CLINICAL AND HISTOLOGICAL STUDY OF THE EFFECTS OF UTERINE INFECTIONS ON THE PREGNANCY OF DAIRY COWS IN SULAIMANI REGION

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ABSTRACT
The proposal of the present study was conducted to investigate the effects of uterine infections on the pregnancy rate; dairy cows were culled as results of infertility were evaluated by the histopathological lesions of infected uterus. Throughout the clinical study, a total of 75 dairy cows were examined, 19(25.33%) were pregnant, 40(53.33%) were normal uterus and 16(21.33%) were suffered from uterine infection. Out of 150 bovine genital tracts during macroscopic examination, 45(30%) were found pregnant uterus, 78(52%) were found normal non pregnant uterus. The results of the microscopical and histopathological studies reached 27(18%) of endometritis represented by acute and chronic endometritis which was 8(29.62%), and 19(70.37%) respectively. Microscopic examination of acute endometritis revealed infiltration of PMN (polymorphonuclear cells) in the submucosal and among the uterine glands. The microscopical examination of chronic endometritis appeared infiltration of mononuclear inflammatory cells represented by plasma cells, macrophage and lymphocyte in submucosal layers with proliferation of fibrin, as well as presence of necrotic debris inside the lumen of uterine glands. The present results indicated that histopathological and clinical studies are still common methods for the diagnosis of endometritis in dairy cows.

KEY WORDS: clinical, macroscopic, endometritis, histopathology, dairy cows.

INTRODUCTION
In the last decade, milk yield per dairy cows has increased considerably due to continuing genetic selection and improvement of national and herd management. Simultaneously with this selection for production characteristic, dairy cow fertility has declined significantly (Butler, 2003). In addition, a cow’s reproduction performance plays an important role in culling decisions (Rajala-Schults and Garanu, 2001). So; good fertility positively affects a cow active herd life and plays an important role in dairy economics. Commercial dairy managers spend much time and effort trying to achieve pregnancy rate and tare quite reluctant to cull animals once they become pregnant. For example non pregnant cows were 7.5 times more likely to be culled than pregnant cows (Gronu et al., 1998). Cattle are deemed fertile when they are neither normally fertile nor completely sterile or infertility which represent the diminish or absent capacity to produce viable offspring (Roberts, 1986). Uterine disorders, primarily nonspecific infections, reduce reproductive efficiency of dairy cows. In some herd, 40% of the postparturient cows may be diagnosed with and treated for uterine infections. In addition reducing reproductive cost, often reduce food consumption, cause a noticeable reduction in milk production, reduce pregnancy rate and force producers to cull cows that would otherwise be productive and remained in the herd (Bartlet et al., 1986, Olson et al., 1986, Dhaliwal et al., 2001, Hammon et al., 2006). Clinical signs, rectal palpation of the uterus, vaginoscopic examination and uterine biopsy are techniques available for the diagnosis of uterine infections (Drillich et al., 2001, LeBlanc et al., 2002, Kasimanikam et al., 2004).

MATERIALS & METHODS
This study involved two parts: The first part, the study was conducted clinically on a small private field of cross and native dairy cows. Whereas the second part of the study was done through the gross investigation of the female reproductive tract in the slaughter house of Sulaimani abattoir. A total 75 lactating cows were involved in this study. The ages of cows were various ranging between 3 to 12 years. Cows are examined clinically depending upon the case history and rectal palpation to detect the pregnancy and uterine infections. Infected cows with uterine infections are examined vaginally by sterile vaginal speculum as well as by massage of the reproductive tracts through rectal palpation to evaluate the nature of the discharge based on the color and volume of pus. If the vaginal discharge shows clear translucent mucus character, the score is 0 and when it shows clear mucus pus containing flecks of white pus character, the score is 1 and when it contains ≤50% white or yellow pus character, the score is 2, and when it contain ≥50% white, yellow or bloody pus character, the score is 3 (Sheldon et al., 2006). Through the macroscopic study of a total 150 specimens of the female genital tracts, the uteri are classified as pregnant and non pregnant and the non pregnant uterus is incised to examine grossly to identify the infected and non infected uterus. Tissue samples were taken from the abnormal infected uterus for histopathological study. The samples were fixed in 10 %...
neutral buffered formalin and processed for paraffin embedding and stained with haematoxylin and eosin.

RESULTS
Clinical study is conducted on native dairy cows in different villages of Sulaimani district. The result of the study was obtained through the clinical and vaginal examination of cows. Out of 75 cows examined, 19(25.33%) were pregnant, 40(53.33) had not infected uterus and 16(21.33%) suffered from uterine infections table (1). During the gross or macroscopic inspection of 150 specimen of bovine genital tracts which are brought from the Sulaimani abattoir. Out of 150 bovine genital tracts, 45(30%) were found pregnant, figure (1), 78(52%) were found with non infected uterus (normal uterus) figure (2), and 27(18%) were found with infected uterus with endometritis, figures, (3, 4). The result showed that 19 (29.68%), and 45(70.31%) of the pregnancy rate were seen in the clinical and macroscopic study respectively table (2). A total of 27 specimens of female reproductive tracts showed uterine infections macroscopically according to the type of the discharge which was visible after uterine wall incision. Depending on the histopathological examination, the types of inflammations of the uterus were classified into two forms, 8(29.62%) acute and 19(70.37%) chronic endometritis according to the type of inflammatory exudates and the period of inflammation, table (3). Microscopic examination of the acute endometritis revealed to sloughing of the epithelial lining of the endometrium and capillaries of blood vessels and infiltration of PMN (polymorphic nuclear inflammatory cells) in the sub mucosal and among the uterine glands, figures, (5, 6, 7, and 8). While in chronic endometritis, the microscopic examination appeared infiltration of mononuclear inflammatory cells represented by plasma cells, macrophage and lymphocyte in sub mucosal layer, figure (9), with proliferation of fibrin (collagen fiber), connective tissue around the atrophied uterine gland, figure, (10). It also showed multifocal granular nodules in the sub epithelial layer, in addition to presence of necrotic debrì inside the lumen of endometrial glands, figures (11and12), and narrowing of the uterine arterial due to hyperplasia of the arteriolar smooth muscle, figure (13).

| TABLE 1: Investigation of female reproductive tracts in clinical and macroscopic study of cows |
|---------------------------------|-----------------|-----------------|-----------------|
| Specimens/cows                  | Clinical study  | Macroscopic study | Total           |
| No                             | %               | No              | %               | No              | %               |
| Total examine                   | 75              | 33.33           | 150             | 66.66           | 225             | 100             |
| Pregnant                       | 19              | 25.33           | 45              | 30              | 64              | 28.44           |
| Non uterine infections         | 40              | 53.33           | 78              | 52              | 118             | 52.44           |
| Uterine infections             | 16              | 21.33           | 27              | 18              | 43              | 19.11           |

| TABLE 2: Showing the incidence of pregnancy rate in cows |
|---------------------------------|-----------------|-----------------|-----------------|
| Specimens/cows                  | Clinically gravid uterus | Macroscopically gravid uterus | Total |
| No                             | %               | No              | %               | No              | %               |
| Pregnant                       | 19              | 29.68           | 45              | 70.31           | 64              | 100             |

| TABLE 3: Histopathological classification of endometritis in cows |
|---------------------------------|-----------------|-----------------|-----------------|
| Uterine infection               | Acute endometritis | Chronic endometritis | Total |
| No                             | %               | No              | %               | No              | %               |
| Endometritis                    | 8               | 29.62           | 19              | 29.68           | 27              | 100             |

**FIGURE 1:** Pregnant uterus (black arrow) (twin).

**FIGURE 2:** Non pregnant (normal uterus)
FIGURE 3: Mucus in uterine cavity (black arrow).

FIGURE 4: Purulent discharge in uterine horns.

FIGURE 5: Histomicrograph of the uterus showing sloughing of epithelial lining of the endometrial layer (arrow, a), infiltration of inflammatory cells in the submucosal layer (arrow, b) and in the lumen of uterine gland (arrow, c) (H&E stain) X100.

FIGURE 6: Histomicrograph of the uterus showing infiltration of polymorphonuclear inflammatory cells in the submucosal layer of the endometrium (arrow a & b) (H&E stain) X100.

FIGURE 7: Histomicrograph of the uterus showing infiltration of polymorphnuclear cells (arrow, a) and in the lumen of endometrial gland (arrow, b) (H&E stain) X100.

FIGURE 8: Histomicrograph of the uterus showing infiltration of polymorphonuclear cells (arrow, a) and presence of necrotic debr in submucosal layer of endometrium (arrow, b) (H&E stain) X400.

FIGURE 9: Histomicrograph examination of the uterus showing infiltration of mononuclear inflammatory cells in the submucosal layer of the endometrium (arrow, a & b) (H&E stain) X100.

FIGURE 10: Histomicrograph examination of the uterus showing infiltration of mononuclear inflammatory cells in the submucosal layer of the endometrium (arrow, a) and atrophy of uterine glands (arrow, b) (H&E stain) X100.
DISCUSSION

The objectives of this study are to investigate the effects of uterine diseases on the pregnancy rate, to know the incidence of slaughtered dairy cows due to infertility and conducting the histopathological study of uterine infections. Clinical signs, rectal palpation of the uterus, ultrasonography and visual inspection of the vulva for purulent discharge are common methods for the diagnosis of endometritis in dairy cows (Roberts, 1986, Drillich et al., 2005, Risco et al., 2007, Gumen et al., 2012). During the period of the present study, a total of 75 cows were examined clinically, 19(25.33%) are found pregnant. This incidence is considered lower in comparison with other researches (White et al., 1989, Rosenbaun and Warnik, 2004, Frick, 2002). The pregnancy rate is decreased in dairy farms when postpartum diseases such as metritis, clinical and subclinical endometritis, and pyometra are represented as causes of reproductive inefficiency (Lewis, 1997, Quintela et al., 2004), through the effect on reproductive performance as increased service preconception and reduce pregnancy rate. In addition it could mean significant economic loss for the dairy industry (Bartlett et al., 1986, Fourichon et al., 2000, Foldi et al., 2006). However, cows with positive subclinical endometritis had a relative pregnancy rate 21.4% with high significant association compared to cow free of subclinical endometritis (Moges and Jebar, 2012). The results of the clinical study demonstrated (16) 21.33% of uterine infections represented by endometritis. This incidence is nearly similar to that recorded by (Kasimanickam et al., 2004, Gilbert et al., 2005), who considered the conditions of endometritis as a prevalent problem affection 35% to %51% of cows at 40 to 60 days in milk, with substantial decrease in pregnancy rate. While Sheldon et al., (2006) mentioned that the percentage of endometritis in his study reached 16.9%. Furthermore subclinical endometritis, puerperal metritis and endometritis are found to be major reproductive problems with a prevalence rate of 51.8%, 21% and 15%-7, respectively ( Hammon et al., 2006).The discrepancy of the incidence rate may be attributed to the methods of diagnoses used for uterine lesions, population of cows from year to year and from breed to breed( Herenda 1987, Hatipoglu et al., 2002), and the classification of the uterine infections selected( Curtis et al., 1985, Markusfeld, 1987). Conditions of uterine inflammations result in inadequate hygiene condition in the postpartum period during parturition, retained placenta, abortion and traumatic laceration due to dystocia ( McEntee, 1990, Arthur et al., 1996, Youngquist and Shore,1997, Hafez and Hafez, 2000, Fluid et al., 2006). Through the investigation of macroscopic study, a total of 150 bovine genital tracts were examined, 45(30%) of the cows slaughtered were pregnant and this agrees with the result of Durgut et al., (2003), that the incidence reached 31%, while the percentages were considered higher than mentioned by (Al-dahash, 1976, Herenda, 1987, Fathalla et al.,, 2000), which were (23.36%, 6.6%, 13.5), respectively. This difference is probably due to bad economic condition, in addition to the difficulty of animal management by the owners, besides the value of cows for slaughtering has
increased in the last few years and depended upon the laws of the countries which prevent slaughtering pregnant cows. The total incidence in the present macroscopic study is represented by 27(18%) endometritis and this is considered lower than those observed by (Herenda, 1987, Fathalla et al., 2000, Haptipoglu et al., 2002, Durgut et al., 2003) which was 5%, 3.50%, 13%, and 20.23% respectively. The variation of the incidence among these studies may be due to many factors involved; poor sanitation during parturition, retained placenta and absence of suitable treatment for uterine infections which absolutely results in increases in the incidence of cows culling. The results of the microscopical and histopathological studies reached 27(18%) of endometritis represented by acute and chronic endometritis which were 8(29.62%), and 19(70.37%) respectively. The prevalence of endometritis during the study was lower than that observed in other studies that based the diagnosis of endometritis on rectal palpation of genital tract after 26 days post partum (Drlilich et al., 2005, Gilbert et al., 2005, Bademkiran et al., 2009). In addition, macroscopic examination cannot depend on the limitation, if the specimens are intact or infected (Durgut et al., 2003, Al-Zebary, 2008). Endometritis has been diagnosed by detection of fluid in the uterus using ultrasonography (kasimanickam et al., 2004), palpation of the genital tract per rectum (LeBlanc et al., 2002). Nonetheless, these methods were found to be less sensitive than endometrial cytology (Barlund et al., 2008 Sheldon et al., 2006, Prieto et al., 2012), and endometrial biopsy examination (Ahmad et al., 2003, Garoussi et al., 2010). Finally, the incidence of endometritis depends on the sensitivity of the diagnostic method and the time of postpartum when the examination was performed (Drlilich et al., 2005, Gilbert et al., 2005). Endometritis, through the microscopic examination of pathological lesion, is characterized by inflammation of the endometrium with atrophy of the arterial and uterine glands as well as infiltrations of polymorphonuclear cells in the submucosal layer and among the uterine gland (McEntee, 1990, Sheldon et al., 2006, Oral, 2009).

REFERENCES


Histological study of the effects of uterine infections on the pregnancy of dairy cows


