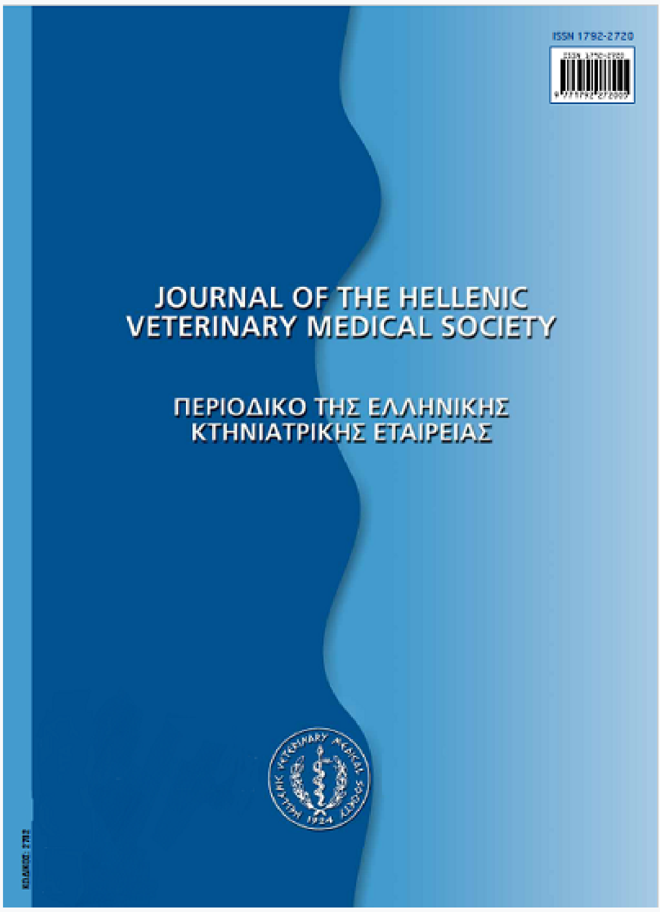
Journal of the Hellenic Veterinary Medical Society

Vol 68, No 4 (2017)




# The effects of four protocols for the treatment of retained placenta on reproduction performance and milk yield in Holstein cows

*M. MELLADO, R. SOLANO, F. G. VELIZ, A. DE SANTIAGO, L. GAYTAN, J. E. GARCIA*

doi: 10.12681/jhvms.16030

J HELLENIC VET MED SOC 2017, 68(4): 513-520
ΠΕΚΕ 2017, 68(4): 513-520

Research article
Ερευνητικό άρθρο

# The effects of four protocols for the treatment of retained placenta on reproduction performance and milk yield in Holstein cows

**Mellado, M.[1], Solano, R.[2], Veliz, F.G.[2], de Santiago, A.[2], Gaytan L.[1], Garcia, J.E.[1]**

*[1]Autonomous Agrarian University Antonio Narro, Department of Animal Nutrition, Saltillo, Mexico*

*[2]Autonomous Agrarian University Antonio Narro, Department of Veterinary Science, Torreon, Mexico*

**ABSTRACT.** In a first study, 231 cows with retained placenta (RP) were assigned to four groups and their reproductive performance after treatment was studied. Group 1 received 2.0 mg/kg ceftiofur hydrochloride (CH), 10 mg estradiol cypionate (ECP) and 100 IU oxytocin for three consecutive days (CEO-3; n= 63). Group 2 received the same treatment for six consecutive days (CEO-6; n= 48). Group 3 was treated with CH and oxytocin for three consecutive days (CO-3; n= 68). Group 4 was subjected to the previous treatment for six consecutive days (CO-6; n=52). The control group (n= 62) did not present RP. Conception rates (CR) did not differ between treatments with values of 36.5, 25.0, 30.9, 30.8 and 33.9% for CEO-3, CEO-6, CO-3, CO-6 and control group, respectively. Services per pregnancy did not differ between groups (overall 3.6 ± 2.0; mean ± SD). In a second study, changes in milk production associated with RP were examined in cows receiving the CO-3 protocol. Data came from 360 cows with RP and 1623 served as controls. Mean 305-d milk yield was 283 ± 43 kg lower ($P<0.05$) in cows with RP compared to controls. It was concluded that either prolonged (6-d) or short (3-d) application of CH in combination with oxytocin and ECP were equally effective in preventing a reduction of the reproductive performance in cows suffering RP. Additionally, RP was associated with a 3% decrease in 305-d milk production.

***Keywords:*** Retained placenta; Oxytocin; Ceftiofur hydrochloride, Estradiol cypionate; Conception rate; Services per pregnancy; Milk yield

*Corresponding Author:*
Jose E. Garcia
Autonomous Agrarian University Antonio Narro
Department of Animal Nutrition Saltillo, Coah. 25315 Mexico
Tel: 52 (844) 4-11-03-24
Fax: 52 (844) 4-17-37-84
Email: edugarmartz@gmail.com

*Date of initial submission: 29-8-2016*
*Date of revised submission: 16-9-2016*
*Date of acceptance: 18-10-2016*

## INTRODUCTION

Retained placenta (RP) in dairy cows reduces reproductive efficiency (Kasimanickam et al., 2004; Gilbert et al., 2005), increases the risk of culling (Leblanc, 2008) and causes potential losses in milk production (Laven and Peters, 1996; Rajala and Grohn, 1998). To date, there is no data supporting the beneficial effects of intrauterine antibiotics for RP (Drillich et al., 2006, 2007; Beagley et al., 2010), although intrauterine ozone flush seems to be an efficacious and cost-effective treatment option for this reproductive disorder (Zobel and Tkalcic, 2013). It has been suggested that while placement of antibiotics into the uterus can be beneficial in treating metritis, it is unlikely that they will result in an earlier release of membranes, prevent metritis or improve reproductive outcome in cows with RP (Goshen and Shpigel, 2006; Drillich et al., 2007; Beagley et al., 2010). Some authors have found that systemic administration of ceftiofur hydrochloride (CH) offers better results in the treatment of RP when compared to prostaglandins and estradiol, but this treatment has not improved the reproductive efficiency in dairy cows (Risco and Hernandez, 2003; Leblanc, 2008). There are conflicting results regarding the treatment of RP, likely due to several factors such as the differences in criteria used to diagnose uterine complications derived from RP, the postpartum stage of the animals when medication is applied, the outcome variables being measured, and the frequency and route of administration of the diverse drugs used in each experiment (Smith and Risco, 2002). Treatment of retained fetal membranes with CH on three consecutive days is not always effective to prevent uterine infections in cows; therefore, longer treatments of antibiotic are often required (Drillich et al., 2003). In fact, 13% of cows treated for RP with CH (x 5 days) developed metritis (Risco and Hernandez, 2003). That is why in the present study it was considered pertinent to explore the extension of the CH treatment to 6 days in cows with RP. On the other hand, the use of estradiol cypionate (ECP) for RP or prevention of postpartum metritis is controversial, because the benefits of this hormone in cows have not been entirely substantiated by objective research. The limited information on the benefits of estradiol to treat cows with RP is another compelling reason to conduct this controlled field trial. Additionally, very limited information is available on the combination of three medications: cephalosporin, ECP and oxytocin, for the treatment of RP (Beagley et al., 2010; Kaya et al., 2012).

RP also affect overall productivity of dairy cow by reducing milk production (Rajala and Grohn, 1998) and this negative effect of RP on milk yield occurs for several weeks after calving (Lucey et al., 1986; Rowlands and Lucey, 1986). No information is available on the effect of RP in cows receiving medication for this reproductive disorder, subjected to three milkings per day and exposed to intense heat stress. Therefore, it was considered appropriate to find out to what extent RP affect milk yield under the circumstances of the present study.

It was hypothesized that systemic treatment with CH in combination with ECP and oxytocin during six consecutive days would prevent a reduction of reproductive performance of dairy cows diagnosed with RP, via a reduction of cows developing uterine infections. A second hypothesis was that RP reduces 305-d milk yield. Thus, a filed study was designed to evaluate whether treatment of RP with the combination of CH, ECP and oxytocin for three or six days, starting 24 hours after calving, prevents fertility reduction in Holstein cows. An additional objective was to assess the effect of RP after treatment on 305-d milk yield in high-yielding Holstein cows under heat stress.

## MATERIAL AND METHODS

### Study herd, housing and feeding

The experimental procedures and animal care conditions were approved by the Ethics Committee of the Research Department of the Agrarian Autonomous University Antonio Narro. The first field trial was conducted on a commercial dairy farm located in northern Mexico (latitude 26° N; mean maximum annual ambient temperature of 32.3°C). The herd consisted of 3800 lactating Holstein cows housed in open-dirt pens with fans and sprinklers for forced evaporative cooling during the warm weather.

Cows were fed diets formulated to provide recommended total daily nutrients for 660-kg dairy cows producing >35 kg of milk/d (NRC, 2001). Cows were fed total mixed diets (49% forage and 51% concentrate; DM basis) that were formulated to provide at least 1.62 Mcal/kg NEL and contained 18% crude protein. Diets contained soybean meal and ground shelled corn as the base ingredients of the concentrate mix; the forage portion of the diet was 50% corn silage and 50% alfalfa hay (DM basis). Cows were fed ad libitum for a daily feed refusal of approximately 10% of that offered, 4 times daily at 6:00, 10:00, 12:00, and 16:00 h.

Cows included in the first study were in their first to seven lactation, with body condition score ranging from 2.75 to 3.5 (scale 1 to 5). Cows were milked thrice daily (04:00, 12:00, and 20:00 hours), and the annual rolling herd average was about 11600 kg throughout the duration of the study.

**Reproductive management**

All cows were routinely vaccinated against diseases that impair reproduction functions, such as brucellosis (*B. abortus* strain RB51 vaccine), bovine viral diarrhea, infectious bovine rhinotracheitis, bovine respiratory syncytial virus and para-influenza (CattleMaster Gold FP5®, Zoetis, Mexico D.F., Mexico). Cows were vaccinated twice a year against leptospirosis using a pentavalent leptospiral vaccine (LEPTAVOID-H**®**; Merck Sharp & Dohme Corp., Mexico, D.F.). The herd veterinarian examined fresh cows weekly to identify and treat cows with postpartum reproductive disorders such as RP, pyometra and metritis. The voluntary waiting period was set at 50 days postpartum. Cows were inseminated by experienced AI technicians based on visual observation of estrous behavior, following the standard a.m./p.m. rule. Pregnancy diagnosis was carried out by the same veterinarian throughout the study period by rectal palpation of the uterus between 43 and 46 d post-insemination. Commercial non-sexed frozen-thawed semen from multiple sires of known acceptable fertility was used across all months of the year for artificial insemination (AI) of cows.

**Treatments and data collection**

For the reproductive performance study, 231 Holstein cows that calved in 2013 and were diagnosed with RP (fetal membranes retained for more than 12 h) were included in the study. These cows were alternately assigned to four treatment groups. Cows in group 1 were treated with 2.0 mg/kg BW CH (Actynoxel® Lab. PISA, S.A. De C.V.; Guadalajara, Mexico), 10 mg estradiol cypionate (ECP®; Lab. Pharmacia & Upjohn, Mexico) and 100 IU oxytocin (Triparox®, Lapisa S.A. de C.V., La Piedad Michoacan, Mexico) i.m. for three consecutive days (CEO-3; n= 63). Cows in group 2 received the same treatment as group 1 but for six consecutive days (CEO-6; n= 48). Cows in group 3 were treated with CH and oxytocin for three consecutive days (CO-3; n= 68), while those in group 4 were subjected to the same treatment as in group 3 for six consecutive days (CO-6; n=52). One cow without RP that had calved on the same day with each treated cow was enrolled in a healthy control group (n = 62). These cows received 2 mL of sterile saline solution i.m. during 6 consecutive days. Treatments started within 24 hours of parturition. For economical and ethical/welfare reasons, it was not possible to leave a group of cows untreated as controls, as cows with RP and toxic early lactation metritis complex may die (Gardner et al., 1990). It should be noted that estradiol cypionate is legally permitted in farm animals in Mexico.

No manual removal of fetal membranes or intrauterine antibiotics application was conducted; only cutting of the visible fetal membranes approximately 10 cm from the vulva was performed. The occurrence of metritis in treated cows was not considered, due to technical difficulties in obtaining data on uterine infections of cows during the puerperium in this particular dairy operation. However, only cows which presented clear and translucent cervico-vaginal mucus with healthy viscosity and without bad odor at estrus (grade 0 for clinical endometritis; Sheldon et al., 2009) were used in this study. Interval from parturition to first postpartum AI was calculated for each cow. Conception rate was defined as the number of cows that conceived out of the ones that were detected in estrus, also known as heat, which is the physiological and behavioral phenomena which precedes and accompanies ovulation in female mammals. The interval between parturition and pregnancy was calculated for each individual cow.

In a second trial, the effect of RP on 305-d milk yield was assessed. Individual cows were removed from the data set if the lactation length was less than 305 d, if lactations were hormonally induced or cows experienced calving difficulties and health problems post-calving. Also, individual heifers calving before 22 months of age or after 31 months of age were removed from the data set. A total of 1986 cows from the same dairy operations were used, 363 with RP and 1623 without this condition (data from 2012-2013). All cows with RP were treated with the CO-3 protocol, because, from the first study, this therapy proved to be practical and efficacious (no significantly different from the control group) to avoid fertility depression in cows with RP. Additionally, the exclusion of ECP in this therapy reduces the cost of treatment and results in a more practical choice for dairy producers.

Milk yields were recorded for individual cows once every 14 days throughout lactation and 305-d milk yield was calculated by multiplying the average between successive milk measurements by the number of days between milk recordings, and summing the products until reaching 305 days in milk. All cows completed their lactation and when cows were milked longer than 305 days, their yield for their first 305 d was taken as the lactation yield. Milk yield was adjusted for number of lactation and month of calving by multiplicative correction factors.

**Statistical analyses**

CR was analyzed as binomial data with the LOGIT function of the PROC GENMOD statement of SAS (SAS Inst., Inc., Cary, NC, USA). The model statement contained the effect of treatment; number of lactation and month of calving were incorporated as covariates. After limiting the number of services per pregnancy to cows with a confirmed pregnancy diagnosis, the effect of treatment on the number of services per pregnancy was evaluated by the bivariate Wilcoxon rank sum test (PROC NPAR1WAY; SAS) without adjustment for confounders. Both the interval between calving to first insemination and calving to pregnancy were analyzed by the LIFETEST procedure of SAS using both strata and time statements (SAS). The 305-d milk production, peak milk yield and days to peak were analyzed using PROC MIXED with group (cows with or without RP) as a fixed effect and year and month of initiation of lactation as covariates. Means and standard deviations for continuous reproductive traits and milk production variables were determined. The experimental unit of the study was the cow over one lactation period. Statistical differences were considered significant at $P<0.05$.

**Table 1.** Reproductive performance of high milk-yielding Holstein cows from a large commercial herd in a hot environment receiving different treatment protocols for retained placenta.

| Treatment | Conception rate | Services per pregnancy | Calving to pregnancy interval | Calving to first AI interval |
|---|---|---|---|---|
| CEO-3 | 36.5 (23/63) | 3.3 ± 1.9 | 134 ± 97 | $65 \pm 42^{a}$ |
| CEO-6 | 25.0 (12/48) | 4.4 ± 2.3 | 142 ± 84 | $76 \pm 45^{ab}$ |
| CO-3 | 30.9 (21/68) | 3.4 ± 2.3 | 158 ± 108 | $90 \pm 78^{b}$ |
| CO-6 | 30.8 (16/52) | 3.4 ± 1.9 | 132 ± 61 | $59 \pm 32^{a}$ |
| Control | 33.9 (21/62) | 3.3 ± 1.8 | 136 ± 86 | $65 \pm 27^{a}$ |

*CEO-3= Ceftiofur hydrochloride, estradiol cypionate, oxytocin for three consecutive days.*
*CEO-6= Ceftiofur hydrochloride, estradiol cypionate, oxytocin for six consecutive days.*
*CO-3= Ceftiofur hydrochloride, oxytocin for three consecutive days.*
*CO-6= Ceftiofur hydrochloride, oxytocin for six consecutive days.*
$^{a,b}$ *Within columns, values ± SD followed by different superscript letter differ significantly (P<0.05).*

## RESULTS

### Incidence of RP and reproductive performance

During the year where the study took place, 242 cases of RP were recorded from a total of 2419 calvings, which gives an incidence of 10.0%. The descriptive reproductive performance of cows subjected to different treatments combining systemic antibiotic and hormones for three or six days are given in Table 1. Conception rate (CR) did not differ between groups (treated and control) at the day of pregnancy diagnosis. The lowest CR was found in cows of the CEO-6 group; this value was 6 percentage points lower than control. Overall conception rates were similar between cows receiving ECP or those that did not receive steroid therapy (30.8% for CEO-3 and CEO-6, combined vs. 30.9% for CO-3 and CO-6 combined, respectively). Services per pregnancy did not differ significantly among cows in the different treatments. Likewise, none of the treatments had a significant effect on calving to pregnancy interval. Calving to first service interval was higher ($P<0.05$) in the CO-3 group (90±78 days) compared to control group (65±27 days).

### Milk yield

There was a reduction ($P<0.05$; Table 2) on 305-d milk yield in cows with RP subjected to the CO-3 treatment compared to the control group.

## DISCUSSION

### Incidence of RP and reproductive performance

The incidence of RP in dairy cows varies from 5 to 10% (Kelton et al., 1998; Leblanc, 2008). The overall incidence of RP in the present study (10%) falls within this range. The overall CR in this study (31.7) was very close to the value of 33% found for artificially inseminated high milk-yielding Holstein cows in this zone (Mellado et al., 2013). The low CR observed in the present study was due to a prolonged exposure of cows to high temperatures for the most part of the year.

In other trials with cows experiencing RP, pregnancy rate was reduced in cows treated with antibiotics (Mcdougall, 2001; Gilbert et al., 2005; Gunay et al., 2011); however, in the present study reproductive performance of cows with RP receiving antibiotic and hormone treatment were not affected, which is in line with previous observations (Drillich et al., 2006; Beagley et al., 2010). Likewise, it has been observed that treating cows with RP systemically for five days with 2.2 mg/kg BW, CH prevented metritis when compared with no treatment or administration of ECP, although reproductive performance of cows has not improved (Risco and Hernandez, 2003).

RP reduces fertility in two ways: by a direct effect through an ovarian dysfunction (Liu et al., 2011) and indirectly through metritis (Han and Kim, 2005). Thus,

**Table 2.** Effect of retained placenta (RP) on milk yield in high milk-yielding Holstein cows from a large commercial herd in a hot environment[1].

| Item | Control | Retained placenta |
|---|---|---|
| Number of cows | 1623 | 360 |
| 305 d-milk yield, kg | $9755 \pm 1501^{a}$ | $9472 \pm 1543^{b}$ |
| Days to peak | $63 \pm 20^{a}$ | $63 \pm 23^{a}$ |
| Milk yield at peak, kg | $47 \pm 12^{a}$ | $46 \pm 14^{a}$ |

*[1]All cows with RP were treated with ceftiofur hydrochloride and oxytocin for three consecutive days pospartum.*
*[a,b]Within rows, values ± SD followed by different superscript letter differ significantly ($P<0.01$).*

therapy for RP should focus on preventing postpartum uterine infections. In the present study, the systemic antibiotic application in combination with adjunctive stimulators of uterine contractions apparently was effective in preventing complications by metritis, as this therapy resulted in reproductive performance similar to that of cows without this postpartum reproductive complication. Thus, systemic CH in combination with oxytocin appears to be beneficial in aiding cows with RP to return to normal reproductive function.

Both services per pregnancy and calving to pregnancy interval did not differ among treatment groups. According to previous studies, calving to conception interval was longer in cows with retained placenta compared with their healthy herd mates (Maizon et al., 2004; Han and Kim, 2005). The mean calving to pregnancy interval is one of the most suitable overall indexes of reproductive performance in dairy herds, where calvings are fairly spread over the year. Thus, in the present study cows with RP treated with systemic antibiotics and adjunctive drugs to enhance placental detachment and expulsion seems to recover satisfactory, since treatment led to a similar calving to pregnancy interval compared with their herd mates.

In this study, time to first AI was significantly longer in CO-3 cows compared with all other groups, except CEO-6. Endometritis is associated with increased intervals from calving to first service (LeBlanc et al., 2002: Williams et al., 2005). Therefore, the elimination of uterine bacterial contamination derived from RP could have taken longer in cows subjected to the simpler and shortest treatment. This treatment might have altered the postpartum ovarian function (Sheldon et al., 2002) and apparently prolonged the calving to first service interval. The prolonged calving to pregnancy interval in the present study is comparable to that reported in another study in a neighboring dairy operation (Mellado et al., 2012). This response is ascribed to a prolonged and severe heat stress experienced by cows for the most part of the year.

### Milk yield

Another objective of the present study was to establish the effect of RP on 305-d milk yield of a cow, disregarding the milk loss during the recovery period. The cumulative loss of milk was almost 3% in 305-d lactation of cows with RP compared with milk production of healthy herd mates. This milk yield loss due to RP is close to the 300–500 kg loss in cows with RP compared with their healthy herd mates observed by others (Cesarini et al., 2003; Goshen and Shpigel, 2006). However, it is much lower than the decrease of 759 kg per lactation experienced by cows with both RP and metritis (Wittrock et al., 2011; Dubuc et al., 2011). These differences in results may originate from the fact that in the present trial only one herd was studied, whereas the aforementioned authors have used several herds and different frequency of milk production measurements. Additionally, the different measures of milk production, the analysis of milk loss by different statistical methods, the sample size (power) and the duration of the follow-up period, may also have contributed to different results among studies.

Given that cows with RP in the present study were subjected to preventive antibiotic therapy against metritis, milk yield reduction could be attributed to the occurrence of RP. There is evidence that the effects of metritis and RP on milk yield are different and additive when both are experienced by multiparous cows (Dubuc et al., 2011). It is uncertain how RP alone would cause a decrease in milk yield. It is possible that cows with RP have a lower feed intake (Bareille et al., 2003). Additionally, RP may be linked with a reduction in milk yield that persists even after resolution of the problem, via subclinical metabolic disorder (Han and Kim, 2005). This has been reported by other authors, who documented a negative effect of RP on milk yield during several weeks after calving (Lucey et al., 1986; Rowlands and Lucey, 1986; Rajala and Grohn, 1998).

## CONCLUSIONS

The combination of systemic administration of ceftiofur hydrochloride, oxytocin and ECP either for three or six days was equally effective in preventing impairment of reproductive function in cows with retained placenta. The 6-days therapeutic regimen was not associated with an improvement in fertility, a result that does not support a prolonged antibiotic therapy combined with ecbolic agents in cases of retained placenta. Additionally, prompt treatment of RP in cows with a combination of antibiotics and oxytocin did not prevent the reduction of 305-d milk yield.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

## REFERENCES


Bareille N, Beaudeau F, Billon S, Robert A, Faverdin P (2003) Effects of health disorders on feed intake and milk production in dairy cows. Livest Prod Sci 83: 53-62.

Beagley JC, Whitman KJ, Baptiste KE, Scherzer J (2010) Physiology and treatment of retained fetal membranes in cattle. J Vet Int Med 24: 261–268.

Cesarini F, Sturaro E, Contiero B, Bittante G, Gallo L (2003) Effects of health disorders on milk yield and calving interval in Italian Holstein cows. Italian J Anim Sci 2: 160-162.

Drillich M, Klever N, Heuwieser W (2007) Comparison of two management strategies for retained fetal membranes on small dairy farms in Germany. J Dairy Sci 90: 4275–4281

Drillich M, Mahistedt M, Reichert U, Tenhagen BA, Heuwieser W (2006) Strategies to improve the therapy of retained fetal membranes in dairy cows. J Dairy Sci 89: 627–635.

Drillich M, Pfutzer A, Sabin HJ, Sabin M, Heuwieser W (2003) Comparison of two protocols for treatment of retained fetal membranes in dairy cattle. Theriogenology 59: 951–960.

Dubuc J, Duffield TF, Leslie KE, Walton JS, LeBlanc SJ (2011) Effects of postpartum uterine diseases on milk production and culling in dairy cows. J Dairy Sci 94: 1339–1346.

Gardner IA, Hird DW, Utterback WW, Danaye-Elmi C, Heron BR, Christiansen KH, Sischo WM (1990) Mortality morbidity case fatality and culling rates for California dairy cattle as evaluated by the National Animal Health Monitoring System 1986-87. Prev Vet Med 8: 157-170.

Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M (2005) Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 64: 1879–1888.

Goshen T, Shpigel NY (2006) Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membrane in dairy cows. Theriogenology 66: 2210-2218.

Gunay A, Gunay U, Orman A (2011) Effects of retained placenta on the fertility in treated dairy cows. Bulg J Agric Sci 17: 126-131.

Han YK, Kim IH (2005) Risk factors for retained placenta and the effect of retained placenta on the occurrence of postpartum diseases and subsequent reproductive performance in dairy cows. J Vet Sci 6: 53-59.

Kasimanickam R, Duffield TF, Foster RA, Gartley CJ, Leslie KE, Walton JS, Johnson WH (2004) Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 62: 9–23.

Kaya D, Ay SS, Kucukaslan I, Beceriklisoy HB, Agaoglu AR, Findik M, Ozyurtlu N., Aslan S (2012). The effectiveness of combined preventive treatment with Ceftiofur, Oxytocin and PGF(2 alpha) on fertility parameters in cows. Rev Med Vet 163: 302-308.

Kelton DF, Lissemore KD, Martin RE (1998) Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. J Dairy Sci 81: 2502–2509.

Laven RA, Peters AR (1996) Bovine retained placenta aetiology pathogenesis and economic loss. Vet Rec 139: 465-471.

Leblanc SJ (2008) Postpartum uterine disease and dairy herd reproductive performance A review. Vet J 176: 102–114.

LeBlanc SJ, Duffield TF, Leslie KE, Bateman KG, Keefe GP, Walton JS, Johnson WH (2002) Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J Dairy Sci 85: 2223–2236.

Liu WB, Chuang ST, Shyu CL, Jack A, Peh HC, Chan JPW (2011) Strategy for the treatment of puerperal metritis and improvement of reproductive efficiency in cows with retained placenta. Acta Vet Hung 59: 247–256.

Lucey S, Rowlands GJ, Russell A (1986) Short-term associations between disease and milk yield of dairy cows. J Dairy Res 53: 7-15.

Maizon DO, Oltenacu PA, Gröhn YT, Strawderman RL, Emanuelson U (2004) Effects of diseases on reproductive performance in Swedish Red and White dairy cattle. Prev Vet Med 66: 113-126.

Mcdougall S (2001) Effect of intrauterine antibiotic treatment on reproductive performance of dairy cows following periparturient disease. NZ Vet J 49: 150-158.

Mellado M, Sepulveda E, Meza-Herrera C, Veliz FG, Arevalo JR, Mellado J, De Santiago A (2013) Effects of heat stress on reproductive efficiency of high yielding Holstein cows in a hot-arid environment. Rev Colomb Cienc Pecu 26: 193-200.

Mellado M, Zuñiga A, Veliz FG, de Santiago A, Garcia JE, Mellado J (2012) Factors influencing pregnancy per artificial insemination in repeat-breeder cows induced to ovulate with a CIDR-based protocol. Anim Reprod Sci 134: 105–111.

NRC (2001) National Research Council: Nutrient Requirements of Dairy Cattle 7th rev ed Natl Acad Press Washington DC.

Rajala PJ, Grohn YT (1998) Effects of dystocia retained placenta and metritis on milk yield in dairy cows. J Dairy Sci 81: 3172–3181.

Risco CA, Hernandez J (2003) Comparison of ceftiofur hydrochloride and estradiol cypionate for metritis prevention and reproductive performance in dairy cows affected with retained fetal membranes. Theriogenology 60 47–58.

Rowlands GJ, Lucey S (1986) Changes in milk yield in dairy cows associated with metabolic and reproductive disease and lameness. Prev Vet Med 4: 205-221.

Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth HJ (2009) Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol Reprod 81: 1025–1032.

Sheldon IM, Noakes DE, Rycroft AN, Pfeiffer DU, Dobson H (2002) Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reprod123: 837–845.

Smith BI, Risco CA (2002) Predisposing factors and potential causes of postpartum metritis in dairy cattle. Comp Cont Educ 24: 74-79.

Williams EJ, Fischer DP, Pfeiffer DU, England GC, Noakes DE, Dobson H, Sheldon IM (2005) Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63: 102-117.

Wittrock JM, Proudfoot KL, Weary DM, von Keyserlingk MAG (2011) Short communication: Metritis affects milk production and cull rate of Holstein multiparous and primiparous dairy cows differently. J Dairy Sci 94: 2408-2412.

Zobel R, Tkalcic S (2013) Efficacy of ozone and other treatment modalities for retained placenta in dairy cows. Rep Dom Anim 48: 121–125.