EFFECT OF TWO DIFFERENT TREATMENTS ON THE CLINICAL SIGNS AND INFLAMMATORY PARAMETERS IN CASE OF EXPERIMENTALLY INDUCED ACUTE PUERPERAL METRITIS IN DAIRY COWS

Julia Jeremejeva[1, *], Toomas Orro[2], Andres Waldmann[3], Raivo Lindjärv[4], Kalle Kask[1]
[1]*Department of Therapy,*
[2]*Department of Animal Health and Environment,*
[3]*Department of Reproductive Biology,*
[4]*Department of Infectious Diseases, Institute of Veterinary Medicine and Animal Sciences*
Estonian University of Life Sciences, Kreutzwaldi 62, Tartu, 51014, Estonia
Tel. +37253332242; E-mail: tjulia@emu.ee

Summary. The aim of the study was to test the effect of two treatments on clinical cure and inflammatory response in the case of acute puerperal metritis (APM) associated with retained foetal membranes. Late gestation healthy cows (n=21) were divided into three groups (A, B and C), seven animals in each group. In order to obtain RFM followed by APM, induced parturitions were used. Treatments were started on the third day post-partum (PP). Group A was treated with an oxytocin analogue carbetocin and intrauterine administration of cephapirin. Group B treatment was intramuscularly treated with ceftiofur followed by two injections of $PGF2_{\alpha}$ at an interval of 8 h on the eighth day PP. Group C served as controls. In order to analyze acute phase proteins (APP) as markers of inflammatory response, blood samples were collected twice a week. General health status, body temperature, and vaginal discharge characteristics were recorded daily. Uterine involution was followed by ultrasonographic examination once a week. Uterine biopsies for bacteriological analyses were taken once a week for seven weeks PP. Milk samples for the analysis of progesterone were taken twice weekly.

Body temperature decreased more rapidly in group A than in group C (P = 0.014). Uterine involution in the treated groups A and B occurred earlier than in group C (P = 0.012 and P = 0.002, respectively). No significant differences were found between the groups with regard to vaginal discharge, the time changing patterns of APP, the start of ovarian activity, and the length of the first luteal phase PP. Decrease of bacterial growth in uterine biopsies was lower in group B than in group C (P = 0.009). Levels of APP were high in all the groups and declined to the baseline after the third week PP.

Treatments had no clear effect on the improvement of clinical signs and inflammatory parameters.

Keywords: dairy cow, puerperal metritis, RFM, treatment.

SKIRTINGO GYDYMO EFEKTYVUMAS MELŽIAMŲ KARVIŲ POGIMDYVINIO METRITO KLINIKINIAMS POŽYMIAMS IR UŽDEGIMO RODIKLIAMS

Julia Jeremejeva[1, *], Toomas Orro[2], Andres Waldmann[3], Raivo Lindjärv[4], Kalle Kask[1]
[1]*Terapijos skyrius*
[2]*Gyvūnų sveikatingumo ir ekologijos skyrius*
[3]*Akušerijos skyrius*
[4]*Užkrečiamųjų ligų skyrius, Veterinarinės medicinos ir gyvūnų mokslų institutas*
Estijos gyvybės mokslų universitetas, Kreutzwaldi 62, Tartu 51014, Estija
tel. +372 5333 2242; el. paštas: tjulia@emu.ee

Santrauka. Atliktas bandymas lyginant dvejopą gydymą Palygintas jo efektyvumas melžiamų karvių pogimdyvinio metrito klinikiniams požymiams ir uždegimo rodikliams. Kliniškai sveikos karvės prieš veršiavimąsi buvo suskirstytos į tris grupes (A, B ir C) (n=21), po septynias kiekvienoje grupėje. Norint nustatyti pogimdyvinio metrito tikimybę, buvo skatinamas veršiavimasis. Gydyta 3 dienos po veršiavimosi. A grupės karvės buvo gydomos oksitocino analogo karbetocino injekcijomis ir cefarinu į gimdą. B grupės karvės buvo gydomos intraraumeninėmis ceftiofuro injekcijomis ir dviem $PGF2_{\alpha}$ injekcijomis su 8 val. intervalu aštuonias dienas po veršiavimosi. C grupės karvės gydomos nebuvo (kontrolė). Norint nustatyti uždegimo mastą, du kartus per savaitę imti kraujo mėginiai ir tirtas aštrios fazės baltymų lygis. Be to, bandymo metu kasdien buvo stebima bendra gyvulių sveikatos būklė, matuojama kūno temperatūra, tiriamos vaginalinės išskyros. Gimda buvo tiriama ultragarsu kartą per savaitę. Taip pat kartą per savaitę 7 savaites po veršiavimosi bakteriologiškai buvo tiriami gimdos biopsijos mėginiai. Progesterono kiekiui ištirti du kartus per savaitę buvo imami pieno mėginiai.

Nustatyta, kad kūno temperatūra greičiau atsistatė A ir B grupių karvių palyginti su kontrole (C grupė) (p = 0,014). Be to, A ir B grupių karvių gimda atsigavo greičiau negu C grupės karvių (atitinkamai p = 0,012 ir p = 0,002,). Ženklių skirtumų tarp bandomųjų ir kontrolinės grupės karvių tiriant vaginalines išskyras aštrios fazės baltymų kiekį, kiaušidžių aktyvumą ir geltonojo kūno susidarymą nenustatyta. B grupės karvių gimdos biopsijos mėginiuose bakterijos augo žymiai lėčiau negu C grupės. (p = 0,009). Ištirta, kad aštrios fazės baltymų kiekis buvo didelis visose grupėse ir iki fizio-

loginės normos sumažėjo trečią savaitę po veršiavimosi.

Tyrimai parodė, kad skirtingas gydymas neturėjo ženklaus efekto melžiamų karvių pogimdyvinio metrito klinikiniams požymiams ir uždegimo rodikliam.

Raktažodžiai: pogimdyvinis metritas, gydymas, melžiamos karvės.

Introduction. Uterine infections in the dairy cow are common complications during the post-partum period (PP). Acute puerperal metritis (APM), which is also called acute post-partum or toxic puerperal metritis, is a serious problem during this period, which is often associated with retained foetal membranes (RFM) (Drillich et al., 2001). The disease causes low fertility, an increase in days open, involuntary culling from the herd, a decrease in milk production, and the expenses of treatment and costs of uncollected milk, which all cause high economic losses (Bar and Ezra, 2005). A number of studies conducted in Estonia have shown that post-partum metritis is common, and in some herds up to 40% of cows may suffer from APM (Kask et al., 2003). The high prevalence of metritis could be a reason why, in Estonian herds, the period of days open and the calving interval are longer than economically accepted (142 and 421 days, respectively) (Results of animal recording in Estonia 2009). Common methods of treating uterine inflammation in Estonia include a combination of intrauterine antibiotics with injections of oxytocin or its agonistic analogue, parenteral antibiotic injections with or without the administration of $PGF2_{\alpha}$ or non-steroidal anti-inflammatory drugs (NSAID) and repeated flushings of the uterus. However, based on the reports obtained from veterinarians working in field conditions, the treatment results are often of doubtful efficacy, and subsequent chronic inflammation may be found.

Because post-partum infections cannot be eliminated from dairy herds, there is a need to treat the disease to minimize the economic losses. However, the efficiency of different treatment methods is questionable, and some studies suggest that treatment of early post-partum metritis may not be efficient (Drillich et al., 2007; Dolezel et al., 2008). A number of researchers have compared the different treatment schemes of APM in recent years (Smith et al., 1998a; Chenault et al., 2004; Goshen and Shpigel, 2006; Drillich et al., 2007).

The objective of this study was to test the physiological response of two APM treatment methods used in practice:

1. intramuscular administration of antibiotics, in combination with a $PGF_{2\alpha}$ analogue during the earlier stage of inflammation,
2. oxytocin administration during the earlier stage of inflammation in combination with intrauterine antibiotic during the later stage of inflammation.

Materials and methods

Farm and animals. The study was conducted from November 2006 to February 2007 on a tie-stall commercial dairy farm with 450 cows. The cows were fed an identical total mixed ration (TMR). The TMR consisted of grass silage and a concentrate mix. The cows were milked twice a day; the milking system used was a machine pipeline system.

The study was conducted on multiparous (parity number between 2 and 5) late gestation Estonian Holstein Friesian cows with mean milk production during the previous lactation of 10.250 kg energy-corrected milk. All late gestation cows on the farm expected to calve during a two-week period (n = 21) were chosen and randomly divided into three groups, seven in each group. In order to obtain RFM followed by APM, parturitions were induced in all cows two weeks before term by means of two $PGF2_{\alpha}$ injections (Dinolytic®, Pfizer Animal Health) as described by Kask et al. (2000). A single injection dose was 25 mg, and the interval between injections was 24 h. Foetal membranes were defined as retained if they were not expelled within the first 24 hours after delivery (Noakes et al., 2001).

Diagnosis and treatment. No treatment of RFM was given after calving. Diagnosis of APM was made on the third day PP clinical examination according to Sheldon et al. (Sheldon et al., 2006; Sheldon et al., 2008). Considering the results from previous studies where in some cases no pyrexia was found in case of APM with severe bacterial infection (Sheldon et al., 2004; Benzaquen et al., 2007), in our study increased body temperature (≥ 39.5°C), if detected, was used as an additional sign. Treatment began immediately after diagnosing metritis (day three PP). The first group (A) was treated using the conventional treatment method for the investigated herd: 0.35 mg carbetocin (Hypophysin ® LA, Veyx-Pharma GmbH) intramuscular (im) for three consecutive days, starting on the third day PP and intrauterine (iu) administration of 500 mg cephapirin (Metricure®, Intervet international) between days 15 and 17 PP. Animals from the second group (B) were treated by im injection of one mg/kg ceftiofur (Excenell RTU®, Pharmacia Animal Health) for five days followed by two injections of 25 mg dinoprost ($PGF2_{\alpha}$ analogue) (Dinolytic®, Pfizer Animal Health) at an interval of 8 h on day eight PP. Ceftiofur was chosen for parenteral administration due to lack of milk withdrawal time and the positive effects described in previous clinical studies (Drillich et al., 2002; Risco and Hernandez, 2003; Chenault et al., 2004). The third group (C) served as the control group without any treatment.

Clinical and ultrasonographic examination. The cows were observed daily for morning body temperature (BT), appetite and health problems. Body temperature measurements began on the day of parturition, but statistical analysis was performed on the results obtained from day three PP. Temperature measurements were performed during the first two weeks PP. Appetite and health problems were recorded, starting from two weeks before parturition until the end of the experimental period, at seven weeks PP. Evaluation of the existence and character of vaginal discharge on the vulva, perineum, or tail was per-

formed daily for each animal for seven weeks PP. The appearance of the vaginal discharge was scored according to the scale proposed by Bekana et al. (1994) as follows: 5 = watery mucohaemorrhagic malodorous secretion; 4 = viscous haemopurulent discharge; 3 = viscous purulent material; 2 = mucus with the presence of pus; 1 = clear mucus; 0 = no discharge. Uterine involution was determined using ultrasound (US) beginning at the third week PP. US examination was performed every week until the end of the experiment. The US equipment was a real time B-mode linear array scanner (Hondex HS-120, Honda Electronics Co., Ltd., Aichi, Japan) with 5 and 7.5 MHZ transducers, supplied with an image freezer facility and electronic callipers for taking measurements. The diameters of both uterine horns and the thickness of the uterine wall were measured. Uterine involution was considered as complete if the difference in diameter between the previous pregnant and non-pregnant horn was ≤ 1 cm, the diameters of both horns were ≤ 4.5 cm and did not increase later, and the thickness of the uterine wall was ≤ 0.6 cm.

Bacteriological sampling and examination of uterus. Biopsies from uterine endometrium were collected from all experimental animals once a week over a period of seven weeks. The first biopsy was taken between days three and seven PP. A total 137 samples were collected according to the method of Bekana et al. (1994) and Kask et al. (1998). The biopsies were immediately placed in 10ml of a freshly prepared thioglycollate medium (LAB M Bury, England) for transportation to the laboratory. A 0.01 ml of samples (thioglycollate medium) of 0.01 ml was spread on Columbia blood agar plates containing 5% bovine blood. The aerobically and anaerobically cultivated plates were examined after 24 and 72h respectively. Identifications were made according to Bergey`s Manual of Systematic Bacteriology (Holt et al., 1994). To determine the species of the isolates, we used the BBL Crystal™ (Becton, Dickinson and Company, Maryland, USA) miniaturized biochemical test systems (Gram positive, Enteric/nonfermenters and Anaerobe ID kits (BD BBL Crystal™ Identification Systems)). The measures of bacterial growth on plates were classified as follows: 0 = no isolated bacteria, 1 = mild growth (1 to 20 isolated colonies per 10 µl of cultivated material), 2 = moderate growth (21 to 40 isolated colonies per 10 µl of cultivated material), 3 = heavy growth (≥41 isolated colonies per 10 µl of cultivated material).

Blood and milk samples. Blood samples for APP (serum amyloid-A (SAA) and haptoglobin (Hp)) analyses were taken from coccygeal vessels using heparinized venoject glass tubes (Terumo Europe N. V. Leuven, Belgium). From all cows one blood sample was collected 4.8 ± 0.5 days before the induction of parturition. After parturition collection was performed twice a week for seven weeks. After immediate centrifugation about 5 ml of plasma was removed and stored at –18° C until the analyses were performed.

In order to determine progesterone (P_4), milk samples were collected twice a week starting from approximately one week after calving. To avoid the effect of time of milk extraction on P_4 concentration (Waldmann et al., 1999), milk sampling was performed within 60 min after morning milking. The samples were collected into plastic tubes containing potassium dichromate as preservative, stored at 4ºC for one week, and frozen at –18ºC until analysis.

Methods of APP analysis. Plasma Hp was determined using the haemoglobin binding assay described by Makimura and Suzuki (1982) with the modification that tetramethylbenzidine (0.06 mg/ml) was used as a chromogen (Alsemgeest et al., 1994). Pooled and lyophilized aliquots of bovine acute phase serum were used to create standard curves. To calibrate the assay, a bovine plasma sample with a known Hp concentration provided by the European Commission Concerted Action Project (number QLK5-CT-1999-0153) was used. The range of the standard curve was 0.04 to 1.16 g/l. If the Hp concentration of a sample was higher, the sample was diluted with isotonic saline and reassayed. The intra- and inter-assay coefficients of variations were <12% and <11%, respectively. Serum amyloid A concentrations in plasma were measured with a commercially available ELISA kit (Phase SAA kit, Tridelta Development Ltd.) according to the manufacturer's instructions. The detection limit of the assay for bovine samples is 0.3 mg/l. The intra- and inter-assay coefficients of variations were <7% and <14%, respectively.

Methods of milk P_4 analysis. Milk P_4 was determined by enzyme immunoassay (Waldmann, 1993), which was modified by using the second antibody coating technique. The specificity of the monoclonal antibody 9C11 has been described previously (Waldmann, 1999). The inter-assay and intra-assay coefficient of variation was below 10%. The limit of sensitivity, using a 20-µl sample, was < 0.5 ng/ml. Milk P_4 concentration was used to determine days to the first luteal phase PP, defined as the first two consecutive measurements of P_4 concentrations > 3 ng/ml. Day when P_4 concentrations exceeded the level 3 ng/ml was estimated using the graph of P_4 concentration. Luteal phase was defined as the time during a cycle with P_4 concentrations > 3 ng/ml. Based on the definitions by Opsomer et al. (1998) the duration of the luteal phase was considered to be prolonged if P_4 levels remained elevated for > 20 days, without a preceding insemination. A luteal phase ≤ 11 days was defined as a short luteal phase (Eger et al., 1988).

Statistical analysis. Linear random-intercept models were used to explore differences in APP concentrations and BT between the treatment groups. Differences in bacterial growth from endometrium biopsies and vaginal discharge scores were tested using generalized linear mixed models in which a Poisson distribution was used for response variables. The cow was included as a random factor. Polynomials for time in ascending order and their interactions with the treatment groups served as fixed factors and were added until significant, for modelling changes in time. Overall time-trend differences between the groups were tested by means of an F-test. As there were different intervals between sampling, isotropic spatial exponential correlation structures were used to model serial correlations of repeated measurements within cows.

Logarithmic transformation of APP was used. The nlme-package with statistical software R 2.7.0 (http://www.R-project.org) was used to fit linear random-intercept models, and generalized linear mixed models were fitted using the GLIMMIX procedure with the SAS/STAT 9.1 (SAS Institute Inc., Cary, NC, USA) software.

The Cox proportional hazards model was used to explore group differences in the duration of uterine involution, at the time of the first P_4 rise over 3 ng/ml and in the duration of the first regular cycle. The statistical software Stata 9.2 (Stata Corp, Texas, USA) was used for these models. Data are presented as means ± SEM.

Results

Calving data and exclusions. After induction of parturition all the animals calved between 268 to 276 days of gestation, four cows needed some assistance during the calving process. Altogether 19 living calves were born and two cases of foetal death were detected. All cows had retention of placenta, and severe APM as a complication. One cow from group A was culled from the herd and excluded from the study during the third experimental week because of polyarthritis.

Clinical and ultrasonographic examination. On the third day PP all 21 animals were anorectic, had enlarged and atonic uteruses with poorly smelling vaginal discharge. However one animal from group A and three cows from group B did not show pyrexia. Fig. 1 shows changes in the group mean body temperatures after calving. Temperature increased in groups B and C for three days and in group A for four days after parturition. Considering that the day of parturition was day 0, peak BT was seen on the second day in groups B and C and on the third day in group A. The maximal BT of groups A and C was ≥ 39.5°C, BT of group B did not exceed 39.1°C. The analysis of the time-trend differences between the treatments showed that BT decreased more rapidly in group A than in group C (P = 0.014). The BT difference in animals in groups A and B and in groups B and C was not significant.

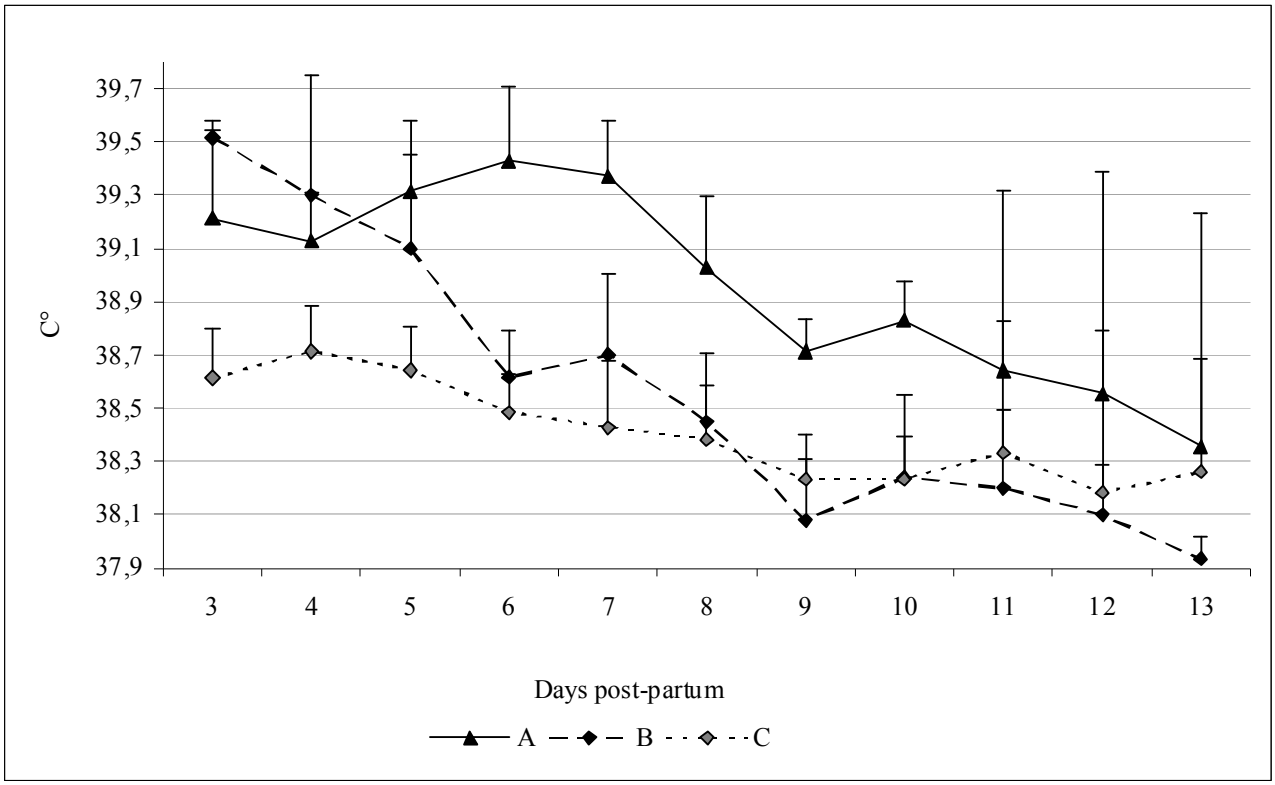

Figure 1. **Mean** (±SEM) **daily body temperature of cows treated with a combination of intrauterine antibiotic and oxytocin analogue** (group A) **and with a combination of parenteral administration of antibiotic and PGF2α** (group B) **in comparison with the non-treated control group** (group C)

All animals had a watery, fetid sanguinopurulent vaginal discharge during the first week PP week. After the third week PP vaginal discharge of those cows that had been treated by using a combination of parenteral antibiotic and $PGF2_{\alpha}$ was clearer and consisted of less pus than the discharge of non-treated animals and those animals that had been treated with oxytocin in combination with intrauterine antibiotic. However, this difference was not significant (Fig. 2).

According to US, involution of the uterus was more rapid in groups A and B, and it was the slowest in group C. Constant values of uterine parameters were reached on day 27.5 ± 1.6, 26.0 ± 1.1 and 36.7 ± 2.4 in groups A, B, and C, respectively There were significant differences between groups B and C and between groups A and C (P = 0.002 and P = 0.012, respectively).

Bacteriology. The results of bacteriological growth are presented in Fig. 2. A total 136 biopsies were collected, of which 82 were bacteria-positive and the remaining 54 biopsies were found to be bacteriologically negative. No totally negative animals were found. Four samples were missing due to problems during collection. A total of 28% positive samples showed mixed infections: six samples from group A, six from group B, and 11 samples from group C. The mixed cultures contained mainly *Enterobacter spp., Bacteroides spp. and Bacillus spp*., *Arcanobacterium pyogenes, Fusobacterium necrophorum*, and *Escherichia coli.* The most frequent isolates were *Bacteroides spp*. and *F. necrophorum*. These organisms were isolated in 43.9% and 20.7%, respectively, of the total positive samples. *A. pyogenes* and *Enterobacter spp.* were present in the same number of biopsies – 13.4% each.

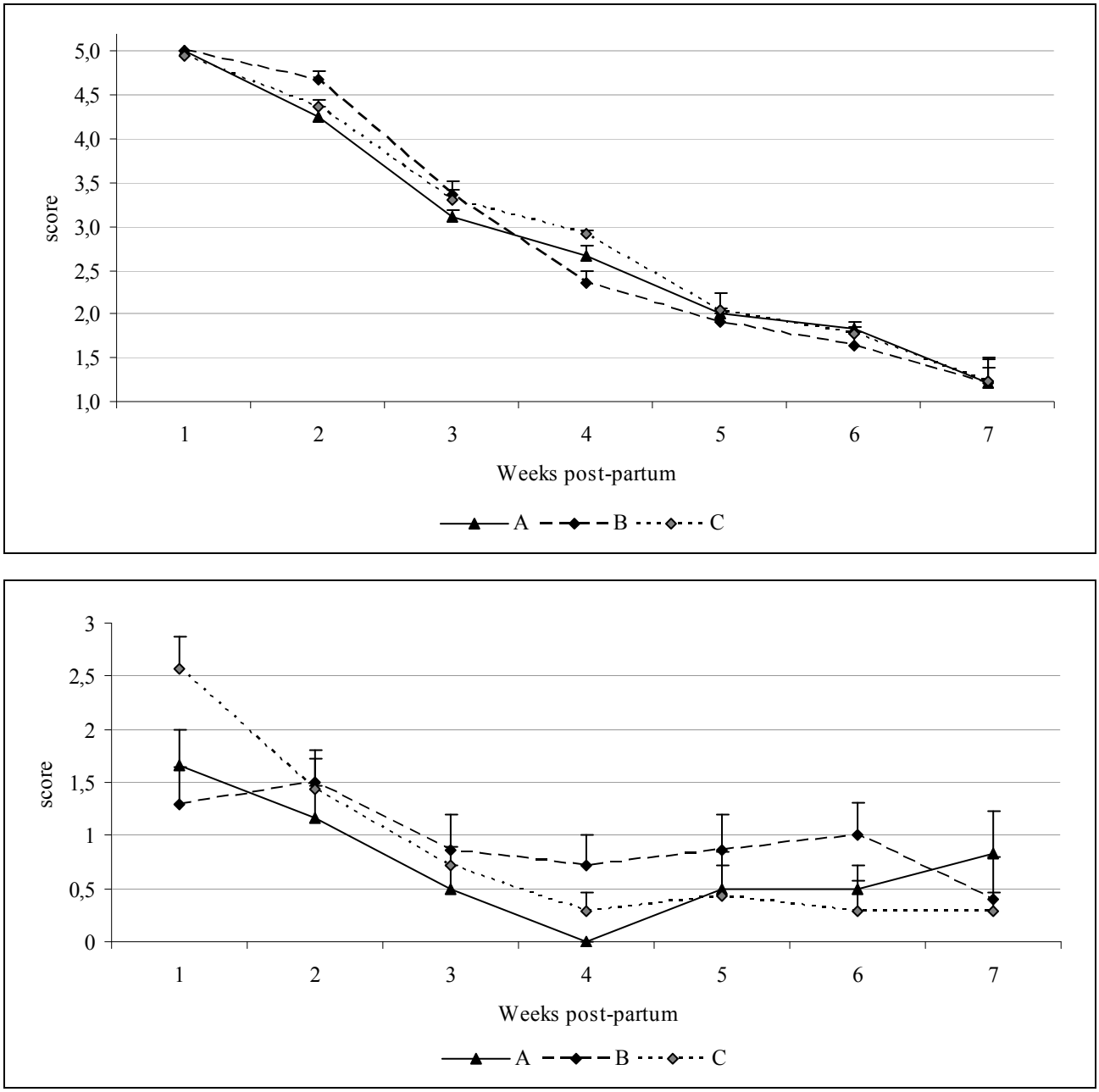

Figure 2. **Mean** (±SEM) **score of vaginal discharge** (above) **and bacterial growth** (below) **in cows treated with a combination of intrauterine antibiotic and oxytocine analogue (group A) and with a combination of parenteral administration of antibiotic and PGF2α** (group B) **in comparison with the non-treated control group** (group C)**. Vaginal discharge was scored as follows: 5 = watery mucohaemorrhagic malodorous secretion; 4 = viscous haemopurulent discharge; 3 = viscous purulent material; 2 = mucus with the presence of pus; 1 = clear mucus; 0 = no discharge. The number of bacterial colonies was scored as follows: 0 = no isolated bacteria, 1 = mild growth** (1–20 isolated colonies)**, 2 = moderate growth** (21–40 isolated colonies)**, 3 = heavy growth** (≥41 isolated colonies)

Animals from group A showed the shortest duration of uterine infection; all the biopsies collected from those animals were bacteriologically negative in the fourth PP week, but bacterial growth returned and again increased in the fifth week PP. Animals from group B constantly revealed a high incidence of bacterial growth during the six weeks PP, and a decrease to the minimum was seen only in the seventh week PP. *Bacteroides spp.* constituted the major bacteria found in group B, representing 70% of all the micro-organisms isolated in this group. The treatment had no effect on the elimination of these bacteria. An increase in *Bacteroides spp.* was observed from the first to the third week PP and then stabilized. A similar situation was detected in group C, where the number of isolated bacterial colonies increased during the first three weeks PP, but then, unlike the treated group B, it started to decrease. The slower decrease in bacterial growth over time was seen in cows from group B, compared with group C ($P = 0.009$).

Acute phase proteins. The highest concentration of SAA (275.98 ± 50.71 mg/l) was found in group C in the first week PP. At the same time the concentration of SAA in groups A and B was slightly lower (238.44 ± 42.56 and 226.6 ± 45.3 mg/l, respectively), but this difference was not significant. After this peak SAA in plasma started to decrease gradually in all the groups. It did not reach the basal level by the end of the experiment but was stabilized by the third week PP. A slight increase in SAA concentration was seen in the fifth week PP in the untreated group (Fig. 3).

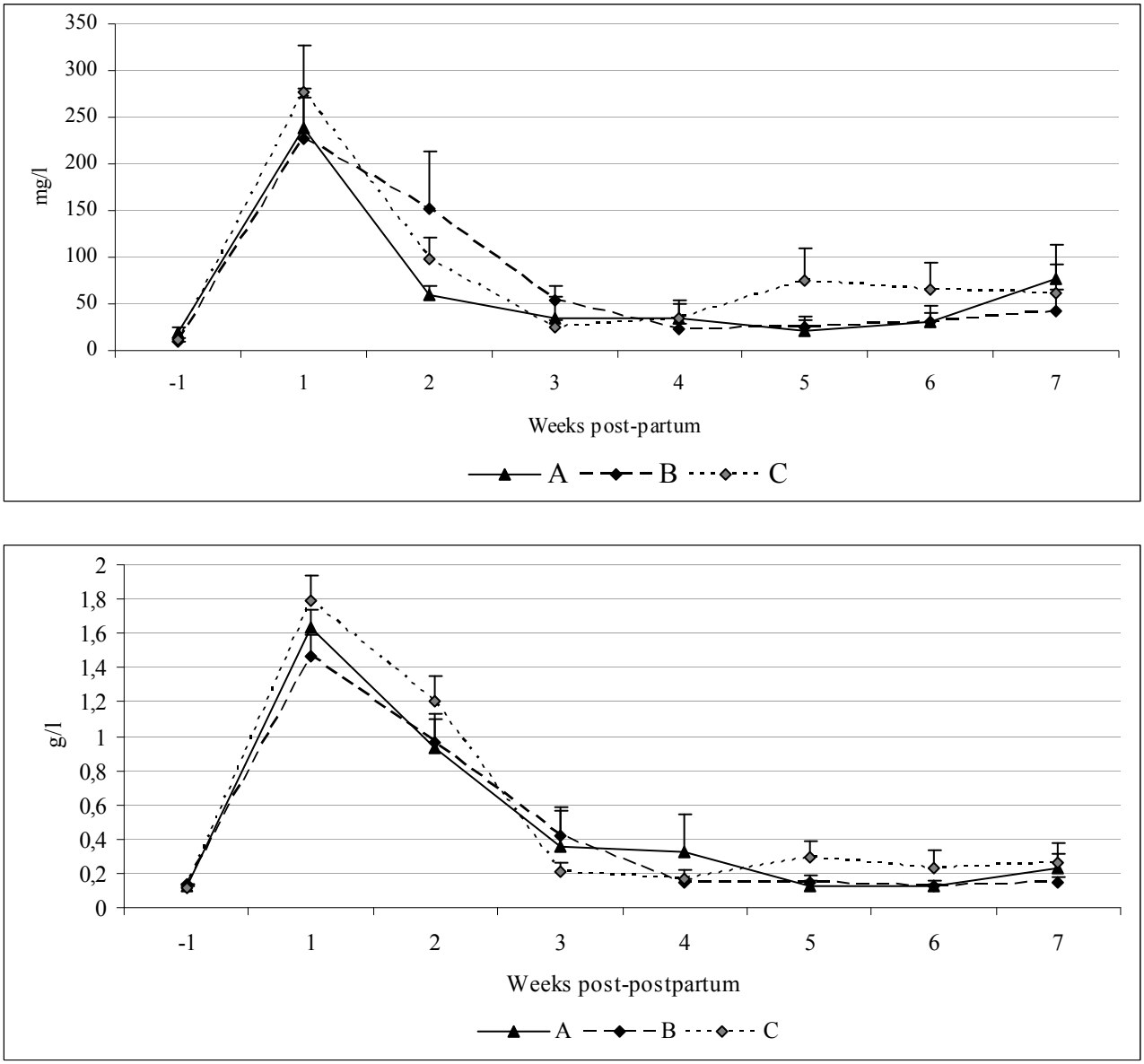

Figure 3. **Mean** (±SEM) **concentration of serum amyloid A** (SAA) **above and Haptoglobin** (Hp) **below in cows treated with a combination of intrauterine antibiotic and oxytocin analogue** (group A) **and with a combination of parenteral administration of antibiotic and PGF2α** (group B) **in comparison with the non-treated control group** (group C).

The same trend was found for the concentration of plasma Hp. The maximum levels were detected during the first week PP (1.6 ± 0.1, 1.5 ± 0.1 and 1.8 ± 0.1 g/l in groups A, B, and C, respectively). The difference in Hp concentration between the non-treated group and group B at that time was significant. After the peak concentration of Hp a rapid decrease was observed. The Hp concentration decreased to the stable level within three weeks PP and reached the pre-partum level by the sixth week PP. No significant differences in the concentrations of both APP were found in the time changing patterns between the groups (Fig. 3).

Progesterone. All the animals had low levels of P_4 during three weeks PP, and then an increase of more than 3 ng/ml was observed in all the groups. The first day of the first luteal phase was recorded on day 40 ± 3.4, and on days 44 ± 5 and 34 ± 5.3 PP in groups A, B, and C, respectively. One cow from group A did not show any normal luteal activity during the whole experimental period. According to P_4 levels (P_4 rise above the 3.0 ng/ml), the luteal phase of the first cycle was considered to be prolonged in two cows from group A, three cows from group B, and two cows from group C. One cow from group A and one cow from group B showed a short luteal phase, and the remaining 10 animals showed a normal luteal phase. No statistical difference between the groups was found with regard to the time to start of ovarian activity and the length of the luteal phase.

Discussion. One of the criteria for evaluating the efficiency of APM treatment is weakness or disappearance of symptoms of systemic illness and normalization of the vaginal discharge. The APM treatment was started on the third day PP, and it was therefore rational to start the analysis of the range of BT in time after the third day PP. The difference of BT between groups on the third day PP is random and this was taken into consideration. A more rapid decrease of BT in group A could be explained by the fact that the maximum BT in this group was observed on day three PP in comparison with groups B and C where the maximum BT was measured on day two PP. Considering the fact that the BT peaked in group A on day three PP, in the other groups (C and B) a decrease in BT, which had then been already observed, this may have been the cause of the changes of BT curve in time. Thus, the most rapid decrease of BT was observed in group A. Chenault et al. (2004), however, detected a more rapid reduction in BT in cows treated with ceftiofur hydrochloride than in the control group, which was not in agreement with the results of Smith et al. (1998a) and Drillich et al. (2001) who noticed no difference between the groups.

No significant difference was found between the groups with regard to the process of normalization of vaginal discharge. Nor did Chenault et al. (2004) detect any statistical difference in vaginal discharge when comparing the effect of using different concentrations of ceftiofur hydrochloride administered parenterally with the affected non-treated control group. The positive effect of $PGF2_{\alpha}$ analogues has been well documented in previous studies (Sheldon and Noakes, 1998; Smith et al., 1998b; Azawi et al., 2008). It is known that concentrations of endogenous prostaglandins are high during the first seven days post-partum. These levels are higher in animals with uterine infections or RFM (Lindell et al., 1982; Risco et al., 1994) and a decrease to the basal concentration will start after the first week PP. Considering this fact, the effect of exogenous administration of prostaglandins during the first seven days PP is uncertain. In the present study two injections of $PGF2_{\alpha}$ analogues were used at eight hour intervals between the administrations on day eight PP. However, in the present study no effect of systemic administration of PGF2α in combination with the antibiotic on the parameters of vaginal discharge was detected. Azawi et al. (2008), on the other hand, found that vaginal discharge in buffaloes treated by parenteral injections of oxytetracycline and PGF2α was improved and changed into clear mucus, showing the same result as the negative control group. However, Hendricks at al. (2005), using PGF2α twice a day on days seven and 14 and once on days 22 and 35 PP, did not detect any difference in the prevalence of purulent discharge between the treated and non-treated groups.

Both PGF2α and oxytocin, which were used in the present study, stimulate myometrial contraction (Gajevski et al., 1999; Kaczmarowski et al., 2004) and help uterine cleaning from lochial debris and discharge containing micro-organisms. Intrauterine and parenteral antibiotics should reduce bacterial growth in the inflamed uterus. In our study a high level of bacterial growth was detected in all experimental groups during the first two weeks PP, findings also found elsewhere (Makimura and Suzuki, 1982; Kaczmarowskin et al., 2004) In the fourth week PP all the uterine biopsies from group C were found bacteriologically negative. The reasons why bacteria returned after the fifth week are not clear. The main bacterial isolates found included *A. pyogenes*, *Bacteroides spp.*, *F. necrophorum*, and *Enterobacter spp*. The most frequently isolated bacteria were *Bacteroides spp.*, most of which were isolated from uterine biopsies in group B. Presence and number of these bacterium colonies did not decrease after treatment. An increase was seen from the first to the third week PP and then stabilized. A similar situation was detected in group C, where the number of isolated bacterial colonies increased during the first three weeks PP, but then, in contrast to group B, it started to decrease. Samitz et al. (1996) reported poor activity of ceftiofur against *Bacteroides spp.* It is interesting that the growth intensity of *Bacteroides spp.* is higher in the treated than in the non-treated group. The current authors believe that it could be explained by the elimination of some antagonistic microbes after treatment that can allow the growth of *Bacteroides spp*. Berg (1978) showed that the strictly anaerobic *Eubacterium sp*. and *Fusobacterium sp*. that colonized gnotobiotic mice caused a reduction in the *in vivo* population levels of the strictly anaerobic *Bacteroides sp*. However, this question has been insufficiently investigated and requires further research. Incidence of bacterial growth, which is one of the most important criteria for presence and severity of uterine infections, was more stable and lower in group C after the fourth week PP. It is

possible that the regulation of such mechanisms as cytokines, the function of antibody production of leukocytes, and endotoxin response is more active in non-treated animals (Földi et al., 2006).

According to the US examination, uterine involution was more rapid in group B. Previous studies, where prostaglandins were used (Kask et al., 2000; Melendez et al., 2004), had similar results. Oxytocin is also an uterotonic product, which helps to decrease the time of uterine involution. A more prolonged time of uterine involution was observed in the non-treated group C, where no uterotonics or antibiotic were used.

Sheldon et al. (2002) hypothesized that uterine infections have a direct negative effect on ovarian function. This finding is in agreement with our study, where the earliest start of ovarian activity was seen in group C (day 34 PP). Also, a more rapid elimination of bacteria was seen in group C than in group B, where a delayed start of P_4 rise was observed. We detected no significant difference in the time to start of ovarian activity between the groups, but this is possibly an effect of the small sample of animals used in the present study. Previous studies have shown an increase in APP concentration, indicating an acute PP uterine inflammation (Smith et al., 1998a; Sheldon et al., 2001; Melendez et al., 2004; Drillich et al., 2007). A slight increase in SAA concentration during the fifth week in the control group in the present study can be explained by the individual reaction of one cow due to polyarthritis, which was subsequently diagnosed. Stabilization of APP concentration was seen between the third and the fourth weeks PP. This result is in agreement with a study where the concentration of plasma Hp was measured in ewes affected by experimentally induced metritis (Regassa et al., 2002). In 1993–1995 Smith et al. (1998a) compared the treatment efficacy of various intrauterine and intramuscular antibiotics. In their study Hp was also used for the measure of the success of treatment strategies, and no difference was detected in APP concentration between the groups. In the present study no effect of treatment on APP levels was detected using clinical parameters. The efficiency of different treatment methods of post-partum metritis is questionable, and this has been found not only in our study (Drillich et al., 2007; Drillich et al., 2008).

Conclusions

Body temperature decreased more rapidly in group A than in group C. Uterine involution in the treated groups A and B occurred earlier than in group C. Bacterial growth in uterine biopsies decreased lower in group B than in group C. No significant differences were found between the groups with regard to vaginal discharge, the time changing patterns of APP, the start of ovarian activity, and the length of the first luteal phase PP.

The findings of the present study indicate that the treatments used did not have clear effect on the improvement of clinical signs and inflammatory parameters during APM.

Acknowledgements

The study was financially supported by the Estonian Ministry of Agriculture (research projects No. T6043 and T8010), the Estonian Science Foundation (grants No. 5733 and 7891), and the Estonian Ministry of Education and Research (target-financed project No.B0045VL07).

References

1. Alsemgeest S.P., Kalsbeek H.C., Wensing T., Koeman J.P., van Ederen A.M., Gruys E. Concentrations of serum amyloid-A (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. Vet. Q., 1994. T. 16. P. 21–23.

2. Azawi O.I., Omran S.N., Hadad J.J. A study on postpartum metritis in Iraqi Buffalo cows: bacterial causes and treatment. Reprod. Domest. Anim., 2008. T. 33. P. 77–85.

3. Bar D., Ezra E. Effects of common calving diseases on milk production in high yielding dairy cows. Israel J. Vet. Med., 2005. T. 60. P. 106–111.

4. Bekana M., Jonsson P., Ekman T., Kindahl H.Intrauterine bacterial findings in postpartum cows with retained fetal membranes. J. Vet. Med. A., 1994. T. 41. P. 663–670.

5. Benzaquen M.E., Risco C.A., Archbald L.F., Melendez P., Thatcher M.-J., Thatcher W.W. Rectal temperature, calving-related factors, and the incidence of puerperal metritis in postpartum dairy cows. J. Dairy Sci., 2007. T. 90. P. 2804-2814.

6. Berg R.D. Antagonism among the normal anaerobic bacteria of the mouse gastrointestinal tract determined by immunofluorescence. Appl. Environ. Microbiol., 1978. T. 35. P. 1066–73.

7. Chenault J.R., McAllister J.F., Chester J.S.T., Dame K.J., Kausche F.M., Robb E.J. Efficacy of ceftiofur hydrochloride sterile suspension administered parenterally for the treatment of acute postpartum metritis in dairy cows. J. Am. Vet. Med. A., 2004. T. 224. P. 1634–1639.

8. Drillich R., Vecera M., Palenik T., Cech S., Vyskocil M. Systematic clinical examination of early postpartum cows and treatment of puerperal metritis did not have any beneficial effect on subsequent reproductive performance. Vet. Med., 2008. T. 53. P. 59–69.

9. Drillich M., Bergmann J., Falkenberg U., Kurth A., Heuwieser W. Effect of the intensity of a post partum examination on the fertility performance of high yielding dairy cows. Dtsch Tierärztl Wochenschr, 2002. T. 10. P. 386–390 (in German).

10. Drillich M., Voigt D., Forderung D., Heuwieser W. Treatment of acute puerperal metritis with flunixin meglumine in addition to antibiotic treatment. J. Dairy Sci., 2007. T. 90. P. 3758–3763.

11. Eger S., Shemesh M., Schinder H., Amir S., Foote Rh. Characterization of short luteal cycles in the early post-partum period and their relation to reproductive performance of dairy cows. Anim. Reprod. Sci., 1988. T. 16. P. 215–224.

12. Földi J., Kulcsar M., Pecsi A., Huyghe B., de Sa. C., Lohuis J.A.C.M., Cox P., Huszenicza Gy. Bacterial complications of postpartum uterine involution in cattle. Anim. Reprod. Sci., 2006. T. 96. P. 265–281.

13. Gajevski Z., Thun R., Faundez R., Boryezko Z. Uterine motility in the cow during puerperium. Reprod. Domest. Anim., 1999. T. 34. P. 185–191.

14. Goshen T., Shpigel N.Y. Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membranes in dairy cows. Theriogenology, 2006. T. 66. P. 2210–2218.

15. Hendricks K.E.M., Bartolome J.A., Melendez P., Risco C., Archbald L.F. Effect of repeated administration of PGF2α in the early post partum period on the prevalence of clinical endometritis and probability of pregnancy at insemination in lactating dairy cows. Theriogenology, 2005. T. 65. P. 1454–1464.

16. Holt J.G., Krieg N.R., Sneath P.H.A., Staley J.T. Williams ST. Bergey`s Manual of Determinative Bacteriology. 1994. 9th edn. Baltimore, Williams & Wilkins., P. 299.

17. Kaczmarowski M., Malinowsk E., Markiewicz H. Influence of various treatment methods on bacteriological findings in cows with puerperal endometritis. Pol. J. Vet. Sci., 2004. T. 7. P. 171–174.

18. Kask K., Kindahl H., Gustafsson H. Bacteriological and histological investigation of the postpartum bovine uterus in two Estonian dairy herds. Acta vet. Scand., 1998. T. 39. P. 423-432.

19. Kask K., Gustafsson H., Gunnarsson A., Kindahl H. Induction of parturition with prostaglandin F2 alpha as a possible model to study impaired reproductive performance in the dairy cow. Anim. Reprod. Sci ., 2000. T. 59. P. 129–139.

20. Kask K., Kurykin J., Lindjärv R., Kask A., Kindahl H. Assessment of early postpartum reproductive performance in two high producing estonian dairy herds. Acta Vet. Scand., 2003. T. 44. P. 131–143.

21. Lindell J.O., Kindahl H., Jansson L., Edqvist L.E. Postpartum release of prostaglandin F2 alpha and uterine involution in the cow. Theriogenology, 1982. T. 17. P. 237–245.

22. Makimura S., Suzuki N. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory disease. Jpn. J. Vet. Sci., 1982. T. 44. P. 15–21.

23. Melendez P., McHale J., Bartolome J., Archbald L.F., Donovan G.A. Uterine involution and fertility of Holstein cows subsequent to early postpartum $PGF_{2\alpha}$ treatment for acute puerperal metritis. J. Dairy. Sci., 2004. T. 87. P. 3238–3246.

24. Noakes D.E., Parkinson T.J., England G.C.W., Arthur G.H. Arthur`s veterinary reproduction and obstetrics. 2001. Eighth edition. Saunders.

25. Opsomer G., Coryn M., Deluyker H., de Kruif A. An analysis of ovarian dysfunction in High yielding dairy cows after calving based on progesterone profiles. Reprod. Dom. Anim., 1998. T. 33. P. 193–204.

26. Regassa F., Sheldon I.M., Noakes D.E. Effect of experimentally induced metritis on uterine involution, acute phase response and PGFM secretion in the postpartum ewe. Vet. Rec., 2002. T. 150. P. 605–607.

27. Results of animal recording in Estonia 2009. ELMATAR 2010. p. 17–18. Available at: http://www.jkkeskus.ee/english/sisu/aastaraamat_2009 . pdf Accessed: 2010-03-22. (in English)

28. Risco C.A., Drost M., Thatcher W.W., Savio J., Thatcher,M.J. Effect of calving-related disorders on prostaglandin, calcium, ovarian activity and uterine involution in postpartum dairy cows. Theriogenology, 1994. T. 42. P. 183–203.

29. Risco C.A., Hernandez J. Comparison of ceftiofur hydrocloride and estradiol cypionate for metritis prevention and reproductive performance in dairy cow affected with retained fetal membranes. Theriogenology, 2003. T. 60. P. 47–58.

30. Samitz E.M., Jang S.S., Hirsh D.C. In vitro susceptibilities of selected obligate anaerobic bacteria obtained from bovine and equine sources to ceftiofur. J. Vet. Diagn. Invest., 1996. T. 8. P. 121–123.

31. Sheldon I.M., Noakes D.E. Comparison of three treatments for bovine endometritis. Vet. Rec., 1998a. T. 142. P. 575–579.

32. Smith B.I., Donovan A., Risco C., Littell R., Young C., Stanker L.H., Elliott, J. Comparison of various antibiotic treatments for cows diagnosed with toxic puerperal metritis. J. Dairy Sci., 1998b. T. 81. P. 1555–1562.

33. Sheldon I.M., Noakes D.E., Rycroft A., Dobson H.Acute phase protein responses to uterine bacterial contamination in cattle after calving. Vet. Rec., 2001. T. 148. P. 172–175.

34. Sheldon I.M., Noakes D.E., Rycroft A.N., Pfeiffer D.U., Dobson,H. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reprod., 2002. T. 123. P. 837–845

35. Sheldon I.M., Rycroft A.N., Znou C. Association between postpartum pyrexia and uterine bacterial infection in dairy cattle. Vet. Rec., 2004. T. 154. P. 289–293.

36. Sheldon I.M., Lewis G.S., LeBlanc S., Gilbert R.O. Defining postpartum uterine disease in cattle.

Theriogenology, 2006. T. 65. P. 1516–1530.

37. Sheldon I.M., Williams E.J., Miller A.N., Nash D.M., Herath S. Uterine diseases in cattle after parturition. Vet. J., 2008. T. 176. P. 115–21.

38. Smith B.I., Donovan A., Risco C.A., Young C., Stanker L.H. Serum haptoglobin concentrations in Holstain dairy cattle with toxic puerperal metritis. Vet. Rec., 1998. T. 142. P. 83–85.

39. Waldmann A. Enzyme immunoassay (EIA) for milk progesterone using a monoclonal antibody. Anim. Reprod. Sci., 1993. T. 34. P. 19-30.

40. Waldmann A. Monoclonal antibodies to progesterone: Characterization and selection for enzyme immunoassay (EIA) in bovine milk. Hybridoma, 1999. T. 18. P. 289–296.

41. Waldmann, A., Ropstad, E., Landsverk K., Sorensen K., Solverod L., Dahl E.Level and distribution of progesterone in bovine milk in relation to storage in the mammary gland. Anim. Reprod. Sci., 1999. T. 56. P. 79–91.

Received 28 July 2010
Accepted 29 October 2010