Kafkas Üniv Vet Fak Derg
15 (1): 25-33, 2009

Effect of Immunomodulatory Treatment with Levamisole on Uterine Inflammation and Involution, Serum Sialic Acid Levels and Ovarian Function in Cows

Ş. Metin PANCARCI * ✎ Kutlay GÜRBULAK ** Hasan ORAL *
Mahmut KARAPEHLİVAN *** Recai TUNCA **** Armağan ÇOLAK *****

* Department of Obstetrics, Faculty of Veterinary Medicine, University of Kafkas, Kars - TURKEY
** Department of Obstetrics, Faculty of Veterinary Medicine, University of Erciyes, Kayseri - TURKEY
*** Department of Biochemistry, Faculty of Veterinary Medicine, University of Kafkas, Kars - TURKEY
**** Department of Pathology, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydın - TURKEY
***** Department of Obstetrics, Faculty of Veterinary Medicine, University of Atatürk, Erzurum - TURKEY

Yayın Kodu (Article Code): 2008/47-A

Summary

The effects of immunomodulatory treatment with levamisole on uterine involution, ovarian function and total sialic acid (TSA) concentrations during postpartum period in lactating dairy cows were investigated. In Group I (n=20), levamisole hydrochloride (Actipar®) was weekly administered at immunomodulating doses (2.5 mg/kg; IM) starting from five or six weeks until two weeks prior to expected calving. In Group II (n=13), physiologic saline was administered during the same injection schedule. All cows (n=33) were examined on days 16±3, 23±3, 30±3 and 37±3 postpartum including external inspection, vaginoscopy, and transrectal ultrasonography. Relative risk of cows in control group having a pathological vaginal discharge (7/11; 63.6%) was 1.9 (0.8-4.4; P>0.05) times higher than that in levamisole treated group (5/15; 33.3%). There is an interaction effect of treatment by vaginal discharge scores on the involution of cervix uteri (P<0.05), and levamisole treatment accelerated the involution of the cervix uteri in cows with normal vaginal discharges compared to those with pathological discharges. There was an earlier recruitment of the follicular wave in cows with normal vaginal discharge compared to those with pathological vaginal discharge. Serum TSA concentrations were higher in cows with pathological vaginal discharge than those with normal vaginal discharge (P<0.01). Consequently, facilitation of involution of the cervix uteri and earlier recruitment of the follicular wave in cows with normal vaginal discharge reveals beneficial effect of immunomodulatory treatment with levamisole, and warrants further research regarding the immunomodulatory treatment with levamisole during dry period prior to breeding protocols. In addition, relationships between vaginal discharge scores and TSA concentrations could be used for evalulation of postpartum reproductive health.

Keywords: *Cow, Immunomodulation, Involution, Ovarian function, Sialic acid*

İneklerde Levamizol ile İmmunomodulatör Tedavinin Uterus Yangısı ve İnvolüsyonu, Serum Sialik Asit Düzeyleri ve Ovaryum Fonksiyonu Üzerine Etkileri

Özet

Sağmal ineklerde levamizol ile immunomodülatör tedavinin postpartum dönemde uterus involüsyonu, ovaryum fonksiyonu ve toplam sialik asit (TSA) konsantrasyonları üzerine olan etkileri araştırıldı. Group I'deki (n=20) ineklere levamisole hydrochloride (Actipar®) haftada bir kez olmak üzere immunomodulasyon dozunda (2.5 mg/kg; IM) beklenen doğumdan beş veya altı hafta öncesinden başlanılarak doğuma iki hafta kalana kadar uygulandı. Group II'deki (n=13) ineklere serum fizyolojik aynı zamanlama ile uygulandı. Bütün inekler (n=33)16±3, 23±3, 30±3 ve 37±3 günlerde inspeksiyon, vajinoskopi ve transrektal ultrasonografik olarak muayene edildiler. Kontrol grubundaki ineklerde göreceli olarak patolojik vajinal akıntı olma riski (7/11; %63.6) levamizol uygulanan gruba (5/15; %33.3) göre 1.9 (0.8-4.4; P>0.05) kat daha fazla bulundu. Vajinal akıntı skoru ile tedavinin etkileşiminin serviks uterinin involüsyonu üzerine etkisi belirlendi (P<0.05) ve levamizol tedavisi patolojik akıntısı olanlara nazaran normal vajinal akıntısı olan ineklerde serviks uterinin involüsyonunu hızlandırdı. Patolojik akıntısı olanlara nazaran normal vajinal akıntısı olan ineklerde erken folikül dalgası gelişimi gözlendi. Patolojik vajinal akıntısı olan ineklerde serum TSA konsantrasyonları normal akıntılı ineklere göre yüksek bulundu (P<0.01). Sonuç olarak, normal vajinal akıntısı olan ineklerde serviks uterinin involüsyonunun hızlandırılması ve erken folikül dalgası gelişimi, levamizol ile immunomodülatör tedavinin faydalı etkilerini ortaya koymakta olup suni tohumlama protokollerinden önce kuru dönemde levamizol ile immunomodülatör tedavinin kullanımına ilişkin araştırmalara ihtiyaç duyulduğunu göstermektedir. Ayrıca, vajinal akıntı skoru ve TSA konsantrasyonları arasındaki ilişkinin postpartum reprodüktif sağlığın değerlendirilmesinde kullanılabilirliğini göstermektedir.

Anahtar sözcükler: *İnek, İmmunomodulasyon; İnvolusyon, Ovaryum fonksiyonu, Sialik asit*

✎ İletişim (Correspondence)
☎ +90 474 242 68 07/1054
✉ smpancarci@hotmail.com

INTRODUCTION

The existence of bacteria in the uterus results in inflammation of the endometrium and postpones uterine involution [1,2]. Moreover, uterine bacterial infection or bacterial products suppress LH secretion from pituitary, and disrupt postpartum ovarian follicular growth and function leading to disruption of ovulation in cows [3-6].

Levamisole is commonly used as an anti-nematodal [7] and immunomodulatory agent [8-10] in domestic animals and humans. It is known that levamisole stimulates cell-mediated immune reactivity by potentiating the rate of T-lymphocyte differentiation, the sensibility to antigens and mitogens and activity of phagocytes [11,12]. Administration of a single dose (2.5 mg/kg) of levamisole results in immunomodulatory effect for about 48 hours [13]. Intermittent treatment with one-third of the anthelmintic dose (2.5 mg/kg) results in more effective immune response [12,13].

In the cattle industry, clarifying the relationships between the postpartum uterus and ovary, and decreasing the effect of uterine infection are the reproductive challenges to be faced at the beginning of the twenty-first century [14]. There is little convincing data for the benefit of any intrauterine antimicrobial use on reproductive performance in cows with endometritis [15,16]; however, most comparative clinical investigations [15,17] revealed that PGF2α is at least as effective as or is preferable to intra uterine infusion of antibiotics for treatment of endometritis and reduction of interval between calving to pregnancy [18,19]. Immunomodulatory treatment with levamisole in dairy cows with retained foetal membranes or purulent uterine discharge resulted in enhanced cell immune reaction leading to significant association between neutrophil and eosinophil granulocytes in peripheral blood on day 12 postpartum [20]. Weekly injections of levamisole during dry period for six weeks significantly decreased the incidences of mastitis, fetal deaths and metritis due to its immunopotentiating activity in cows [21-23]. Similarly, beneficial effect of levamisole treatment with immunostimulative doses at the last period of pregnancy on the health state of sows after parturition was reported [24]. However, most of the studies focused on effect of levamisole on the prevention of metritis rather than its mechanism.

Sialic acid (SA) is an acetylated derivative of neuraminic acid, and it is a significant cell-surface component of biological membranes in bacteria and animals [25]. It has been reported that serum SA concentrations increase rapidly following inflammation and injury [26]. It has been suggested that serum SA concentrations, particularly lipid bound SA, are an indicator of inflammatory diseases [27]. Similarly, increase in serum total sialic acid (TSA) concentrations have been reported in diseases such as pneumonia in calves [28], dermatophytosis in calves [29], traumatic reticuloperitonitis [30] and infectious bovine keratoconjunctivitis in calves [31].

Therefore, the first objective of this study was to investigate the effect of immunomodulatory treatment with levamisole on uterine inflammation and involution by examining uterine biopsies and serum TSA concentrations during postpartum period in lactating dairy cows. The second objective of this study was to determine the effect of immunomodulatory treatment with levamisole during prepartum period on postpartum resumption of cyclicity.

MATERIAL and METHODS

This experiment was conducted on 33 cows (3 primiparous and 30 multiparous) at University of Ataturk, Faculty of Agriculture, Dairy Research Farm, Erzurum, Turkey, during December 2004 and May 2005. Cows were housed in free stall barns accessed to a feeding lot, and fed with total mixed ration including concentrated feed (4.5 kg; CP %18.2, ME 2428 kcal/kg), grass hay (8 kg), alfalfa hay (4 kg) and grass silage (5 kg) per animal basis as fed. Rolling herd average daily milk production was 10.2 L during experimental period. Cows were blocked based on breed (Brown Swiss [n=18] and Holstein [n=15]), and then randomly assigned into two groups. In Group I (n=20; [7 Holstein and 13 Brown Swiss]), levamisole hydrochloride (Actipar®; Alke Ilac San. 34896, Pendik, İstanbul-TÜRKİYE) was weekly administered at immunomodulating doses (2.5 mg/kg; IM) starting from five or six weeks until two weeks prior to expected calving. In Group II (n=13; [8 Holstein and 5 Brown Swiss]), physiologic saline was administered during the same injection schedule. Cows injected more than this interval were removed from this study. Therefore, cows in both groups got mainly four injections; while few of

them got five injections during dry period.

Uterus and ovaries were scanned with transrectal ultrasonography (Pie Medical/100 Falco Vet equipped with 6.0-8.0 MHz rectal linear probe) weekly for a month starting at two weeks after calving on days 16±3, 23±3, 30±3 and 37±3 postpartum. Diameters of cervix and uterine horns at approximately five cm away from the bifurcation of the uteri were measured to evaluate uterine involution. Ovaries were scanned for follicles and presence of corpus luteum (CL) to investigate ovarian function. Monitored follicles were classified based on their sizes into three classes [class I (2-5 mm), II (6-9 mm) and III (>10 mm)] as described by Lucy et al.[32]. Moreover, vaginal discharges observed at visual examinations and vaginoscopy were scored at 16±3 days postpartum in four scales (score: 0, clear or translucent mucus; score: 1, mucus containing flecks of white or off-white pus; score: 2, <50 mL exudate containing <50% white or off-white mucopurulent material; and score: 3, >50 mL exudate containing >50% purulent material, usually white or yellow, but occasionally sanguineous) as described by Williams et al.[33]. Because none of the animals had vaginal discharge score of one, vaginal discharge scores were used as normal (0) and pathologic (2 and 3) in the analyses. Dystocia scores were recorded based on severity of dystocia using scores ranging from 1 (no problem) to 5 (extreme difficulty) as described by Djemali et al.[34].

Biopsy samples were collected from endometrium at dorsal site of the uterine body between intercaruncular areas at 2±1 day postpartum. Biopsy samples were evaluated based on intensity of inflammatory cells, necrosis and hyperemia, and scored with four scales (0: normal; 1: few lymphocytes and neutrophyle leucocytes; 2: moderate inflammatory cell infiltration and hyperemia; and 3: severe inflammatory cell infiltration, necrosis, hyperemia and bleeding) in a double blind study. Blood samples were collected weekly prior to ultrasonographic examinations via cocygeal vein puncture to investigate serum sialic acid concentrations. Serum total sialic acid concentrations were determined colorimetrically using a spectrophotometer (UV-1201, Shimadzu, Japan) according to the methods reported by Sydow [35].

Vaginal discharge and biopsy scores were analyzed using Mann Whitney analyses in Minitab program (Minitab 12 for windows). Diameters of the uterine horns and cervix, and the numbers of Class I, II and III follicles were analyzed using the repeated measures analysis of the mixed procedure of SAS [36]. Ovulation rate based on presence of CL was analyzed by the logistic regression procedure of SAS. The Logistic Regression-Stepwise Selection Procedure was utilized to determine independent variables at pre-determined significance levels (variable entered with $P<0.30$ and stayed with $P<0.20$ in the model) among all variables and all-possible interactions with treatment. The mathematical model included treatment, parity, breed, week of the enrollment (replicate), scores of dystocia, calf sex, metritis and interactions. However, relative risk ratio values along with 95% confidence limits obtained from the Proc Freq procedure of SAS were reported to be more realistic as opposed to odds ratio values. The Proc Freq procedure of SAS was utilized to obtain frequency values for each variable in data set.

RESULTS

All of the animals in both groups calved (dystocia scores were 1 to 3 in range) and expelled fetal membranes normally. Based on clinical examinations including purulent vaginal discharges (score 3), enlarged uterine diameter and visualization of pus in uterus by ultrasonography, relative risk for having metritis in control group (4/13; 30.8%) was 1.5 (0.5-5.1) times higher than that in levamisole group (4/20; 20%) during the monitored period; however, this difference is not statistically significant ($P>0.05$). For the examination of vaginal discharges, seven cows had missing observations. The median scores of vaginal discharge were 0 and 3 in levamisole and control groups, respectively ($P<0.07$). Relative risk of cows in control group having a pathological vaginal discharge (7/11; 63.6%) was 1.9 (0.8-4.4) times higher than that in levamisole treated group (5/15; 33.3%); however, this difference is not statistically significant ($P>0.05$). Similarly, the median scores of endometrial biopsies were 1 and 2 in levamisole and control groups; respectively, and this numerical difference is not statistically significant ($P>0.05$).

No effect was observed on the involution of the previously pregnant uterine horn. Surprisingly, there is a significant ($P<0.05$) effect of vaginal discharge scores on the rate of involution of the previously non-pregnant uterine horn. There is an

interaction effect of treatment by vaginal discharge scores on the involution of cervix uteri (*Figure 1*, P<0.07). Analyses of cows with normal vaginal discharges (n=14) indicated a significant (P<0.05) effect of treatment on the involution of cervix uteri; however, there is no treatment effect when cows with pathological discharges (n=12) were analyzed separately *(Figure 1)*. These analyses indicate that levamisole treatment accelerated the involution of the cervix uteri in cows with normal vaginal discharges compared to those with pathological discharges.

Weekly ultrasonographic examinations revealed that none of the cows (n=29) in both groups ovulated at 16±3 days following calving based on detection of CL. Ovulation rates at 23±3 days following calving did not differ between control (4/12; 33.3%) and levamisole treated (4/17; 23.5%) groups. However, at 23±3 days following calving there is a breed effect on presence of CL, and ovulation rate was 0.44 (0.24-0.80) times less (P<0.05) in Holstein cows (1/14; 7.1%; [one control cow]) than that in Brown Swiss cows (7/15; 46.7%; [three control and four levamisole treated cows]). At the end of the monitored period (37±3 days postpartum) three cows had missing observations, and ovulation rates did not differ between control (5/12; 41.7%) and levamisole treated (8/14; 57.1%) groups, however, there are breed and metritis effects on ovulation rate. In this regard, overall ovulation rate was 0.30 (0.11-0.85) times less (P<0.01) in Holstein cows (3/13; 23.1%; [two control and one levamisole treated cows]) than that in Brown Swiss cows (10/13; 76.9%; [three control and seven levamisole treated cows]). At the end of the monitored period, overall ovulation rate was 6.0 (0.84-43.13) times higher (P<0.05) in cows without metritis (12/19; 63.2%; [four control and eight levamisole treated cows]) than that in cows with metritis (1/7; 14.3%; [one control cow]).

Number of Class I follicles was significantly lower (P<0.05) in cows with normal vaginal discharge compared to those with pathological vaginal discharge at days 16±3 postpartum *(Figure 2)*. In contrast, number of Class II follicles was significantly higher (P<0.05) in cows with normal vaginal discharge compared to those with pathological vaginal discharge at days 16±3 postpartum *(Figure 2)*. Number of Class III follicles was significantly higher (P<0.05) in cows with normal vaginal discharge compared to those with pathological vaginal discharge at days 37±3 postpartum *(Figure 2)*. Moreover, average diameter of the largest follicle monitored at days 37±3 postpartum was significantly larger (P<0.01) in cows with normal vaginal discharge compared to those with pathological vaginal discharge *(Figure 3)*. These results indicate earlier recruitment of the follicular wave in cows with normal vaginal discharge compared to those with pathological vaginal discharge following calving.

Serum TSA concentrations were higher in cows with pathological vaginal discharge than those with normal vaginal discharge (P<0.01; *Figure 4*). There is an interaction effect (P<0.05) of treatment by week by biopsy score for serum TSA concentrations *(Figure 5)*. In this regard, serum TSA concentrations in cows with biopsy score 3 in levamisole group are higher than that in control group on day 16±3 postpartum. However, serum TSA concentrations were abruptly decreased in cows with biopsy score 3 in levamisole group.

DISCUSSION

In present study, levamisole treatment accelerated the involution of the cervix uteri in cows with normal vaginal discharges as oppose to those with pathological discharges. This result implies that immunomodulatory treatment could be more effective in healthy cows for involution of the cervix uteri. Similarly, shorter period for uterine involution has been reported in cows treated with levamisole during dry period [37]. It has been reported that the presence of purulent uterine discharge or cervical diameter >7.5 cm after 20 days postpartum, or mucopurulent discharge after 26 days postpartum revealed clinical endometritis in cows, and among postpartum history, inspection, vaginoscopy, and palpation, only the presence of purulent discharge and cervical diameter were significantly associated with impaired fertility [38]. Moreover, the presence of purulent discharge on vaginoscopy is highly correlated with active bacterial uterine infection [39,40]. Furthermore, it has been pointed out that the presence of purulent uterine discharge should be a good indicator for determination of postpartum uterine health [38]. Likewise, the interaction effect of treatment by vaginal discharge scores on the rate of involution of cervix uteri in present study supports that uterine discharge could be a good indicator for determination

of postpartum uterine involution. Involution of the cervix is slower than the uterus; however, both uterus and cervix should reach a diameter of <5 cm by 25 days in milk in normal cows [41]. In a study investigating the association of postpartum uterine discharge and cervical size with reproductive performance, significantly decreased risk of pregnancy at first insemination and significantly longer days open were reported in cows with a large cervix [42]. Likewise, it has been concluded that the optimum time and optimum threshold of the cervical diameter to determine cervical involution were three weeks postpartum and 6 cm, respectively [42]. It has been reported that cervical diameter of <5 cm is apparently not associated with impaired reproduction; however, the critical threshold of cervical diameter lies between 6 and 7.5 cm, and they made an assumption that an enlarged cervix should estimate reduced pregnancy rate independent of uterine findings due to less variability in the size of the fully involuted cervix than in the location or size of the uterus after involution [38]. In all the cows, cervical diameter was <5 cm at 16±3 days postpartum in currrent study. Because cervical diameter was measured by transrectal ultrasonography in present study, involution of the cervical diameter was monitored more accurately, and the interaction effect of vaginal discharge by treatment on cervical involution was detected.

It has been postulated that the presence of mildly purulent uterine discharge in the first month postpartum likely reveals a successful immune response to a bacterial challenge [38]. It has been reported that cows with endometritis were more likely to have no palpable ovarian structures than healthy cows [38]. Furthermore, first ovulation takes place 9 to 19 days later in cows with slow involution [43], dystocia, twins, or retained placenta [41,44] than that in normal cows. It has been concluded that there was no effect of postpartum uterine bacterial contamination on plasma FSH concentration and initial ovarian follicle wave emergence; however, high bacterial contamination of the uterus leads to a contemporaneous localized effect on ovarian follicle selection with fewer first and second dominant follicles in the ovary ipsilateral to the previously gravid uterine horn and slower growth of subsequent dominant follicle along with reduced oestradiol secretion following calving [3]. Similarly, it has been reported that ovarian follicles were smaller after calving with a uterine bacterial infection [5]. Moreover, the hormonal interactions for the control of normal ovarian function are disturbed by inflammatory mediators such as bacterial endotoxin and immune mediators such as cytokines [45-47].

Administration of inflammatory mediators disrupts GnRH release from the hypothalamus and LH secretion from the pituitary. However, inhibition of follicular oestradiol secretion in the presence of sufficient plasma LH concentrations following administration of endotoxin reveals a direct effect of inflammatory mediators at the ovarian level [48,49]. Therefore, effect of uterine bacterial contamination on follicle growth and function could be resulted from disruption of LH secretion or ovarian function [49]. Lower plasma LH concentration suppressed the growth rate and oestradiol secretion of dominant follicles after follicle selection [50]. Although weekly examination of ovaries is not adequate to investigate follicular dynamics, earlier recruitment and faster growth of the follicles in cows with normal vaginal discharge reveal that vaginal discharge scores could be an indicator for the postpartum follicular dynamics in the present study. Furthermore, present study supports previous studies and provides evidence for an effect of the pathologic uterine discharge on ovarian follicular growth after calving. Because dairy cows are expected to have their first postpartum ovulation between 14 and 28 days in milk in average [41,51,52], most of the cows were expected to reinitiate cyclicity during the examination period. Accordingly, first ovulations were detected on 23±3 days postpartum in current study. Overall ovulation rate was significantly higher in cows without metritis, and levamisole treatment numerically decreased the rate of metritis in present study. This result reveals that levamisole treatment during dry period could be beneficial for postpartum uterine health leading to earlier initiation of ovarian cyclicity.

In response to tissue damage and inflammation, heptaocytes produce acute phase proteins [53]. Serum SA concentrations have been used to determine the acute-phase protein response [54]. Serum SA is a relatively unspecific marker of inflammation, and its elevation could be explained by enhanced levels of richly sialylated acute phase glycoproteins [55]. Because peripheral plasma acute phase protein concentrations enhance to maximum levels between days 1 and 3 postpartum

followed by decrease within two weeks to basal concentrations, acute phase protein response have been used to monitor the process of uterine involution [56-58]. Because uterine bacterial contamination also elevates the plasma concentrations of acute phase proteins [56] and SA [59], interpretation of these data could be complicated. Higher serum TSA concentrations in cows with pathological vaginal discharge may also reflect the presence of inflammation or co-existence of bacterial infection in current study. Similarly, numerically higher total sialic acid concentrations in cows with metritis compared to those in healthy cows has been reported [60].

In conclusion, facilitation of involution of the cervix uteri and earlier recruitment of the follicular wave in cows with normal vaginal discharge implies beneficial effect of immunomodulatory treatment with levamisole during dry period on postpartum involution. Thereby, immunomodulatory treatment with levamisole during dry period could be alternative prevention program for postpartum uterine disorders. Overall, these results warrant large fertility trials with immunomodulatory treatment with levamisole prior to breeding protocols. Furthermore, relationships between vaginal discharge scores, TSA concentrations, postpartum follicular dynamics and rate of involution of cervix uteri reveal that vaginal discharge scores and TSA concentrations could be good tools for evalulation of postpartum reproductive health.

Acknowledments

Levamisole hydrochloride (Actipar®) was generously donated by Alke Ilac San., Pendik, İstanbul-Turkey. Authors gratefully thanks to the farm personnel at Ataturk University, Faculty of Agriculture, Dairy Research Farm, Erzurum, Turkey.

REFERENCES

1. **Bonnett BN, Martin SW, Gannon VP, Miller RB, Etherington WG:** Endometrial biopsy in Holstein–Friesian dairy cows. III. Bacteriological analysis and correlations with histological findings. *Can J Vet Med,* 55, 168–173, 1991.
2. **Sheldon IM, Noakes DE, Rycroft AN, Dobson H:** The effect of intrauterine administration of estradiol on postpartum uterine involution in cattle. *Theriogenology,* 59, 1357–1371, 2003.
3. **Sheldon IM, Noakes DE, Rycroft AN, Pfeiffer DU, Dobson H:** Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. *Reproduction,* 123, 837–845, 2002.
4. **Opsomer G, Grohn YT, Hertl J, Coryn M, Deluyker H, de Kruif A:** Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: A field study. *Theriogenology,* 53, 841–857, 2000.
5. **Peter AT, Bosu WTK:** Relationship of uterine infections and folliculogenesis in dairy cows during early puerperium. *Theriogenology,* 30, 1045–1051, 1988.
6. **Peter AT, Bosu WTK, DeDecker RJ:** Suppression of preovulatory luteinizing hormone surges in heifers after intrauterine infusions of Escherichia coli endotoxin. *Am J Vet Res,* 50, 368–373, 1989.
7. **McDonald LR, Roberson EL:** Chemotherapy of parasitic diseases, 897–902. **In,** Booth NH, McDonald LE (Eds): Veterinary Pharmacology and Therapeutics. Ames-Iowa: Iowa State University Press, pp 1238, 1991.
8. **Lowe RJ:** Levamisole as an immunostimulant. *Vet Rec,* 106 (17): 390, 1980.
9. **Treurniet-Donker AD, Meischke-de Jongh ML, van Putten WL:** Levamisole as adjuvant immunotherapy in breast cancer. *Cancer,* 59 (9): 1590-1593, 1987.
10. **Mojzisova J, Paulik S, Bajova V, Baranova D:** Immunomodulatory effect of levamisole and administration of amitraz in dogs with uncomplicated generalized demodicosis. *Vet Med (Praha),* 42, 307-311, 1997.
11. **Brunner CJ, Muscoplat CC:** Immunomodulatory effects of levamisole. *J Am Vet Med Assoc,* 176, 10 (2): 1159-1162, 1980.
12. **Einstein R, Jones RS, Knifton A, Starmer GA:** Levamisole, 504-505. **In,** Principles of Veterinary Therapeutics, Longman Scientific and Technical, Essex, UK, pp 591, 1994.
13. **Anderson JC:** Levamisole and bovine mastitis. *Vet Rec,* 11, 138-140, 1984.
14. **Sheldon IM, Dobson H:** Reproductive challenges facing the cattle industry at the beginning of the 21st century. *Reproduction (Suppl),* 61, 1-13, 2003.
15. **Steffan J, Agric M, Adriamanga S, Thibier M:** Treatment of metritis with antibiotics or prostaglandin F2 alpha and influence of ovarian cyclicity in dairy cows. *Am J Vet Res,* 45 (6):1090-1094, 1984.
16. **Thurmond MC, Jameson CM, Picanso JP:** Effect of intrauterine antimicrobial treatment in reducing calving-to-conception interval in cows with endometritis. J Am Vet Med Assoc, 203 (11):1576-1578, 1993.
17. **Sheldon IM, Noakes DE:** Comparison of three treatments for bovine endometritis. *Vet Rec,* 142 (21): 575-579, 1998.
18. **Etherington WG, Martin SW, Bonnett B, Johnson WH, Miller RB, Savage NC, Walton JS, Montgomery ME:** Reproductive performance of dairy cows following treatment with cloprostenol 26 and/or 40 days postpartum: A field trial. *Theriogenology,* 29, 565–575, 1988.
19. **Risco CA, Archbald LF, Elliott J, Tran T, Chavatte P:** Effect of hormonal treatment on fertility in dairy cows with dystocia or retained fetal membranes at parturition. *J Dairy Sci,* 77 (9): 2562-2569, 1994.
20. **Vojtic I:** Levamisole-caused association between neutrophil and eosinophil granulocytes in dairy cows after parturition. *Veterinarski Arhiv,* 68 (4): 135-142, 1998.
21. **Flesh J, Harel W, Nelken D:** Immunopotentiating effect of levamisole in the prevention of bovine mastitis, fetal.death and endometritis. *Vet Rec,* 17, 56-57, 1982.
22. **Gurbulak K, Kılıcarslan MG:** The effect of levamisole administration on the postpartum mastitis, metritis and foetal death in the pregnant cows (in Turkish). *Istanbul University Journal of Veterinary Faculty,* 30, 35-46, 2004.
23. **Kacar C, Yiidiz S, Ozturkler Y:** The effects of levamisole on certain peripartum reproductive diseases in cows. *Indian Vet J,* 84, 1255-1256, 2007.
24. **Wawron W:** Effect of immunostimulating agents on the occurrence of puerperal diseases in swine. *Dtsch Tierarztl*

Wochenschr, 104 (6): 220-223, 1997.
25. **Schauer R:** Chemistry, metabolism and biological functions of sialic acid. *Advances in Carbohydrate Chemistry and Biochemistry,* 40, 131-234, 1982.
26. **Haq M, Haq S, Tutt P, Crook M:** Serum total sialic acid and lipid-associated sialic acid in normal individual patients with myocardial infarction and their relationship to acute phase proteins. Annals of Clinical Biochemistry, 30, 383-386, 1993.
27. **Motoi Y:** Determination and clinical evaluation of sialic acid and mucoprotein in bovine blood. *National Institute of Animal Health,* 37, 643-649, 1984.
28. **Karapehlivan M, Atakisi E, Citil M, Kankavi O, Atakisi O:** Serum sialic acid levels in calves with pneumonia. *Vet Res Com,* 31, 37-41, 2007.
29. **Karapehlivan M, Uzlu E, Kaya N, Kankavi O, Ural K, Citil, M:** Investigation of some biochemical parameters and the antioxidant system in calves with dermatophytosis. *Turk J Vet Anim Sci.,* 31 (2): 85-89, 2007b.
30. **Citil M, Gunes V, Karapehlivan M, Atalan G, Maraslı, S:** Evaluation of serum sialic acid as an inflammation marker in cattle with traumatic reticulo peritonitis. *Rev Med Vet,* 155 (7): 389-392, 2004.
31. **Gunes V, Karapehlivan M, Citil M, Atalan G, Maraslı S:** Relationship between serum sialic acid levels and eye lesions in calves with infectious bovine keratoconjunctivitis. *Rev Med Vet,* 155 (10): 508-511, 2004.
32. **Lucy MC, Savio JD, Badinga L, De La Sota RL, Thatcher WW:** Factors that affect ovarian follicular dynamics in cattle. *J Anim Sci,* 70, 3615-3626, 1992.
33. **Williams EJ, Fischer DP, Pfeiffer DU, England GCW, Noakes DE, Dobson H, Sheldon IM:** Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology*, 63, 102–117, 2005.
34. **Djemali M, Freeman AE, Berger PJ:** Reporting of dystocia scores and effects of dystocia on production, days open, and days dry from dairy herd improvement data. *J Dairy Sci,* 70 (10): 2127-2131, 1987.
35. **Sydow G:** A simplified quick method for determination of sialic acid in serum. *Biomedica Biochimica Acta,* 44,1721-1723, 1985.
36. **Littell RC, Pendergast J, Natarajan R:** Modelling covariance structure in the analysis of repeated measures data. *Statist Med,* 19, 1793-1819, 2000.
37. **Sattar A, Lodhi LA, Qureshi ZI, Ahmad I, Naz NA:** Effects of immunopotentiators on postpartum reproductive performance in Sahiwal cows. *Pakistan Vet J,* 23 (3):130-133, 2003.
38. **LeBlanc SJ, Duffield TF, Leslie KE, Bateman KG, Keefe GP, Walton JS, Johnson WH:** Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J Dairy Sci,* 85, 2223–2236, 2002.
39. **Miller HV, Kimsey PB, Kendrick JW, Darien B, Doering L, Franti C, Horton J:** Endometritis of dairy cattle: diagnosis, treatment, and fertility. *Bovine Practitioner,* 15, 13–23, 1980.
40. **Dohmen MJW, Lohuis JACM, Huszenicza G, Nagy P, Gacs M:** The relationship between bacteriological and clinical findings in cows with subacute/chronic endometritis. *Theriogenology*, 43, 1379–1388, 1995.
41. **Morrow DA, Roberts SJ, McEntee K, Gray HG:** Postpartum ovarian activity and uterine involution in dairy cattle. *J Am Vet Med Assoc,* 149, 1596–1609, 1966.
42. **Oltenacu PA, Britt JH, Braun RK, Mellenberger RW:** Relationships among type of parturition, type of discharge from genital tract, involution of cervix, and subsequent reproductive performance in Holstein cows. *J Dairy Sci,* 66, 612–619, 1983.
43. **Madej A, Kindahl H, Woyno W, Edqvist LE, Stupnicki R:** Blood levels of 15-keto-13,14-dihydro-prostaglandin F2 during the postpartum period in primiparous cows. *Theriogenology*, 21, 279–287, 1984.
44. **Fonseca FA, Britt JH, McDaniel BT, Wilk JC, Rakes AH:** Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of the cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. *J Dairy Sci,* 66, 1128–1147, 1983.
45. **Rivest S, Lee S, Attardi B, Rivier C:** The chronic intracerebroventricular infusion of interleukin-1b alters the activity of the hypothalamic–pituitary–gonadal axis of cycling rats. I. Effect on LHRH and gonadotropin biosynthesis and
46. secretion. *Endocrinology*, 133, 2424–2430, 1993.
Battaglia DF, Beaver AB, Harris TG, Tanhehco E, Viguie C, Karsch FJ: Endotoxin disrupts the estradiol-induced luteinizing hormone surge: interference with estradiol signal reading, not surge release. *Endocrinology*, 140, 2471–2479, 1999.
47. **Williams CY, Harris TG, Battaglia DF, Viguie C, Karsch FJ:** Endotoxin inhibits pituitary responsiveness to gonadotrophin-releasing hormone. *Endocrinology*, 142, 1915–1922, 2001.
48. **Xiao E, Xia-Zhang L, Barth A, Zhu J, Ferin M:** Stress and the menstrual cycle: Relevance of cycle quality in the short- and long-term response to a 5-day endotoxin challenge during the follicular phase in the rhesus monkey. *J Clin Endocrin Metabolism,* 83, 2454–2460, 1998.
49. **Battaglia DF, Krasa HB, Padmanabhan V, Viguie C, Karsch FJ:** Endocrine alterations that underlie endotoxin-induced disruption of the follicular phase in ewes. *Biol Reprod,* 62, 45–53, 2000.
50. **Ginther OJ, Bergfelt DR, Beg MA, Kot K:** Follicle selection in cattle: Role of luteinizing hormone. *Biol Reprod,* 64, 197–205, 2001.
51. **Leslie KE:** The events of normal and abnormal postpartum reproductive endocrinology and uterine involution in dairy cows: A review. *Can Vet J,* 24, 67–71, 1983.
52. **Stevenson JS, Call EP:** Fertility of postpartum dairy cows after administration of gonadotropin-releasing hormone and prostaglandin F2alpha: A field trial. *J Dairy Sci,* 71, 1926-1933, 1988.
53. **Baumann H, Gauldie J:** The acute phase response. *Immunology Today,* 15 (2): 74-80, 1994.
54. **Taniuchi K, Chifu K, Hayashi N:** A new enzymatic method for the determination of sialic acid in serum and its application for a marker of acute phase reactants. *Kobe J Med Sci,* 27, 91-102, 1981.
55. **Sillanaukee P, Pönniö M, Jaaskelainen IP:** Occurence of sialic acids in healthy humans and different disorders. *Eur J Clin Invest,* 29, 413-425, 1999.
56. **Sheldon IM, Noakes DE, Rycroft A, Dobson H:** Acute phase protein response to postpartum uterine bacterial contamination in cattle. *Vet Rec,* 148, 172-175, 2001.
57. **Regassa F, Noakes DE:** Acute phase protein response of ewes and the release of PGFM in relation to uterine involution and the presence of intrauterine bacteria. *Vet Rec,* 144, 502-506, 1999.
58. **Alsemgeest SP, Taverne MA, Boosman R, van der Weyden BC, Gruys E:** Peripartum acute-phase protein serum amyloid-A concentration in plasma of cows and fetuses. *Am J Vet Res,* 54, 164-167, 1993.
59. **Crook M:** The determination of plasma or serum sialic acid. Clinical Biochemistry, 26, 31-38, 1993.
60. **Musal B, Seyrek K, Ulutaş PA:** Serum sialic acid, total protein and albumin levels in cows with metritis treated with intrauterine oxytetracycline (in Turkish). Uludag University Journal of Faculty of Veterinary Medicine, 23, 71-75, 2004.

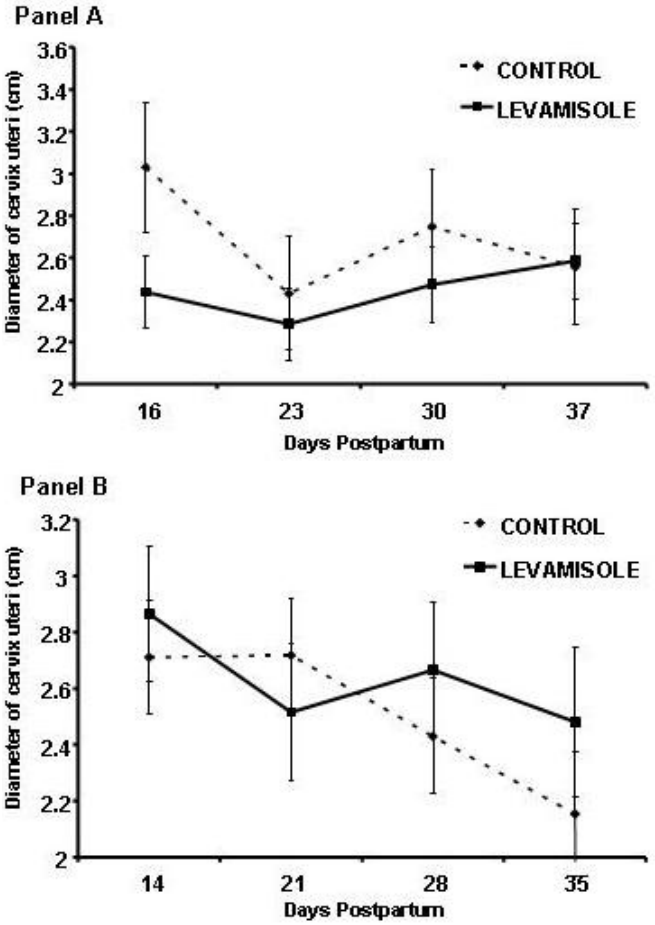

Fig 1. Least squares means and SEM of diameters of cervix uteri of cows with normal (Panel A, n=14) and pathological (Panel B, n=12) vaginal discharges. Interaction of treatment by vaginal discharge is tended to be significant (P<0.07)

Şekil 1. Normal (Panel A, n=14) ve patolojik (Panel B, n=12) vajinal akıntılı ineklerde serviks uteri çaplarının ortalama ve standart hata değerleri. Tedavi ile vajinal akıntı arasındaki etkileşim kısmen önemli bulundu (P<0.07)

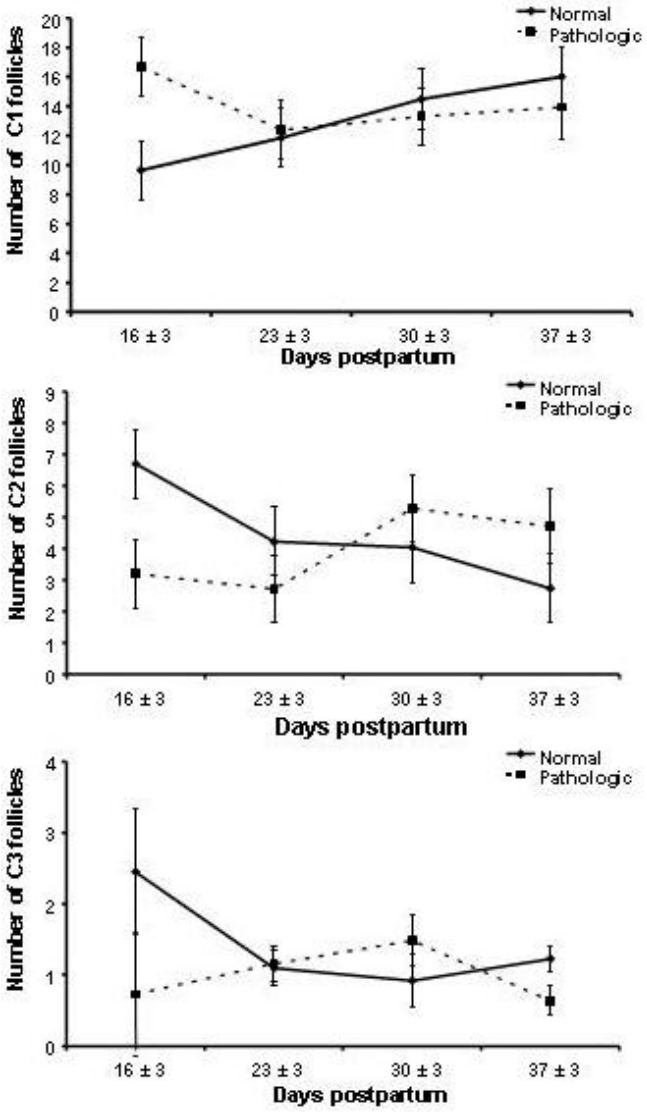

Fig 2. Least squares means and SEM of numbers of Class I (C1), Class II (C2) and Class III (C3) follicles in cows with normal or pathological vaginal discharges

Şekil 2. Normal ve patolojik vajinal akıntılı ineklerde 1. sınıf (C1), 2. sınıf (C2) ve 3. sınıf (C3) folikül sayılarının ortalama ve standart hata değerleri

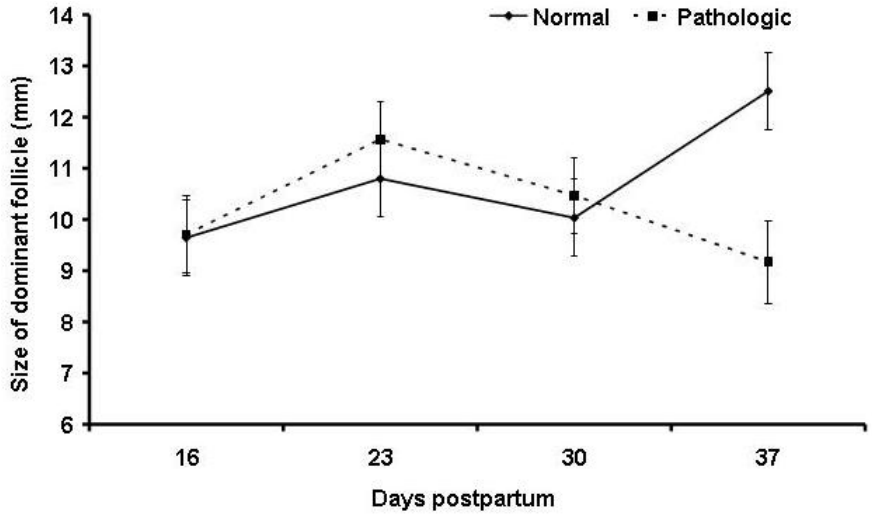

Fig 3. Average diameter of the largest follicle monitored at days 37±3 postpartum was significantly larger (P<0.01) in cows with normal vaginal discharge compared to those with pathological vaginal discharge

Şekil 3. Postpartum 37±3 günde ölçülen en büyük folikülün ortalama çapı normal vajinal akıntılı ineklerde patolojik akıntılılara gore istatistiksel olarak büyük bulundu (P<0.01)

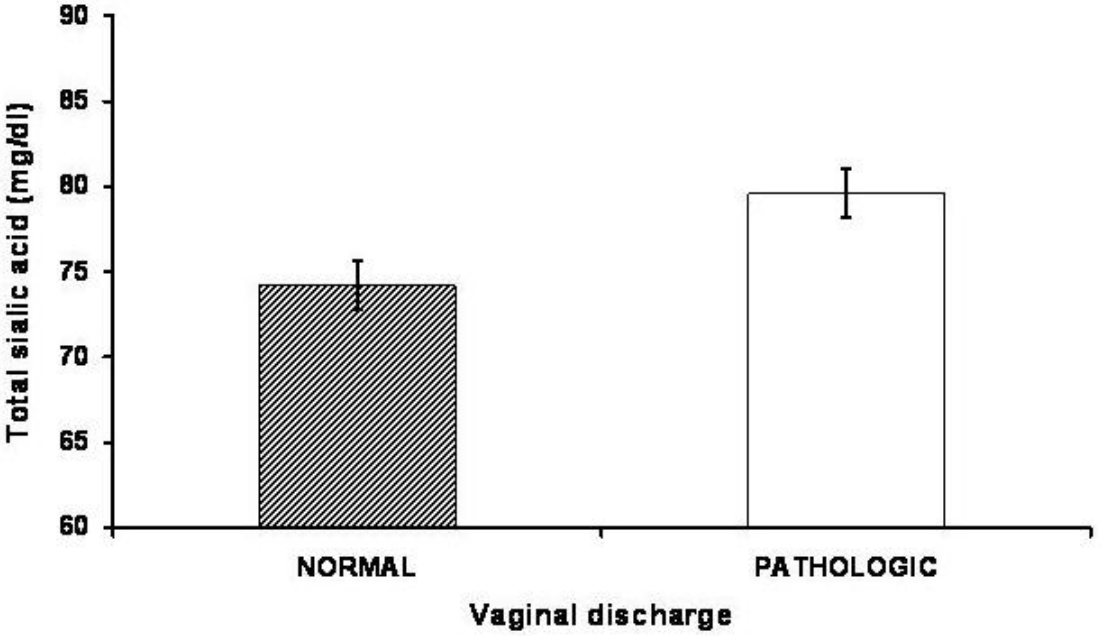

Fig 4. Serum total sialic acid concentrations were higher in cows with pathological vaginal discharge than those with normal vaginal discharge (P<0.01)

Şekil 4. Serumdaki toplam sialik asit konsantrasyonları patolojik vajinal akıntılı ineklerde normal vajinal akıntılı olanlara nazaran daha yüksek bulundu (P<0.01)

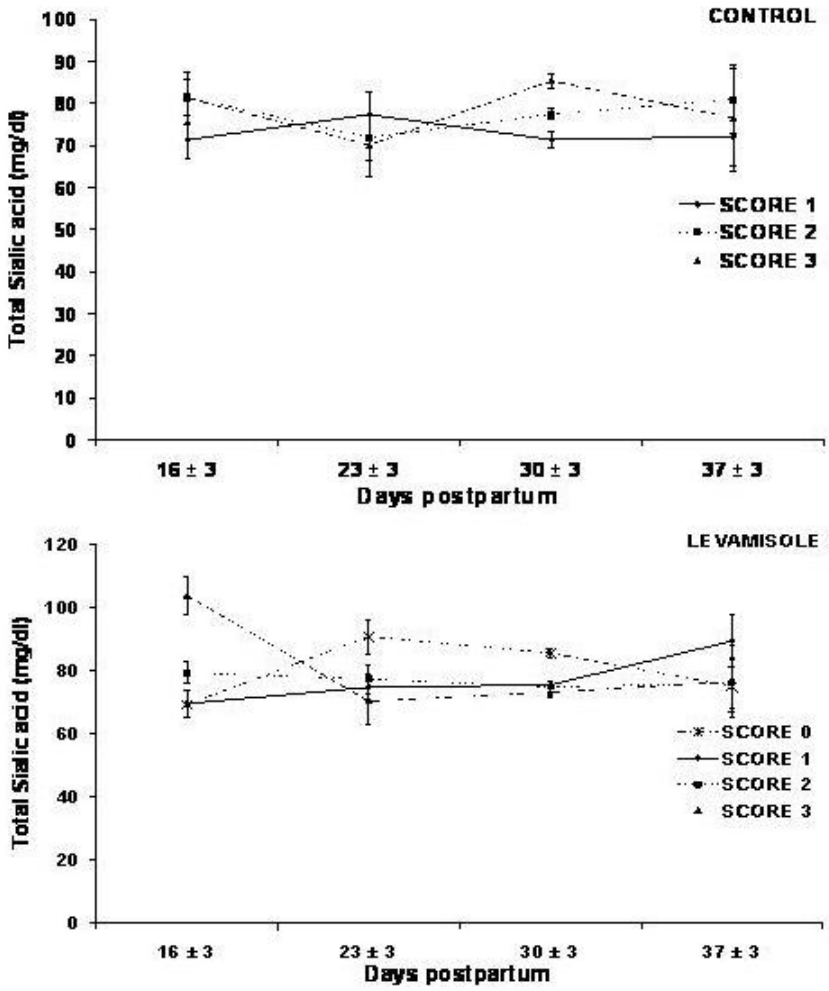

Fig 5. Least square means and SEM of serum total sialic acid concentrations. There is an interaction effect of treatment X postpartum week X biopsy score (P<0.05)

Şekil 5. Serumdaki toplam sialik asit konsantrasyonlarının ortalama ve standart hata değerleri. Tedavi ile postpartum hafta ve biopsi skorları arasında etkileşim bulundu (P<0.05)