

**INFLUENCE OF DIFFERENT THERAPY APPROACHES - WITH OR WITHOUT MANUAL
EXTRACTION - OF RETAINED PLACENTA ON REPRODUCTIVE PERFORMANCE IN
SIMMENTAL COWS**

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The aim of the research was to determine the influence of different methods used in the therapy of retained placenta on conception rate and duration of days open in cows. Research was performed on 241 Simmental cows aged 2-8 years divided in three groups. The first group of cows (group A) (n=82) with retained placenta was treated with manual extraction of placenta 12-24 h after parturition combined with application of intrauterine antibiotics. The second group (group B) (n=79) with retained placenta was treated with intrauterine antibiotics only 12-24 h after parturition and repeated 2-3 times in 48 h intervals. The third group (group C) (n=80) was the control group and had physiological puerperium (without retained placenta). To assess the reproductive performance of Simmental cows, interval from calving to first insemination (days open to first service, DOFS), interval from calving to pregnancy (days open to pregnancy, DOP), relative pregnancy rate (%), first service conception rate (FSCR, %) and all service conception rate (ASCR, %) were measured. The estimate of hazard ratio for DOFS in group A relative to cows in group B was 2.20 ($p < 0.0001$), implying that cows in group B had oestrus earlier and were open shorter until the first insemination, that is, the relative rate of first service decreased by 54.5% in the group A while holding all other variables constant. The variable that had a significant influence on DOFS was lactation ($HR = 0.99$; $p < 0.0001$). The estimate of hazard ratio for DOP in group A relative to cows in group B and C was 3.53 ($p < 0.0001$) and 1.73 ($p < 0.0001$), respectively, implying that cows in group B and C were open longer until pregnancy, that is, the relative rate of pregnancy in group A decreased by 71.6% and 42.2% in comparison with group B and C while holding all other variables constant. Variables that had a significant influence on DOP were lactation ($HR = 0.99$; $p < 0.0001$) and peripartal diseases ($HR = 0.48$; $p = 0.02$). The estimate of hazard ratio for DOFS and DOP between group B and C was not significant. Cows treated with intrauterine foaming tablets after RFM became pregnant earlier and had a better

conception rate in contrast to cows treated with manual extraction of placentae.

Key words: days open, manual extraction, retained placenta, Simmental cow

INTRODUCTION

Factors influencing fertility rate include environment, management, general health and genetics (Emanuelson and Oltenacu, 1998; Gröhn and Rajala-Schultz, 2000; Roche, 2006). In the latest time is obvious that different conditions and diseases in periparturient cows (dystocia, retained fetal membranes, aseptic laminitis, metritis, and ovarian cysts) also play an important role and could have a negative impact on the fertility rate in dairy herds (Fourichon *et al.*, 2000).

Different metabolical conditions in the periparturient interval such as for example hypocalcaemia, nutrition, A, D, E vitamins, selenium, iodine and zinc deficiency could alone or combined cause retained placenta (Laven and Peters, 1996; Gupta *et al.*, 2004; Han and Kim, 2005). Retained placenta delays uterine involution and could lead to endometritis and metritis resulting with subfertility (Gröhn and Rajala-Schultz, 2000; Maizon *et al.*, 2004). Postparturient endometritis, usually not pathognomonic, could lead to a significant decrease of fertility of cows (Kim and Kang, 2003; Gautam *et al.*, 2009). The incidence of postparturient uterine inflammations is 10 till 50 % (Lewis, 1997), usually as a result of dystocia and/or retained placenta in additional 20-25% cases (Joosten *et al.*, 1988; Rajala-Schultz and Gröhn, 1998; Bruun *et al.*, 2002). Structural tissue changes caused by enzymatic activity of collagenolysis and proteolysis combined with myometrial contractions are crucial for successful placental separation (Noakes, 2009).

Decrease of number of neutrophils in the circulation decreases placental adhesion and represents the primary cause of retention. Decreased interleukin-8 level as potential chemoattractant of neutrophils in the blood and the subsequent decrease of immune defense is described in cows with retained placenta (Kimura *et al.*, 2002).

Evaluation of reproductive efficiency in cows is based on the duration of days open, intercalving interval, conception rate and non-return rate (Prandi *et al.*, 1999). One of the main goals of cattle production is to shorten days open (Tomaskovic *et al.*, 2007).

Retained placenta is a pathological condition caused by expulsion failure during the third stage of parturition. Physiologically, placenta is expelled in 6-8 hours after parturition in the majority of cows (77.3%) according to Van Werven *et al.* (1992). Placenta is considered to be retained if not expelled in the first 12 hours after parturition (Noakes *et al.*, 2009). Incidence of retained placenta in cows is 5-10%, depending on herd (LeBlanc, 2008) or on average 8.6% (1.3-39.2%) (Kelton *et al.*, 1998). There are numerous factors influencing placenta expulsion: hereditary, nutritional, immunological and pathological factors (Tomaskovic *et al.*, 2007). Complete understanding of the process is still unclear, but the main cause

of retained placenta is definitely connected with structural changes in the fetoplacental unit and hormonal changes (Laven *and* Peters, 1996).

There are different approaches in the treatment of retained placenta. Traditionally, it is treated by manual extraction combined with intrauterine dry therapy using foaming antibiotic tablets (Tomaskovic *et al.*, 2007). Manual extraction of placenta can cause uterine injuries, prolong involution and restitution of the uterus resulting in the recovery of cyclicity and consequently decrease uterine immune defense mechanisms. (Bolinder *et al.*, 1988). It is recommended to leave the placenta to separate alone from the uterus wall and gently remove it approximately 10 days after parturition (Bolinder *et al.*, 1988). Unfortunately, there are no accurate data to support the recommended approach by research performed on a significant amount of cows with retained placenta. Intrauterine antibiotic therapy should prevent putrefaction and puerperal endometritis (Lewis, 1997). Since puerperium influences the duration of days open and fertility rate, our goal was to determine the influence of different therapy approaches (with or without manual extraction of retained placenta) on conception rate and duration of days open in Simmental cows.

MATERIALS AND METHODS

The study was done on 241 Simmental cows divided in three groups. The first group consisted of cows with retained placenta treated using manual extraction and dry therapy once (n=82). The second group consisted of cows with retained placenta (n=79), treated with repeated dry therapy (every 48h until placenta expulsion, but not later than 12 days postpartum). The third group consisted of cows with physiological puerperium (n=80). Cows were picked randomly in Koprivnica-Krizevci region in northwestern Croatia. Cows in the control group were picked randomly from herds which gave animals from both experimental groups. Some of the cows from experimental groups were treated parenterally, if needed. Some of them received additionally antibiotics IM, oxytocine and antiinflammatory drugs. Intrauterine dry antibiotic therapy consisted of 2 foaming tablets (Geomycin® F, Veterina d. o. o., Croatia) (1 intrauterine foaming tablet contains 1 g oxitetracycline hydrochloride with foaming diluent).

Examinations and data collections

All cows were examined, including vaginal and transrectal uterine palpation, and treated (only the third group was not treated) within 12-24 h after calving. All cows were again examined 35–45 days post partum, and 45 days after insemination with transrectal ultrasonography with a 5 MHz linear-array transducer (SonoVet 2000, Medison, Seoul, South Korea) until confirmation of pregnancy.

After the first examination, cows with diseases endometritis, ovarian cyst diseases, anestrus or ovarian atrophy were managed according to the usual herd management procedure (hormonal therapy, intrauterine application or systemic

therapy). All reproductive data such as date of parturition, parity, pregnancy and culling, lactation, postpartal diseases (recumbency, ketosis, ovarian cyst diseases, endometritis, mastitis, etc.), interval from calving to first insemination (days open to first service, DOFS), interval from calving to pregnancy (days open to pregnancy, DOP), relative pregnancy rate (%), first service conception rate (FSCR, %) and all service conception rate (ASCR, %) were collected.

Cows were artificially inseminated with frozen-thawed semen by experienced inseminators. Reproductive performance and culling data of individual animals were collected for a minimum of 8 months after enrolment (until confirmed pregnant or culled).

Statistical analysis and modelling

Statistical analyses was performed using SAS 9.1.3. software (SAS Institute Inc., 2002-2003). To assess the reproductive performance of Simmental cows, the interval from calving to first insemination (days open to first service, DOFS), the interval from calving to pregnancy (days open to pregnancy, DOP), the relative pregnancy rate (%), the first service conception rate (FSCR, %) and all service conception rate (ASCR, %) were measured.

The non-parametric, log-rank test and Wilcoxon test of equality across strata (PROC LIFETEST) was done for each categorical variable separately. The Kaplan-Meier curves shape was verified and survival estimates were used to calculate the median days open. Values were censored when observations were terminated for reasons beyond investigator's control. The peripartal diseases (assisted calving, recumbency, endometritis, mastitis) were pooled into a single category because the number of occurrences was small. For continuous variables, a semi-parametric model, Cox proportional hazard regression (PROC PHREG) was done. The final model was built using stepwise regression analysis (PROC PHREG) with p value <0.10 to remain and <0.15 to enter in the model.

To verify the assumption of proportionality in Cox proportional hazards regression, time-dependent covariates were included in the model and interactions with logarithm (time) were used. If one of the predictors was not proportional, parametric regression model (PROC LIFEREG) was used. To verify if the fitted model was correct, the Kaplan-Meier estimates of cumulative hazard function were calculated using the Cox-Snell residuals (PROC LIFETEST) and plotted against the residuals (PROC GPLOT). Since the Weibull distribution fitted correctly, the hazard ratio parameters were estimated by multiplying the Weibull Shape parameter with the negative of the accelerated failure time (AFT) parameter.

The hazard ratio was interpreted as the relative daily probability of conception or relative pregnancy rate (Fourichon *et al.*, 2000).

The binary variables (FSCR and ASCR) were analysed using GENMODE procedure with log link function to obtain the relative risk (RR) and binomial distribution with significance covariates included in the model.

RESULTS

The percent of censored values was 4.88 %, 2.53 % and 3.75 % for group A, B and C respectively. Table 1 describes the reproductive performance of group A, B and C (control group) of cows.

Table 1. Differences of reproductive performance between the groups

	Group		
	A	B	C
N	82	79	80
MDOFS (95%, CI)	78 ^a (76, 86)	79 ^a (74, 82)	81 ^a (77, 88)
MDOP (95%, CI)	98 ^a (93, 114)	89 ^b (80, 95)	96 ^{ab} (89, 99)
FSCR(%)	29.2 ^a	56.9 ^b	55.0 ^b
ASCR(%)	43.5 ^a	60.1 ^b	53.1 ^b

Different superscript letters in the same row ($p < 0.05$)

MDOFS – Median days open to first service; MDOP – Median days open to pregnancy; FSCR – First service conception rate; ASCR – All service conception rate; CI – Confidence interval.

The median days open to first service (MDOP) was significantly different between groups A and B. After 98 days (MDOP of group A) the estimated proportion of pregnant cows in group A was $51.2\% \pm 5.5\%$, but in Group B the estimated proportion of pregnant cows was $65.0\% \pm 5.4\%$ ($p < 0.0001$). The FSCR and ASCR were significantly lower for cows in Group A. The relative risk for cows in Group A to become pregnant after the first insemination was 49.1% (RR=0.509; $p < 0.0001$) lower in comparison with cows in Group B and C. Regarding ASCR, the relative risk for the cows in Group A to become pregnant was 25.8% (RR=0.784; $p = 0.05$) and 21.6% (RR=0.742; $p < 0.05$) lower comparing with cows in Group B and C, respectively. Cows treated with intrauterine foaming tablets after RFM became pregnant earlier and had a better conception rate in contrast to cows treated with manual extraction of placentae. The effectiveness of the therapy with intrauterine foaming tablets is evident by the fact that there was no significant difference in MDOP, FSCR and ASCR from the cows in the control group. However, MDOFS was not different between the groups.

Table 2 shows the hazard ratio of Group A treated with manual extraction of placenta relative to Group B and control group (Group C) with days open to first service and days open to pregnancy.

The estimate of hazard ratio for DOFS in Group A relative to cows in Group B was 2.20 ($p < 0.0001$), implying that cows in Group B had oestrus earlier and were open shorter until the first insemination, that is, the relative rate of first service decreased by 54.5% in Group A while holding all other variables constant. Variable that had a significant influence on DOFS was lactation (HR=0.99; $p < 0.0001$). Variables that did not have a significant influence on DOFS were parity, peripartum diseases and additional systemic treatment. Differences

between Group A and C were close to significant ($p=0.08$). The estimate of hazard ratio for DOP in Group A relative to cows in Group B and C was 3.53 ($p<0.0001$) and 1.73 ($p<0.0001$), respectively, implying that cows in Group B and C were open longer until pregnancy, that is, the relative rate of pregnancy in the Group A decreased by 71.6% and 42.2% in comparison with Group B and C while holding all other variables constant. Variables that had a significant influence on DOP were lactation ($HR=0.99$; $p<0.0001$) and peripartum diseases ($HR=0.48$; $p=0.02$). Variables that did not have a significant influence on DOFS were parity and additional systemic treatment. The estimate of hazard ratio for DOFS and DOP between the Group B and C was not significant.

Table 2. Hazard ratio of group A relative to group B and C with days open to first service and days open to pregnancy

Reproductive measures	Group A relative to group					
	B			C		
	No	Estimate (95% CI)	p	No	Estimate (95% CI)	p
DOFS	161	HR=2.20 (1.60-3.02)	<0.0001	162	HR=1.15 (0.98-1.34)	0.08
DOP	161	HR=3.53 (2.56-4.85)	<0.0001	162	HR=1.73 (1.47-2.03)	>0.0001

DOFS – Days open to first service; DOP – Days open to pregnancy; HR – Hazard ratio; CI – Confidence interval

Figure 1 shows the relationship between the proportion of open cows and days open to pregnancy for Groups A and B with Kaplan-Meier survival curves

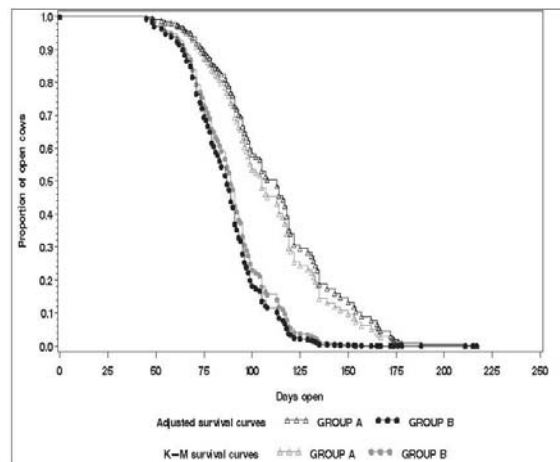


Figure 1. Kaplan-Meier and adjusted survival curves: relationship of proportion of open cows and days open to pregnancy for Group A and B

and adjusted survival curves with equal mean of covariates. The adjusted survival curves shows that difference between the groups was even greater. Similar relationship between the proportion of open cows and days open for Groups A and C is shown in Figure 2.

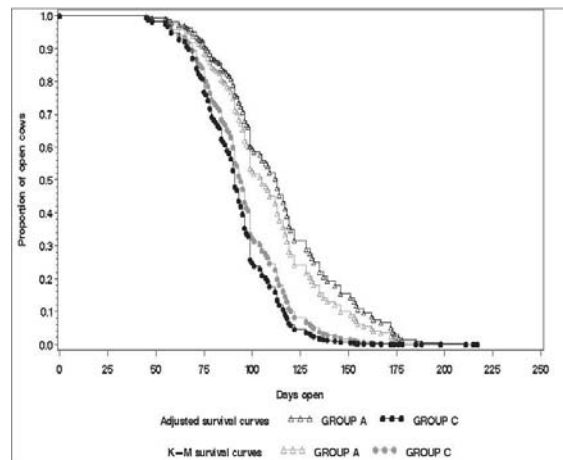


Figure 2. Kaplan-Meier and adjusted survival curves: relationship of proportion of open cows and days open to pregnancy for Group A and C

DISCUSSION

Despite extensive research, treatment of retained placenta has not changed much in many years. Kozdrowski and Twardon (2003) were discussed about the advantages and disadvantages of manually removing the placenta, as well as its treatment without manual removal. Some authors concluded that early oxytetracycline treatment of retained fetal membranes in the cow did not shorten the uterine involution or uterine infection but it did slow down the detachment process of the retained placenta (Konigsson *et al.*, 2001). Previous authors were concluded that oxytetracycline treatment after placental shedding might shorten the uterine infection, but otherwise did not affect the clinical results which is opposite to our investigation. Dobranic *et al.* (2006) described the negative connection between retained placenta with conception rate, duration of days open and intercalving interval. Duration of days open was longer in both groups of cows, in our research, with retained placenta compared to the control group of cows. When comparing different treatments, cows with manual extraction of placenta had prolonged days open, if compared with antibiotic therapy only. It is probably caused by small lesions of the uterus during manual extraction (Bolinder *et al.*, 1988). It is determined that such lesions decrease phagocytic activity and neutrophil number in the blood consequently decreasing local and general resistance to infections (Cai *et al.*, 1994). Local antibiotic therapy causes decrease of microbial population resulting in decreased putrefaction and absence of

unpleasant smell (Brooks, 2001; Drillich *et al.*, 2006). This was also the case in our research. Considering the conception rate it was obvious that cows treated with local antibiotic therapy only, obtained better results. Their conception rate was much higher than the conception rate of cows after manual extraction of the placenta and was comparable with the conception rate of the control healthy group. According to the literature (Tomaskovic *et al.*, 2007), such cows are considered fertile, while cows after manual extraction of placenta ended up as subfertile animals. During this research we didn't find any significant differences according to parity of cows in any of the observed groups. Similar results were already obtained by Gilbert *et al.*, (2005) who also did not find any correlations between parity and incidence of puerperal diseases. Our results are opposite to LeBlanc *et al.*, (2002) and Gautam *et al.*, (2009) who determined a connection between parity and endometritis in dairy cows.

CONCLUSIONS

Simmental cows treated with intrauterine foaming tablets after RFM became pregnant earlier and had a better conception rate in contrast to cows treated with manual extraction of placentae. We could finally conclude that our research suggested to treat retained placenta with local antibiotic therapy only, every 48h. After such treatment the cows easily drop out the retained placenta 7-10 days postpartum. Thus, antibiotic local therapy only in cows with retained placenta is a method of choice by all means as it results with practically identical conception rate and duration of days open as in the healthy population.

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UTICAJ RAZLIČITIH TERAPEUTSKIH PRISTUPA – SA ILI BEZ MANUELNE EKSTRAKCIJE ZAOSTALE PLACENTE NA REPRODUKTIVNE SPOSOBNOSTI SIMENTALSKIH KRAVA

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SADRŽAJ

Cilj istraživanja bio je odrediti utecaj različitih metoda korištenih u lečenju zaostale placente na procenat koncepcije i trajanje servis perioda u krava. Istraživanje je provedeno na 241 kravi simentalске pasmine u dobi od 2-8 godina podeljenih u tri grupe.

Prva grupa krava (grupa A) (n=82) sa zaostalom placentom lečena je manualnim vađenjem placente 12-24 h nakon poroda u kombinaciji sa aplikacijom antibiotičkih intrauterinih obleta. Druga grupa (grupa B) (n=79) sa zaostalom placentom lečena je aplikacijom samo intrauterinih antibiotičkih obleta 12-24 h nakon poroda te ponovljena 2-3 puta svakih 48 h. Treća grupa (grupa C) (n=80) bila je kontrolna grupa krava koje su imale fiziološki puerperij (bez zaostale placente). Za analizu reproduktivnih parametara krava simentalске pasmine određivan je interval od poroda do prvog osemenjavanja (servis period do prvog osemenjavanja, DOFS), interval od poroda do graviditeta (servis period do graviditeta, DOP), relativni postotak graviditeta (%), procenat koncepcije nakon prvog osemenjavanja (FSCR, %) i indeks osemenjavanja stada (ASCR, %). Procena omjera rizika za DOFS u grupi A u odnosu prema kravama grupe B iznosila je 2,20 ($p < 0.0001$), što ukazuje da su krave grupe B bile u estrusu ranije i imale kraće razdoblje od poroda do prvog osemenjavanja, odnosno, relativni procenat perioda do prvog osemenjavanja krava u grupi A je bio manji za 54,5% uz zadržavanje ostalih varijabli konstantnim. Varijabla koja je imala statistički značajan efekat na DOFS bila je laktacija (HR=0.99; $p < 0.0001$). Procena omjera rizika za DOP u grupi A u odnosu na krave grupe B i C bila je 3,53 ($p < 0.0001$) i 1,73 ($p < 0.0001$), pomenutim redosledom, što ukazuje da su krave u grupi B i C imale dulji period do graviditeta, odnosno, relativni procenat perioda do graviditeta u krava grupe A je umanjen za 71,6% i 42,2% u poređenju sa grupom B i C uz zadržavanje ostalih varijabli konstantnim. Varijable koje su imale statistički značajan efekat na DOP bile su laktacija (HR=0.99; $p < 0.0001$) i oboljenja u peripartalnom periodu (HR=0.48; $p = 0.02$). Procena omjera rizika za DOFS i DOP između grupe B i C nije bila statistički značajna.

Zaključno, krave lečene intreuterinim obletama nakon zaostajanja placente ostale su gravidne ranije i imale su bolji postotak koncepcije u odnosu na krave lečene manualnim vađenjem placente.