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Evaluation of Intramammary Platelet Concentrate Efficacy as a Subclinical Mastitis Treatment in Dairy Cows Based on Somatic Cell Count and Milk Amyloid A Levels [1][2]

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Abstract

The intramammary administration of platelet concentrate is expected to treat subclinical mastitis and prevent disease recurrence effectively; therefore, it was aimed to evaluate its efficacy in terms of somatic cell count (SCC) and Milk Amyloid A (MAA) measurements. A total of 120 cow mammary lobes with subclinical mastitis were randomly assigned to one of the following three groups: Antibiotic Group (ABG, n=40), Platelet Concentrate Group (PCG, n=40) or Combined Group (CG, n=40). Platelet concentrates were prepared by the double centrifugation method from blood collected from donor cows. All groups received intramammary treatments for 3 days. Analysis of MAA using a commercially available ELISA method and measurement of SCC were performed from milk samples collected on days 0, 7, 14, and 21. Treatment success and absence of recurrence were found to be statistically significant for all three treatment protocols (P<0.001). It is concluded that intramammary platelet concentrate administration can be an effective alternative to intramammary antibiotic use for the treatment of subclinical mastitis.

Keywords: *Subclinical mastitis, Platelet concentrate, Milk amyloid A, Somatic cell count, Dairy cow*

Sütçü İneklerde Subklinik Mastitis Tedavisinde Meme İçi Platelet Konsantresi Etkinliğinin Somatik Hücre Sayımı ve Süt Amiloid A Seviyeleri İle Değerlendirilmesi

Öz

Meme içi platelet konsantresi uygulamasının, subklinik mastitisi tedavi etme ve hastalığın rekürrensini engellemede etkin olabileceği beklenmekte olup; uygulamanın etkinliğinin somatic hücre sayısı (SHS) ve Süt Amiloid A (Milk Amyloid A: MAA) ölçümlerine dayandırılarak değerlendirilmesi amaçlanmıştır. Subklinik mastitisli ineklere ait 120 meme lobu rastgele Antibiyotik Grubu (ABG, n=40), Platelet Konsantresi Grubu (PKG, n=40) ve Kombine Grup (KG, n=40) olmak üzere üç gruba ayrıldı. Platelet konsantreleri donör ineklerden alınan kandan çift santrifüj metoduyla hazırlandı. Gruplara 3 gün boyunca meme içi tedavi uygulandı. Çalışmanın 0.,7.,14. ve 21. günlerinde alınan süt örneklerinde SHS ölçümü ve ELISA yöntemiyle MAA analizi yapıldı. Uygulanan her üç tedavi protokolü için de iyileşme başarısı ve rekürrens görülmemesi durumu istatistiksel açıdan önemli bulundu (P<0.001). Subklinik mastitis tedavisinde meme içi platelet konsantresi uygulamasının meme içi antibiyotik kullanımına alternatif olabileceği sonucuna ulaşıldı.

Anahtar sözcükler: *Subklinik mastitis, Platelet konsantresi, Süt amilod A, Somatik hücre sayısı, Sütçü inek*

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INTRODUCTION

Milk and dairy products are important sources of food consumption for the vast majority of the world's population [1]. The inflammation of mammary tissue is called mastitis, usually caused by intramammary bacterial infections [2]. Subclinical mastitis is characterized by inflammation with no clinical findings in milk or mammary tissues [3,4].

Mastitis is the most common pathology seen in the dairy industry and is the main cause of antibiotic use and economic loss [1,5,6]. Most cases of mastitis are subclinical with almost 20-50 cases of subclinical mastitis recorded for every clinical mastitis case [7]. Intramammary administration of antibiotics is used to reach the highest drug concentration in cases of clinical mastitis and in almost all subclinical mastitis cases, except for those where the mammary tissue has excessive swelling or fibrosis [8]. Mastitis is difficult to treat due to various direct and indirect issues including treatment costs, unusable milk, labor and time loss, recurrent mastitis, a decrease in milk production and quality, increase in the number of discarded animals, affected animal welfare, etc. [1,3,5]. Among the problems caused by administering antibiotics to animals produced for consumption are antibiotic residue in food and micro-organisms acquiring antibiotic resistance [1,6].

Platelet activation is the first step of healing process that occurs after tissue damage. This process consists the release of several bioactive factors that plays role in the recruitment of cells associated with healing to the damaged tissue. New treatment protocols of platelet concentrate administration, so the highly concentrated bioactive factors, has become popular in recent years. It has several advantages as being safe, easy and has a wide range of application area [9]. Platelets contain growth factors, chemokines, cytokines and active metabolites which are required for rapid wound healing and tissue regeneration. Local application of these growth factors at high concentrations through platelet concentrate increases the repair rate of the tissue by optimizing the area of healing [10]. Alpha-granules of platelets contain various factors as transforming growth factors (TGF), platelete-derived growth factors, epidermal growth factors, insulin-like growth factor-1, chemokins and cytokines which play role in healing process and tissue regeneration [11]. Throughout these factors, TGF-α was reported to have an impact on mammary epithelial proliferation and morphogenesis of the mammary gland [12] which may play important role during healing from mastitis.

Blood products containing intensive amounts of platelets have become widely used in many areas of human medicine. Although there is limited information on their use in veterinary medicine, platelet-rich plasma applications have also become popular in recent years, especially for tendon injuries in equine medicine. The use of platelet concentrate obtained through a double centrifugation method for the treatment of mastitis is of current research interest as an alternative method to stimulate the regeneration of glandular tissue by providing growth factors at supraphysiological concentrations [10].

Regarding the evaluation of mastitis, the use of various parameters such as haptoglobin, milk amyloid A (MAA), lactoferrin, lysozyme, lactate dehydrogenase enzyme, nitric oxide, and heat shock proteins have become widespread in bovine medicine [13]. One of these parameters, MAA, is a specific isoform of serum amyloid A and is secreted only in the presence of inflammation directly from mammary epithelial cells. It is a highly sensitive marker that allows the detection of subclinical mastitis in milk [13,14].

In addition to the use of antibiotics for the treatment of mastitis, which is the most important and common problem in dairy farms, there is a need for new antimicrobial treatment methods. Optimally, such new methods would not create a basis for bacterial resistance and would not leave antibiotic residue in nature and animal-origin food and, therefore, would not threaten human health. Considering the growing interest in organic livestock and organic animal-origin food production in recent years, the development of a novel approach to mastitis treatment could be regarded as a milestone. The absence of information on the use of platelet concentrate in subclinical mastitis screening in the literature confirms the authenticity of our study and the lack of knowledge on this subject.

The intramammary administration of platelet concentrate, which was previously prepared for use and stored in a laboratory environment, is expected to treat subclinical mastitis and prevent disease recurrence effectively; therefore, it was aimed to evaluate its efficacy in terms of somatic cell count (SCC) and MAA measurements.

MATERIAL and METHODS

Study Design

The study was approved by Istanbul University Local Committee on Animal Research Ethics (Permit no. 2016/79).

Milk samples were collected from 3- to 6-year-old cows in the 2nd lactation period [15] with subclinical mastitis, at a private Holstein dairy cattle farm in Istanbul. The mean milk yield of the animals were 24±3.4 L/day. They were kept in 10 m^2 barns per animal and milked by machine twice per day in milking parlors. The machine milking procedure applied in the private farm was consisted of these following steps: preparation of the staff, cleaning of teats with a pre-milking germicide dip solution, drying of teats, foremilk stripping, application of the machine, milking, detaching of the machine and finally post-milking teat germicide dipping [16]. The animals received a complete diet prepared according to their nutritional requirements by the Department of Animal Breeding. The study was

performed during autumn (October-December).

Mastitis screening was performed at a pre-visit (Dpre) to the farm by evaluating clinical examination findings and California Mastitis Test (CMT) results. Milk samples were aseptically collected from 210 mammary lobes with no clinical findings, pre-diagnosed with subclinical mastitis by evaluating CMT scores as +, ++, and +++. Subclinical mastitis diagnoses were confirmed in 187 samples having ≥200.000 cells/mL according to SSC results. Bacteriological analysis was performed on milk samples for isolation of microbial pathogens and antibiotic selection. Of these, 120 mammary lobes with bacterial growth were randomly selected to create study groups consisting of the Antibiotic Group (ABG, n = 40), the Platelet Concentration Group (PCG, n = 40), and the Combination Group (CG, n = 40) which was a combination of both treatment protocols, AB and PC. On the initial day of treatment (D0), two milk samples were taken from each mammary lobe following aseptic conditions. After the mammary lobes were completely emptied, depending on their assigned group, the applications, (intramammary antibiotics, intramammary platelet concentrate, or intramammary antibiotics + platelet concentrate) were performed. Intramammary antibiotic solutions were applied using commercial injectors (amoxicillin + clavulanic acid, Synulox LC, Pfizer). The platelet concentrate in a 5-mL sterile syringe was administered intramammary after the sterile teat catheter was advanced halfway into the teat [10]. The treatment was continued for 12 days at 3-day intervals and teat dipping was applied after each application. Milk sampling was repeated on the 7th, 14th, and 21st (D7, D14, D21) days of the study. SCC and MAA measurements were performed on the milk samples.

Milk Sample Collection

Before taking the milk samples, the mammary quarter was washed, cleaned with 70% alcohol, dried, and the first three milking streams were discarded. Milk samples were collected in sterile tubes under aseptic conditions, preserved cold and delivered to the laboratory within 2 h. Milk samples were collected for SCC measurement and microbiological analysis on Dpre and for SCC and MAA measurements on D0, D7, D14, and D21.

SCC Measurement

Somatic cell count measurements were performed on milk samples for the verification of subclinical mastitis diagnosis on Dpre and to evaluate treatment success on D0, D7, D14, and D21. SCC measurements were performed using a Fossomatic 90 cell counter (Foss Electric, Hillerod, Denmark) after heat treatment at 40°C for 15 min.

Bacteriological Analysis

Milk samples were double inoculated onto 5% sheep blood Columbia Agar, MacConkey agar, and Sabouraud dextrose agar. The inoculants were incubated at 37°C for 24-72 h under aerobic and microaerobic conditions. Morphology and colony characteristics formed at the end of incubation were examined and the samples with three or more types of colonies were evaluated as contaminated. Gram staining was performed on the colonies in the samples suitable for isolation and identification. The isolated microorganisms using pure culture were identified by classical methods [17].

Preparation of Platelet Concentrates

Platelet concentrates were prepared in the Accredited Blood Bank Laboratory of Istanbul University-Cerrahpasa, Faculty of Veterinary Medicine.

To prepare allogeneic platelet concentrate, whole blood from v. jugularis of healthy, non-pregnant, non-lactating cows that received no antibiotic treatment in the last two months, was transferred with 16-gauge needles into 450 mL blood transfusion bags containing citrate-phosphate-dextrose-adenine (CPDA-1) [10,18]. Blood samples were taken to the laboratory within two hours under cold conditions. The blood bags were weighed on a precise balance (Precisa XT 6200C, Dietikon, Switzerland) and confirmed to be of equal weight. The blood bags were placed perpendicularly in a centrifuge (Beckman Coulter J6-M1, JS-4.2, California, USA) set at 4200g for 5 min at 22°C with acceleration and deceleration cycles set to 5 min. The blood bags were then carefully removed from the centrifuge, placed in a plasma extractor (Terumo Teruflex ACS201, Tokyo, Japan) from which platelet-rich plasma samples were transferred into 50 mL Falcon tubes. The second centrifugation for preparing the platelet concentrate was performed at 1500 g for 10 min at 4°C. Cell counts of prepared platelet concentrates were performed using a blood counting device (Abacus Junior Vet, Diatron, Budapest, Hungary) and platelet concentrates with the standard of 1×10^9 platelets/mL [10] were stored in 50 mL sterile Falcon tubes.

The prepared platelet concentrates were frozen three times at -80°C (Sanyo MDF-U2086S, Tokyo, Japan), and thawed at 37°C in a plasma heater (DH2 QuickThaw Plasma Thawing System, Helmer, Noblesville, IN, USA) to release platelet-derived factors, then stored at -20°C [10]. Various preparation stages of platelet concentrate are shown at *Fig. 1*.

MAA Analysis

Milk Amyloid A concentrations of D0, D7, D14, and D21 milk samples were determined using a commercial ELISA kit according to the manufacturer's instructions (Milk Amyloid A (MAA) ELISA Kit, Cat. No.: TP-807, Tridelta Development Ltd., Ireland). The sensitivity of the assay is 0.10 µg/mL, the intra-assay and inter-assay coefficient of variations are 6.62% and 9.99%, respectively. Optical densities were read on an automatic plate reader (BioTek EL×808 Absorbance Microplate Reader, USA) at 450 nm and reference 630 nm. MAA concentrations were

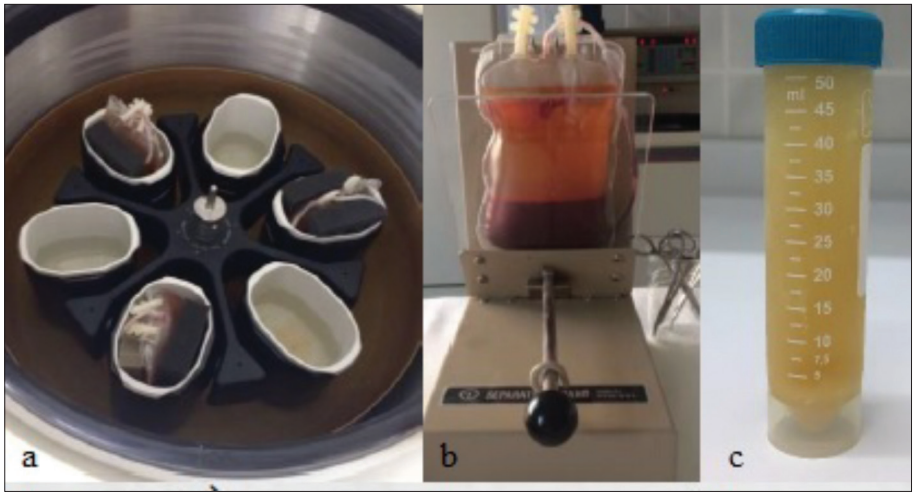

Fig 1. Platelet concentrate preparation. a- blood bags placed in the centrifuge; b- blood bags placed in the plasma extractor after the first centrifugation; c- platelet concentrate obtained after the second centrifugation stored in a Falcon tube

Table 1. *SCC measurement (×10³ cell/mL) per study groups according to sampling day*

Groups	Days				Significance
	D0 Mean±SE	D7 Mean±SE	D14 Mean±SE	D21 Mean±SE	
ABG	748.48±37.27^{Aa}	402.98±22.66^{Ba}	265.23±14.84^{C}	223.38±12.89^{C}	***
PCG	623.83±32.14^{ab}	350.45±19.33^{Bab}	256.05±13.19^{C}	213.68±8.68^{C}	***
CG	667.77±33.27^{ab}	318.75±19.69^{Bb}	228.08±9.23^{C}	206.70±7.45^{C}	***
Significance	*	*	NS	NS	

*SE: Standard Error; NS: Not Significant (P>0.05); * P<0.05; *** P<0.001*
^{a,b} Indicates the significance controls in the same column; ^{A,B,C} Indicates the significance controls in the same row

Table 2. *MAA measurement results of the study groups according to sampling day*

Groups	Days				Significance
	D0 Mean±SE (ng/mL)	D7 Mean±SE (ng/mL)	D14 Mean±SE (ng/mL)	D21 MEan±SE (ng/mL)	
ABG	6198.38±228.49^{Aa}	2851.28±238.70^{B}	1530.00±150.60^{C}	1113.85±109.88^{C}	***
PCG	4762.78±358.85^{Ab}	2935.13±292.56^{B}	1826.08±215.40^{C}	1344.68±160.68^{C}	***
CG	4999.83±309.64^{Ab}	2828.35±262.57^{B}	1497.75±134.88^{C}	1137.38±93.64^{C}	***
Significance	***	NS	NS	NS	

*SE: Standard Error; NS: Not Significant (P>0.05); * P<0.001*
^{a,b} Indicates the significance controls in the same column; ^{A,B,C} Indicates the significance controls in the same row

calculated based on a standard curve using references provided by the manufacturer. Samples were diluted 1:50 as mentioned in the manufacturer's instructions.

Statistical Analyses

Statistical analyses of the collected data were performed to evaluate the success and recurrence status of the intramammary treatments. The results of the MAA measurements were evaluated using a Kruskal-Wallis test, while SSC results were evaluated by one-way ANOVA. The significance control of the groups was tested by the Duncan method and the statistical software program SPSS 13.0 was used for the analyses.

RESULTS

Microbiological analysis of the milk samples revealed *Staphylococcus* sp. (71.5%), *Streptococcus* sp. (14.5%), *Escherichia coli* (3.5%), Pasteurella sp. (3.5%), *Corynebacterium* sp. (3.5%), and *Enterococcus* sp. (3.5%). In the study, for mammary lobes assigned to ABG and CG, an intramammary suspension (Synulox LC, Pfizer) containing amoxicillin + clavulanic acid was preferred as an antibiotic. The compatibility between the bacterial species that amoxicilin + clavulonic acid combination has bactericidal effect and the microbiological results obtained from our study was effective at our antibiotic selection.

Treatment groups were formed with 120 mammary lobes selected from the milk samples with SCC over 200×10^3 cells/mL on Dpre. The SCC measurement results of the collected milk samples at the beginning of the treatment and at the follow-up days are detailed in *Table 1*.

The MAA measurement results of the collected milk samples at the beginning of the treatment and on the follow-up days are detailed in *Table 2*.

In the milk samples taken from ABG mammary lobes, SCC, which was $748.48\pm37.27 \times 10^3$ cells/mL before treatment, decreased to $402.98\pm22.66 \times 10^3$ cells/mL on D7 as a result of intramammary antibiotic administration ($P<0.001$). Also, MAA measurements for the same days decreased from 6198.38±228.49 ng/mL to 2851.28±238.70 ng/mL ($P<0.001$). SCC and MAA measurements of milk samples taken on the 14th and 21st days after the beginning of treatment to evaluate the recurrence status of disease were similar to each other and significantly lower than those obtained on D0 and D7 ($P<0.001$).

For the PCG samples, SCC measurements and MAA concentrations at D0 and D7 significantly decreased from $623.83\pm32.14 \times 10^3$ cells/mL to $350.45\pm19.33 \times 10^3$ cells/mL ($P<0.001$), and from 4762.78±358.85 ng/mL to 2935.13±292.56 ng/mL ($P<0.001$), respectively. SCC and MAA measurements for D14 and D21 were similar to each other but significantly lower than those obtained on D0 and D7 ($P<0.001$).

Similar results were obtained for CG samples as those for the other treatment protocols. SCC which was determined to be $667.77\pm33.27 \times 10^3$ cells/mL on D0 decreased to $318.75\pm19.69 \times 10^3$ cells/mL ($P<0.001$) on D7. The MAA concentration also decreased from 4999.83±309.64 ng/mL to 2828.35±262.57 ng/mL ($P<0.001$). SCC and MAA measurements on D14 and D21 which evaluated the recurrence status with the combined treatment were similar to each other and lower than those on D0 and D7 ($P<0.001$).

DISCUSSION

This study was planned considering the need for new alternative approaches in the treatment of sub-clinical mastitis. The efficacy of intramammary platelet concentrate treatment was evaluated through SCC and MAA measurements. According to the current literature, although it is recognized that the application of intra-mammary platelet concentrate provides a very new perspective in the treatment of clinical mastitis [10], no studies were found on animals with subclinical mastitis. On the other hand, the presence of studies examining methods such as the application of various fruit and plant extracts [8,19-21], photodynamic therapy [22], and nitric oxide-releasing solutions [23] for subclinical mastitis treatment indicates the search for alternative methods to antibiotic use worldwide. The lack of new information on mastitis treatment by platelet concentrate applications shows the original value of this study.

The causes of mastitis are divided into two as environmental or contagious microorganisms. *Klebsiella* sp., *E. coli*, and Streptococci are the most frequently isolated environmental factors. *Staphylococcus aureus* and *Str. agalactiae* are the most frequently isolated contagious microorganisms [4,5]. In our study, the most detected species was *Staphylococcus* sp., (71.5%) followed by *Streptococcus* sp. (14.5%). The fact that the majority of the identified microorganisms were contagious factors suggests that the disease may spread due to inadequate sanitation.

Intramammary antibiotics are used as a routine protocol to treat mastitis and to prevent the spread of infectious factors causing this disease [1,5,6,8]. Blood products containing high levels of platelets are thought to exhibit antibiotic action by secreting antimicrobial peptides as well as inducing cell regeneration by stimulating cell proliferation, angiogenesis, and cell migration [10,24]. In an *in vitro* study on the antimicrobial efficacy of human platelet-rich gelatin, it was shown that this application was highly effective on various microorganisms, especially *S. aureus* [25]. In addition, anti-inflammatory and analgesic effects have also been reported [24]. In a study by Lange-Consiglio et al.[10], where the results were evaluated according to SCC, combined treatment in clinical mastitis was reported to be more successful than sole antibiotic or sole platelet concentrate applications, and it was stated that platelet concentrate application was as effective as intramammary antibiotic administration. According to the data obtained from the present study, all three protocols, antibiotic, platelet concentrate, and combined treatment were found to be successful in the treatment of subclinical mastitis. In all three application groups, a decrease of nearly half was detected between pre-treatment (D0) and post-treatment SCC measurements (D7) ($P<0.001$), and this decrease continued at other measurement days. Although SCC did not decrease below 200×10^3 cells/mL, it was determined to be very close to this value. This decrease in SCC since the beginning of the treatment was evaluated as recovery. Examining the recurrence status of the chronic cases in the study mentioned previously, sole platelet concentrate application was more successful than other treatment applications [10]. In our study, the three treatment protocols were also found to be successful in terms of controlling subclinical mastitis recurrence. This new treatment protocol may easily replace antibiotic usage considering the stages of platelet concentrate preparation. It is a simple, uncomplicated and economically affordable method. It can be performed in the office setting of the farm with very limited consumable material supply when the infrastructure is created as well as the laboratory. However, attention must be paid to work sterile. Lysing or damaging platelets should be avoided during process.

Considering that mastitis cases are mostly subclinical [7], it is obvious that the detection of this disease, which can spread to the herd without being noticed, is the most important step. Accordingly, many parameters can be evaluated including CMT; SCC measurements; factor isolation and identification; evaluation of electrical conductivity, density, freezing point and mineral percentage of milk [26,27]. Among these parameters, the one most frequently evaluated is SCC. However, it should be remembered that SCC may vary depending on the species of microbial agent, lactation period and the number of lactations, age and breed of the cow, milk yield, milking frequency, season and the geographical region that the herd is located, as well as non-inflammatory factors [28]. According to the results of a study by Risvanlı and Kalkan [29], there was no strong correlation between age and breed of cows and SCC values in subclinical mastitis. In the present study, factors affecting SCC were not evaluated. However, since animals were kept at the same farm, being the same breed, of a similar age range and lactation period, all milked twice a day, and not affected by any disease other than subclinical mastitis, we decided that SCC results were minimally affected by other factors and accurately reflect subclinical mastitis levels.

In recent years, studies on acute phase protein measurements that give more precise results than SCC for subclinical mastitis diagnosis and follow-up have become widespread. In the present study, MAA, as well as SCC, were preferred for evaluating the success of subclinical mastitis treatment protocols and recurrence status. In a study in which amyloid A was evaluated by a serum ELISA test kit in serum samples and by both serum and milk ELISA test kits in milk samples, it was determined that the most sensitive kit for subclinical mastitis diagnosis was the milk MAA ELISA test kit [30]. In a study on the relationship between subclinical mastitis pathogens and MAA measurements, the lower limit value was 3.9 µg/mL for major pathogens and 1.6 µg/mL for all other pathogens [31]. The MAA data obtained from the present study were consistent with the published literature data and MAA concentrations of AB, PCG, and CG before treatments were 6198.38±228.49 ng/mL, 762.78±358.85 ng/mL, and 999.83±309.64 ng/mL, respectively. Statistically significant reductions were determined in MAA concentrations after treatment in all three study groups ($P<0.001$). MAA concentrations continued to decrease at D14 and D21. D14 and D21 measurements of each group were similar ($P>0.05$) and significantly lower than D0 and D7 ($P<0.001$). All three treatment protocols showed improvement and no recurrence was determined during the 21-day period from the start of treatment.

Examining the relationship between D0 measurement results of all treatment groups, it was seen that the ABG had the highest SCC and MAA results ($P<0.05$ and $P<0.001$, respectively). As a result of this coincidental outcome, which was due to the randomization of the groups, it was decided that the SCC and MAA measurements actually showed a similar pattern in detecting mammary tissue inflammation and also MAA measurements yielded much more sensitive results. Since SCC and MAA measurements are both indicators of healing from mastitis, bacteriological examination was not repeated in this study.

Platelet concentrate heals the mammary tissue by peptide growth factors which lead to tissue protection and/or repair processes through stimulation of glandular tissue regeneration. Additionally, these growth factors increase the infiltration of neutrophils and macrophages to fight against the microorganisms related to mastitis [11,32]. In case of complete healing from mastitis and the lack of recurrence, the factors secreted after inflammation and platelet concentrate administration are expected to be decreased. However, SCC and MAA measurements were the target parameters for treatment evaluation in this study. Nevertheless, repeating the microbiological examinations are considered to be useful for future correspondence.

In mastitis cases, the migration of polymorphonuclear neutrophils (PMNs) from blood to mammary glands is triggered following the invasion of the pathogenic factor into the mammary gland. Activated PMNs, while destroying pathogens, can cause tissue damage as a result of producing reactive oxygen metabolites and granular enzyme release, resulting in the disruption of mammary function. Antibiotics frequently used for the treatment of mastitis can not protect the mammary gland from damage [2]. Therefore, it is understood that damage caused at mammary epithelial cells can not be repaired by using antibiotics, but it is possible to repair damage due to platelet concentrate, which is rich in cytokines, chemokines, and various growth factors. The authors of this study suggest that, although there are no visible pathological changes in subclinical mastitis, changes in the cellular basis that occur during treatment with intramammary platelet concentrate after the invasion of the mammary gland by pathogenic factors can be studied with invasive methods. Reexamination of bacteriological evaluation is considered to be needed to detect the treatment efficiency of platelet concentrate with histopathological examinations for future correspondence.

Results of the present study suggest that intramammary platelet concentrate may be an alternative to antibiotic use in the treatment of subclinical mastitis. It is predicted that the risk of antibiotic residues can be reduced in nature as in the milk offered for human consumption, and the resistance of microorganisms to antibiotics can be partially prevented. In addition, this method can become a treatment protocol for management systems that produce organic milk and dairy products by organic livestock farming, which have become increasingly popular in recent years.

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Conflict of Interest

There is no conflict of interest in the present study.

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