Histopathological and microbiological evaluation of uterus in repeat breeder cows shipped to slaughterhouse

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Kesime sevk edilen repeat breeder ineklerde uterusların histopatolojik ve mikrobiyolojik değerlendirilmesi

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Öz

Amaç: Bu çalışmada, repeat breeder ineklerde endometritislerin histopatolojik muayene ile belirlenmesi ve endometritise neden olabilecek bakterileri araştırmak amaçlanmıştır.

Gereç ve Yöntem: Konya ilindeki mezbahalara kesimciyle getirilen 53 baş repeat breeder inekten ve 10 baş döltutma problemi olmayan inekten alınan toplam 63 uterus örneği makroskobik, histopatolojik ve mikrobiyolojik yöntemlerle incelendi.


Öneri: Ineklerde repeat breederin etyolojisinde önemli faktörlерden biri olan endometritislerin belirlenmesinde histopatolojik muayene etkili olduğu görülmüştür. Uygun tedavi protokollerinin oluşturulmasında klinik muayene bulgularının, histopatolojik ve mikrobiyolojik bulgularla birlikte değerlendirilmesinin daha faydalı olduğu kanaatine varılmıştır.

Anahtar kelimeler: Endometritis, Histopatoloji, İnek, Mikrobiyoloji, Repeat breeder

Abstract

Aim: The purpose of this study is to determine endometritis in repeat breeder cows by histopathological examination and to investigate the bacteria that may cause endometritis.

Materials and Methods: A total of 63 uterus specimens taken from 53 repeat breeder cows brought to the slaughter houses in Konya province for slaughtering and from 10 cows without any reproductive problem were examined by macroscopic, histopathological and microbiological methods.

Results: Histopathological examination showed that 22 out of 63 samples were diagnosed as endometritis. The types of the endometritis were determined which that 5 catarrhal endometritis, 5 purulent endometritis, 6 chronic nonpurulent endometritis, 1 chronic purulent endometritis and 1 granulomatous endometritis were found. In the microbiological examination, it was found that while bacteria were isolated in 11 uteri, mostly Streptococcus spp. and Escherichia coli were isolated. According to the antibiogram results, all of the identified bacteria were determined to be susceptible to Amoxicillin/Clavulanic acid and Florfenicol. It is also found that isolated bacteria were found as increased penicillin G resistance.

Conclusion: It has been determined that endometritis is major causing factor in the etiology of repeat breeder in cows. Histopathological exams are key to diagnose endometritis causing repeat breeder. It has been concluded that when clinical findings are combined with histopathological and microbiological findings, the effective treatment protocol can be established.

Keywords: Endometritis, Histopathology, Cow, Microbiology, Repeat breeder
Introduction

One of the most important factors affecting the sustainability of the dairy cattle farms and production is reproduction. There are many factors that adversely affect reproduction in dairy cattle farms and one of the most important factors is the Repeat breeder (RB) problem. Cows that giving birth, younger than 10 years of age, have estrus at regular intervals, clinically healthy and are not getting pregnant after breeding at least three times with a fertile bull or after performing artificial insemination even though are defined as RB (Aköz and Dinç 2001, Alaçam 2005, Saraswat and Purohit 2016).

Repeat breeder rates in dairy cattle farms are reported to be between 5-36% depending on variable factors (Purohit 2008, Perez-Marin et al 2012, Saraswat and Purohit 2016). Luperto Telli (2017) reported when the data received five-year average of five dairy cattle business in different regions in Turkey RBA rate varied between 4.8% and 21.38. He also emphasized that this ratio should be below 15% for a successful farms.

Two main reasons of RB having a multifactorial and complex etiology is the lack of the formation of fertilization and early embryonic deaths (Alaçam 2005, Saraswat and Purohit 2016). Other factors playing a role in etiology, play an important role in the emergence of RB problems by accompanying or leading to these two main causes (Maurer and Echternkamp 1985, Lafi and Kaneene 1988, Dinç 1990). These factors can be regarded as ovulation defects, genital canal pathologies, hormonal imbalances, genetic disorders, management and estrus detection deficiencies, insemination mistakes, sperm quality, infectious agents (bacteria, virus, and fungi) and endometritis (Alaçam 2005, Perez-Marin et al 2012, Saraswat and Purohit 2016). High milk yield, season, age, heat stress and immunological reactions are among the factors affecting the incidence of RB (Alaçam 2005, Purohit 2008).

It is reported that while the changes caused by the subclinical endometritis in the uterine environment have negative effects on embryonic life, subclinical endometritis sometimes due to the changing pH in the uterus environment prevents the formation of fertilization with its effects during the transportation of spermatozoons (Dinç 1990, Sheldon et al 2009, Ahmed and Elsheikh 2014).


It was stated that microbiological examination used in the diagnosis of subclinical endometritis alone is not sufficient for diagnosis and more accurate results can be obtained when it is evaluated together with histopathologic findings (Polat 2008, Doğruer and Güler 2010).

By considering the etiologic factors in the treatment of repeat breeder cases, it is seen that the combined or individual use of hormone applications and intrauterine antibiotic and antiseptic applications are more preferred (Aköz and Dinç 2001, Perez-Marin et al 2012, Raval et al 2018).

The aim of the present study is to determine endometritis with histopathologic findings, microbiological agents that may cause endometritis and the efficiency of antibiotics in repeat breeder cows which were sent for slaughtering in Konya province

Materials and Methods

Animal material

The study was carried out between February and April 2017. The material of the study was consisted of 53 repeat breeder cows taken to slaughter houses around Konya province. Also the uteri of 10 cows without any reproductive disorders was evaluated as the control group.

Clinical examination

Clinically, the reproductive system of the animals were examined according to the abnormal vaginal discharge.

Postmortem examination

Immediately after slaughtering, genital organs were macroscopically examined in terms of pathological structures (follicular cyst, luteinised cyst, tumor-like structures, atrophy, etc.). The presence of CL and follicle on ovaries was examined in terms of ovarian activity.

Microbiological examination

Samples were taken from cornu and corpus uteri cavities of the uteri collected after slaughtering with transport swabs with sterile cotton and transferred to the Bacteriology Laboratory of Konya Veterinary Control Institute in the cold chain for bacterial lawn. Swab samples were incubated at 37 °C in aerobic and microaerophilic conditions for 48 hours with 7% sheep blood agar and MacConkey agar. Preparates prepared from isolated colonies were stained using Gram stain method (Biomerieux 1992). The isolates were identified by placing with the Gram positive and Gram negative test cards into the Vitec 2 compact device (Biomerieux) in accordance with the procedure.
The antibiogram susceptibility test of the identified bacteria was conducted using the disc diffusion method explained by Kirby-Bauer.

Histopathological examination

For histopathological examination, tissue samples taken from the uterine bifurcation region were determined with 10% formalin and then embedded into paraffin blocks after routine follow-up. Microtome sections with 5μ thickness taken from the prepared paraffin blocks were stained with hematoxylin-eosin according to the procedure and examined by light microscope (Olympus, BX51) (Luna 1968). Degeneration, desquamation, and neutrophil infiltration in lamina epithelialis; neutrophil and mononuclear cell infiltration, connective tissue increase, hyperemia, oedema and haemorrhage in lamina propria, dilation in endometrial glands, neutrophil infiltration in gland lumen observed in histopathologic examinations were scored and evaluated. Classification of endometritis was carried out histopathologically according to inflammatory changes in the endometrium reported by Hatipoglu et al (2002).

Results

The age of the RB cows in the study ranged between three and nine years old. Table 1 shows the distribution of the ages of the RB cows used in the study.

Macroscopic findings

In the examination of genital organs of 63 cows after slaughtering, follicular cysts in the ovaries of two RB cows and a cystic structure on the mesovarium of a cow in the control group were observed. After the macroscopic examination, it was determined that the activity continued at least one of the ovaries of all cows.

In one of the examined uterus haemorrhage in uterine lumen and in the other’s uterine mucosa white diffuse and hard small blisters were detected.

<table>
<thead>
<tr>
<th>Age</th>
<th>Control Group</th>
<th>RB</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total (n)</td>
<td>10</td>
<td>53</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Endometritis Type</th>
<th>RB</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catarrhal</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Purulent</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Chronic Purulent</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Chronic Nonpurulent</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Granulomatous</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total (n)</td>
<td>21</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 1. a. Purulent endometritis, degeneration and desquamation in epithelial cells (black arrow), neutrophil infiltration (white arrow), (H&E stain, 100x). b. Chronic nonpurulent endometritis, degeneration and desquamation in epithelial cells (black arrow), mononuclear cells infiltration (white arrow), (H&E stain, 100x). c. Granulomatous endometritis, granuloma in lamina propria and cell aron infiltration (arrow), (H&E stain, 100x). d. Chronic purulent endometritis, desquamation in epithelial cells (white arrow), neutrophil infiltration (black arrow), (H&E stain, 100x).

In this study in the histopathologic examination performed, that 22 out 63 uteri were detected endometritis (Figure 1), one of them belonged to control group. The endometritis types are shown in Table 2. Among 41 uteri was no findings of endometritis were found, neither macroscopic nor microscopic findings were observed in 8 uteri, 7 of them belonged to RB cows. The findings of hyperemia, haemorrhage, oedema, increased number of glands were observed in 33 uteri.

Microscopic findings

In the macroscopic examination, a small blisters was observed in the lumen and granuloma was detected in the histopathological examination (Figure 1.c.). Table 3 shows the distribution of histopathological and microbiological examination results according to study groups.

Microbiological findings

In the microbiological examination, bacteria were isolated in 10 (18.86%) of RB cows and in one (10%) of the control group (Table 4). While one type of bacteria was isolated from 8 of them, 2 types of bacteria were isolated from 3 of them. 5 of the isolated bacteria could not be identified.
It was determined that all of the bacteria identified according to the antibiogram result were susceptible to amoxicillin/clavulanic acid and florfenicol and all bacteria except for Streptococcus spp. were resistant to penicillin G. Table 5 shows antibiogram results of the identified bacteria.

While in this study endometritis was detected 70% of the bacteria isolated samples in RB cows, was not detected in the control group. Table 6 shows the distribution of the samples isolated from bacteria in terms of endometritis.

Discussion

Subclinical endometritis is an important factor in the etiology of RB cows, which leads to embryonic deaths and fertilization. The evaluation of clinical and postmortem examination findings with the histopathological and microbiological findings is important in determining the etiology of RB and in developing more appropriate treatment protocols (Dinç 1990, Azawi et al 2008, Ferreira et al 2008, Kaya 2008, Polat 2008, Ergün et al 2009, Cannazik and Polat 2015).

Endometritis is the inflammatory reaction occurs against various factors (parturition, mating, infection, etc.) in the endometrium of uterus. Histopathologically, it is characterized by degeneration, desquamation and inflammatory cell infiltration in the lamina epithelialis, different degrees of inflammatory cell infiltration, vascular, glandular and stromal changes in the lamina propria. (Alaçam 2007, Kaya 2008). However, it is reported that there may be a small number of neutrophils in the endometrium in the prooestrus, oestrus and metoestrus stages in healthy cows and this situation should be considered in the evaluations to be made in the endometrium (Alaçam 2007, Kaya 2008, Espejel and Medrano 2017).

In this study, endometritis was detected in 39% of repeat breeder cows. Other studies reported rates ranging from 12% to 100% (Messier et al 1984, Javed and Khan 1991, Dogan et al 2002, Garoussi et al 2010, Pothmann et al 2015). The reason why endometritis has a very wide range in other studies is thought to be related about the number of selected animals, the difference in management of the enterprises and time.

Table 3. Distribution of histopathological and microbiological findings obtained from uterus samples

<table>
<thead>
<tr>
<th>Presence of Endometritis</th>
<th>RB</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>119</td>
<td>111</td>
<td>230</td>
</tr>
<tr>
<td>-</td>
<td>11</td>
<td>11</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 4. Prevalence of bacteria obtained from the uterus samples as a result of microbiological examination

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>RB</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3.77</td>
<td>-</td>
<td>3.17</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>5.66</td>
<td>-</td>
<td>4.76</td>
</tr>
<tr>
<td>Methylobacterium spp.</td>
<td>1.88</td>
<td>1.88</td>
<td>3.17</td>
</tr>
<tr>
<td>Globicetella sulfidaciens</td>
<td>1.88</td>
<td>-</td>
<td>1.58</td>
</tr>
<tr>
<td>Sphingomonas paucimobilis</td>
<td>1.88</td>
<td>-</td>
<td>1.58</td>
</tr>
<tr>
<td>Unidentified</td>
<td>7.54</td>
<td>1.88</td>
<td>7.93</td>
</tr>
</tbody>
</table>
Table 5. Antibiotic susceptibilities of the identified bacteria

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>AMC</th>
<th>AMP</th>
<th>TE</th>
<th>GN</th>
<th>SXT</th>
<th>TIL</th>
<th>FFC</th>
<th>ENR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus spp.[1]</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td>Streptococcus spp.[2]</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td>Escherichia coli [1]</td>
<td>S</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Methylobacterium spp. [1]</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Methylobacterium spp. [2]</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Globicetella sulfidifaciens</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Sphingomonas paucimobilis</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
</tbody>
</table>


Table 6. Distribution of the microorganisms in the uterus samples with and without endometritis

<table>
<thead>
<tr>
<th>Antibiotic Susceptibility</th>
<th>Catarhal</th>
<th>Purulent</th>
<th>Chronic purulent</th>
<th>Chronic nonpurulent</th>
<th>Granulatous</th>
<th>Non-endometritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus spp.</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylobacterium spp.</td>
<td>1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Globicetella sulfidifaciens</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingomonas paucimobilis</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified</td>
<td></td>
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</tr>
</tbody>
</table>

Haemorrhage was determined in the lumen of one uterus sample in the macroscopic examination and it was thought in the histopathological examination that neutrophil infiltration along with mononuclear cell infiltration and increased connective tissue indicated a chronic endometritis and the determined haemorrhage may be associated with metoestrus.

Degeneration and desquamation at different grades were detected in lamina epithelialis in 25 out of the uterus samples evaluated in the study. Similar findings were determined in cases of endometritis in other studies (Dogan et al 2002, Hatipoglu et al 2002, Azawi et al 2008, Doğruer and Güler 2010). Furthermore, degeneration and desquamation were detected in lamina epithelialis in two of uterus samples of the control group but no inflammatory cells were found in the study. In these two examples, the changes observed in lamina epithelialis are thought to be caused by the errors in the preparation stages.

In the study, increased connective tissues at different grades were observed around the glands in lamina propria of 12 uterus samples. Dogan et al (2002) determined periglandular fibrosis of 13.33% in their study. It was reported that severe periglandular fibrosis may cause embryonic death by inhibiting the glandular functions required by the embryo (Kenney 1978, Polat 2008).

It was thought in the study, the histopathological findings such as hyperemia, haemorrhage, oedema etc. in the endometrium of 33 (52.34%) uterus samples that is not detected endometritis were the changes based on cycle phase. Dogan et al (2002) stated in their study that they found similar findings in both fertile and RB cows and these were the normal histological changes that did not affect fertility. In addition, it was also reported that the haemorrhage and stromal oedema in endometrium may result from the estrogenic effects in the follicular phase (Ohtani et al 1993, Espejel and Medrano 2017).

It is stated that depending on its anatomical, histological and functional structure, uterus can be more sensitive to the changes and therefore uterine infections have a significant
negative effect on fertility (Ferreira et al 2008). Physical factors, hormonal effects, humoral and cellular immune mechanisms affect the resistance of uterus against infections (Foster 2007). In the luteal phase where the uterus is under the influence of the progesterone hormone, immunosuppressive substances are secreted to the uterine luminal and this inhibit lymphocyte proliferation and makes the uterus susceptible to many nonspecific bacteria (Mili 1998, Özdemir and Erer 2017).

Genital organ examinations, erroneous artificial insemination practices or natural insemination are the preparatory reasons for the genital canal to be contaminated by bacteria. Elimination of the resistance of uterus against infections increases the production and pathogenicity of bacteria and results in the formation of inflammation in the endometrium. The formed inflammation can affect the viability and motility of spermatozoa, thus preventing the formation of fertilization or causing early embryonic deaths (Rahman et al 1996, Gani et al 2008, Bhat et al 2013).

It was reported in the microbiological examinations conducted with the uterus of repeat breeder animals by Messier et al (1984), Aköz and Dinç (2001), Azawi et al (2008), Gani et al (2008), Ergün et al (2009), Doğruer and Güler (2010), Yılmaz et al (2012), Bhat et al (2013) and Potheim et al (2015) that Trueperella pyogenes, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium spp. and Pseudomonas spp. were commonly isolated. They reported in the histopathological examination of the uterus samples of these animals that they detected endometritis at different intensities. It is also stated in the studies that most of these bacteria are isolated from the uterus of fertile cows (Gani et al 2008, Bhat et al 2013).

While bacteria were isolated in 18.86% of the swab samples taken from RB cows in the study. The bacteria isolation rate in swap samples of uterus was reported between the ranges of 52.9 and 75 (Gani et al 2008, Bhat et al 2013, Potheim et al 2015, Raval et al 2018). The reason why the rate obtained in the present study is lower than the other studies is thought to be associated with the use of antibiotics which samples were taken from cows since the treatment history is not known.

In this study, it was thought that other bacteria, except Streptococcus spp. and Escherichia coli were nonspecific bacteria and due to their contamination isolated from the uterus. Azawi et al (2008) and Gani et al (2008) suggest that nonspecific uterine bacteria can be transmitted during insemination and when the uterine resistance decreases, their virulence can increase and cause endometritis. In the study, endometritis were not detected in 3 of the bacteria isolated from uterus specimens and in 15 samples with detected endometritis, the bacteria were not isolated. Yilmaz et al (2012) reported similar results. Gilbert et al (1998) reported that inflammations in the uterus not related to bacterial infection adversely affect the continuation of the embryonic process.

Raval et al (2018) reported that bacteria isolated against penicillin were resistant to 94.7%, and this rate was 77.7% in our study. It was reported in the previous studies that resistance against penicillin was detected as 50% by Gani et al (2008) and 64.50% by Ergün et al (2009). This increase is thought to be due to unconscious antibiotic use.

Conclusions

It has been determined that endometritis is major causing factor in the etiology of repeat breeder in cows. Histopathological exams are key to diagnose endometritis causing repeat breeder. It has been concluded that when clinical findings are combined with histopathological and microbiological findings, the effective treatment protocol can be established.

Acknowledgements

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