EARLY VERSUS LATE POSTPARTUM TREATMENT OF CLINICAL ENDOMETRITIS IN DAIRY COWS USING A COMBINATION OF SYSTEMIC ADMINISTRATIONS OF CEFTIOFUR WITH PROSTAGLANDIN F2A

*Julia Jeremejeva[1, *], Toomas Orro[1], Andres Waldmann[2], Marina Aunapuu[3,4], Ivar Blank[3], Andres Arend[4], Kalle Kask[1]*

[1]Department of Clinical Veterinary Medicine; [2]Department of Reproductive Biology,
[3]Department of Basic Veterinary Sciences and Population Medicine
Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences
Tartu, 51014, Estonia
[4]Department of Anatomy, University of Tartu, Ravila 19, Tartu, 50411, Estonia

**Corresponding author: Julia Jeremejeva*
Tel.: +372 53332242; E-mail: tjulia@emu.ee
Postal address: Fr. Kreutzwaldi 62, Tartu 51014, Estonia

Abstract. The aim of this study was to test treatment of clinical endometritis in the early postpartum (PP; group A; 5-10 day after calving) and late PP (group B; 30-35 day PP) using systemic administrations of ceftiofur with two injections of PGF2α at intervals of 8 h. Examination of vaginal discharge, determination of plasma progesterone (P_4), measurement of acute phase proteins (APP), histological examination of uterine biopsies and fertility parameter data were used for evaluation of treatment success. No significant differences in improvement of vaginal discharge, the start of ovarian activity, the length of the first luteal phase measured by P_4, or in the time-trends of APP and presence of subclinical endometritis on days 43-45 PP, based on histological examination, were detected. Fertility parameters of group A were better than in group B. However, this difference was not significant, possibly because of the small experimental groups.

Keywords: Early postpartum, endometritis, late postpartum, PGF2α, treatment

Introduction. Regardless of developments in therapy, and the use of biosecurity measures, postpartum (PP) clinical endometritis (CE), affects up to 20% of dairy cows (LeBlanc, 2008). The most common treatment methods for PP uterine inflammation, which occur without such signs of systemic illness as pyrexia, anorexia and signs of toxaemia, involve the administration of PGF2α to enhance uterine defence and repair mechanisms, and systemic or intrauterine administration of antibiotics to reduce the pathogenic bacterial load. PGF2α is a hormone that causes muscular contractions of the uterine myometrium, luteolysis of the corpus luteum and, in the absence of a corpus luteum, increases the uterine immune response (Kasimanickam et al. 2005). The proposed benefits of PGF2α use are an uterotonic effect, which helps uterine cleaning of local debris and discharge-containing microorganisms, and induction of oestrus in cows with a responsive corpus luteum, causing evacuation of uterine contaminants. In addition to an uterotonic effect, PGF2α results in a decrease in P4, which reduces the immune function of the uterus, and increases the oestrogen level, which improves uterine immune function (Lewis, 1997). A further positive effect of PGF2α on uterine immune function is its effect on increasing leukotriene B4 secretion by the uterus, which supports chemotaxis, cell-mediated cytotoxicity, phagocytosis and lymphocyte function (Lewis, 2003).

It has been reported that the administration of two luteolytic doses of PGF2α at 8 h intervals has a significantly positive effect on the reproductive performance of dairy cows with uterine inflammation (Archbald et al. 1993; Salasel et al. 2003; Melendez et al. 2004; Jeremejeva et al. 2012).

The choice of antibiotics used for treatment of PP uterine inflammations is broad. Ceftiofur is one of the most widely investigated parenteral antibiotics, which has no milk withdrawal time, and positive effect, which have been described in previous clinical studies (Chenault et al. 2004).

One possibly effective treatment for PP CE could be parenteral administration of ceftiofur, followed by two doses of PGF2α at intervals of 8 h. However, the optimum timing of this treatment is unclear. One possible treatment time is immediately after diagnosis in the early PP, when CE is in its acute phase. Based on the reports of veterinarians working in field conditions (Toni et al. 2015), veterinarians from some countries prefer to treat CE after the fourth week PP in cases where clinical signs remain, when CE enters the chronic phase.

The hypothesis was that treatment of CE in the early PP period, when uterine inflammation is in the acute phase and treatment could be more productive, improves clinical signs, inflammatory parameters and fertility parameters of dairy cows significantly better than treatment in the late PP, when some of the animals became healthy and some animals have been developing chronic phase of the inflammation. The purpose of this experiment was to determine the optimal time of CE treatment, diagnosed in the early PP period, using

systemic administrations of ceftiofur with two injections of PGF2α at intervals of 8 h.

Materials and methods

Farm and animals

Sixty seven multiparous late-pregnant cows, expected to calve during the subsequent three months, were used. Animals were loose-housed on a dairy farm with 600 cows, and were fed the same diet. Animals with puerperal metritis, clinical mastitis, arthritis, hoof problems or other clinical diseases besides CE were not included.

Diagnosis and treatment

Clinical endometritis was defined as the presence of a purulent uterine discharge without foul odour, and atonic and enlarged uterus without changes in general health condition (body temperature <39.5°C, without depression, anorexia or other signs of toxaemia). The diagnosis of CE was made on the 5th day PP using vaginal and rectal examination, and measurements of body temperature. Vaginal examination was made using vaginoscopy, and manual examination of vaginal discharge. To ensure minimal variation of the initial response to the inflammation of the uterus among the treatment groups stratified randomisation, on the basis of fibrinogen (Fb) in plasma measurements on day 5 PP, was used. Group A (n = 20) began treatment on the 5th day PP by s.c. injection of 1 mg/kg Ceftiofur (Excenel RTU®, Pharmacia Animal Health, Poland) for five days, followed by two injections of 25 mg PGF2α (Dinoprost; Dinolytic®, Pfizer Animal Health Belgia), with an interval of 8 h, on the 10th day PP. Animals in group B (n = 35) were reexamined on day 28 PP, and in the case of confirmation of CE diagnosis, using the same diagnostic criteria as on the 5th day PP, (group B2) were treated using the same scheme as group A, commencing on day 30 PP: injection of 1 mg/kg Ceftiofur (Excenel RTU®, Pharmacia Animal Health, Poland) for five days followed by two injections of 25 mg PGF2α (Dinoprost; Dinolytic®, Pfizer Animal Health, Belgium), with an interval of 8 h, on the 35th day PP. Clinically recovered animals (group B1) were not treated. A negative control group C (n = 12) was composed of healthy cows.

Clinical examination

The cows were observed daily for health problems. Evaluation of vaginal discharge on the vulva, perineum, tail or in the vulva (by manual examination) was performed daily for each animal for seven weeks PP. The appearance of vaginal discharge was scored as follows: 0 = no discharge; 1 = clear mucus; 2 = mucus with the presence of pus; 3 = viscous purulent material; 4 = viscous haemopurulent discharge; 5 = watery mucohaemorrhagic malodorous secretion.

Histological sampling and examination of the uterus

Biopsies from the uterine endometrium for histological examination were collected from all cows, once at 43-45 days postpartum. Slides for histological examination were prepared by the paraffin section method, using haematoxylin-eosin staining. The diagnosis of subclinical endometritis (SE) was based on the presence of fibrosis and infiltration of inflammatory cells in the epithelium, stratum compactum and stratum spongiosum. Two independent observers performed the evaluation blind.

Blood samples

Plasma samples for analysis of acute phase proteins (APP) (serum amyloid-A (SAA) and haptoglobin (Hp)), used as indicators for acute inflammatory processes, were taken from nine days before term and were performed once before parturition and once a week for seven weeks after parturition. Whole blood for Fb analyses was taken once a week during the entire experimental period, starting on the 5th day PP. For the determination of progesterone (P4), plasma samples were collected twice a week commencing from day 10 PP and until day 75 PP. The plasma P4 concentration was used to determine the number of days to the first luteal response PP, defined as the first two consecutive measurements of P4 concentrations > 1 ng/ml.

Methods of analysis of APP

The fibrinogen concentration in plasma was measured by the heat precipitation method. Plasma Hp was determined using haemoglobin binding assay, as described by Makimura and Suzuki (1982), with the modification of tetramethylbenzidine (0.06 mg/ml) used as a chromogen (Alsemgeest et al. 1994). Concentrations of serum amyloid A in plasma were measured according to the manufacturer's instructions with a commercially available ELISA kit (Phase SAA kit, Tridelta Development Ltd.). The intra- and inter-assay coefficients of variation for Hp were <12% and <11%, and for SAA <7%, <14%, respectively.

P4 analysis

Plasma P4 was determined according to the manufacturer's instructions with a commercially available ELISA kit (EIA-1561, DRG Instruments GmbH, Germany). The intra-assay precision at P4 concentrations of 0.36 and 6.16 ng/ml were 4.26 and 1.74 %, respectively. The inter-assay precision at P4 concentrations of 0.29 and 5.52 ng/ml were 10.19 and 4.93%, respectively.

Fertility analysis

To evaluate fertility performance, the interval from calving to first insemination (days to first service, DFS), first service conception rate (FSCR, %), two services (first and second) conception rate (TSCR, %), interval from calving to successful insemination (days open, DO) and number of services per pregnancy (NSP) were recorded for all cows.

Statistical analyses

Linear random-intercept models were used to explore differences in APP concentrations between treatment groups. Logarithmic transformations of APPs were used. Differences in vaginal discharge among groups were tested using generalized linear mixed models, in which a Poisson distribution was used. Treatment group, sampling time (day) and their interactions were included as fixed factors in the initial models. Logistic regression was used to evaluate differences among treatment groups for histological examination results, days to the first luteal response (before or after 50 days PP), first service conception (pregnant or not) and second service

conception results. Poisson regression was used to evaluate group differences in NSP, and linear regression was used to evaluate group differences in DO after logarithmic transformation of DO. These analyses were made using STATA 10.0 (Stata Corporation, Texas, USA) software. Data are presented as means ± SEM. Analyses of the minimal number of animals in experimental groups for evaluation of treatment effect to fertility parameters was made retrospectively.

Results

Two animals from group A and four from group B were culled from the herd before the end of the sampling period because of health problems, not associated with the uterus, and were excluded from the study. Thirteen cows from group B were clinically healthy on day 28 PP and were assigned to group B1 and the remaining 18 cows from this group were assigned to group B2. Vaginal discharge from negative control cows (group C) was clear and did not occur after day 26 PP. Vaginal discharge in groups A, B1 and B2 became clear on days 31, 22 and 32 day PP, respectively. There was no difference between treatment groups in the improvement of vaginal discharge. The start of ovarian activity, measured by P4, was detected on the 37.8 ± 5.9, 36.0 ± 4.6, 30.2 ± 3.6 and 32.3 ± 4.8 day PP in groups A, B1, B2 and C, respectively. There was no significant difference either in the start of ovarian activity or in the length of the first luteal phase. Concentrations of Hp, SAA and Fb were maximum in all groups during the 1st week after parturition, after which time they started to decrease. There were no differences in the time-trends of APP between groups. The percentages of animals showing presence of SE, based on histological examination, were 54%, 46%, 42% and 50% in groups A, B1, B2, and C. There were no significant differences between groups.

Table 1 provides a summary of the parameters of reproductive performance. Regardless of the better fertility parameters in group A (early treated cows) in comparison with group B2 (animals treated in the late PP), this difference was not significant.

Table 1. **Fertility parameters of animals treated in the early postpartum (group A), cows recovered to 28th day postpartum (B1), animals treated in the late postpartum (B2) and control group (group C) in case of clinical endometritis.**

Experimental group (number of animals)	Appearance of subclinical endometritis (%)	Number of days to first service	First service conception rate (%)	Two service conception rate (%)	Number of days open	Number of services per pregnancy
A (n=18)	54	83.6 ± 7.9	64	93	100.7 ± 9.6	1.4 ± 0.2
B1 (n=13)	46	88.1 ± 10.7	46	85	118.9 ± 12.4	1.7 ± 0.2
B2 (n=18)	42	85.6 ± 6.5	50	71	125.2 ± 14.1	2.2 ± 0.4
C (n=12)	50	84.7 ± 6.7	50	75	113.0 ± 13.4	1.8 ± 0.6

Discussion

This positive effect of early administration of PGF2α has been found by numerous researchers (Nakao et al. 1997; Melendez et al. 2004; Jeremejeva et al. 2012). Nakao et al. (1997) treated endometritic animals with PGF2α on days 7 to 10 PP, and showed that the fertility parameters of treated cows were the same as in healthy control animals. Melendez et al. (2004) studied the effect of PGF2α on animals with acute puerperal metritis. They treated animals with an i.u. administration of ceftiofur with two doses of PGF2α, 8 h apart, on d 8 PP. Primiparous treated cows had smaller uterine diameters and lower uterine scores, reduced concentration of α1-acid glycoprotein, and increased first service conception rate. Multiparous cows with METRITIS were not affected by treatment. In a previous study of Jeremejeva et al. (2012) the use of two injections of PGF2α, with an interval of 8 h, on the 8th day PP for treatment of acute puerperal metritis and endometritis had a positive effect on fertility parameters, which were as good as those in healthy animals.

In this study no significant differences in any of the clinical or physiological parameters were found between experimental groups. However, TSCR in the early treated group was better than in the group treated in the late PP (93% and 71% in groups A and B2, respectively). The same tendency was seen in number of DO (100.7 ± 9.6 and 125.2 ± 14.1 in groups A and B2) and in NSP (1.4 ± 0.2 and 2.2 ± 0.4 in groups A and B2, respectively). But those differences were not significant, possibly because of not enough number of studied animals. The analysis of optimal sample size showed that this difference could be significant in case of more significant number of studied animals (at least 55 animals per group).

This positive effect of using this treatment in the early PP could be explained by changes in PP physiology as a result of administration of PGF2α. The concentration of PGF2α metabolites in dairy cows increases around parturition and, in the case of normal parturition and puerperium, returns to base levels on the10th-20th day PP (Guilbault et al. 1984). In cows with normal uterine involution, the duration of PGF2α release is negatively correlated with the time of completed uterine involution (Lindell et al. 1982). The situation is different in the case of PP uterine inflammations, such as clinical endometritis. After a period of higher increase than in normal cows

(Kindahl et al. 1992), the concentrations of prostaglandin metabolites decline drastically at 7-10 days PP, while healthy cows show a longer period of increased prostaglandins levels (Nakao et al. 1997). The shorter duration or release of PGF2α could be partly responsible for the prolonged period of uterine involution in cows with an abnormal PP period (Nakao et al. 1997). Administration of exogenous PGF2α after the endogenous PGF2α peak on day 4 PP in cows with abnormal puerperium (Nakao et al. 1997) could elongate the period of elevated PGF2α and improve the fertility of animals with inflamed uterus.

However, Hendricks et al. (Hendrickset al. 2006) treated cows twice with PGF2α at 8 h intervals on days 7 and 14 PP and once on days 22 and 35 PP and found no positive effect of PGF2α on fertility parameters. Others studies have shown a positive effect of late administration of PGF2α on animals with PP uterine inflammation. Salasel and Mokhtari (2011) used two doses of PGF2α 8 h apart on day 20 PP for treatment of cows with puerperal health problems, and found that PGF2α treatment increased the first service conception rate of treated animals, reduced the mean number of services per conception and the mean open days, and increased the possibility of pregnancy by 150 days PP. Drillich et al. (2005) treated animals with uterine inflammation at 21-27 days PP using two strategies: administration of PGF2α on days 21-27 and 35-41 and secondly, in cases of signs of uterine inflammation, at 35-41 days PP and 49-55 days PP. Treatment with PGF2α resulted in the same fertility parameter values as those found in healthy cows. Drillich et al. (2005) suggested that PGF2α is the treatment of choice for chronic endometritis in dairy cattle.

The results of the current study also agree with other studies which have demonstrated a negative, or nil, effect of PGF2α administered in the late PP. Mejía and Lacau-Mengido (2005) treated endometritic cows with PGF2α every 20 days, if endometritis was rediagnosed during rechecking by rectal palpation, starting on days 30-50 PP, and reported a negative effect of PGF2α on days to first service and days open. Feldmann et al. (2005), in a study of the treatment of chronic bovine endometritis, treated animals with prostaglandins over 21 days PP. Two weeks following the first treatment, cows were reexamined. The treatment group did not differ with respect to the clinical outcome or reproductive performance. In cases of confirmation of diagnosis at the second examination, the treatment was repeated. Animals after a single treatment had a higher cure rate and first service conception rate, and lower pregnancy index when the treatment was performed after day 42 PP. Le Blanc et al. (2002) found that administration of PGF2α between 20 and 26 days PP, to cows with endometritis that did not have a palpable corpus luteum, was associated with a significant reduction in pregnancy rate. However, there was no difference in pregnancy rate between animals treated in the period between 27 and 33 days PP and untreated cows.

The rate of normalization of vaginal discharge, levels of APPs, start of ovarian activity and fertility parameters of animals in the present study did not differ between experimental groups. So, neither early nor late administration of PGF2α had any effect on clinical or biochemical parameters, or the presence of SE in animals with PP CE. Previous studies have shown that prevalence of SE in clinically healthy cows can very from 12% to 68% (Barański et al 2013, Bicalho et al. 2015). The presence of SE in the control group C of present study was quite high (50%). This could have been because of a high incidence of SE and poor hygiene in this herd generally. Fertility parameters in group A (early treated cows) were better in comparison with group B2 (animals treated in the late PP), however this difference was not significant. But the analysis of optimal sample size showed that it could be significant in case of using at least 55 studied animals per experimental group.

In conclusion, treatment of CE in the early PP period, when uterine inflammation is in its acute phase, and at a time when uterine defence mechanisms actively contend with uterine pathogens and inflammation, the use of systemic administration of ceftiofur with two injections of PGF2α at an interval of 8 h, could be more preferable to using the same treatment in the late PP.

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, SF0180012s11 and project No. T9001VLVL).

Conflict of interest

The authors declare that they have no competing interests.

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. The Veterinary quarterly, 1994. T. 16. P. 21–23.

2. Archbald L.F., Risco C., Chavatte P., Constant S., Tran T., Klapstain E., Elliot J. Estrus and pregnancy rate of dairy cows given one or two doses of prostaglandin F2 alpha 8 or 24 hours apart. Theriogenology. 1993. T. 40. P. 873-884.

3. Barański W., Łukasik K., Skarżyński D., Sztachańska M., Zduńczyk S., Janowski T. Secretion of prostaglandins and leukotrienes by endometrial cells in cows with subclinical and clinical endometritis. Theriogenology. 2013. T. 80. P. 766-772.

4. Bicalho M.L.S., Lima F.S., Machado V.S., Meira E.B. Jr., Ganda E.K., Foditsch C., Bicalho R.C., Gilbert R.O. Associations among *Trueperella pyogenes*, endometritis diagnoosis, and pregnancy outcomes in dairy cows. Theriogenology. 2016. T. 85. P. 267-274.

5. Chenault J.R., Mc Allister 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. Journal of the American Veterinary Medical Association .2004. T. 224. P. 1634–1639.

6. Drillich M., Raab D., Wittke M., Heuwieser W. Treatment of chronic endometritis in dairy cows with an intrauterine application of enzymes. A field trial. Theriogenology. 2005. T. 63. P. 1811-1823.

7. Feldman M., Tenhagen G., Emming S., Hoedemarker M. Treatment of chronic bovine endometritis and factors for treatment success. Dtsch Tierarzti Wochenschr. 2005. T. 112. P. 10-6. (In German).

8. Guilbault L.A., Thatcher W.W., Drost M., Hopkins S.M. Source of F series prostaglandins during the early postpartum period in cattle. Biology of Reproduction. 1984. T. 31. P. 879-887.

9. Hendricks K.E.M., Bartolome J.A., Melendez P., Risco C., Archbald L.F. Effect of repeated administration of $PGF_{2\alpha}$ in the early post partum period on the prevalence of clinical endometritis and probability of pregnancy at first insemination in lactating dairy cows. Theriogenology. 2006. T. 65. P. 1454-1464.

10. Jeremejeva J., Orro T., Waldmann A., Kask K. Treatment of dairy cows with PGF2α or NSAID in combination with antibiotics in cases of postpartum uterine inflammation. Acta veterinaria Scandinavica. 2012. T. 54. P. 45.

11. Kasimanickam R., Duffield T.F., Foster R.A., Gartley C.J., Leslie K.E., Waltson J.S., Johnson W.H. The effect of a single administration of cephapirin or cloprostenol on the reproductive performance of dairy cows with subclinical endometritis. Theriogenology. 2005. T. 63. P. 818-830.

12. Kindahl H., Odensvik K., Aiumlamai S., Fredriksson G. Utero-ovarian relationships during the bovine postpartum period. Animal Reproduction Sciences. 1992. T. 28. P. 363-369.

13. LeBlanc J. Postpartum uterine disease and dairy herd reproductive performance: A review. The Veterinary Journal. 2008. T. 176. P. 102-114.

14. LeBlanc S.J., Duffield T.F., Leslie K.E., Bateman K.G., Keefe G.P., Walton J.S., Johnson W.H. The effect of treatment of clinical endometritis on reproductive performance in dairy cows. Journal of dairy sciences. 2002. T. 85. P. 2237-2249.

15. Lewis G.S Symposium: health problems of the postpartum cow. Journal of dairy sciences. 1997. T. 80. P. 984-994.

16. Lewis G.S. Steroidal regulation of uterine resistance to bacterial infection in livestock. Reproductive biology and endocrinology. 2003. T. 1. P. 117-125.

17. Lindell J.O., Kindahl H., Jannon L., Edqvist L.E. Postpartum release of prostaglandin $F_{2\alpha}$ and uterine involution in the cow. Theriogenology. 1982. T. 17. P. 237-245.

18. Makimura S., Suzuki N. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory disease. The Japanese journal of veterinary sciences. 1982. T. 44. P. 15–21.

19. Mejía M.E., Lacau-Mengido I.M. Endometritis treatment with a PGF2α analog does not improve reproductive performance in a large dairy herd in Argentina. Theriogenology. 2005. T. 63. P. 1266-1276.

20. Melendez P., Mc Hale 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. Journal of dairy sciences. 2004. T. 87. P. 3238–3246.

21. Nakao T., Gamal A., Osawa T., Nakada K., Moriyoshi M., Kawata K. Postpartum plasma PGF metabolite profile in cows with dystocia and/or retained placenta, and effect of fenprostalene on uterine involution and reproductive performance. The Journal of veterinary medical sciences. 1997. T. 59 P. 791-794.

22. Salasel B., Mokhtari A. Effect of early postpartum $PGF_{2\alpha}$ treatment on reproductive performance in dairy cows with calving and puerperal traits. Theriogenology. 2011. T. 76. P. 1723-1729.

23. Toni F., Vincenti L., Ricci A., Schukken Y.H. Postpartum uterine diseases and their impacts on conception and days open in dairy herds in Italy. Theriogenology. 2015. T. 84. P. 1206-1214.

Received 11 October 2016
Accepted 01 December 2016