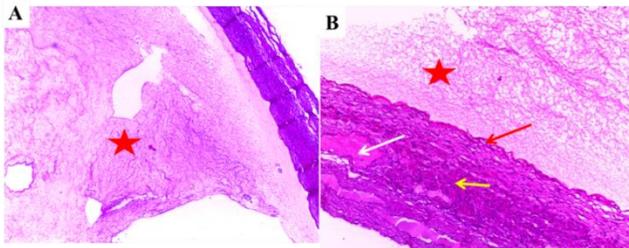


Figure 5: Photomicrograph of a follicular cyst. A) A Cyst filled with fluid (red star) and surrounded by thin wall thin layers of granulosa cells (H&E stain, 4X). B) High magnification cyst is surrounded by proliferated theca layer cells (yellow arrow), with the marked proliferation of granulosa cells within the ovarian cyst (red arrow) congested of blood vessels (H&E stain, 20X).

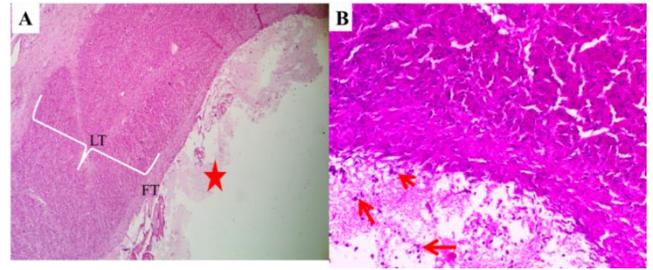


Figure 6: Photomicrograph of Luteal cyst. A) The lumen of the cyst is lined by fibrous tissue (FT), and FT is lined by massive proliferated luteal tissue (white line). The cyst filled with fluid (red star, H&E stain, 4X). B) High magnification cyst is surrounded by Luteinised tissue (LT), with infiltration of inflammatory cells into the cystic lumen (red arrows, H&E stain, 20X). Luteinised tissue (LT), Fibrous tissue (FT).

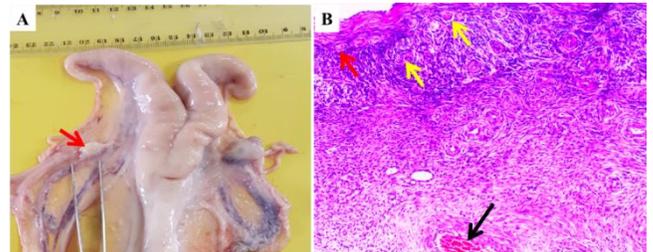


Figure 7: Photomicrograph of ovarian atrophy. A) Ovarian atrophy characterised by small in size and smooth ovary with devoid from follicles and corpus luteum (red star); B) Histological section of ovarian atrophy, the cortex of the ovary surrounded by fibrous connective tissue. The cortex contains numerous un developed follicles, primordial follicle (red arrow), primary follicles (yellow arrows). The medulla of the ovary had numerous congested blood vessels (black arrow), H&E 20X.

Adhesion between ovaries

The results showed that 0.4% (1/270) of severe adhesion was seen between ovaries (Table 1, Figure 1). Grossly, this adhesion appeared a band of fibrous connective tissue.

Uterus

Among the whole reproductive tracts were studied, only about 3% (8/270) of various abnormalities were seen in the uterus (Table 1). These abnormalities are listed below:

Hydrometra (Pseudopregnancy)

The rate of this condition in ewe was 0.4% (1/270) observed in this study (Table 1). Both uterine horns were distended with clear watery fluid about 180 ml. Uterine wall became thin and translucent due to distension pressure (Figure 3). The foetus or foetal membranes were not seen in the uterus.

Endometritis

This study found about 2.2% (6/270) the total number of reproductive tracts was examined. Grossly, the pus was drainage from the cervix (Figure 3), and pyometra was seen in the in both horns (Figure 3). Microscopically, uterine endometrial epithelium was sloughed into the uterine lumen with infiltration of inflammatory cells in the lamina propria into the uterine lumen (Figure 8). Diagnosis of endometritis only by microscopic but pyometra diagnosis by macroscopic.

Mummified foetus

The results of the present found only one case of mummified fetus 0.4% (Table 1) form the total number of reproductive tracts were examined.

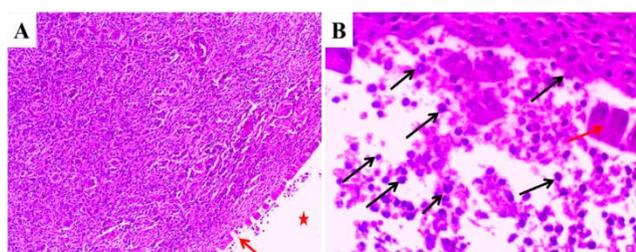


Figure 8: Photomicrograph of endometritis. A) Endometritis showed with sloughing endometrial epithelial (red arrow) into the uterine lumen (red star), H&E stain, 4X. B) High magnification, endometritis characterised by sloughed endometrial epithelial into uterine lumen (red arrow), with infiltration of inflammatory cells in sub epithelial layer and into the lumen of the uterus (black arrows), H&E stain, 20X.

Discussion

The present study reported various numbers of pathological conditions in ewe's reproductive organs. The overall incidence rate of gross reproductive abnormalities in ewes was 24.1%, these abnormalities were predominantly occurring in ovaries 17.8% followed uterine tubes 3.3% then uterus 3.0%. This rate of incidence was nearly the same rate as reported in the previous studies, in India the incidence was 23.33% (10), in Sulaimani 21.64% (11), in Iran 28.44% (2). However, the rate of pathologic abnormalities of reproductive tracts found in this study was higher compared to the previous studies 3.32% as reported by (4) and 9.4% by (12). These variations in the incidence may be related to the nutritional status of the animals, breeds, age of animals or the environmental conditions of the places. The current study investigated that the most important abnormalities in the ovary was ovarian cysts such as follicular and luteal cysts. In agreement with the previous studies reported that the most predominant ovarian diseases in small ruminant was cystic ovarian diseases due to disturbance in hormonal balance (10). Cystic ovarian diseases regarded as an important

disease leading to infertility, in cattle. However, in small ruminants its substantial effect remains unknown compared to cattle (13). It should be noted that some authors reported that luteal cysts have no influences on animals ovarian cyclicity and luteal cyst secrete enough progesterone to maintain the pregnancy (4). It has been reported that animals with polycystic ovarian disease causes imbalances in the hypothalamus-pituitary glands-ovary axis which leads to an irregular oestrous cycle or failure of animals to return oestrous cycle due to high level of oestrogen and lead o reproductive failure (14). Polycystic ovaries unlike single follicular cyst produce a consistent change in the mucosa of uterine tube and uterus as well (1). As a result of rapid secretion of GnRH pulse for LH secretion along with low level of FSH, which leads to an unfavourable ovum to develop and causes failure of ovulation becomes arrested (13).

Another type of cysts was found in this study a case paraovarian cyst 0.4% and two cases of paraoviductal cysts 0.7%. This rate was lower than have been investigated previously by (3) they found paraovarian cysts and paraoviduct cyst about 14.3% and 3.6%, respectively, and 5% of paraovarin cysts in ewes by (6). Furthermore, the rate of paraovarian cyst in this study was much lower than reported in Does (10). These variations might be due to the number of samples used in this study, or might be due to other factors contributing this variation in the rate of paraovarian cysts such as ecological, breed or species of animals. It is interesting to note that these types of cysts neither had no influence on fertility in small ruminants unless these cysts causing pressure on the uterine tubes.

This study investigated 0.7% unilateral ovarian atrophy, which makes animals infertile when this condition occurred in bilateral, however, fertility becomes sub normal when this condition occurred unilateral (15). It has been reported that ovarian atrophy regarded as an important factor decreasing fertility in cows and lead to anoestrous in cattle (15). This disease developed either when animals were treated with toxic compounds which damaged the cellular components of ovary or by blocked or destroy the surface of ovary (15). In the present study ovarian atrophy was characterised by incomplete development of ovary or ovary devoid from primordial follicles. This study could not investigate the causes of this condition, but this may cause anoestrous to animals.

In terms of uterine tubes, this study observed 9 cases of pathological lesions in oviducts. Obstruction of uterine tubes described in 7 cases from the total number of abnormalities occurred in uterine tubes. Uterine tube obstruction occurred unilaterally in this study. Bilateral uterine obstruction cause sterility, however, unilateral obstruction causes varying degrees in the fertility in small ruminants (15). An obstruction of uterine tubes may occur as results of the permanent stage of inflammatory conditions and this may lead to sterility (15).

This study noticed a single case of hydrometra 0.4%. Similarly, to the previous study (6) reported only one case of hydrometra from the whole number (n=600) of examined ewes reproductive tracts. It has been previously reported that hydrometra in small ruminants was associated with some factors, including ovarian cysts, segmental aplasia of the uterus, and genetic. But it should be noted that such relationship between these factors and the incidence rate of hydrometra was not detected currently (16). Small ruminants (ewes and does) with hydrometra (pseudopregnancy) exhibit the same clinical signs as pregnancy. It is interesting to note that the pseudopregnant animal in the current study was depending on an abattoir animal, clinical history of such animals was not provided in this study.

The current study observed that endometritis was the most common pathological lesions finding among uterine abnormalities about 2.2%. This rate was higher than reported 0.3% by (10). It is interesting to note that the rate of endometritis in ewes ranged from 0.5-3% (2,12). It has been reported that endometritis in ewes as in cattle act as an important cause in fertility. This is in agreement with the previous study reported that endometritis in caprine was the most common pathological lesions (10). It has been recently published that endometritis had a negative impact on postpartum ovarian activity and postpartum reproductive performance (17,18). Cows with endometritis had abnormal progesterone profiles particularly luteal phases (18). It has been demonstrated that endometritis induces embryonic loss due to uterine tissue disruption or direct embryo cytolysis (19). It has been recently published cows with endometritis caused by *E.coli* Lipopolysaccharide had small corpus luteum and produced the low level of progesterone in both *in vivo* and *in vitro* (19,20). It is interesting to note that both *in vivo* and *in vitro* *E.coli* Lipopolysaccharide decreased luteal cells angiogenesis and increased luteal cells apoptosis thereby lead to decreased production of progesterone (19). Furthermore, the absorption of bacterial toxin prevents the development of the Graffian follicles and lead to failure of ovulation (21). It has been investigated that *E.coli* is uncommon condition caused endometritis in ewes, but it might be associated with other bacteria (22). The present study observed a case of the mummified foetus 0.4% from whole reproductive tracts was examined. It has been reported that the prevalence of mummified foetus in small ruminants is uncommon (22).

The rate of ovarobursal adhesion in the present study was 14.44% among whole ovarian abnormalities. The incidence of mild ovaro bursal adhesion in this study was 10.37%. This result is higher when compared to Faraidoon (11) and in which 8.2% of specimens presented with extensive ovaro-bursal. It has been reported that animals with mild ovaro bursal adhesions are resolve spontaneously, as they appear to be more common in oestrous (23). Mild ovaro bursal adhesions are assumed to originate from the blood clots and follicular fluid released at ovulation (23). It has been

documented that these minor adhesions may have little significance with respect to fertility and cases have been found that have related to pregnancy (23).

The incidence of sever ovaro bursal adhesion in this study was 4.07% which is higher than reported by Faraidoon (11) and Moghaddam and Gooraninejad (24), in which 7.65% and 7.6%, respectively. However, the results of this study are nearly the same rate reported previously by Millward *et al.* (23) and Saberivand *et al.* (2) and in which 3% and 3.59%, respectively of specimens presented with extensive ovaro-bursal. The main reasons of occurring of sever ovaro-bursal adhesion might be associated with oophoritis, endometritis peritonitis which acts as main causes of an adhesion of ovary with either bursa or with salpinx in small ruminants (19). It has been reported that ovarobursal adhesion may disturb ovulation or blocking the passage of ovum through the uterine tube (10).

Conclusion

This study can be concluded that out of 270 ewes, 24.1% were affected by pathological conditions of the reproductive tracts. Endometritis, ovarobursal adhesion and ovarian cysts were the most common abnormalities of genital tracts of the slaughtered ewes. The current study drive offers basic knowledge on the productive health of the ewes in Duhok Province, which can be used for guiding advance investigations throughout the country or in the application of the best treatments/or control strategies directed to decrease the incidence of infertility in ewes.

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Conflicts of interest

The authors hereby declare that there were no conflicts of interest regarding this research and the publication of the manuscript.

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دراسة التشوهات المرضية للأعضاء التناسلية في النعاج في دهوك، العراق

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الخلاصة

التشوهات الخلقية أو المكتسبة في الأجهزة التناسلية لإنات الأغنام مما يؤدي إل العقم الدائم، والتي لا يمكن ملاحظته ببساطة عن طريق الفحص السريري الروتيني. تعتبر التشوهات في الأجهزة التناسلية من الأسباب الرئيسية التي تؤدي إلى فقدان إنتاج الأغنام. لذلك كان الهدف من هذه الدراسة هو فحص الأجهزة التناسلية لإنات الأغنام عياناً ونسجياً. شملت الدراسة ٢٧٠ عينة من الأجهزة التناسلية لإنات الأغنام البالغة غير حامل في مجزرة خانكي بمحافظة دهوك. تم جمع العينات من مجزرة خانكي خلال الفترة المحصورة من سبتمبر ٢٠١٩ إلى مارس ٢٠٢٠. أظهرت النتائج ٢٤,١٪ تشوهات مكتسبة و ٧٥,٩٪ طبيعي. بلغ معدل التشوهات في الرحم حوالي ٣,٣٪ وفي المبيض ١٧,٥٪ وفي قناة المبيض ٣,٠٪. أهم الإصابات التي سجلت في الرحم هي التهاب بطانة الرحم بنسبة ٢,٢٪ من الحالات المرضية، تلتها الحمل الكاذب ومومياء الجنين ٠,٤ و ٠,٤٪ على التوالي. أهم الإصابات التي سجلت في المبايض كانت الالتصاقات المبيضية الجرابية بنسبة ١٤,٤٪، تليها أكياس المبايض ١,١٪ أكياس جنب المبايض ٠,٤٪. الإصابات التي سجلت في قناة المبيض كانت انسداد أنابيب المبايض بنسبة ٢,٦٪، يليه تجمع السوائل في قناة المبيض بنسبة ٠,٧٪. وقد استنتج من الدراسة، أن الآفات المرضية مثل التهاب بطانة الرحم وأكياس المبايض وتجمع السوائل في قناة المبايض في النعاج يمكن أن تؤدي إلى العقم أو العقم من النعاج مما يؤدي إلى خسائر اقتصادية للمزارعين.