HISTOPATHOLOGICAL AND HORMONAL INVESTIGATION OF NON-PREGNANT EWES DURING BREEDING SEASON IN BASRAH PROVINCE

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ABSTRACT

The study was conducted to investigate the causes of infertility in ewes at the end of breeding season in Basra province. The study was conducted from November to December 2020 on 66 non-pregnant ewes, which were isolated for slaughtering after found non pregnant post breeding season. The isolated reproductive systems were subjected to gross and histopathological examination and hormonal evaluation to determine the main cause of infertility. The study revealed that 66.66% ewes had abnormal lesions in reproductive system. The most common pathological conditions of the ovary were inactive ovaries, follicular cyst and luteal cyst which were found in 10.60%, 10.60% and 9.09% ewes, respectively. Oviductal lesion abnormalities included oviductal occlusion and hydrosalpinx that was found in 4.56% and 6.06% ewes, respectively. The common uterine abnormalities that were found in infertile ewes are endometritis (19.69%) and pyometra (6.06%). The histopathological abnormalities of the genital tract were noted in 80.3% cases. The most common microscopical conditions of the ovary were ovarian fibrosis (10.60%), follicular cyst (10.60%), luteal cyst (9.09%), cystic corpus luteum (6.06%) and peri-corpus luteum fibrosis (6.06%). Histopathological lesions in oviduct included salpingitis and epithelial cell hyperplasia in 7.58% and 3.03% ewes, respectively. The most common pathological conditions of the uterus included endometritis (19.69%), pyometra (6.06%) and adenomyoma (1.52%). The hormonal analysis for LH, FSH, estrogen and progesterone in ovarian lesions showed a significant decrease values ($p<0.05$) as compared control. While significant decrease ($P<0.05$) in LH and progesterone were reported in grossly normal reproductive tract ewes as compared to control. In conclusion, the hormonal disturbances and uterine infections play important causes for non-pregnancy in ewes.

***Key words*:** Pathological, hormonal, ewes, Basrah

Introduction

The sheep is considered as one of the most important livestock (Esteves *et al*., 2018) and its importance comes in order of human consumption, as it was noted that the local consumer prefers sheep meat than others because of milch purpose, short pregnancy duration and multiple births (Prache *et al*., 2021). Ewes are considered as seasonal breeders, which exhibit estrus cycles when day lenghts become shorter (Simões *et al*., 2021). It means they are seasonal poly estrus animals (Clarke *et al*., 2009). Another researchers have also indicated that estrus cycles in ewes can continue throughout the months of the year (Gracia *et al*., 2019), however the pregnancy, lambing and weaning remain closed (Freitas-de-Melo *et al*., 2018).

The reproductive problems in ewes may lead to a temperory infertility or permanant sterility which leads to a decrease in the level of production and reproduction (Noakes *et al*., 2019). Many studies were carried out in the field of ewes reproduction which include issues of estrus, its stages and methods of using different hormones to increase the number of births per year (Hansel and Convey, 1983). However, the study of reproductive problems which leads to infertility were not deeply covered (Noakes *et al*., 2019). Infertility or sterility plays a major role in reducing the level of pregnancy in ewes, especially during the reproductive season. Several reasons that contribute to reduce the chance of pregnancy in ewes during the reproductive season include managemental, nutritional, hormonal and infectious causes (Ali *et al*., 2019).

Because of economic importance of ewes, it is necessary to take practical and scientific steps that lead to enhancement of the breeding and reproduction in order to improve sheep population, and increase the level of reproduction by early diagnosis of reproductive problems to reduce their occurrence and carrying out periodic examinations of ewes, especially pregnancy diagnosis to find out non-pregnant and infertile ewes to fix an appropriate medication for them.

The lack of studies on the causes of infertility in ewes and its relationship with non-pregnancy during breeding season in Iraq, and in order to determine the main pathological causes of temporary and permanent infertility, the current study was designed to study the following:

1. Identify some of temporary and permanent causes of infertility in ewes which may occur during breeding season that is accompanied by failure of pregnancy.
2. Investigate the most important pathological lesions in reproductive system of non- pregnant infertile ewes during breeding season.
3. Determine the hormonal and bacteriological causes in non-pregnant infertile ewes during breeding season.

Ethical Statement

The study was approved by Department of Pathology and poultry diseases, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Materials and Methods

Animals studied

The study included 66 non-pregnant matured ewes despite the presence of a fertile male during the reproductive season (during November and December 2020). The experimental animals were collected from the sheep farm owners of the Hay-Alhusain and Al-Qarma regions after they decided to slaughter non pregnant ewes. Absence of pregnancy was diagnosed by external examination in addition to clinical

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signs and case history from the owners.

Sample Collection

Blood samples

Blood samples in non EDTA vials were collected for serum isolation before slaughter for hormonal profile (FSH-LH-progesterone-estrogen).

Hormonal Analysis

1. Hormonal Analysis of FSH and LH

FSH and LH levels were estimated by sandwich immune detection method as described by Goldstein and Kosasa (1975) (AFIAS kit) (Boditech, Germany). The procedure of analysis was carried out as per manufacturer's guidelines. Assay sensitivity for FSH and LH were 0.05 ng/ml.

2. Hormonal Analysis of Progesterone and estrogen

Based on the principle of the immune-chromatographic assay, a special progesterone and estrogen kits were used (fluorecare®) (Biotech, Germany) to detect the concentration of progesterone and estrogen in the serum by competitive immunodetection method. The procedure of analysis was carried out as per manufacturer's guidelines. Assay sensitivity for progesterone was 0.05 ng/ml, while for estrogen 5 pg/ml.

Reproductive system samples

Specimen's of reproductive organs of ewes were stored in a cool box with normal saline and ice and transported to the pathology department /university of Basrah, College of Veterinary Medicine. Morphological examinations of collected specimens were recorded in detail. The amount and quality of the fluid or pus, as well as the organ's size, presence of ovarian cysts, abnormal gross lesion as well as the occlusion of oviduct by injecting the stained solution inside the oviduct were carried out. The lesions were cut and fixed in 10% natural buffered formaldehyde for three days before being prepared for histopathology procedures (James Zachary and McGavin, 2013).

Pathological study

1. Macroscopic Examination

A post-mortem examination was performed immediately after the animals were slaughtered, including optical inspection, palpation of the reproductive system texture, observation of any abnormal discoloration on the reproductive system, and making an incision for recording any lesion according to the methods described by (Collins and Huey, 2014).

2. Histological procedure

The collected specimens were processed histopathologically as per Bancroft *et al. (*2018).

Statistical analysis

SPSS version 25 was used to conduct the statistical analysis. Number and percentage were used to represent the data. One way ANOVA model was used to analyze the results of hormonal study. Differences were compared by Tukeys multiple comparison post hoc test. All data were representing as mean ± SEM, and the differences were considered as significant at $P<0.05$.

Results and Discussions

Gross reproductive tract abnormalities of non-pregnant ewes

The result of ovarian lesions showed inactive ovaries in 10.60%, follicular cyst in 10.60% and luteal cyst in 9.09% ewes. The result of oviductal lesion showed hydrosalpinix in 6.06% while oviductal occlusion in 4.56% cases (Table 1). The result of uterine lesion showed endometritis as most frequent cause in 19.69% ewes while the pyometra in 6.06% ewes.

Histopathological changes of reproductive tract in non –pregnant ewes

The results of ovarian lesion showed ovarian fibrosis and follicular cyst in 10.60% and luteal cyst in 9.09% ewes. The oviductal lesion showed highest frequency of salpingitis (7.58%) followed by epithelial cell hyperplasia (3.03%). The uterine lesion in our results showed that the highest value is endometritis which reached (19.70%) while the pyometra showed (6.06%) as in Table 2.

While the normal grossly appearance genital systems and during histo-pathological experiment showed 13.64% pathological lesions which included cystic corpus luteum and peri-corpus luteum fibrosis that reached 6.06% for both of them and adenomyoma (1.52%), while 19.70% from these system didn't show pathological lesions as in the Table 2.

The result of hormonal analysis in non-pregnant ewes suffered from ovarian lesions or normal appearance

The hormonal study revealed that the ovarian fibrosis showed a significant decrease ($p<0.05$) in FSH, LH, progesterone and estrogen as compare to control, while the follicular cyst revealed a significant increase ($p<0.05$) in the level of FSH (27.14± 4.22) as compare to control (12.64±1.83), while the level of LH, progesterone showed a significant decrease and level of estrogen didn't show any difference with control (table 3). In luteal cyst, the level of FSH, estrogen exhibited a significant decrease ($p<0.05$) as compare to control group while the LH level (11.61±3.28) found significantly higher ($p<0.05$) in compared control, not like progesterone which didn't show significant changes as compare to control. In cases of cystic corpus luteum and peri-corpus luteum fibrosis, the levels of FSH, LH, estrogen and progesterone showed significant decreases ($p<0.05$) as compared to control (Table 3).

The hormonal study of normal appearance cases showed that 10 cases have a significant decreases ($p<0.05$) in the level of LH and progesterone and no any variation occurred in the level of FSH and estrogen in compared control while 3 cases didn't reveal any differences in compared control as in Table 3.

Pathological result

Ovary

The microscopic lesions of ovary shows an ovarian fibrosis which characterized by a dense bland spindle like fibrotic structure particularly located in the ovarian cortex and in the area of perifollicular region. In other section of ovary shows an follicular cyst which characterized by a proliferating cyst lining that composed of an inner layer of granulosa cells and an outer layer of luteinized theca cells.

In addition pericorpus luteum fibrosis is characterized by

a dense bland spindle like fibrotic structure particularly in the pericorpal luteum region. Besides, the cystic corpus luteum is characterized by a proliferating cyst lining that composed of an inner layer of granulosa cells and an outer layer of theca cells that has a granulosa cells which are polygonal in shape, with abundant eosinophilic cytoplasm and central round nuclei, also outer theca cells are smaller in size prominent inner layer of fibrous tissue. While the luteal cyst which characterized by a cyst lined by a layers of luteinized cells that has a clear to pink cytoplasm, also it has no theca and granulosa layers, which shows a focal nuclear atypia particularly enlarged, pleomorphic and hyperchromatic nuclei associated with degenerative changes and covered by obvious fibrotic layer.

Oviduct

The histopathological section of oviduct shows an oviduct hyperplasia which is characterized by a benign proliferation of inner epithelial layer with an increases in columnar layer size and numbers, also it shows a degree of apoptotic processes. In addition, salpingitis which is characterized by infiltration of inflammatory cells mainly mono-nuclear cells which localized in the mucosal and submucosal layer; as well as to localized de-epithelization of mucosal layer.

Uterus

The histopathological section of uterus shows endometritis which is characterized by infiltration of inflammatory cells that localized in the mucosal and submucosal layer; as well as to localized increase the thickness of mucosal layer that may revealed to mucosal hyperplasia. Pyometra is also observed which is characterized by infiltration of inflammatory cells mainly polymorphic nuclear cells which localized in the submucosal layer, also it appeared in some area as a micro-abscesses. The adenomyoma which is characterized by atypical infiltration of uterine glands in the myometrium, and surrounded by a marked fibrous tissue were also observed.

Distribution of pathological study in reproductive system of non-pregnant ewes

The current study showed approximately 66% gross lesion from the total non-pregnant ewes. These lesions involved ovarian, oviduct and uterine lesions. While another study (Aljaafary, 2002) recorded a lower percentage of gross lesions (46%) as compare our results. This elevation in gross lesion in our study may be related with our samples which are of non-pregnant ewes no like the samples of other studies which include fertile and non-fertile ewes.

The ovarian lesion takes the higher percentage from the total lesion as compared to the other lesions which take approximately 30% from other lesions. Aljaafary (2002) recorded higher percentage of ovarian lesions which reached 67% as compared to our results. There are many reasons for the high percentage of ovarian lesions like hormonal defect, bad management, nutrition which play an important role for ovarian defect and consequently infertility (Beena *et al*., 2016).

In our results the uterine lesion had the second high value from the total gross lesion which involved 25%. Many studies indicated that the uterine lesion is an important cause for infertility in many species of animals. Aljaafary (2002) and (Hussien, 2019) were recorded a higher percentage of uterine lesion as compare to our results which take approximately 25.75% from another lesions. Aljaafary (2002) recorded 46% of uterine lesions while Hussien (2019) recorded 71% uterine lesions. There are many reasons for the high percentage of uterine lesions which perhaps due to the difference in the management system in which the animal lives, as well as inflammatory changes that may cause a lack of fertility and infertility (Hussien, 2019).

The oviductal lesions take the low percentage from the total lesions which take approximately 10.62%. Mohammed (2021) recorded a lower percentage of oviduct lesion which reached 3% in compared our result. The low percentage of oviductal lesion in his study may be as results of the nature and the origin of inflammation that extended from the uterus (Noakes *et al.,* 2019). In our results the high percentage of oviductal lesion confirms that there is a high proportion of uterine infection in the experimental ewes which extend to the oviduct and produce this defect.

The current study showed approximately 66% histopathological lesion from the total non-pregnant ewes. These lesions involved ovarian, oviduct and uterine lesions. While another study (Dokhan, 2000 and Abou-Rawash *et al.,* 2008) recorded a lower percentage of histopathological lesions (29%) as compare to our results. This elevation in gross lesion in our results may be related to the difference in the incidence which can be attributed to the difference in sheep breeds, breeding systems, management systems, localities and environment (Abou-Rawash *et al*., 2008).

Histopathological changes of reproductive tract in non –pregnant ewes

Ovaries

The microscopical result of ovary shows an follicular cyst which characterized by a proliferating cyst lining that composed of an inner layer of granulosa cells and an outer layer of luteinized theca cells, these results may occur due The incidence of cystic ovaries (follicular and luteal cystic) these results may occur due Insufficient luteinizing hormone before or during ovulation, animal age, and food insufficiency may all play a role in the development of cystic ovaries. The role of endotoxins produced by gram-negative bacteria in preventing preovulatory LH surge and ovulation, resulting in persistent ovarian cysts, could explain the effect of uterine infection on ovarian function. This result agree with (Karim *et al*., 2017) who mention follicular cystic characterized by a large fluid-filled cyst surrounded by a thick layer of theca cell layer with granulosa cell layer development in the cyst lumen in the ovarian stroma.

The microscopic results of the luteal cyst which is characterized by a cyst lined by a layers of luteinized cells that has a clear to pink cytoplasm, also it has no theca and granulosa layers, which shows a focal nuclear atypia particularly enlarged, pleomorphic and hyperchromatic nuclei

associated with degenerative changes and covered by obvious fibrotic layer, these results may occur due to cystic ovarian disorders are recognized as a major cause of infertility due to a disruption in hormonal balance. these result agree with (Silva *et al*., 2021) who mention The luteinized cyst, also evaluated, showed areas with a layer of lutein cells in the cyst wall with a yellowish color in the absence of the granular layer internal teak fibrosis and partial luteinization of the cystic wall these result agree with (Zeravan, 2021). Infiltration of the inflammatory cell into the cystic lumen was lined with a thin layer of fibrous connective tissue and surrounded by a thick layer of luteinised tissue.

The microscopical results of ovary shows an ovarian fibrosis which is characterized by a dense bland spindle like fibrotic structure particularly located in the ovarian cortex and in the area of peri-follicular region, these results may occur due to high oestrogen levels, problems in the hypothalamus-pituitary glands-ovary axis might result in an irregular oestrous cycle or failure of animals to return to oestrous cycle, leading in reproductive failure. These result agree with (Zeravan, 2021) who mention the ovary's stroma was fibrosed, and the ovary's cortex was surrounded by fibrous connective tissue. Numerous undeveloped follicles, known as primordial follicle, can be found in the cortex.

The microscopical result of the cystic corpus luteum which is characterized by a proliferating cyst lining that composed of an inner layer of granulosa cells and an outer layer of theca cells that has a granulosa cells are polygonal in shape, with abundant eosinophilic cytoplasm and central round nuclei, also outer theca cells are smaller in size prominent inner layer of fibrous tissue, these results may occur Caused by a lack of prostaglandin hormone at the end of the estrous cycle. these result agree with (El-Nesr *et al*., 2017) who mention shows small eosinophilic cell- bodies surrounded by granulosa cells in the shape of rosette within the theca externa, some of which seemed larger in size with conspicuous nuclei, and these result agree with (Hatipoglu *et al*., 2002) who mention showed that the cystic corpora lutea had a zone of fibrous connective tissue between the luteal tissue and the cystic cavity.

The microscopical study of ovary shows an peri-corpus luteum fibrosis which characterized by a dense bland spindle like fibrotic structure particularly in the pericorpal luteum region, these result agree with (Adenowo *et al*., 2005) who mentioned a mild fibrosis of the ovarian stroma with extensive fibrous connective tissue which results from the extension of infection from another part of genital system.

Oviduct

The microscopical study of oviduct shows an oviduct hyperplasia which characterized by a benign proliferation of inner epithelial layer in which an increases of the columnar layer size and numbers, also it shows a degree of apoptotic processes. The occurrence of these disorders can be attributed to various pathological factors such as infections, metabolic diseases or poisoning of some elements, as well as hormonal disorders. the estrogen has a role in the development of the epithelial cells lining the oviduct, unlike progesterone, which leads to weakness and the growth and decline of the sizes and diameters of these cells with the appearance of degenerative changes these result agree with Ali and Madboli (2013) who mention the presence of scattered areas of epithelium hyperplasia lining the cilia of the oviduct and these result agree with (Morales *et al.,* 2006) who mention the oviduct accompanied by infiltration of inflammatory cells and apoptotsis.

The microscopical result of uterus shows salpingitis which is characterized by infiltration of inflammatory cells mainly mononuclear cells which localized in the mucosal and submucosal layer (red arrows); as well as to localized de-epithelization of mucosal layer, these results may occur due the cases of oviduct inflammation in ewes as a result of the spread of infections from the endometrium, which can lead to infertility or permanent infertility if left untreated. The oviduct inflammation in ewes are mostly secondary that spread from the uterus with a non-specialized infection indicated that there are two facts that lead to the emergence of infections of the oviduct, namely, hydrops oviducts and tubular adhesions to the bursa or to the ovary or to any part around the oviduct, oviduct inflammation can lead to infertility depending on the severity of the infection. These result agree with Karim *et al.* (2020) who mention the uterine tube shows mild to moderate infiltration of chronic inflammatory cells within the subepithelial fibromuscular stroma, focal sloughing of the lining epithelium.

Uterus

The microscopical result of uterus shows endometritis which is characterized by infiltration of inflammatory cells that localized in the mucosal and submucosal layer (yellow arrows); as well as ocalized increase in the thickness of mucosal layer that may revealed mucosal hyperplasia, these results may occur due the endometritis as the most important common disease lesion of uterine abnormality indicating that this condition plays role in infertility. These results agree with Waheed and Kareem (2019) who mention endometritis characterized by inflammatory cells and epithelial hyperplasia, oedematous fluid, and uterine wall thickening.

The microscopical results of uterus shows pyometra which is characterized by infiltration of inflammatory cells mainly polymorphic nuclear cells which is localized in the submucosal layer, also it appeared in some area as a micro-abscesses structure, these results may occur due to some bacteria which were also isolated from sheep uterine infections and identified as important sources of uterine infection. These result agree with Zachary and McGavin (2012) who mention definite increased uterine wall thickness, particularly in the endometrium, and inflammatory cell infiltration with exudate.

The microscopical study of the uterus showed adenomyoma. The adenomyoma is characterized by atypical infiltration of uterine glands in the myometrium, and surrounded by a marked fibrous tissue, these results may occur due to made up of hyperplastic glands with uneven shapes Endometrial glands that are benign and have no architectural abnormalities, but are dangerously arranged in the smooth muscle. These findings are in agreement with Tuna and Doan (2008) who mention that uterine adenomyomas are technically benign. They are seen in the endometrium, lower uterine region, and occasionally the endocervix, and come in both common and uncommon types. They resemble endometrial polyps in appearance. The benign endometrial glands are haphazardly grouped inside the smooth muscle with no architectural abnormalities, but the atypical adenomyoma is made up of irregularly grown hyperplastic glands.

Table 1: Gross reproductive tract abnormalities of non-pregnant ewes

Gross lesion	No.	Macroscopic lesions	No.	Percentage (%)
Ovarian lesion	20	Inactive ovaries	7	10.60
		Follicular cyst	7	10.60
		Luteal cyst	6	9.09
Oviductal lesion	7	Oviductal occlusion	3	4.56
		Hydro salpinix	4	6.06
Uterine lesion	17	Endometritis	13	19.69
		Pyometra	4	6.06
Normal appearance	22	No lesion	22	33.34
Total	66		66	100

Table 2: Histopathological changes of reproductive tract in non – pregnant ewes

Lesion site	No.	Histo-pathological changes	Cases (No.)	Percentage (%)
Ovarian lesion	20	Ovarian fibrosis	7	10.60
		Follicular cyst	7	10.60
		Luteal cyst	6	9.09
Oviductal lesion	7	Salpingitis	5	7.58
		Epithelial cell hyperplasia	2	3.03
Uterine lesion	17	Endometritis	13	19.70
		Pyometra	4	6.06
Normal appearance	22	Cystic corpus luteum	4	6.06
		Peri-corpus luteum fibrosis	4	6.06
		Adenomyoma	1	1.52
		Normal appearance	13	19.70
Total	66		66	100

Table 3: The result of hormonal analysis in non-pregnant ewes suffered from inactive ovaries or showed normal appearance

Lesion site	No.	Histo-pathological changes	Cases (No.)	Percentage (%)
Ovarian lesion	20	Ovarian fibrosis	7	10.60
		Follicular cyst	7	10.60
		Luteal cyst	6	9.09
Oviductal lesion	7	Salpingitis	5	7.58
		Epithelial cell hyperplasia	2	3.03
Uterine lesion	17	Endometritis	13	19.70
		Pyometra	4	6.06
Normal appearance	22	Cystic corpus luteum	4	6.06
		Peri-corpus luteum fibrosis	4	6.06
		Adenomyoma	1	1.52
		Normal appearance	13	19.70
Total	66		66	100

Hormonal study

Our results revealed increasing in the level of FSH and LH in control group in compared infertile ewes. The FSH play an important role for follicular development and increasing in the level of estrogen from Graffian follicles, while the LH act as an ovulatory hormone for these follicles and developing the corpus luteum (Legan *et al.*, 1977).In the present study the ewes which suffered from ovarian fibrosis, cystic corpus luteum and peri- corpus luteum fibrosis showed a significant decrease in the level of FSH, LH, estrogen, progesterone in compared control group. This phenomenon play an important role in prevents any signs of estrus which followed with abnormal anestrus. These results were in agreement with many researchers (Petroviæ *et al*., 2012 and Khodakaram-Tafti and Davari, 2013) which proved the role of these hormones in ewe reproduction.

The ovarian cyst is regarded an important cause for infertility in many species of animals, which interfere with follicular waves in many species of animals, as a result of abnormal steroidal hormone production (Nanda *et al*., 1991). In follicular cyst the absent of LH hormone promote the follicular enlargement and increasing in the level of estrogen which interfere with hormonal control in these animals (Oz *et al*., 1985).In our results the cases of follicular cyst showed a significant decrease in the LH hormone with increasing in the estrogen. The results were in agreement with many studies which involved ovarian cyst (Oz *et al*., 1985 and Nanda *et al.*, 1991 and Christman *et al*., 2000). Like follicular cyst, the infertile ewes which suffered from luteal cyst, the significant decrease in the LH hormone also play a role in occurrence of this case (Christman *et al*., 2000). The low levels of ovulatory surge of LH hormone act as main cause for the incidence of luteal cyst which followed with high level of progesterone as our results. Many researchers fixed the role of LH for the occurrence of ovulation and the incidence of luteal cyst in case of any abnormal secretion for LH (Oz *et al*., 1985 and Christman *et al*., 2000).

Also, in our results the infertile ewes which didn't show any abnormalities in the macroscopical and microscopical examination, showed a significant decrease in the level of LH hormone. This result improve these ewes suffer from delay or absent of ovulation which prevent the chance of fertilization which was in agreement with researchers (Ozturk *et al*., 1998 and Dobson and Smith, 1998). This case occur frequently in animals which suffered from mal-nutrition and decreasing in the mechanism of protein hormone synthesis (Dobson and Smith, 1998). While the other ewes which didn't show any abnormalities in the macroscopical and microscopical examination and also didn't show any differences in the level of hormones (FSH, LH, estrogen and progesterone) in compared control, these cases may be occurred as a result of infertile male or didn't inseminate with fertile male during breeding season. These results were in agreement with many studies about the role of fertile male and the number of each ram with the number of fertile ewes (Cushwa *et al.*, 1992 and Ungerfeld *et al*., 2008).

The current study concluded that the ovaries have the highest percentage of lesions among other genital organs in non-pregnant ewes in compared uterine lesion while the hormonal disorder play a very important role for non-pregnancy in ewes. Also the delay in ovulation is regarded one of the causes that lead to infertility in ewes and consequently prevent the occurrence of pregnancy as well as the ratio between the number of fertile males with the number of female is a critical factor for the occurrence of pregnancy in ewes. Bacteriological study concluded that the *Pseudomonas spp.* and *Shigella spp.* are the main bacteria which infected the genital system of ewes.

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