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# The effect of a single administration of parenteral oxytetracycline and flunixin meglumine combination on the reproductive performance of dairy cows with subclinical endometritis

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**Abstract:** This study explored the effects of a single administration of flunixin meglumine (acting as a non-steroidal anti-inflammatory drug) and oxytetracycline combination on the reproductive performance of cows diagnosed with subclinical endometritis during the postpartum period. Cows aged 3 to 9 at 40 to 60 days postpartum were studied. Endometrial samples were collected from cows that had a uterine cornu (middle of the cornu) width of greater than 3.5 cm in the ultrasonographic examination, and 40 cows diagnosed with subclinical endometritis were included in the study. Cows in group 1 (n = 20) received a single dose of 20 mg of flunixin meglumine and 300 mg of oxytetracycline IM per 10 kg of body weight, whereas the cows in group 2 (n = 20) served as controls and were administered placebo simultaneously with the animals in group 1. Following the application, cows in both groups were artificially inseminated during the first detected heat. Twenty-five percent of cows in the control group and 55% of cows in the treatment group conceived. As a consequence, it was concluded that clinical and subclinical endometritis causes delays in conception or embryonic deaths in cows, and that a single IM administration of oxytetracycline + flunixin meglumine to cows with subclinical endometritis may yield favourable results in enhancing the pregnancy rate.

**Key words:** Subclinical endometritis, cow, postpartum, conception rate, reproduction

## Subklinik endometritisli sütçü ineklerde tek doz parenteral oksitetrasiklin ve flunixin meglumine tedavisinin reprodüktif performans üzerine etkisi

**Özet:** Çalışmada postpartum dönemde subklinik endometritis teşhis edilen ineklerde tek doz nonsteroid antiinflamatuar olan flunixin meglumin ile oksitetrasiklin kombinasyonunun reprodüktif performans üzerine etkilerini araştırmak amaçlandı. Bu amaçla 3-9 yaşlı, postpartum 40-60 gün arasında bulunan inekler materyal olarak kullanıldı. Ultrasonografik muayenede uterus cornularının genişliği (cornunun orta bölgesi) >3,5 cm olan ineklerden endometrial örnek alındı ve subklinik endometritis oldukları teşhis edilen kırk adet sütçü inek çalışmaya alındı. Grup 1'deki ineklere

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(n = 20) tek doz 10 kg canlı ağırlığa 20 mg flunixin meglumine ve 300 mg oksitetrasiklin kombinasyonu IM uygulandı. Grup 2'deki inekler (n = 20) ise kontrol olarak bırakılarak 1. gruptaki hayvanlar ile eş zamanlı olarak plasebo uygulandı. Uygulamaları takiben Grup 1 ve Grup'2'deki inekler tespit edilen ilk kızgınlıkta suni olarak tohumlandılar. Kontrol grubu ineklerinde % 25 gebelik oranı elde edilirken, tedavi grubundaki ineklerden % 55 gebelik oranını elde edildi. Sonuç olarak; klinik ve subklinik endometritisin ineklerde gebe kalmanın gecikmesine ya da embriyonik ölümlerde artmaya neden olduğu, subklinik endometritisli ineklerde oxitytetracyclin + flunixine meglumine'in IM tek doz verilmesinin gebe kalma olasılığına fayda sağlayabileceği kanaatine varıldı.

**Anahtar sözcükler:** Subklinik endometritis, inek, postpartum, gebelik oranı, reprodüksiyon

## Introduction

High reproductive efficiency is requisite for the success of a dairy farm and the attainment of the desired calving interval (1,2). The successful identification of oestrus and the use of a good insemination technique as well as high quality semen in healthy uteruses are the critic components for a high reproductive efficiency (3).

Before calving, the uterine lumen is considered sterile. Although numerous bacteria of various species found on the uterine mucosa, which becomes contaminated by ascendance from the surroundings and the animal's own skin and faeces, are isolated from 90% of cows, the physiologic control mechanisms of the uterus may eliminate these bacteria. On the other hand, defects in the said mechanisms arising mainly due to some particular endocrinal disorders; selenium, vitamin E, vitamin A, and β-carotene deficits; failure of the calf to suckle; reasons reducing the myometrial contraction such as hypocalcaemia; and an unhygienic environment result in diseases (4,5).

Clinical endometritis is the inflammation of the endometrium characterised by a delay in uterine involution without the animal displaying any systemic symptoms (4,6). In subclinical endometritis, endometrial inflammation is diagnosed by means of a cytological examination revealing the non-presence of a purulent material in the vagina (7).

In animals, non-symptomatic subclinical endometritis is diagnosed by measuring the neutrophil rate in samples collected from the uterine lumen (8,9). In subclinical endometritis, the neutrophil rate is reported to be greater than 18% in samples collected on days 20 to 33 postpartum, and greater than 10% in samples collected on days 34 to 47 postpartum (9).

Various techniques including but not limited to transrectal uterine palpation (10), vaginascopic examination (11), uterine discharge culture (12), uterine biopsy (13), and uterine cytology (7) are available for diagnosing endometritis. In diagnosing clinical and subclinical endometritis, vaginascopic examination is a more specific and accurate method than transrectal palpation in detecting abnormal uterine discharge (11).

Intrauterine or parenteral antibiotics and PGF2α are used for treating postpartum uterine diseases. During the normal uterine involution process, the diameter of the uterus decreases and the lochia is expelled as a result of increasing uterine contractions due to constantly high concentrations of PGF2α (14,15).

In the present study, the aim was to examine the effects of a single administration of flunixin meglumine (acting as a non-steroidal anti-inflammatory drug) and oxytetracycline combination on the reproductive performance of cows diagnosed with subclinical endometritis during the postpartum period.

## Materials and methods

Selection of animals

Randomly selected cows of the Holstein breed aged 3 to 9 and kept under similar care and feeding conditions at 40 to 60 days postpartum were studied. Cows at 40 to 60 days postpartum were subjected to vaginal examination by vaginascopy and those not displaying an abnormal vaginal discharge underwent

ultrasonographic examination by means of endometrial cytology.

Samples were collected from cows that had a uterine cornu (middle of the cornu) width of greater than 3.5 cm and an oedema in their cornu lumens during ultrasonographic examination (8) by using special materials produced for endometrial swab and the swabs were subjected to vaginal cytology (9), bacterial isolation, and antibiotic susceptibility tests. Forty dairy cows diagnosed with subclinical endometritis on the basis of ultrasonographic and cytological examination results were included in the study.

Bacteriological studies

*Isolation:* The swab samples were subjected to general bacteriological examination and also investigated to detect the presence of Brucella spp. and Mycoplasma spp. For the general bacteriological examination, the swabs were streaked onto blood agar containing 10% of sheep blood and MacConkey agar plates and were inoculated into nutrient broths. The plates and broths were incubated aerobically and microaerobically at 37 °C for 24 to 72 h. For Brucella spp., the swabs were streaked onto Brucella agar plates and incubated microaerobically at 37 °C for 7 days. The isolates were identified by using routine bacteriological techniques (16,17). For Mycoplasma spp., the swabs were inoculated in tubes containing 4 mL of Eaton liquid medium (21 g of PPLO broth base, 100 mL of yeast extract, 200 mL of horse blood serum, 10 g of glucose, 200,000 IU/mL penicillin, 12.5 mL of 0.2% phenol red, 0.02 g of DNA) and left to incubate under microaerobic conditions at 37 °C. The media were checked every day for growth and contamination. Passages were made to liquid media from media not exhibiting any growth during the 3-day incubation period and the media were then left to incubate under the same conditions. The procedures were repeated twice more in cases when no growth was observed during the first passage. In case of contamination following the first inoculation, the cultures were first strained using a 0.45 mm filter to eliminate contamination and then passed over to liquid media. Three days after the last passage to the liquid media, the cultures were passed onto Eaton's agar plates and were incubated under microaerobic conditions and at 37 °C for 21 days. All plates were examined by microscope for the detection of colonial growth from day 4 onwards (18).

*Antibiotic susceptibility tests:* The susceptibility of isolated bacteria against antibiotics used for treatment purposes in the cows was determined by using the Kirby-Bauer disc diffusion technique (19,20). Ampicillin (AMP 10), amoxicillin (AML 25), amoxicillin/clavulonic acid (AMC 30), cefoperazone (CFP 75), enrofloxacin (ENR 5), erythromycin (E 15), gentamicin (GN 10), neomycin (N 10), oxytetracycline (OT 30), penicillin G (P 10), rifaximin (RAX 40), streptomycin (S10), ampicillin/sulbactam (SAM 20), sulfamethoxazole/trimethoprim (SXT 25), and tetracycline (TE 30) discs were used in the study.

Clinical study

Forty cows diagnosed with subclinical endometritis were numbered and divided into 2 equal groups. Cows numbered 1 to 20 were designated as group 1, whereas the remaining cows numbered 21 to 40 served as group 2. Cows in group 1 (n = 20) received a single 1 mL per 10 kg of live weight IM administration of the solution containing 20 mg of flunixin meglumine and 300 mg of oxytetracycline per millilitre (Flunited® Bayer), whereas the cows in group 2 (n = 20) served as controls and were administered placebo simultaneously with the animals in group 1.

Following the application, cows in both groups were artificially inseminated during the first detected heat. Repeat breeder cows were considered to have (-) conception whereas the others were subjected to pregnancy tests by means of rectal palpation on day 45.

Statistical analysis

The calving–first oestrus interval and the attained conception rates were established for cows in groups 1 and 2. The difference between the groups was compared statistically by using the chi-square test.

## Results

Bacteriological results

Out of 40 swab samples collected from the animals, bacterial growth was observed in 39 samples. One species of bacteria was isolated from 16 samples,

2 species from 13 samples, and 3 or more species from 10 samples. Out of a total of 75 isolated strains, the respective order of the most frequently isolated species was as follows: Bacillus spp. (25.3%), coagulase positive Staphylococcus spp. (12%), Streptococcus spp. (12%), *Escherichia coli* (10.7%), *Klebsiella pneumoniae* (9.3%), Micrococcus spp. (9.3%), coagulase negative Staphylococcus spp. (6.7%), Enterobacter spp. (6.7%), and Corynebacterium spp. (2.7%). Brucella spp. and Mycoplasma spp. were not isolated from any samples (Table).

The antibiotic susceptibility tests revealed that 44 (59%) of the isolates were susceptible and 15 (20%) were moderately susceptible while 16 (21%) were resistant to oxytetracycline. Out of the 16 isolates resistant to oxytetracycline, 14 were found to be susceptible to ampicillin/sulbactam; 13 to amoxicillin/clavulonic acid and enrofloxacin; 12 to amoxicillin; 10 to neomycin; 8 to cefoperazone; 7 to streptomycin; 5 to gentamicin and rifaximin; 4 to ampicillin, penicillin G, and sulfamethoxazole/trimethoprim; 3 to erythromycin; and 2 to tetracycline.

Clinical results

The first oestrus after calving took 63.2 ± 15.7 days in cows in group 1 and 78.3 ± 17.6 days in cows in group 2. The examination of the interval between treatments – first oestrus revealed that the oestrus occurred earlier in group 1 than in the control group. A statistically significant difference was observed between the groups ($P < 0.01$).

When cows in both groups were considered, the conception rates of the cows in the treatment group were better than those of the cows in the control group. The comparison of the conception rates for cows in groups 1 and 2 showed a statistically significant difference ($P < 0.01$). Conception rates of 25% and 55% were obtained for the cows in the control group and the treatment group, respectively. Accordingly, cows in group 1 conceived faster than cows in group 2, which were left untreated.

## Discussion

Normally, the uterine involution process concludes within 4 to 5 weeks from birth with the continuous secretion of PGF2α. The normal uterine involution process involves the reduction of the uterine size and expulsion of the lochia (21). Ultrasonography and endometrial cytology can be used to diagnose cows with subclinical endometritis. Cows with a neutrophil rate of greater than 18% and fluid accumulation in their endometrium are identified as cows with subclinical endometritis (8).

Flunixin meglumine is a strong non-steroidal anti-inflammatory drug reducing the biosynthesis of PGF2α by hindering the cyclo-oxygenase enzyme in the arachidonic acid cycle (22).

Although the administration of flunixin alleviates the clinical symptoms of endometritis, it may be claimed to have a negative effect on the subsequent reproductive performance of the cows. However, the reproductive performance findings obtained in our study do not support this hypothesis. Even though flunixin serves to repress PGF' alpha for 4 h after the administration, it never totally represses it (23). Meanwhile, it is also reported that even a long-term daily administration of flunixin does not imperil uterine involution (24).

In our study, the calving – first oestrus interval for group 1 was shorter than that in the control group. In concordance with the evaluations of Bosu et al. (25), this delay in the cows in the control group was associated with the infection in the uterine endometrium.

McDougall (14) reported that embryonic survival in an infected uterus causes a fall in the amount of uterine milk as well as a reduction in the toxic effects of the bacterial components, which in turn tend to affect the probability of conception. In our study, the conception rate for cows in group 2, which were left untreated, was 25%. This lower conception rate in comparison to cows in group 1 was congruent with the arguments set forth by McDougall (14).

This study proves the advantages of treating cows with subclinical endometritis. In general, the comparison of cows treated with a single administration of flunixin meglumine and oxytetracycline to cows in group 2 reveals a higher conception rate. It was gratifying that Brucella and Mycoplasma spp., causing specific infections, and *Arcanobacterium pyogenes*, which is accountable for serious infections, infertility, and even embryonic death (26), were not isolated. The fact that micro-

Table. Isolation results from animals.

| *COW NO.* | *Bacteria* | *COW NO.* | *Bacteria* |
|---|---|---|---|
| 1 | *Bacillus pulvifaciens* | 21 | *Bacillus coagulans*<br>*Streptococcus bovis* |
| 2 | Bacillus spp. | 22 | *Staphylococcus simulans*<br>Bacillus spp.<br>*Escherichia coli* |
| 3 | Micrococcus spp. | 23 | *Bacillus circulans*<br>*Staphylococcus intermedius*<br>*Streptococcus bovis* |
| 4 | *Bacillus licheniformis* | 24 | Bacillus spp.<br>Bacillus spp.<br>*Escherichia coli* |
| 5 | Bacillus spp. | 25 | Bacillus spp.<br>*Escherichia coli* |
| 6 | *Bacillus licheniformis* | 26 | *Bacillus megaterium*<br>*Staphylococcus intermedius*<br>*Streptococcus canis*<br>Acinetobacter spp. |
| 7 | *Streptococcus bovis* | 27 | *Escherichia coli*<br>*Bacillus laterosporus*<br>Micrococcus spp.<br>Streptococcus spp. |
| 8 | *Bacillus* spp. | 28 | *Klebsiella pneumuniae* |
| 9 | *Escherichia coli*<br>*Bacillus licheniformis* Corynebacterium spp. | 29 | Shigella spp. |
| 10 | *Escherichia coli* | 30 | *Klebsiella pneumuniae* |
| 11 | *Streptococcus bovis* | 31 | *Klebsiella pneumuniae*<br>*Staphylococcus intermedius*<br>Micrococcus spp. |
| 12 | *Streptococcus sangius*<br>*Bacillus licheniformis* | 32 | *Citrobacter freundii*<br>*Staphylococcus intermedius* |
| 13 | No isolation | 33 | *Klebsiella pneumuniae*<br>*Staphylococcus intermedius* |
| 14 | *Streptococcus dysgalactiae*<br>*Bacillus licheniformis* | 34 | *Klebsiella pneumuniae*<br>*Staphylococcus saprophyticus*<br>*Staphylococcus caprae* |
| 15 | *Escherichia coli*<br>*Bacillus licheniformis* | 35 | *Klebsiella pneumuniae*<br>*Staphylococcus intermedius* |
| 16 | Micrococcus spp. | 36 | *Klebsiella pneumuniae*<br>*Staphylococcus intermedius* |
| 17 | *Escherichia coli*<br>Corynebacterium spp. | 37 | *Enterobacter liquefaciens* |
| 18 | Micrococcus spp.<br>*Bacillus circulans*<br>*Streptococcus canis*<br>*Enterobacter agglomerans* | 38 | *Enterobacter clocae*<br>Micrococcus spp. |
| 19 | *Staphylococcus saprophyticus* | 39 | *Enterobacter agglomerans*<br>*Staphylococcus intermedius*<br>Micrococcus spp. |
| 20 | *Bacillus coagulans*<br>*Staphylococcus intermedius* | 40 | *Enterobacter agglomerans*<br>*Proteus* spp. |

organisms of numerous species were isolated as a result of bacteriological examination reveals the importance of isolation of the causative agent not only in diagnosing clinical endometritis but also subclinical endometritis.

In cows, the uterine milieu is contaminated because of inadequate uterine cleaning and weak neutrophil functional capacity. The variation in the prevalence of uterine disorders in a herd demonstrates that managerial and environmental factors play an important role in protection and treatment (8). Efforts to prevent endometritis by focusing on these factors will most likely result in better protection against endometritis and a higher reproductive performance.

The rate of occurrence of clinical and subclinical endometritis during the period of 40 to 60 days postpartum is 53% and this causes delays in conception or an increase in the embryonic death rate (9). When this rate is taken into account, it is bound to result in economic losses arising out of delays in conception or the increasing embryonic death rate in cows with clinical and subclinical endometritis in a herd.

It was concluded that the administration of a single IM administration of oxytetracycline + flunixin meglumine to cows with subclinical endometritis may yield favourable results in enhancing the pregnancy rate.

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