

## RETAINED BOVINE PLACENTA – VARIOUS TREATMENTS AND THEIR EFFECTS

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### Abstract

Given the influence of *retentio secundinarum* (RS) on the reproductive performance of cows, the current work aimed to assess the effect of various approaches to the treatment of retained placenta on the main reproductive parameters, primarily on the interval from parturition to the first service, and the insemination index. The trial involved 120 dairy cows in which the placenta was not expelled within 24 h postpartum. The first group (G1, n=60) contained cows in which the retained placenta was not extirpated, but they were administered antibiotics. The second group (G2, n=60) comprised cows in which the placenta was removed manually, and were also administered antibiotics. At the end of the study, 53 cows remained in G1 and 54 cows in G2. According to medicament therapy, both groups were divided into three sub-groups treated with: procaine benzylpenicillin, chlortetracycline hydrochloride or ceftiofur. Comparison of the performance of G1 and G2 cows showed significantly better fertility parameters occurred in the animals from which the retained placenta was extracted manually. The

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pregnancy rate in the G2 group was remarkably higher than in the G1 group. The average service interval was significantly lower ( $p < 0.05$ ) in the G2 group compared to the G1 group. The best results were obtained with the i.m. administration of ceftiofur after the removal of the placenta.

**Key words:** antibiotics, Holstein cows, placenta, retention, treatment

## INTRODUCTION

In intensive dairy production, various conditions and diseases in cows in the periparturient period (dystocia, placental retention, aseptic laminitis, metritis and ovarian cysts) can have a negative influence on the reproductive parameters (Fourichon et al., 2000). Retained placenta (*retentio secundinarum* – RS) is a condition of dairy cows in the periparturient period, the incidence of which ranges from 4% to 11%, although it can be even higher in problematic herds (Eiler, 1997). Many causes can lead to RS, either infective or un-infective, but it is frequently considered to be a syndrome owing to its extremely complex pathogenesis (Gross et al., 1986). RS affects unfavourably the health and reproductive condition of the animals, leading to economic losses. It has been estimated that cows with RS and consecutive clinical metritis produce 300-500 kg less milk annually in comparison with their unaffected counterparts (Laven and Peters, 1996).

The key element of the pathogenesis of RS in cows is the omission of the timely disruption of cotyledon-caruncle linkage in the third phase of parturition, which results from the disturbed mechanism of detachment within the placentomes (McNaughton and Murray, 2009). Structural changes in the placentome tissues generated by the enzyme activity of collagenase and other proteases in combination with the myometrium contractions are essential for the successful placenta detachment (Peter, 2013). For the timely separation in the placentomes, placenta maturation is necessary, which includes hormonal changes (Laven and Peters, 1996), primarily the conversion of the placental progesterone into oestrogen, which enables the expression of oxytocin receptors on the myometrium cells and increased prostaglandin F<sub>2α</sub> synthesis (McNaughton and Murray, 2009). Besides hormonal changes, an important role in the process of placenta detachment is played by the activation of the maternal immune response on the foetal membrane antigens (McNaughton and Murray, 2009). Any disturbance in the complex mechanism of the disruption of foetal-maternal connection leads to RS (Takagi et al., 2002).

There are several approaches to the therapy of this condition that include local and/or systemic antibiotic treatment with or without manual placenta removal and hormone therapy. Although the traditional, manual treatment of RS includes its manual removal combined with intrauterine therapy (Tomašković et al., 2007), abundant literature data suggest that significantly better results can be achieved when the manual removal is excluded from the treatment protocol. Cephalosporins and penicillins are the most

frequently applied systemic antibiotics, whilst tetracyclines are more often applied locally as tablets for intrauterine use (Brooks, 2001; Biner et al., 2015).

Given the influence of RS on the reproductive performance of cows, the current work aimed to assess the effect of various approaches to the treatment of RS on the main reproductive parameters, primarily on the interval from parturition to the first service, and the insemination index.

## MATERIALS AND METHODS

The research was conducted on a dairy cow farm in the vicinity of Belgrade, with about 1900 Holstein cows. They were kept in a free-range system of intensive production, all under the same hygienic conditions. The average milk production was 7500 L per animal in a standard 305-day lactation cycle. The trial involved 120 dairy cows aged between 4 and 7 years, in which the placenta was not expelled within 24 h postpartum. The first group (G1, n=60) contained cows in which the retained placenta was not extirpated, but the animals received antibiotics. According to medicament therapy, these were divided into three sub-groups, treated as follows:

- Sub-group KP1 (n=20) – procaine benzylpenicillin (Neopen, FM Pharm d.o.o., Subotica, 300,000 IU/ml) was administered daily, intramuscularly, 21,000 IU/kg BW, for five consecutive days;
- Sub-group KH1 (n=20) – chlortetracycline hydrochloride, intrauterine tablets (Neocyclin, FM Pharm d.o.o., Subotica, 1,000 mg) were administered twice, one tablet in the morning and the other one in the evening, for one day.
- Sub-group KC1 (n=20) – ceftiofur (Cefokel, Kela, Belgium, 50 mg/ml) was administered daily, subcutaneously, 1 mg/kg body weight (BW), for five consecutive days.

The second group (G2, n=60) comprised cows in which the placenta was removed manually, and the animals received antibiotics. This group was also divided into three subgroups (KP2, KH2 and KC2) that were administered the same antibiotics as KP1, KH1 and KC1, respectively.

In each G2 cow, the placenta was extirpated carefully, in compliance with all hygienic principles and in a process that did not last for more than 20 minutes. Animals in which it was not possible to complete the extirpation within this time limit were excluded from this study.

At the end of the study, 107 (53 in G1 and 54 in G2) animals remained, as some had had health problems and/or were culled. Among the 107 animals, 53 cows were pregnant over both groups on day 300 after parturition. To determine the fertility of the tested cows, the following parameters of reproductive efficacy were assessed: the length of the service interval, insemination index, conception rate after the first insemination and the number of pregnant cows at the end of the study, i.e. at 300 days after parturition.

Descriptive statistic parameters were calculated for the results obtained and the statistical significance of the differences in the parameters between the groups/subgroups was determined using Student's t-test. Differences with  $p < 0.05$  were considered significant. The data were processed using MS Excel.

## RESULTS

The results of the tested fertility parameters in cows in which the retained placenta was not extirpated manually but which were administered antibiotics are shown in Table 1. The average service interval among the non-extirpated cows was shortest in the KH1 subgroup, which was treated locally with intrauterine chlortetracycline tablets, and their insemination index was the lowest among these three cow subgroups. Out of these KH1 cows ( $n=16$ ), five (32%) were pregnant at the end of the study. In the KP1 subgroup, treated i.m. with penicillin, a somewhat longer service interval was noticeable (187.7 days on average), but also, the largest number of pregnant cows (30%) in KP1 animal subgroups. The worst results were obtained in the KC1 subgroup of cows, which were treated s.c. with ceftiofur; all remained non-pregnant in the research period.

**Table 1.** Fertility parameters in cows treated without (Group 1) or with (Group 2) manual extirpation of retained placenta, and then treated with antibiotic therapy

Group 1	Pregnant after 1st insemination		Total pregnant on day 300		Non-pregnant on day 300		Service interval	Insemination index
	N	%	N	%	N	%	( $\bar{X} \pm \text{SD}$ )	( $\bar{X} \pm \text{SD}$ )
KP1 (n=20)	1	5	6	30.0	14	70.0	187.7 $\pm$ 77.1 <sup>aA</sup>	2.8 $\pm$ 1.3 <sup>aA</sup>
KH1 (n=16)	0	0	5	31.3	11	68.7	179.2 $\pm$ 49.3 <sup>aA</sup>	2.7 $\pm$ 1.4 <sup>aA</sup>
KC1 (n=17)	0	0	0	0	17	100	> 300 <sup>bA</sup>	> 3 <sup>bA</sup>
Total (n=53)	1	1.9	11	20.8	42	79.2	183.8 $\pm$ 63.0 <sup>A</sup>	2.7 $\pm$ 1.3 <sup>A</sup>
<b>Group 2</b>								
KP2 (n=20)	8	40.0	14	70.0	6	30.0	112.0 $\pm$ 33.3 <sup>aB</sup>	1.5 $\pm$ 0.8 <sup>aB</sup>
KH2 (n=18)	2	11.1	13	72.2	5	27.8	162.9 $\pm$ 74.7 <sup>aA</sup>	2.6 $\pm$ 1.0 <sup>bA</sup>
KC2 (n=16)	3	18.8	15	93.8	1	6.2	126.5 $\pm$ 56.4 <sup>aB</sup>	2.2 $\pm$ 1.3 <sup>abB</sup>
Total (n=54)	13	24.1	42	77.8	12	22.2	133.0 $\pm$ 59.4 <sup>B</sup>	2.1 $\pm$ 1.2 <sup>A</sup>

<sup>abc</sup> – Values in the same group with different superscripts are statistically significant ( $p < 0.05$ )

<sup>A,B</sup> – Values in different subgroups with the same antibiotic treatment are statistically significant ( $p < 0.05$ )

The results of fertility parameters in cows with retained placenta extirpated manually followed by antibiotic treatment are shown in Table 1. Among these G2 cows, it is noticeable that KC2 cows treated s.c. with ceftiofur after the placenta was extracted presented favourable results: the average service interval was 126.5 days, the insemination index 2.2, and 93.8% of these cows were pregnant at the end of the

study. Cows with manually extracted placenta and treated locally with chlortetracycline (KH2) had the longest service interval of 162.9 days on average, but also the highest insemination index (2.6). If the length of the service interval and the insemination index are taken into consideration, the most favourable results were obtained in cows after the retained placenta was extracted manually and penicillin was administered i.m. (KP2): their service interval lasted for 112 days and the insemination index was 1.5 on average.

Significantly better fertility parameters were noticeable in the animals from which the retained placenta was extracted manually than in animals with non-extracted retained placentas. After manual placenta removal, in G2 group 54/42 (77.8%) cows were pregnant at the end of the study. In contrast, in G1 group only 11/53 (20.8%) cows with retained placentas had conceived at the end of the study. Comparison of subgroups treated with penicillin (KP1 and KP2) proved that the length of the service interval was significantly longer ( $p < 0.05$ ) in the former group. The average service interval for KP1 cows was 187.7 days, whilst in KP2 cows, it was significantly shorter ( $p < 0.05$ ), 112 days.

The average insemination index did not differ significantly between the two main cow groups (G1 and G2 groups): it was 2.7 and 2.1 respectively. However, between the subgroups of cows treated with penicillin, the difference in the number of animals that conceived after the first insemination was remarkable: only one animal conceived in KP1, but 13 conceived in KP2.

## DISCUSSION

In cows, the incidence of placenta retention after parturition varies between breeds, depends on the season, diet and management, and is considerably more frequent in multiparous cows (Gaafar et al., 2010). The aetiology of RS is complex, multifactorial, and is yet to be explained in detail. The majority of authors do not define it as a disease *per se* but as a syndrome, which is a consequence of, or emerges during some other production, metabolic or infectious diseases. It leads to vast financial losses estimated to reach €200-250 per animal, which is why it has to be perceived as a serious problem in farm production (Dovenski et al., 2016). According to various sources, the conception after RS is delayed from 12 to 32 days or about 15 days on average (Guard, 1999). There is evidence that cows with RS have a considerably higher incidence of metritis (53%) in comparison with those without RS (30%); moreover, a considerably lower conception percentage was observed in cows with RS and metritis (66%) than in animals with metritis only (77%) (Youngquist and Therellfall, 2007). These authors supposed that metritis that follows RS occurs due to the presence of the decayed placental tissue, which is a favourable medium for bacterial colonisation. The presence of coliform bacteria and high endotoxin concentrations in the lochia of cows with RS are powerful inductors of prostaglandin and cytokine, which favour uterine infections (Dohmen et al., 2000). Bacteria or their endotoxins detected in the uterus in the early

puerperium could be related to RS and the secretion of prostaglandin E2, which could predispose the uterus to additional infections (Dovenski et al., 2016). Emanuelson et al. (1993) confirmed that difficult labour and placenta retention frequently lead to endometritis and chronic metritis, which result in reproductive disturbance and, inevitably, huge economic losses due to medication costs, decreased production and poor fertility (Deluyker et al., 1991).

According to the results obtained in this study, significantly better reproductive performance was obtained in cows from which the retained placenta was removed manually and the corresponding antibiotic therapy administered. This does not accord with the findings by Laven (1995) and Paisley et al. (1986), who claimed manual placenta removal is harmful due to the disturbance to the mechanisms of the local immune response in the uterus and longer uterine evolution. Bolinder et al. (1988) considered that manual placenta removal can result in uterine damage and lengthens its involution, which further leads to the decrease of uterine immune defence mechanisms. The same authors recommend that the placenta be removed about 10 days postpartum, when it has detached from the uterine wall and already undergone putrefaction.

In Simmental cows (Đuričić et al., 2011), the effect of manual placenta removal plus intrauterine oxytetracycline tablets applied 12-24 h after parturition were compared with treatment with intrauterine tablets alone 48 h postpartum. Based on the results obtained, it was concluded that the cows treated with tablets only had shorter service intervals, lower insemination index and better conception rates than their counterparts with removed placentas. Similar results were obtained by Gunay et al. (2011) in research that included three groups of cows: in one (67 cows), the problem of RS was solved with manual removal 2-3 days postpartum without antibiotic treatment, in the second (47 cows) and the third (30 cows) groups, the cows were administered 2 g of chlortetracycline by the intrauterine route, plus the last group was given oxytetracycline parenterally on day 4. Intrauterine treatment without the removal of retained placenta led to the best fertility results (service interval 87 days, 27% conception after the first insemination). These results are in sharp contrast with those obtained in our work, because in our group of cows treated with intrauterine tablets without the removal of the placenta (KH1), only 32% of animals conceived, and they had, on average, a 179.2-day-long service period and insemination index of 2.7. In the current study, significantly better results were achieved in cows relieved from their retained placenta, regardless of the medicament therapy.

In work by Drillich et al. (2006), the effects of three antibiotic protocols were tested in cows with pathological vaginal discharge and high body temperature ( $\geq 39.5^{\circ}\text{C}$ ). Groups of cows were administered 600 mg ceftiofur i.m., 2,500 mg cloxacillin in the form of intrauterine tablets, or 6,000 mg ampicillin i.m. All the treatments lasted for three days. Based on reproductive parameter analysis, no significant differences were detected in the efficacy of the three protocols. It was concluded that ceftiofur could successfully replace the local application of antibiotics, and this was justifiable by lower therapy expenses and the shorter milk withholding period in comparison

with the other treatments. The results of Drillich et al. (2006) are not similar to those we obtained in cows with retained placentas but were treated with i.m. ceftiofur: none of these cows conceived. On the other hand, ceftiofur-treated cows after placenta removal achieved the best results in comparison with all the other groups/treatments, as 93.8% of these cows conceived.

Masera et al. (1980) and Bretzlaff et al. (1982) considered that the resorption ability of the uterus affected with endometritis is decreased and, thus, suggest the use of systemic antibiotics owing to better penetration into the endometrium and surrounding tissues and lesser disturbance of the uterine microbiome. These findings that justify the use of systemic antibiotics have been confirmed in our research. Moreover, Oliveira et al. (2020) claimed that ceftiofur-treated cows in the postpartum period produce more milk in the first two months of lactation and are more fertile in comparison with untreated animals.

## CONCLUSION

The results of our research revealed that in cows with RS, significantly better fertility performance can be achieved by manual removal of the retained placenta followed by antibiotic treatment in comparison with the antibiotic therapy alone. The best results were obtained with the i.m. administration of ceftiofur after the removal of the placenta. It can be concluded that longer exposure of the endometrium to placental tissues, with unavoidable bacterial contamination, decreases the effectiveness of both systemically and locally administered antibiotics.

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## Authors' contributions

MM and FS carried out the experimental work, made substantial contributions to acquisition, analysis and interpretation of data and participated in manuscript writing. JB performed the statistical analysis and made a substantial contribution to interpretation of data. AN and SV coordinated experimental performance and were involved in manuscript writing. JVM made substantial contributions to interpretation of data and writing the manuscript. JM conceived and designed the study, made substantial contributions to writing the manuscript, critically revised the manuscript and approved its submission.



## Competing interests

The author(s) declare they have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## ZAOSTALA POSTELJICA KOD GOVEDA – RAZLIČITI PRISTUPI TERAPIJI I NJIHOVI EFEKTI

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### Kratak sadržaj

Uzimajući u obzir uticaj RS (*retentio secundinarum*) na reproduktivne performanse krava, cilj ovog rada je bio da se proceni efekat različitih pristupa lečenju zadržane posteljice na glavne reproduktivne parametre, pre svega na interval od porođaja do koncepcije i indeks osemenjavanja. U ispitivanju je učestvovalo 120 muznih krava kod kojih nije došlo do izbacivanja posteljice 24 sata nakon porođaja. Prva grupa (G1, n = 60) uključivala je krave kod kojih zadržana placenta nije manuelno uklanjana, već je terapija podrazumevala samo aplikaciju antibiotika. Drugu grupu (G2, n = 60) činile su krave kojima je posteljica odstranjena manuelno i uz to primenjena antibiotska terapija. Do kraja eksperimenta je u prvoj ogleđnoj grupi ostalo 53 krave, a u drugoj grupi 54 krave. Prema vrsti medikamentozne terapije, obe grupe su podeljene u tri podgrupe i lečene su prokain-benzilpenicilinom, hlortetraciklin hidrohloridom i ceftiofufom. Upoređivanjem reproduktivnih performansi prve i druge grupe krava, primetni su značajno bolji parametri plodnosti kod životinja kojima je manuelno izvađena zadržana posteljica i kojima su uz to aplikovani antibiotici. Broj gravidnih životinja u G2 grupi bio veći u odnosu na G1 grupu. Prosečno trajanje servis perioda bilo je statistički značajno kraće ( $p < 0,05$ ) u G2 grupi u poređenju sa G1 grupom. Najbolji rezultati postignuti su i.m. primenom ceftiofura nakon uklanjanja placente.

**Ključne reči:** holštajn krave, posteljica, retencija, tretman, antibiotici