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RESEARCH ARTICLE

Investigation of Pathological Lesions and Fetal Losses in Slaughtered Cattle

Serdar Altun[1*], Selim Çomaklı[1], Kübra A. Terim Kapakin[1], Mehmet Cengiz[2]

[1]*Atatürk University, Faculty of Veterinary Medicine, Department of Pathology, Erzurum/Turkey*
[2]*Atatürk University, Faculty of Veterinary Medicine, Division of Clinical Sciences, Erzurum/Turkey.*

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ABSTRACT

In the presented study, we aimed to investigate fetal wastage due to slaughtered pregnant cattle and endometritis ratio in slaughterhouses. For this aim, we examined the corpus parts of uterus tissues of slaughtered cattle by using macroscopic and histopathological evaluation methods to detect pregnancy and endometritis ratio. Routine histopathological processing was performed for collected tissues. All sections were stained with hematoxylin- eosin. After staining, all slides were examined under the light microscope. Out of the 140 cattle, 18 animals were found to be pregnant at different stages of pregnancy. No histopathological changes were observed in 62 samples of 122 non-pregnant cattle and inflammatory uterine lesions were observed in different characters and intensities in 60 samples. In result of this study, we detect the percentage of pregnant animals which are slaughtered is 12,8%. Besides, the percentage of female animals with no pathological lesions that may prevent pregnancy were found to be is 44,2%. According to these findings, we suggest that these rates are a significant loss in terms of animal husbandry in the region.

Kesime Sevk Edilen İnek Uteruslarında Hayvanların Damızlık Değerini Düşüren Patolojik Lezyonların ve Fötal Kayıpların Araştırılması

ÖZ

Bu çalışmada; kesime sevk edilen ineklerin, uterus organlarının makroskobik ve histopatolojik muayenelerinin yapılması, böylece gebe olarak kesime sevk edilen hayvan sayısının belirlenmesi amaçlandı. Ayrıca, toplanan örneklerde yapılacak histopatolojik mueyene ile endometritisli hayvanların, uteruslarında yangı olmayan hayvanlara oranının tespit edilmesi amaçlanmıştır. Bu amaçla dişi hayvan kesimleri rutin olarak takip edildi ve bu hayvanlara ait uterus organları toplandı. Toplanan bu organlar detaylı makroskobik ve histopatolojik muayeneden geçirildi. Tüm kesitler hematoksilen-eozin boyama yöntemi ile boyandı ve histopatolojik muayene ışık mikroskobu kullanılarak yapıldı. Toplam 140 hayvana ait uterus organlarının dış bakısında 18 (%12.8) hayvanın farklı dönemlerde gebe olduğu tespit edildi. Yine uterus organlarına yapılan makroskobik ve histopatolojik incelemeler sonucunda gebe olmayan 122 hayvana ait organların 62' sinde herhangi patolojik bir değişiklik izlenmedi, 60' ında ise farklı karakter ve şiddetlerde yangısal lezyonlar izlendi. Sonuç olarak kesime sevk edilen dişi hayvanlardan gebe hayvan oranının yöremizde %12.8 olduğu, ayrıca kesilen hayvanların %44.2' sinde gebeliğe engel teşkil edecek herhangi bir patolojik lezyona rastlanmadığı görülmüş ve bu oranların bölge hayvancılığımız açısından önemli bir kayıp olduğu kanısına varılmıştır.

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* Corresponding author
E-mail address: serdar.altun@atauni.edu.tr (S. Altun)

Introduction

The birth rate in farms is a very important value in both milk and meat production. Pregnancy and birth of calf which mean the beginning and continuation of lactation for dairy cattle (Gröhna and Rajala, 2000; Inchaisri et al., 2010; Nonga, 2015) and maintain meat production for farms (Kim et al., 2005; Lee et al., 2007; McDougal et al., 2007; Oduguwa et al., 2013). Fertility and infertility problems caused by diseases in cattle are reported as the most important reason for the removal of animals from herd (Lee et al., 2007; McDougal et al., 2007; Potter et al., 2010; Armengol et al., 2015; Barlett et al., 1986; Canisso et al., 2016). There are many pathological conditions that cause infertility in cattle. As a result of these pathological events, the uterus and the connected organs are affected. Endometritis is defined as the inflammations in the endometrium (Milli and Hazıroğlu, 2001; Foster, 2007; Schlafer et al., 2007). These inflammations are usually caused by bacterial agents such as *Streptococcus, Staphylococcus, Escherichia coli, Arcanobacterium pyogenes, Fusobacterium necrophorum* (Sheldon et al., 2006; Schlafer et al., 2007; Potter et al., 2010). These agents are frequently encountered in clinical endometritis cases especially with vaginal discharge. Septic metritis can sometimes lead to problems that can cause death. Brucellosis, Listeriosis, Leptospirosis, Salmonellosis are rarely seen, but important zoonotic and abortive agents such as tuberculosis, as well as protozoans such as *Trichomonas fetus* and many viruses such as *Bovine Viral Diarrhea Virus* cause infection in uterus (Foster, 2007; Schlafer et al., 2007). These infections could prevent the pregnancy and also cause to be completely infertile (Feldmann et al., 2005; Diskin and Morris, 2008; Tsousis et al., 2010). Besides, There are some different conditions such as amnion sickness, umbilical cord or uterine torsion, hormonal effects (progesterone deficiency etc.) nutritional problems (phosphorus, selenium and vitamin E deficiency) and exposure to toxic substances which cause to abortion in animals (Milli and Hazıroğlu, 2001; Foster, 2007; Schlafer et al., 2007). Mild level infections in uterus usually could be developed after the mating (Benbia et al., 2013). Moreover, the presence of inflammatory cell infiltrations at a certain rate in oestrus, pregnancy and postnatal 2-3 days period is accepted as normal or physiological. The most severe inflammations in cows are developed during the puerperal period and the postpartum period of uterus and associated organs (Milli and Hazıroğlu, 2001; Schlafer et al., 2007).

Histopathology is the most reliable method used to determine uterine diseases. Inflammation in the uterus could prevent pregnancy even an animal with physiologically healthy and high breeding value. In this study, it was aimed to identify uterine lesions in animals to detect pathological conditions that may prevent pregnancy of animals referred to slaughter. Besides we aimed to obtain numerical data about slaughtered pregnant animals.

Material and Method

The uterus tissue obtained from the cattle after slaughter were brought to the necropsy hall of the Department of Pathology of the Faculty of Veterinary Medicine with appropriate containers. Tissue samples taken from corpus uteri were stored for 1 day being fixed in a 10% buffered formalin solution for histopathology. Tissue samples were washed with tap water before routine serial treatment of samples with graded alcohol and xylene were performed in Shandon Citadel 2000 tissue system (Minnesota, USA). After routine histopathological processing, all samples were embedded in paraffin block and 5 µm sections were prepared using a rotary microtome (Leica RM 2255, Wetzlar, Germany). All sections were stained with hematoxylin and eosin (H&E) for standard histopathological evaluation. Slides were examined under the light microscopy (Olympus BX51 with DP72 camera attachment, Tokyo, Japan).

Result and Discussion

Macroscopic Findings

In this study, loss of fetus caused by the killing of animals in slaughterhouse not caused by abortion due to infection or other reason. After macroscopic examination of collected uterus tissue, it was determined that 18 out of 140 (12.8%) animals were pregnant (Figre 1). Exudation of different color and odor is encountered in the lumens of some organs. In addition, the palpations made on these organs showed that there was an increase in the consistency of the organs. Diffuse fibrosis was observed in these organs during histopathological evaluaiton (Fig. 5).

Fig. 1. Fetuses found in the uterus lumen.

Histopathological Findings

Uterine inflammations with different severity and character were observed in 60 tissue samples during

histopathological evaluation. chronic endometritis was diagnosed in 32 samples and acute endometritis was diagnosed in 28 cases. In acute cases, intense neutrophil leukocyte infiltrations were observed in the submucosa, mainly in the uterine lumen (Fig. 2). Degenerative and necrotic changes

5), desquamation and fibrosis in the lamina propria to the mucosa were observed. In one case, there was a case of chronic organ tuberculosis characterized by mononuclear cell infiltrations around the caseification necrosis in the center, were observed in the epithelium of the uterine gland (Fig. 3). In chronic cases, inflammatory cells consisting of lymphocytes, macrophages and plasma cells (Fig. 3) and eosinophilic leukocytes (Fig. 4) were observed. Intensive necrosis in the epithelial layer and gland epithelium of the endometrium (Fig. and formation of capsules around it, consisting of Langhans type giant cell formations and surrounding fibrous connective tissue (Fig. 6). Focal mononüclear cell infiltration in pregnant animal endometrium (Fig. 7).

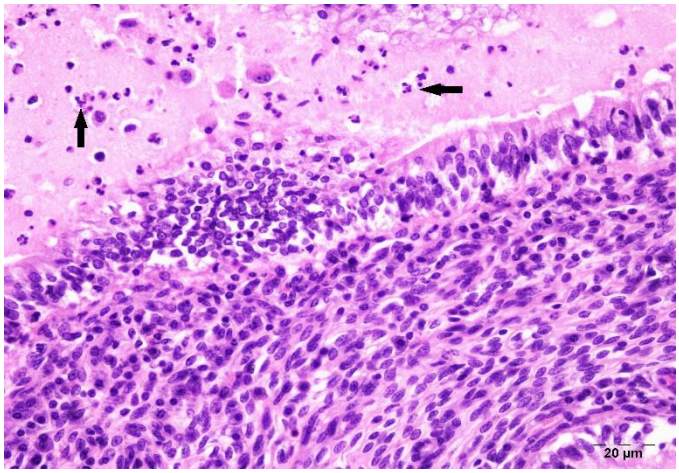

Figure 2. Intensive neutrophil leukocyte (arrows) infiltrations in the uterine lumen, acute endometritis. H&E. 20 µm.

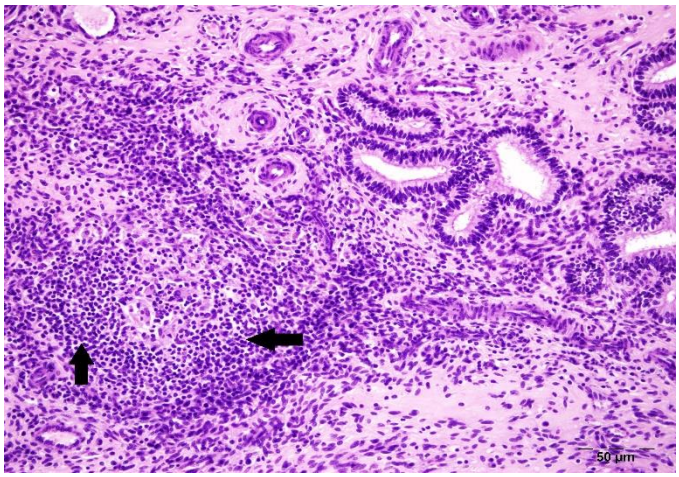

Figure 3. Intensive mononuclear cell infiltration (arrows), chronic endometritis. H&E. 20 µm.

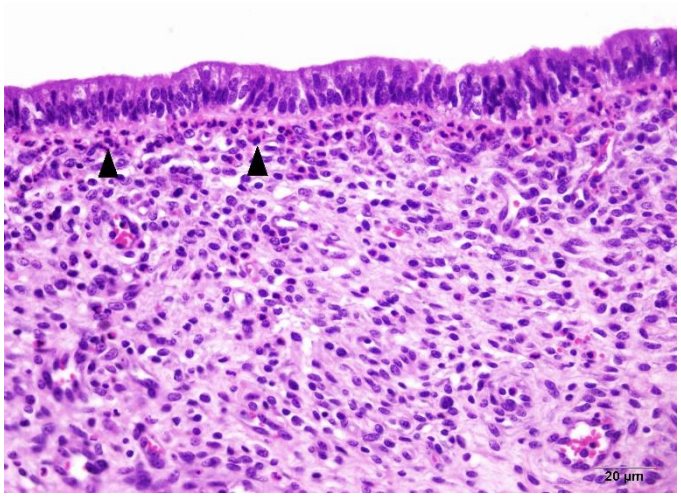

Figure 4. Eosinophil leukocyte infiltration (arrowhead), chronic endometritis. H&E.20µm.

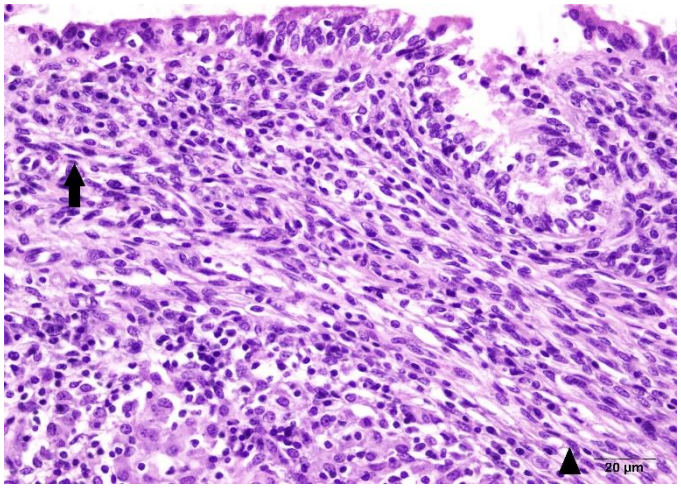

Figure 5. Fibrocyte (arrow) and fibroblast (arrowhead), chronic endometritis, fibrosis. H&E. 20 µm.

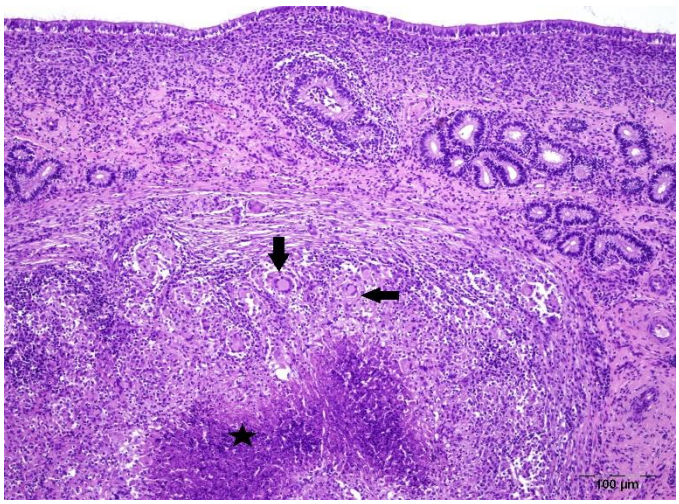

Fig. 6. Langhans-type giant cells (arrows), granuloma consisting of casein (star) necrosis and fibrosis. H&E. 20 µm.

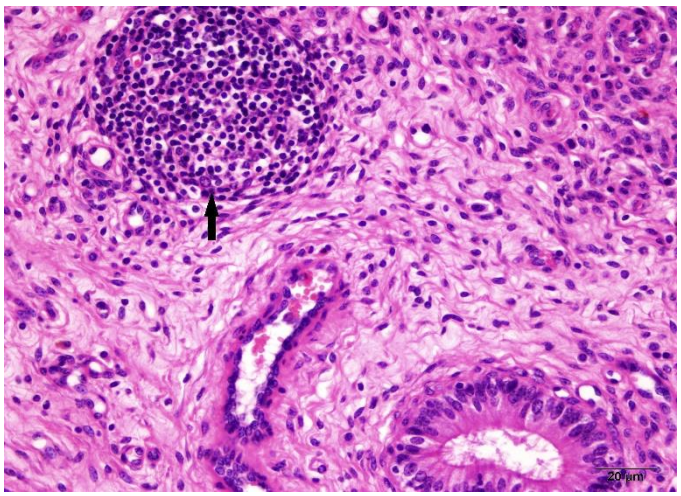

Fig. 7. Focal mononüclear cell infiltration (arrow) in pregnant animal. H&E. 20 µm.

The disease agents found in the uterus reach this organ via mating, blood pathway or postpartum contamination (Schlafer et al., 2007; Potter et al., 2010). Although, the appearance of vaginal discharge containing heavily neutrophil leukocyte in abnormal color and odor (mucoid, purulent, hemorrhagic) in the diagnosis of clinical endometritis is an important display (Sheldon et al., 2006; Singh et al., 2008), there is no exudative discharge in the subclinical endometritis. Methods such as endometrial cytology, ultrasonographic imaging are used in the diagnosis of subclinical endometritis (Knutt et al,. 2000; Sheldon et al., 2006; Potter et al., 2010; Polat et al., 2015). Histopathological examination of biopsy material taken from endometrium in chronic and subclinical cases is shown as the most reliable method in terms of classification of uterine inflammation (Singh et al., 2008; Pleticha and Heuwiser, 2009; Rhyaf, 2010; Benbia et al., 2013). Although, histopathologic examination provides detailed information. This method is not preferred due to destruction and infection predisposition in the endometrium. In this study, samples of uterine tissue were taken from the cows that were slaughtered and the risk of complication of the biopsy known as the most reliable method was eliminated and these samples were evaluated by histopathologic examination. As a result of the evaluation, 60 (42.8%) cases were found to have different characteristic uterine inflammation. At the end of the microscopic examinations, chronic endometritis was diagnosed in 32 cases of uterine tissues and acute endometritis was diagnosed in 28 cases. Intensive neutrophil leukocyte infiltrations were observed in the submucosa, especially in the lumen of the uterus in acute cases (Fig.2). In chronic cases, inflammatory cell infiltrations like lymphocytes, macrophages, plasma cells (Fig.3) and, eosinophil leukocyte (Fig.4) were detected. In a case; granulomas were observed characterized by mononuclear cell infiltrations with consisting of Langhans type giant cell formations around the caesifenation necrosis in the center, and surrounding fibrous connective tissue in this case (Fig.6). In studies conducted to diagnose clinical endometritis; the uterine acute and chronic inflammation is cytologically associated only with the presence of neutrophil leukocyte (Sheldon et al., 2006). The uterine inflammation can not be detected only by the presence of neutrophil leukocytes which are intensely observed in the acute phase and cannot be found in the inflammatory region for a long time. Though neutrophil leukocytes are among the first responding cells to chemotaxis in the inflammation, they are not predominantly among the cells observed in inflammatory reactions that are not exudative in the uterine lumen of chronic inflammations (Milli and Hazıroğlu 2001; Schlafer et al., 2007). To confirm this fact, we have also observed that lymphocytes, monocytes, plasma cells, and fibrosis are evident in the inflammatory areas, while the presence of neutrophil leukocytes in the animals diagnosed with chronic endometritis is not detectable at all.

Histopathological examination of tissue specimens from pregnant animals revealed intense mononuclear cell infiltrations compared to healthy animals. Literature studies have shown that this condition is related to pregnancy (Schlafer et al., 2007; Singh et al., 2008; Armengol and Fraile, 2015; Espejel and Medrano, 2017). Therefore, inflammatory changes in pregnant animals; endometritis was not evaluated in the study.

Literature studies have also reported data on the incidence of slaughetered pregnant animals in some African countries (Oduguwa et al., 2013; Swai et al., 2015). However, considering the worldwide studies, it is seen that the loss of pregnancy which related to infectious and metabolic diseases, external (temperature stress, milk production), maternal (serum progesterone level), genetic (breeding bull, twin pregnancy) factors, is between 0.4% and 10.6% (Lee and Kim, 2007; Benbia et al., 2013; Schlafer et al., 2007; Milli and Hazıroğlu, 2001). Among these reasons, despite the absence of slaughtered pregnant animals, it is reported that this loss is

the most important loss in farms (Knutti et al., 2000; Sheldon et al., 2010; Inchaisri et al., 2010; Benbia et al., 2013; Armengol and Fraile, 2015). With this information, it is thought that this issue should be resolved urgently when it is considered that the basis of animal production is dependent on reproduction.

Conclusion

Considering the fact that severe inflammations in uterus occur after the birth, the animal husbandry enterprises in our region should be made aware for the necessary precautions such as prenatal and postnatal nursing care of pregnant animals in particular. It is recommended to take measures such as the incentive applications for the order to prevent the pregnant animal slaughter. The service of active units using ultrasound for pregnancy examination in slaughterhouses and sacrifice markets, and the prohibition of the sale of animals that do not receive pregnancy examination report as slaughter and sacriface.

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