Effects of the Treatment Method of Reproductive Performance in Cows with Retention of Fetal Membranes

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Abstract. The aim of the research was to find out an appropriate method of treatment of retention of fetal membranes (RFM) in cows for conditions of Latvia. Three protocols for the treatment of retained fetal membranes in dairy cows were evaluated in a field trial. Cows that retained the fetal membranes for more than 24 hours were assigned to three treatment groups. Group 1 – control group; group 2 – cows with RFM, which were removed manually, and cows were intrauterine treated with antibiotics; group 3 – cows with RFM, which were not removed, and cows were intrauterine treated with antibiotics, and group 4 – cows with RFM, which were not removed, and cows were not treated. In animals with a decreased feed consumption or milk production, body temperature above 39.5 °C, systemic therapy was applied with ceftiofur hydrochloride or procaine benzilpenicillin. In order to establish subclinical endometritis, all cows were histologically examined on the 42nd day postpartum (PP). Subclinical mastitis was diagnosed both in cows with RFM and control group cows. Results indicated that better reproductive results were in cows of group 2 and group 4. Actually, results indicated that treatment of RFM without intrauterine manipulation and parenteral treatment could be as effective as conventional treatment including RFM detachment and local antibiotic treatment.
Key words: cows, retained fetal membranes, treatment methods, reproduction performance.

Introduction

Some authors are of the opinion that fetal membranes in cows are considered to be retained if they have not been expelled during 12 hours postpartum (Manspeaker, 2010; Stevens, 1997; Drillich *et al*., 2003; Smith, 2002; Aiello, 1998; Boos *et al*., 2006), while others consider that during 24 hours after calf delivery (Opsomer, 2015; Guard, 1999; Han & Kim, 2005; Könyves *et al.*, 2009; LeBlanc, 2007; Dubuc *et al*., 2011; Kimura *et al*., 2002; Maas, 2004; Risco & Hernandez, 2003, Mordak, 2006; Sheldon *et al*., 2008). Retention of fetal membranes (RFM) in cows during postpartum period (PP) may cause inflammation of the uterus, may inhibit activity of ovaries and the uterus involution, and worsen the total reproductive performance as well. After retention of fetal membranes, most often inflammation of the uterus is metritis that is one of the main causes of decreased reproductive outcomes (Drillich *et al*., 2006). By developing the uterus inflammation (metritis) in animals, the general health condition may become worse; the general intoxication of the body may develop and result in in death if not treated. Researchers in the Netherlands have found out that in case of retention of fetal membranes the milk production decreases for 40%, veterinary expenses increase for 32%, animal culling increases for 19% and the calving interval increases for 9% (Hamali & Karimi, 2008). But Opsomer (2015) mentioned that negative consequences related to retained placenta were: delayed uterine involution, increased time to first insemination, increased number of services per pregnancy, decreased pregnancy rates and increased days open.

In his research, Gröhn (1990) proved that in cows with RFM, the possibility to become ill with endometritis was 4.4 times greater than in cows without RFM as well as there was 1.5 times greater risk of cysts development in the ovaries. LeBlanc (2007) established that cows with RFM got pregnant for 15% less than cows without RFM.

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Santa Skuja, Vita Antāne

*Effects of the Treatment Method of
Reproductive Performance in Cows
with Retention of Fetal Membranes*

RFM treatment methods

It is important to select the most effective method of treatment in cows with RFM. Concerning RFM, many and different methods of treatment are described with a relative effectiveness, often with contradictory results or with a negative effect on cow's further reproductive performance (Drillich *et al*., 2007; Frazer, 2005). Manual removal of retained membranes, intrauterine treatment or systemic administration of antibiotics, prostaglandin or estradiol could be mentioned here (Peters & Laven, 1996; Stevens & Dinsmore, 1997; Drillich *et al*., 2006; Drillich *et al*., 2007). In many parts of Europe and less in the United States of America and Canada, veterinarians apply mainly the manual removal of fetal membranes in cows with RFM and administer antibiotics in the uterine cavity (Drillich *et al*., 2006). Some investigations, however, discover that manipulations in the uterine cavity decrease the uterus protective mechanisms (Paisley *et al.*, 1986; Peters & Laven, 1996) and cause infertility (Drillich *et al*., 2007). Opsomer (2015) had found that cows intrauterine treated with antibiotics did show a reduced incidence of postpartum fever during the first 10 days after calving. Drillich *et al.* (2006a) in their field research, in 501 cows with RFM did not find anything positive of the manual fetal removal or local administration of antibiotics for the purpose of treatment, or combining of both methods. Only in cows with RFM and fever selected at random, when treated with 1mg/kg of ceftiofur, metritis did not develop (Drillich *et al*., 2007). Some researchers support a manual removal of fetal membranes on condition that they remove easily. This method is contraindicated when the animal shows symptoms of septicemia (Garverick & Youngquist, 1993). Drillich *et al.* (2006), Goshen and Shpigel (2006) and Drillich *et al*. (2007) have concluded that the intrauterine administration (infusions or bolus) of antibiotics is a useful treatment of endometritis, but it is unlikely that it will help facilitate the removal process of fetal membranes or will prevent development of endometritis in cows with RFM. Systemic therapy using ceftiofur has given better results in cows with RFM than estradiol and prostaglandin; however, at the same time it has not shown a better reproductive performance in total (Risco & Hermandez, 2003; LeBlanc *et al*., 2005). Also, immediate administration of prostaglandins, oxytocin or calcium preparations postpartum has not been effective for RFM prevention or faster removal and expel process of fetal membranes (Peters & Laven 1996; LeBlanc *et al*., 2005). Nevertheless, Santos *et al.* (2002) in their studies, using 82 cows, established that administering 25mg $PGF_{2\alpha}$ (prostaglandin F2α) during the first hour postpartum the fetus membranes removed faster

(7.7±0.84 h) (P<0.10) than in cows of the control group (10.07±1.09 h) in which medicines were not administered postpartum. There are also authors who describe antibiotic therapy along with $PGF_{2\alpha}$ preparations that give good results in cow reproduction (Polat *et al*., 2009; Drillich *et al*., 2006; Kacmarowski *et al*., 2006). Melendez *et al*. (2004) point out that administration of $PGF_{2\alpha}$ is an often used mode to improve the uterus involution and reproductive outcomes. LeBlanc *et al*. (2002) have found out that $PFG_{2\alpha}$ injections for endometritis treatment from the 20th to 26th day PP do not improve the pregnancy parameters. However, Carlos Antônio Carvalho Fernandes *et al.* (2010) describe that by treating animals with cloprostenol sodium in the first week PP, better reproductive results have been achieved in animals than injecting $PGF_{2\alpha}$ during the third week PP. There are many ways and recommendations how to use $PFG_{2\alpha}$. By injecting $PGF_{2\alpha}$ in animals on the 8th and 21st day PP, the progesterone level in blood increases much faster indicating that ovaries are active and the uterus involution process takes place. Azawi (2008) emphasizes that prostaglandin F2, which is considered a pro-inflammatory molecule, may stimulate the production of pro-inflammatory cytokines that enhance phagocytosis and lymphocytes function. Treatment with $PGF_{2\alpha}$ did not affect the prevalence of subclinical endometritis neither 35 days PP nor 49 days PP; also, it did not affect reproductive performance, for example, days open to the first service. First service conception, however, was affected in all cows as well as in cows with a low body condition; the number of days open to pregnancy decreased (Galvão *et.al*, 2009).

Aim of the research: To select the appropriate method of treatment for the retention of fetal membranes most suitable for conditions of Latvia resulting in favorable effect on further reproductive performance in cows.

Materials and Methods

The research was carried out from September 2007 till January 2009.

Sixty cows of different age of Latvian black-and-white breed from a Joint Stock Company "A" herd with 650 dairy cows and from "B", Ltd., herd with 300 dairy cows were used in the present research. Animal feed ratio was adequate to the standards of dairy cows. On both farms the animal keeping, care and feeding were similar.

Depending on the expulsion of fetal membranes or their retention and the applied treatment, all animals were divided into four groups:

Group 1 (n=15) – control group – fetal membranes were expelled during 24 h after calving.

Santa Skuja, Vita Antāne

*Effects of the Treatment Method of
Reproductive Performance in Cows
with Retention of Fetal Membranes*

Group 2 (n=15) – cows with retention of fetal membranes, which were removed manually, and the cows were treated with antibacterial preparations – gynobiotic bolus (neomycin sulfate 350000 IU, oxitetracycline hydrochloride 500 mg) inserted into the uterus (3 bolus) after removal of fetal membranes.

Group 3 (n=15) – cows with retention of fetal membranes, which were not removed, and the cows were treated with antibacterial preparations – gynobiotic bolus (neomycin sulfate 350000 IU, oxitetracycline hydrochloride 500 mg) inserted into the uterus (3 bolus).

Group 4 (n=15) – cows with retention of fetal membranes, which were not removed, and the cows were not treated into the uterus.

Cows of all groups with a rectal temperature ≥ 39.5 °C and with signs of PP metritis received a systemic antibiotic treatment with ceftiofur hydrochloride (1.1 mg kg^{-1} subcutaneously per day) or procaine benzilpenicillin (10 mg kg^{-1} intramuscularly per day) for 3 to 5 consecutive days. In the control group, there were two cows out of which one was treated with procaine benzilpenicillin and the other with ceftiofur hydrochloride. In Group 2, one cow was treated with procaine benzilpenicillin, but in Group 3, there were 8 cows treated: five of them were treated with procaine benzilpenicillin and three cows with ceftiofur hydrochloride. It should be mentioned that Group 4 was very hard to form because in the first 10 days PP some of those cows had fever (the body temperature increased above 39.5 °C, milk production decreased, the animal became apathetic), so these animals (in total 7) had to be treated and included in Group 3, in which RFM were not removed, but the animals were treated. In some cows of each group, on the day 8 and 21 postpartum synthetic PFG$_{2\alpha}$ was injected – cloprostenol (sodium saline solution) 500 µg/animal once per injection in order to achieve better ovary activity and uterus involution, to decrease development of uterus inflammation, and improve conception rate. In the control group, 8 cows received PFG$_{2\alpha}$ injection, in Group 2 – five cows, in Group 3 – 10 cows, and in Group 4 – six cows were injected PFG$_{2\alpha}$.

All cows involved in the present investigation were clinically examined in 48 hours, on day 6, 14, 16, 18, 22, 24, 26, 28, 30, 32 and 42 postpartum: by evaluating the animal condition visually, measuring the body temperature with a clinical mercury thermometer (Ningbo Huahui, China), evaluating the character of discharge from reproductive organs, examining rectally and determining tonus and size of the uterus. Small uterus – the uterus is of normal shape and tone, indicative of an involution of normal course; the uterus becomes erect at oestrus. Medium size uterus – the uterus is slightly enlarged, has slightly descended towards the abdominal cavity, it is flaccid, pasty to touch. This is indicative of mild subinvolution. Large uterus – the uterus is enlarged, has descended into the abdominal cavity, it is flaccid and palpable, sometimes with abnormal content (Könyves *et al.*, 2009), that enables to determine and prognose the further health condition of the reproductive organs. Starting from the sixth day postpartum, ovaries were examined rectally and their functional condition was determined.

For ultrasonography of cows' reproductive organs Tringa Linear Vet ultrasound (ESAOTEPie Medical, Netherland) was used. The Tringa Linear was specifically engineered for all aspects of bovine and equine reproductive imaging, as well as for basic equine tendon imaging. Ultrasonography of reproductive organs was performed in 7.5 MHz frequency on day 14, 22, 28, and 42 postpartum alongside with the rectal examination. Uterine content was recorded and the following scoring system was used for the amount of the uterine content: A – no fluid detected (no nonechogenic areas detected in the uterine lumen); B – slight amount of fluid (few nonechogenic areas detected); C – moderate amount of fluid (half of the uterine lumen covered with nonechogenic areas); D – large amount of fluid (almost all uterine lumen gives a nonechogenic appearance) (Kask, 1999).

Biopsy samples from cows' uterus were taken on the day 42 PP with original biopsy instrument (Divisible biopsy instrument 60 cm, Kruuse, Denmark). Biopsy samples were taken from ventral side of the dorsal wall of the uterus, intercarruncular places. Each animal was prepared for the procedure of endometrium biopsy sampling by washing and disinfection (70° *spiritus aethylici*) of external part of genital organs. A catheter was inserted into the uterus through the vagina to remove the endometrium samples by gently grasp of tissue with forceps being careful not to crush it. Specimens were snipped free at the base (Sematovica, Pilmane & Jemeljanovs., 2008). Endometrium samples were inserted in labeled containers with 10% formalin solution, pH 7.5 (Humason, 1967) and histological investigations were performed at the Institute laboratory of Pathology of Pauls Stradins Clinical University Hospital. After fixation, tissues were trimmed, embedded in parafin, sectioned at 3 – 4 µm and stained with heamatoxylin and eosin. Investigations were made under the microscope Axiolab (Zeiss, Germany) for presentation of inflammatory cells in endometrium. The histological results were interpreted as follows: normal to slight uterine endometrial inflammation = 0 to 29 inflammatory and mononuclear cells per field;

Santa Skuja, Vita Antāne

*Effects of the Treatment Method of
Reproductive Performance in Cows
with Retention of Fetal Membranes*

medium uterine endometrial inflammation = 30 to 80 inflammatory and mononuclear cells per field; dense uterine endometrial inflammation = more than 80 inflammatory and mononuclear cells per field (Kask, 1999).

Statistical analysis. Data were analyzed with MS Excel descriptive statistics (mean indices and standard deviation). Differences between treatment groups against all variables were analyzed using a Wilcoxon signed rank test, 2-sample z-test to compare sample proportion, t-Test: two - sample assuming equal variances and t-Test: two - sample assuming unequal variances. Results were found statistically significant when $p < 0.05$.

Results and Discussion

Animals under investigation were clinically examined postpartum. Uterus and ovaries were examined rectally and ultrasonographically. In the present study, on the day 14 PP in cows of control group the uterus size had decreased in 93% of cases but in cows with retention of fetal membranes – in 78% of cases, and according to the division, they were medium size uterus. Furthermore, the differences were statistically significant ($p<0.05$). Also Bajcsy *et al.* (2005) emphasize that immediately after calving uterine involution starts, preparing the genital tract for a subsequent conception. Uterine involution involves physical shrinkage, necrosis and sloughing of caruncles, and the regeneration of the endometrium (Sheldon *et al.*, 2008). Puerperal disorders in this early period may cause an extension of complete uterine involution (Bajcsy *et al.*, 2005).

In some cows of each group, on the day 8 and 21 postpartum synthetic $PFG_{2\alpha}$ was injected – cloprostenol (sodium saline solution) 500 µg/animal once per injection to test if it facilitates better uterine involution. Statistically significant differences regarding uterine size were not observed between cows which were injected $PFG_{2\alpha}$ on the day 8 PP and those which were not administered $PFG_{2\alpha}$ injections. While after repeated $PFG_{2\alpha}$ injection, in Group 4 cows a significant uterine involution was observed contrary to cows which were not administered $PFG_{2\alpha}$ injections ($p<0.05$).

Results of ultrasonographic (US) examination of the cows' uterus on the day 14, 22, 28 and 42 PP, the uterine size decreased in parallel with the decrease of the fluid amount. As Sheldon *et al.* (2008) indicate, the sloughed caruncles form the lochial discharge, along with the remains of fetal fluids and blood from the ruptured umbilicus. Uterine smooth muscle activity plays an important role in expulsion of uterine contents, clearing of its cavity and in the reduction of uterine size. These processes, however, can be perturbed, resulting in puerperal disorders, such as retained fetal membranes, or endometritis (Bajcsy *et al.*, 2005). Uterine fluids stop discharging when the mucous membrane is regenerated. And epithelial regeneration is complete by about 25 days after parturition (Sheldon *et al.*, 2008). In the control group of cows of the present study, a complete uterine involution and regeneration of uterine endometrium were observed on the day 28 PP (small uterus by rectal examination; no nonechogenic areas detected in the uterine lumen by ultrasonograph), but in cows with RFM – only in 42 days PP. It is significant to mention that in 64% of cows from groups 1, 2, 3, and 100% of group 4 cows, which were not administered $PFG_{2\alpha}$ injection, on the day 22 PP a slight amount of fluid was detected in the uterus (some nonechogenic areas were detected US).

Ultrasonographic examination of ovaries on the day 14 and 22 postpartum, showed evidence that 38% of cows with RFM and 33% of control group cows had inactive ovaries (oval, small, smooth with many small follicles), whereas on the day 28 and 42 PP the number of RFM cows with inactive ovaries decreased from 38% to 29%, but in the control group, the number of cows was 33%. In total, evaluating cows with inactive ovaries during 42 days PP, significant differences between groups of cows were not observed ($p>0.05$), as well as the average progesterone blood serum level was constantly low. Subclinical and clinical postpartum uterine infection affects ovarian activity (Peter, Vos & Ambrose, 2009). Bajcsy *et al.* (2005) and Könyves *et al.* (2009) also emphasize in their studies that puerperal disorders in early period of postpartum may lead to a delayed resumption of ovarian activity and prolong the interval from calving to the first ovulation.

Evaluating reproductive organs of the study cows on the day 14 PP, in 10% of cases puerperal metritis was diagnosed (the uterus contained a large volume of reddish-brown foul-smelling uterine discharge mostly containing necrotic tissue debris (putrid discharge)) that correspond with Sheldon *et al.* (2006) definitions of uterine diseases: puerperal metritis should be defined as an animal with an abnormally enlarged uterus and a fetid watery red-brown uterine discharge, associated with signs of systemic illness (decreased milk yield, dullness or other signs of toxemia) and fever >39.5 °C, within 21 days after parturition. The average body temperature in cows with metritis was slightly above normal range 39.7±0.06 ºC. It should be mentioned that metritis affected cows were not administered $PGF_{2\alpha}$ injection on the day 8 PP. Melendez *et al.* (2004) mentioned that in the first seven days postpartum, the $PFG_{2\alpha}$ level in blood in cows with RFM and metritis

Santa Skuja, Vita Antāne

Effects of the Treatment Method of
Reproductive Performance in Cows
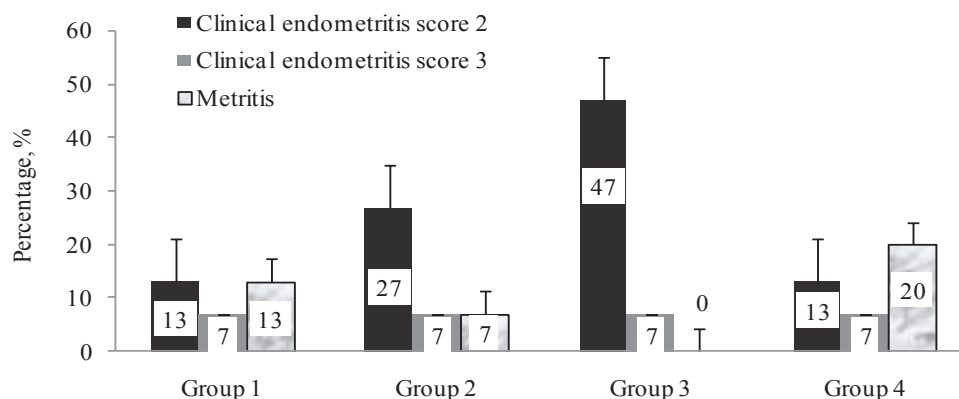*with Retention of Fetal Membranes*

Figure 1. Uterine inflammation in cows on the day 14 after calving. Group 1 - control group;
Group 2 - cows with RFM, which were removed manually, and the cows were treated;
Group 3 - cows with RFM, which were not removed, and the cows were treated;
Group 4 - cows with RFM, which were not removed, and the cows were not treated.

was much higher than that in the control group of cows and administration of $PGF_{2\alpha}$ after this time is an often used mode to improve the uterus involution and reproductive outcomes. Galvão (2011) emphasizes that $PGF_{2\alpha}$ is not only luteolytic but also appears to have pro-inflammatory actions that might enhance neutrophil function. In the present research, the largest number of cows with metritis was in Group 4 (20% of cases) (Figure 1), in cows of the control group, metritis was observed in 13% of cases, and in cows of Group 2, metritis positive cows were in 7% of cases. LeBlanc (2012) emphasizes that the incidence of metritis after calving is usually 10-20%. But in present study, in cow Group 3, there were no cases of metritis because intrauterine administration of antibiotics was used in these animals. And if needed, also intramuscular antibiotic therapy was applied. Some researchers prove that the selective therapy of cows is the best way of treatment for animals and further reproduction (Risco & Hernandez, 2003; Drillich *et al*., 2007, 2006a).

On the 14th day PP, clinical endometritis (discharge containing ≤50% white or off-white mucopurulent material) was established in 25% of cases. That finding was in agreement with score 2 clinical endometritis by I. Martin Shaldon endometritis scoring scheme (Sheldon *et al.*, 2006). Only a little more than half of those animals (53%) were administered $PGF_{2\alpha}$ injection. The lowest occurrence of clinical endometritis was among control and Group 4 cows (13% of cases). A statistically significant larger number of animals affected with clinical endometritis were observed in cows of Group 2 and Group 3 (27% and 47% of cases, respectively) (p<0.05) (Figure 1). The average body temperature in cows with clinical endometritis was within normal range (38.8±0.12 ºC).

Clinical endometritis (discharge containing 50% purulent material, usually white or yellow, but occasionally sanguineous; in scoring scheme – score 3) on the day 14 PP was in total 7% of cases, in each group of cows one sick animal (Figure 1). The average body temperature in sick animals was within normal range (39.3±0.07 ºC), and these animals were not administered $PGF_{2\alpha}$ injection on the day 8 PP.

On the day 22 PP of investigation in some cases (3 cows) clinical endometritis was diagnosed. The body temperature in all animals was within normal range.

In order to establish subclinical endometritis, all cows were histologically examined on the 42nd day PP. Gilbert *et al*. (2005) have mentioned that endometrial biopsy and histopathology may constitute the ideal method of diagnosis of endometritis. Thatcher *et al.* (2006) also have indicated that detection of subclinical endometritis begins between 34 and 47 days postpartum and Opsomer (2015) has mentioned that subclinical endometritis means no clinical signs of endometritis, no purulent or mucopurulent discharge. The authors of the present research considered subclinical endometritis to be diagnosed when chronic inflammatory infiltrates were determined histologically – some lymphocytes and lymphocytic aggregates, macrophages, the plasma cells, eosinophils, some rounded lymphatic follicles. Lymphatic follicles turned out even the multiplication center. In some cases, haemosiderin pellets were found in the subepithelial layer and stroma. The obtained results of histological examination of endometrium showed evidence that in 40% of Group 2, 40% of Group 4, and 47% of Group 3 cows there was subclinical endometritis. Histological changes of endometrium in cows of control group were observed in 33% of cases, and there was a significant difference

Effects of the Treatment Method of
Reproductive Performance in Cows
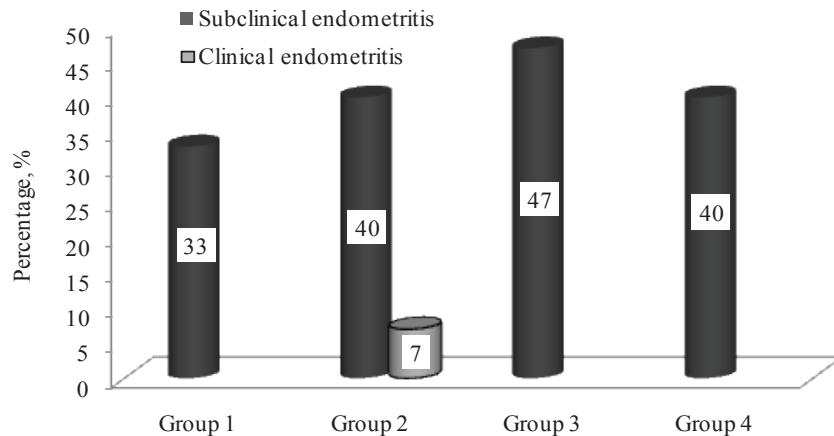*with Retention of Fetal Membranes*

Santa Skuja, Vita Antāne

Figure 2. Uterine inflammation in cows on the day 42 after calving. Group 1 - control group;
Group 2 - cows with RFM, which were removed manually, and the cows were treated;
Group 3 - cows with RFM, which were not removed, and the cows were treated;
Group 4 - cows with RFM, which were not removed, and the cows were not treated.

(p<0.05) between results of RFM cow groups (Figure 2). It should be added that on the day 42 after calving, in one of the cows clinical endometritis was diagnosed, which was developed after ruptures of delivery route uterus cervix and body.

Evaluating reproductive performance in all, it is obvious that independently from the therapy of RFM those were different. The time from parturition to the first insemination differed significantly (p<0.05) between Group 1 (control) and Group 3 (83±9.5 days vs. 120±15.2 days) (Table 1). Until day 100 postpartum, 73% of control group cows and 64% of Group 2 cows were artificially inseminated (AI) that differed significantly (p<0.05) between Group 3 and Group 4 inseminated cows in percentage terms (43% and 31%, respectively) (Table 1).

Evaluating cow services per pregnancy (Table 1), i.e. number of insemination till pregnancy, optimal result was obtained only in the 2nd and 3rd group of cows (1.75±0.37 times vs. 1.86±0.40). The worse results were in cows of the control group (3.27±0.57 times), even though the artificial insemination PP was started earlier (83±9.5 days) than in other research cow groups; also, clinical uterine inflammation on the day 28 PP was not diagnosed. It should be mentioned that on the day 42 postpartum, subclinical endometritis was established in histological samples of uterine mucosa in five cows of control group (33% of cases) resulting in five, six, and even seven times of AI times till pregnancy. In cows of Group 4, services per pregnancy (2.40±0.58 times) exceeded the optimum range, and differed significantly (p<0.05) between parameters of Group 2 and Group 3 cows.

Evaluating pregnancy parameters from the first service conception (Table 1), a comparatively better parameters were in cow Group 4 (38%) and cow Group 2 (29%) which was a significantly higher parameter (p<0.05) in comparison with the control group cows (13%). It should be mentioned that in all 2nd group cows, intrauterine antibacterial treatment was used, and in febrile cows a systemic antibiotic therapy was administered. Drillich *et al.* (2006) and Opsomer (2015) also noted that more successful treatment results in cases of metritis or clinical endometritis, which developed after RFM, were obtained in the cow group in which antibiotics were used only in case of fever, and first service conception in that cow group was greater. Drillich *et al.* (2007) recommended administering intrauterine bolus in all cows, and systemic therapy only in the febrile cows selected randomly.

Conception rate was evaluated in all cows under research expressed as a percentage. The largest number of pregnant cows on the day 150 PP was observed in the 2nd group cows, and their mean parameters were about 43%, which was not so good result (Table 1). The second highest result was in cows of Group 4 (38%). Significant differences (p<0.05) were observed only between the 2nd and 3rd group cows (43% vs. 29%). Conception rate equaled on about the day 200 PP when in all research cows pregnancy rate was within the range of 40% – 50% (Table 1).

In the dairy herd, for evaluation of reproduction, an important parameter is days open, or in other words, in how many days after calving the animal gets pregnant. In a dairy herd, as an optimal time is considered 90-100 days (Daris, 1998). In a highly productive cow herd, this parameter is larger 115-125 days in milk (O`Connor, 2005). In the present research, the mean value of this parameter was within the range from 126±18.08 to 166±24.38 days

Santa Skuja, Vita Antāne

*Effects of the Treatment Method of
Reproductive Performance in Cows
with Retention of Fetal Membranes*

Table 1

Cows reproductive performance summary table

Characteristic values of reproduction	Group 1 n=15	Group 2 n=15	Group 3 n=15	Group 4 n=15
Days open to the first service (days)	83±9.5 [A]	94±12.9	120±15.2 [A]	104±9.1
Bred cows to 100 100 days in milk, %	73[A,B]	64[C,D]	43[A,D]	31[B,C]
Services per pregnancy	3.27±0.57 [A]	1.75±0.37 [A,B]	1.86±0.40 [C]	2.40±0.58 [B,C]
First service conception , %	13[A,B]	29[A]	21[C]	38[B,C]
Pregnancy to 150 days PP, %	33	43[A]	29[A]	38
Pregnancy to 200 days PP, %	47	50	43	46
Days open (days)	166±24.38	126±18.08	148±19.05	160±25.03
Culled cows, %	27[A,B]	47[A,C]	47[B,D]	33[C,D]

[A,B,C,D] - $p < 0.05$. Group 1 – control group; group 2 – cows with retention of fetal membranes, which were manually removed, and the animals were treated; group 3 – cows with retention of fetal membranes, which were not removed, and the animals were treated; group 4 – cows with retention of fetal membranes, which were not removed, and the animals were not treated.

(Table 1), the shortest time was in Group 2 cows (126±18.08 days), the longest time was in cows of Group 4 (160±25.03 days) (p>0.05). This result might be affected negatively by the high proportion (≥40%) of subclinical endometritis.

When evaluating reproductive performance of cows, it is also important to know the number of culled cows and their reasons. Due to different reasons (infertility, anestrous, mastitis, abomasal displacement, hoof problems, fatty degeneration of the liver) 23 cows from 60 were culled in the current lactation. In the control group, four cows were culled (27%, the average age of cows was 3.25±0.48 years and 2.25±0.48 lactations), in the 2nd and 3rd cow group seven cows in each group were culled (47%), but in Group 4, there were five cows culled (33%) (Table 1). RFM group cows were at the age of 3.94±0.45 years and 2.89±0.44 lactations on average. Group of Werven researcers (1992) in their studies on RFM wrote that the older the cow is, the larger percentage of their condemnation is due to various reasons. Larson *et al.* (1985) and Lewis (1997) also indicated that more uterine infections, foot problems, mastitis, infertility were observed in cows with RFM which could be one of the reasons of cow condemnation.

Summarizing reproductive performance in the study cows, it is obvious that in the control group cows, some reproductive parameters are comparatively bad: services per pregnancy were 3.27±0.57 times, first service per pregnancy 13%, pregnancy to 150 days PP - 33%, pregnancy to 200 days PP - 47%, days open were 160±25.03, except days open to the first service were 83±9.5 days PP and bred cows to 100 days in milk were 73% of cows. These facts indicate that the used dairy herds have been facing reproduction problems.

Comparing the obtained results of cow groups with RFM, similar parameters were in the experimental cow Groups 2 and 4. Even though the parameters in Group 4 cows were slightly worse, when the animals were not treated and RFM was not removed, still there were not significant differences between reproductive performances of Group 2 cows.

Conclusions

Evaluating in total the used methods of RFM therapy and their influence on reproductive performance, results are different.

1. There are no significant differences in the days open to the first service, first service

Santa Skuja, Vita Antāne

*Effects of the Treatment Method of
Reproductive Performance in Cows
with Retention of Fetal Membranes*

conception, pregnancy to 150 days PP, pregnancy to 200 days PP and days to conception between treatment Group 2 (RFM are removed manually, and cows are treated with antibacterial preparations) and treatment Group 4 (RFM are not removed, and cows are not treated). Significant differences (p< 0.05) are only in the services per pregnancy (1.75±0.37 vs 2.40±0.58 times) and culling rate (47% vs 33%). It should be mentioned that Group 4 was very hard to be formed because during the first days PP half of those cows had fever, so the animals had to be treated and included in Group 3 where RFM were not removed, and the cows were treated with antibacterial preparations.

2. Reproductive performance of Group 3 (RFM are not removed, and the cows are treated with antibacterial preparations) is rather similar to that of control group cows with the exception of the culling rate that is lower in terms of percentage in control group cows (27% vs 47%; p<0.05).

3. Reproductive performance of the control group cows shows a moderate problem in the herds investigated during the time of research.

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