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COMPARATIVE STUDY OF HORMONAL AND HISTOPATHOLOGICAL CHANGES IN FEMALE *SWISS ALBINO* MICE REPRODUCTIVE SYSTEM DUE TO INFECTION BY *B. ABORTUS* AND B. *MELITENSIS*

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ABSTRACT
The present study aimed to investigate the effect of *B. abortus* and *B. melitensis* on the histological status of female mice reproductive system. This study was carried out at College of Veterinary Medicine- Baghdad, during the period from August to December 2017. Thirty adult females *Swiss albino* mice, aged two months, were divided into three equal groups. First group injected with *B. abortus* antigen. Second group was injected with *B. melitensis* antigen. Third group considered as a negative control. All animals were euthanized and blood samples were taken for hormonal analysis and pieces of ovary, uterus and oviduct were fixed in 10% normal buffer formalin for routine histopathological examination. Results showed that severe pathological lesions in the animals injected with *B. melitensis* antigen compared with animals injected with *B. abortus.* The levels of progesterone (ng), FSH (12.24 ng) and LH (10.10 ng) showed a significant ($P<0.05$) decreasing in the serum levels of the animals injected with *B. melitensis* antigen with mean of 0.236, 12.24, and 10.10 ng/ml respectively and the animals injected with *B. abortus* with corresponding means of 0.212, 14.78, and 18.76 ng/ml as compared with those levels in the control negative group.

KEYWORDS: Histopathology, ovay, Estrogen, *Brucella.*

INTRODUCTION

Brucellosis, which is a zoonosis transmitted by ingesting contaminated food (such as unpasteurized milk products), direct contact with an infected animal, or inhalation of aerosols. Transmission from human to human, for example through sexual intercourse or from mother to child, is exceedingly rare, but possible[1]. Causing chronic disease, which usually persists for life? Four species infect humans and animals: *B. abortus*, *B. canis*, *B. melitensis*, and *B. suis*. *B. abortus* is less virulent than *B. melitensis* and is primarily a disease of cattle. *B. canis* affects dogs. *B. melitensis* is the most virulent and invasive species; it usually infects goats and occasionally sheep. *B. suis* is of intermediate virulence and chiefly infects pigs[2].

Brucella abortus. Various spp of *Brucella* can cause contagious abortion in animals as a natural host, such as cattle, sheep, goats, swine and dogs[3], but rarely induced abortion in women[4] reported that 15 among 60 women suffering from abortion or stillbirth were *Brucella* sero positive, however, the relationship between abortion and stillbirth in human and Brucellosis was controversial[5] this may be due to absence of erythritol in human placenta and fetus in contrast to ruminant placenta, as well as presence of anti-*brucella* activity in human amniotic fluid[7] but in vitro[6] reported that *Brucella* can replicated in human placental trophoblast cells and the pathogen firstly growth in the extracotyledonary trophoblasts then spread to cotyledonary trophoblasts. Microscopic lesions of the reproductive organs infected with Brucella showed degeneration or necrosis in those organs. Congestion or haemorrhage lesions were found to be mild to moderate in all of the reproductive organs of mice in Brucella group, while mice in group has except for ovary which showed moderate to severe congestion. These findings proved that oral inoculation of 0.4 ml×10^9 of *Brucella immunogens* were able to produce cellular changes in the female reproductive organs, as supported by the study of [8].

MATERIALS & METHODS

Experimental design

Thirty adult Swiss Albino mice at the age of two months were divided into three groups (each group ten animals). First group injected I/P with 0.4 ml of bacterial suspension containing 1 x 10^9 CFU/ ml of viable virulent *B. abortus*. Second group was injected with *B. melitensis* I/P with 0.4 ml of bacterial suspension containing 1 x 10^9 CFU/ ml of viable virulent *B. abortus* Third group administrated orally with 0.3 ml of normal saline and served as control negative group.

TREATMENT

Brucellin preparation: This antigen was prepared according to (Mitov).

Hormonal analysis:-

Blood sample should be collected from heart in mice group in each group treated but when die animal that not collected because blood clotting and sera transferred in to epindrof tube after that kept in refrigerator in a stand position then centrifuge at 1500 rpm for 3 minute and kept in the freezer at -20 until used.

Hormonal analysis was done according to [25] this method is called radio immune assay

Histopathology

Pieces of ovary, uterus and oviduct were fixed in 10% buffer formalin for 72 hours for routine histopathological examination[23].

Statistical Analysis

The data were analyzed by one way ANOVA using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). The significance level was designated at $P < 0.05$.

RESULTS

A significant reduction in the serum levels of progesterone, FSH, LH and estrogen in animal injected with *B. melitensis* and *B. Abortus* as compared with the levels in the control negative (Table, 1).

TABLE 1: The effect of *B. melitensis* and *B. abortus* on serum Estrogen, FSH, LH & Progesterone concentration (ng/ml) in adult infected mice for 4 weeks

The Group	Mean ± SE (ng/)			
	Estrogen	progestrogen	LSH	LH
Control	27.82 ±1.04 C a	1.740 ±0.17 BC a	36.26 ±0.44 A a	23.16 ±0.36 B a
Br. Mellitusis	33.02 ±0.53 C a	0.236 ±0.04 D c	12.24 ±1.23 C b	10.10 ±0.36 D d
Br.abortus	29.96 ± 0.25 0-E b	0.212 ±0.04 D b	14.78 ±0.42 C b	18.76 ±0.89 C a

Histopathological examination of uterus of animals injected with *B. melitensis* characterized by neutrophils infiltration with fibrin networks deposition in serosal layer and between muscular layer and in sub epithelial and muscular layer (Figure, 2), in other sections, neutrophils, plasma cells, macrophages and lymphocytes infiltration between uterin gland (Figure, 3), and mucosal glands were seen , as well as between muscular layer in other sections, it was observed squamous metaplasia of epithelial cells of uterine gland with keratine in their lumen (Figure, 5), in addition to hemorrhage in muscular layer (Fig. 6), as well as hyperplasia of epithelial layer of endomaterium (Figure, 5) in other animals, granulomatous lesion consisting from aggregation of macrophages and lymphocytes were seen in subepithelial layer (Figure,6), as well as neutrophils and mononuclear cells in the lumen of dilated uterine glands (Figure,7).The histopathological examination of Oviduct revealed inflammatory cells infiltration in the serosa extended to the oviduct layer (Figure, 8) as well as Inflammatory cells infiltration in the lamina properia (Figure, 3), in addition to folding epithelial lining cells fused together form cystic like structure (Figure,15).Histopathological examination of ovary showed congestion blood vessels with neutrophils in their wall were the main lesions in the ovary (Figure, 9). in addition to Cellular debris in the center of the degenerative follicles (Figure,10),Histopathological examination of animals injected *with B. abortus* of Uterus characterized by congestion of the blood vessels, edema and macrophage, neutrophils and eosinophils infiltration in the sub epithelial layer (Figure,12). In addition to focal squamous metaplasia of epithelial layer of mucosal glands (Figure, 11). The microscopic section of the oviduct revealed inflammatory cells infiltration in the lamina properia (Figure, 15). Histopathological examination of Ovary recorded degenerative changes in the granulose cells with fluid in the lumen of degenerative follicle (Figure, 16).

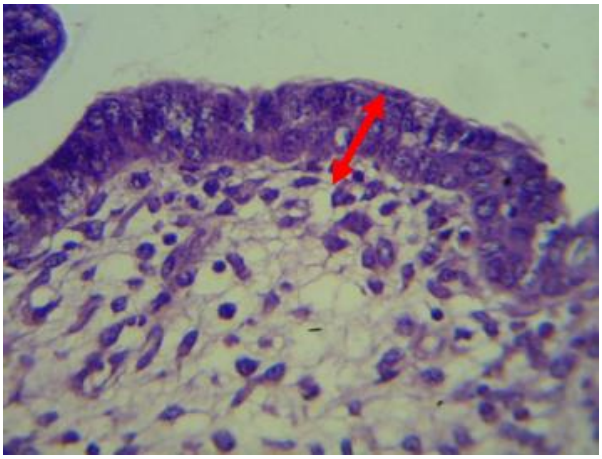


FIGURE 1: Histopathological section in the uterus of infected with *B. melitensis* showed hyperplasia of epithelial layer ⟷ (H&E stain 40X).

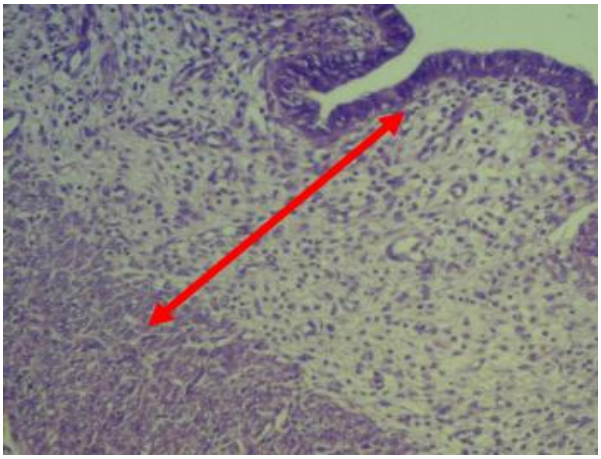


FIGURE 2: Histopathological section in the uterus of infected with *B. melitensis* showed neutrophils infiltration with fibrin networks deposition in sub epithelial and between muscular layer ⟷ (H&E stain 10X)

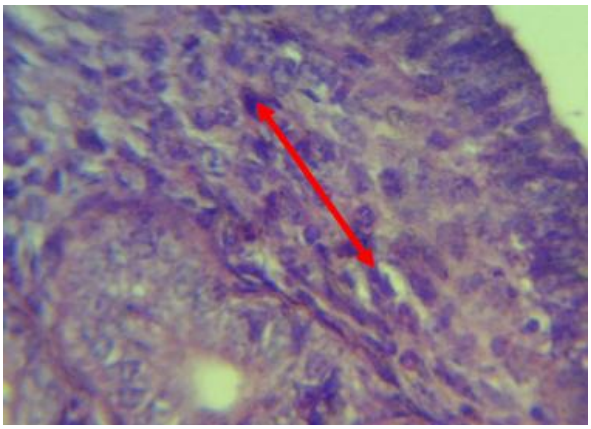


FIGURE 3: Histopathological section in the uterus of infected with *B. melitensis* showed neutrophils,plasma cells, macrophages and lymphocytes infiltration between mucosal glands (H&E stain 40X).

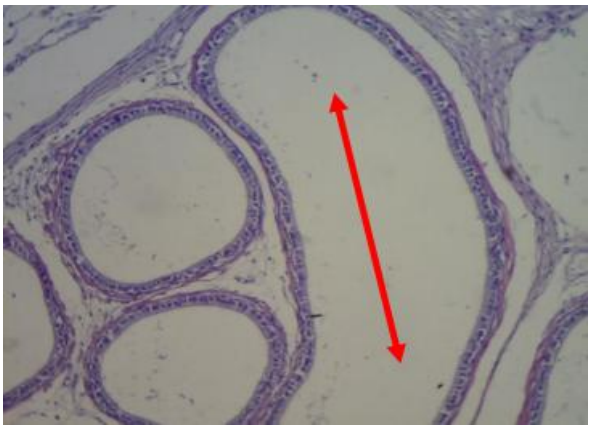


FIGURE 4: Histopathological section in the uterus of infected with *B. melitensis* showed dilated uterine gland (H&E stain 40X).

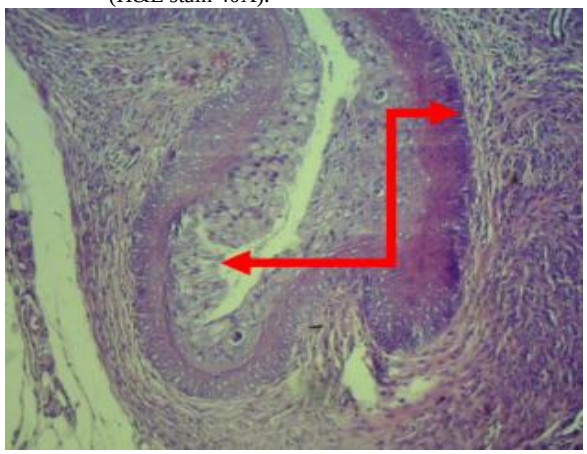


FIGURE 5: Histopathological section in the uterus of infected with *B. melitensis* showed inflammatory cells particularly neutrophils infiltration in serosal and muscular layer (H&E stain 10X).

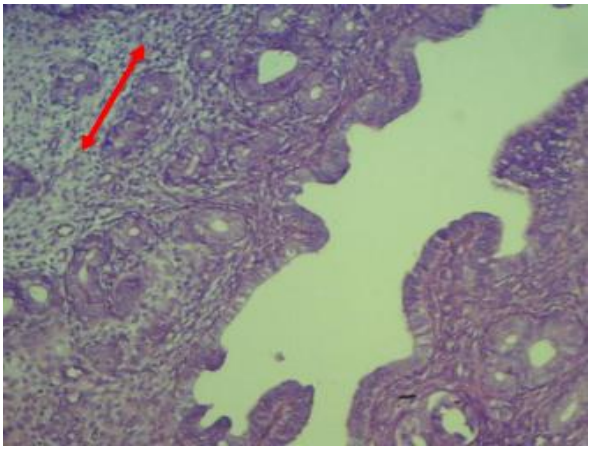


FIGURE 6: Histopathological section in the uterus of infected with *B. melitensis* showed severe inflammatory cells infiltration between uterine H&E stain 40X).

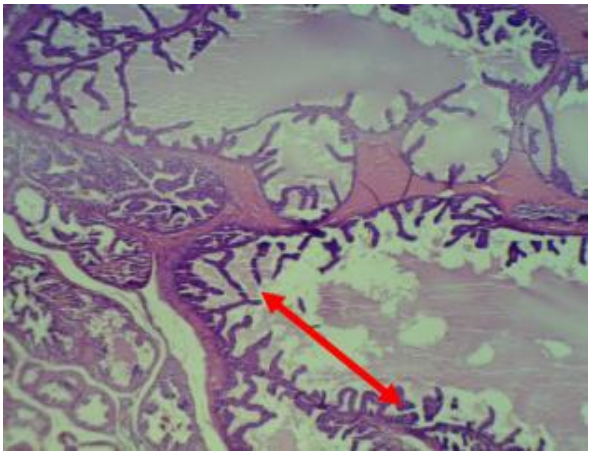


FIGURE 7: Histopathological section in the oviduct of infected with *B. melitensis* the papillary projection of epithelial layer fused together form cystic like structures (H&E stain 40X)

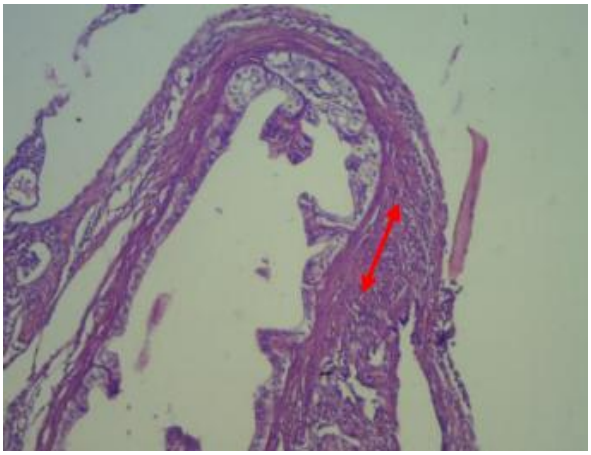


FIGURE 8:-Histopathological section in the oviduct of infected with *B. melitensis* showed inflammatory cells infiltration in the wall and lamina properia of the oviducts (H&E stain 10X)

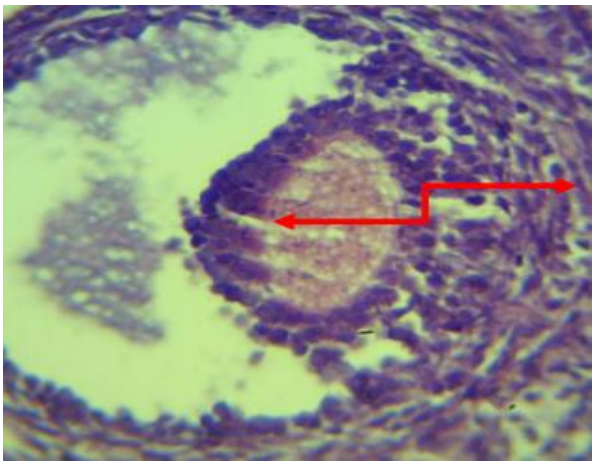


FIGURE 9:-Histopathological section in the ovary of animal with *B. melitensis* showed degenerative follicle with oocyte in antrum (H&E stain 40X)

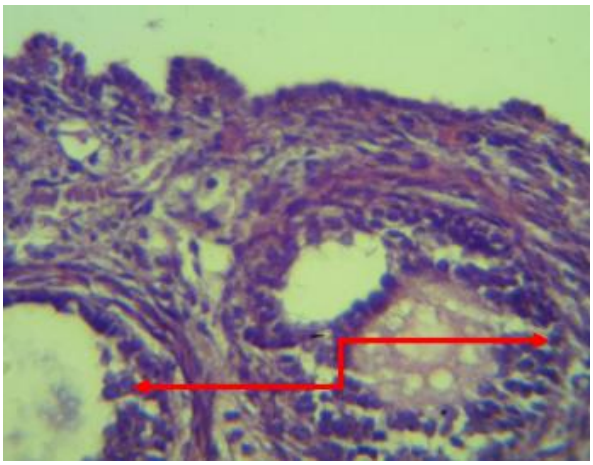
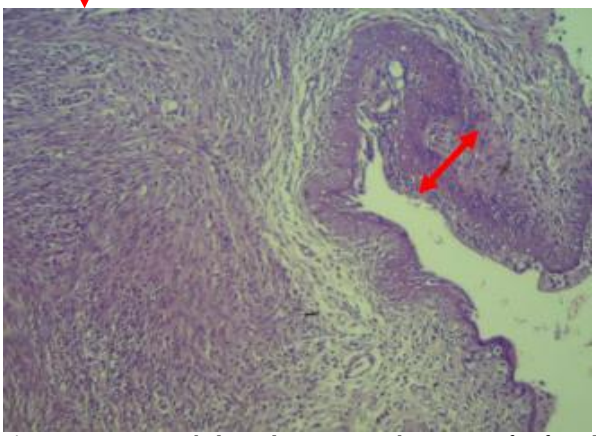
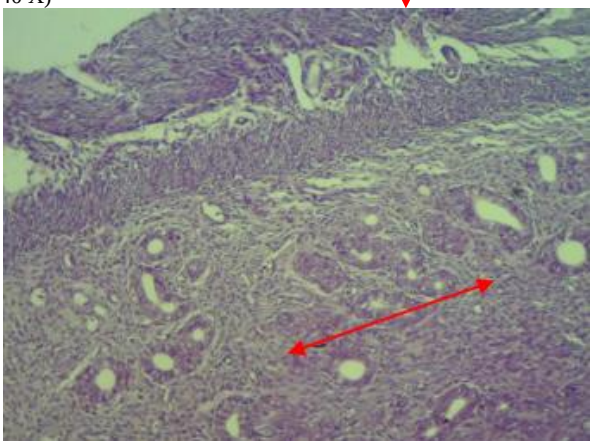


FIGURE 10:-Histopathological section in the ovary of animal with *B. melitensis* showed mononuclear cells and cellular debris in the antrum of the degenerative follicle (H&E stain 40 X)



Figure, 11:-Histopathological section in the uterus of infected with *B. abortus* showed hyperplasia of epithelial layer (H &E stain 10X).



Figure, 12:-Histopathological section in the uterus of infected with *B. melitensis* showed severe inflammatory cells infiltration between uterine gland (H&E stain 40X).

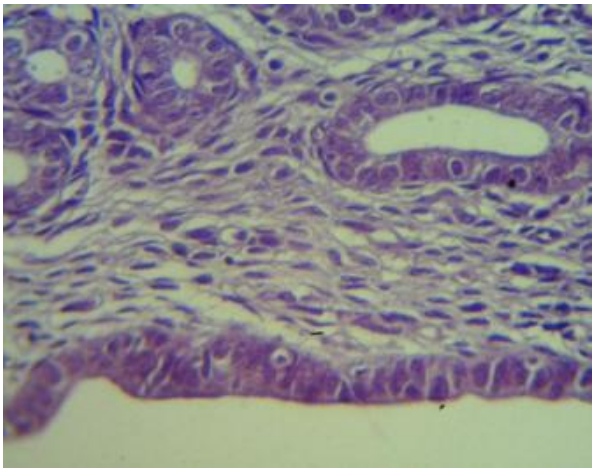


FIGURE 13:-Histopathological section in the uterus of infected with *B. abortus* showed severe inflammatory cells infiltration between uterine gland (H&E stain 40X).

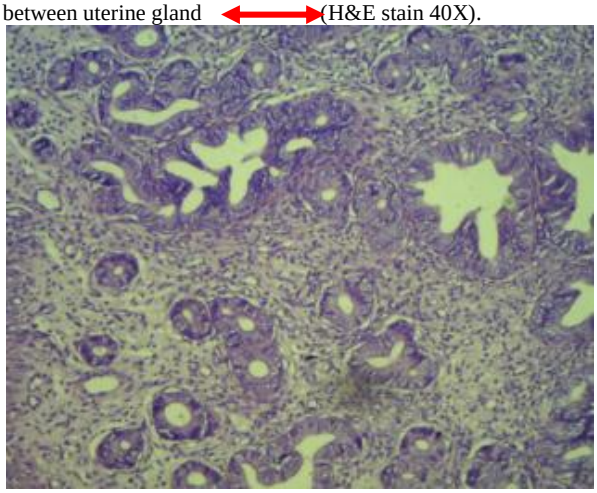


FIGURE 14:-Histopathological section in the uterus of infected with *B. abortus* showed severe inflammatory cells infiltration between uterine gland (H&E stain 10X).

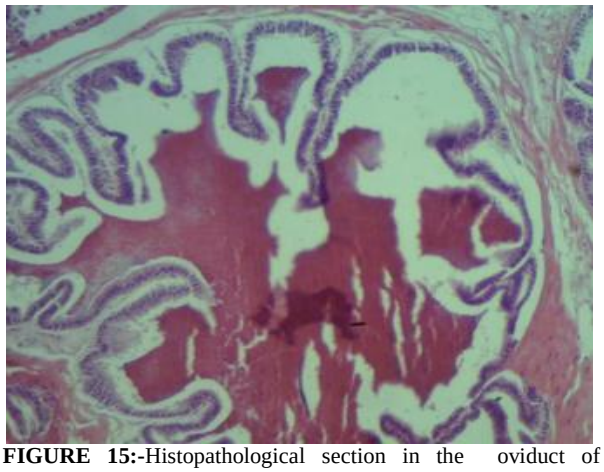


FIGURE 15:-Histopathological section in the oviduct of infected with *B. abortus* the papillary projection of epithelial layer fused together form cystic like structures (H&E stain 10X)

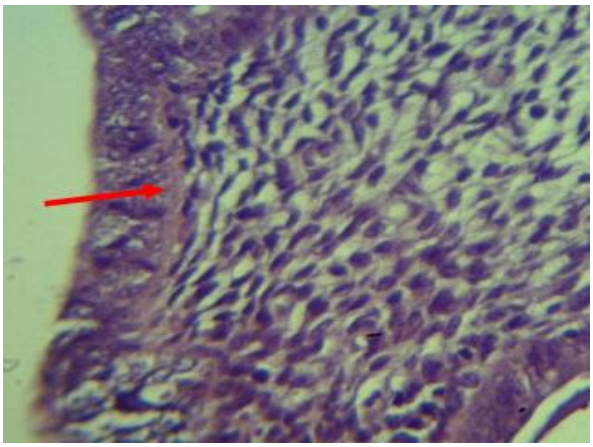


FIGURE 16:-Histopathological section in the uterus of infected with *B. abortus* showed hyperplasia of epithelial layer (H&E stain 10X).

DISCUSSION

The low levels of serum FSH,LH and progesterone in animals infected with *B.abortus* and *B. melitensis* as compared with values of the control negative group may be due to high levels of estrogen in which may cause negative feedback in gonadotropin secretion probably from both direct effects of estrogen on the pituitary gonadotropins to reduce more secret FSH and LH in response to GnRH and indirect effect by stimulating the hypothalamic neurons that secret GnRH with modulation of the frequency and magnitude of the pulses of GnRH[9]. As well as The present finding explained that the serum levels of estrogen, FSH and progesterone in animals infected with Brucella this result may indicate that severe degree of infection of the ovary which posses normal defense mechanism against Brucella infection and this observation also may indicated that severity of Brucella infection may induced impairment of ovary activity[10]**.** Brucella, stimulated a release of free radical that associated with failure of pregnancy and infertility of the females, this idea agreesment with previous reports[19] who investigated that angiogenesis plays a crucial role in the development of ovarian follicles, endometrial growth, embryo development, growth of placental blood vessels and[20] showed that estrogen stimulated angiogenesis in the endometrium by controlling the release of VEGF. ROS were essential for VEGF signaling **,** also ROS stimulat the cyclooxygenase enzyme through activation of transcription factor NFkB resulting in release of prostaglandin F2alpha **.**Free radical causes birth defects[21] and abortion **.** Very severe pathological lesions were noticed in the reproductive tract of animals infected with *B.abortus* and *B. melitensis* this result is in agreement with [11] who showed that *B.abortus* induced abortion in pregnant cows as a result of ,placentitis that occurs due to intracellular replication of *Brucella* within trophoblastic cells and induced placentitis**.**Female reproductive tract, of mice infected I\P with $1X10^9$ *B.abortus* expressed pathological lesions, this result may indicate this dose and route of infection can cause 100% infection of animals, this result is consistent with[12] who found that 100% infected mice after I\P inoculated with1x 10^4 to $1x10^7$CFU\mouse phagocyes were quickly engulfed smooth *Brucella* after I\P infection and the bacteria may reach the blood stream through the peritoneal capillary system14 and the bacteria were distributed throughout the RES testes, joints and placenta during the 1st week [13]. This result agreement with[14] has conducted a similar study in male mice intraperitoneally inoculated with Brucella and its LPS and it has presented significant vagaries in male reproductive organs. Though, information on clinical response and reproductive pathology in female mice orally inoculated with Brucella**.** Very severe pathological lesions were noticed in the reproductive tract of animals infected with *B. melitensis* more than *B. abortus* this result may be due to *B. melitensis* have major virulence factor[15,16]. Either smooth or rough alternatives depending on the appearance of O-Polysaccharides (OPS) as a component of the bacterial outer membrane LPS[17,18]. The smooth LPS (S-LPS) are composed of three domains: the lipid A, the core oligosaccharide and the immunodominant portion of the molecule-the O side chain, also called the O antigen [15]. Apart from the ability to avoid the killing mechanism within macrophages, the low biological activity induced by Brucella S-LPS might be one of the factors contributing to the survival of these pathogens in phagocytic cells[12, 18]. Contributing to the survival of these pathogens in phagocytic cells[24,18].

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