Comparison of two treatment strategies for cows with metritis in high-risk lactating dairy cows

Ramon Armengol [a], Lorenzo Fraile [b,c*]

[a]Departament de Producció Animal, ETSEA, University de Lleida, Lleida, Spain. [b] Agrotecnio Center, Lleida, Spain.

* Corresponding author: Tel.: +34 973702814; fax: +34 973702874.

E-mail address: lorenzo.fraile@prodan.udl.cat

Abstract

Acute puerperal metritis (APM) and clinical metritis (CM) are uterine diseases frequently diagnosed in dairy cows. These diseases are responsible of important economic loss due to its effect not only on reproductive performance but also on milk production. The objective of this study was to assess the impact of two different treatments for metritis on dairy cows by measuring their reproductive performance in the next gestation. The endpoints to measure the reproductive performance included: the conception rate at first artificial insemination (AI), the number of days at conception and the proportion of non-pregnant cows at over 150 days after beginning milk production (>150 DIM). The study was carried out in a high production dairy cow farm located in Lleida (North East Spain). Recordings of 1044 parturitions of 747 Holstein cows were controlled in this farm from 2009 to 2014. Cows were diagnosed as suffering from metritis (APM or CM) if the following parameters were observed: an abnormally enlarged uterus, a fetid watery red-brown uterine discharge with (APM) or without (CM) fever (>39.5 ºC) and presence (APM) or absence (CM) of signs of systemic illness (decreased milk production, dullness or other signs of toxemia) within 21 days postpartum. Afterwards, cows suffering from metritis (APM or CM) were randomly assigned and balanced to two groups: 1) Animals receiving parenteral amoxicillin intramuscularly plus intrauterine infusion with oxytetracycline (P+I group); and 2) Animals receiving only parenteral amoxicillin intramuscularly (P group). Furthermore, reproductive performance of cows without metritis were used as reference (control group).

Metritis was diagnosed in 27.5 % of the total parturitions included in the study (288 out of 1044). In particular, metritis was diagnosed in 30.5% (118 out of 387) and 25.9% (170 out of 657) of parturitions from heifers and multiparous cows, respectively. Reproductive performance was not significantly affected by the parity, the season at first AI, the season at conception, the bull or the inseminator. The P+I treatment was able to significantly reduce the number of days at first insemination and at conception when compared to the P treatment in heifers. In multiparous cows, this significant effect was only observed for days at conception. Additionally, the P+I treatment was

able to significantly increase the percentage of pregnant animals at first insemination and decrease the percentage of non-pregnant cows at >150 DIM for both heifers and multiparous cows when compared to the P treatment.

Key words: Metritis, treatment strategies, reproductive performance, dairy cattle.

1.- Introduction

The reproductive performance of a cow after parturition is affected by many factors such as parity, the number of days without milk production, body weight loss, heat stress, season at first artificial insemination (AI), season at conception, bull used for the AI and the technician that carries out the AI [1]. Moreover, clinical conditions such as clinical ketosis, clinical hypocalcemia, and uterine diseases could negatively affect the future reproductive performance of the cow [2,3].

Metritis can cause important economic losses during the postpartum period due to poor reproductive performance, lower milk production and an increase in the culling rate in comparison with non-affected cows. The animals affected by metritis are unable to control the growth of aerobic and anaerobic bacteria in uterine lumen due to an abnormal post-partum uterine involution that may be triggered by immune, nutritional and management factors [4]. Metritis is characterized by an inflammation of the uterine tract with local and/or systemic implications for the cow [5]. Therefore, diagnosis and treatment of postpartum uterine diseases is common in dairy practice.

It is very important to clearly define the diagnostic criteria for metritis in cows. Thus, two types of metritis are usually observed under field conditions in the early postpartum period (1-21 days after parturition). On one hand, acute puerperal metritis (APM) is diagnosed in cows that have an abnormally enlarged uterus and a fetid, watery, reddish-brown uterine discharge, which is also associated with clinical signs of systemic disease (decreased milk yield, dullness or other signs of toxemia) and fever (>39.5ºC) within 21 days postpartum. On the other hand, clinical metritis (CM) is described in cows that have an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina within 21 days after parturition in the absence of clinical signs of systemic disease [6, 7, 8]. In any case, diagnosis of metritis is challenging for researchers and practitioners under field conditions [6, 7, 9] and the distinction between APM and CM is frequently not straightforward. Fortunately, the basis of the treatment is common for both types of metritis [10].

It has been described in the literature that the treatment for cows suffering from metritis during the early post partum period is important to minimize this risk of low milk production, increase

reproduction performance in the next gestation and reduce culling risk [7]. There are many treatment protocols for cows with metritis. These treatments include various combinations of antimicrobials, administered by intrauterine and/or parenteral routes, and/or hormones [7, 8]. Thus, beta-lactamic antibiotics (mainly amoxicillin and cephalosporins) are efficacious to treat and prevent metritis after their parenteral administration under field conditions [10, 11]. Tetracyclines administered by intrauterine route are extensively used in uterine disease, although their efficacy has been questioned in the literature [12, 13, 14].

The objective of this study was to assess the impact of two different treatments for metritis during early postpartum period (1-21 days) in dairy cows by measuring their reproductive performance in the next gestation. The endpoints to measure the reproductive performance included: the conception rate at first AI, the number of days at conception and the proportion of non-pregnant cows at >150 days in milk production (DIM).

2.- Materials and Methods

2.1- Animals and herd.

During a five-year period (January 2009- January 2014), a total of 1222 cow parturitions were evaluated for routine post-partum examination; of these, 414 corresponded to heifers and 808 to multiparous cows. Parturitions complicated by other diseases that could also affect the reproductive performance (clinical ketosis, left displaced abomasum (LDA), clinical mastitis, lameness, pneumonia, ovarian disorders such as ovarian cysts and other uterine disease such as endometritis and pyometra) were excluded from the study. Culled or dead cows from either group without full reproductive history were also excluded. Eventually, 1044 parturitions were finally included in the study, 387 corresponding to heifers and 657 to multiparous cows. The exact numbers of excluded cows for the various reasons exposed, as well as group allocation for enrolled cows are detailed in

Figure 1.

The study was conducted on a single high production commercial dairy farm in Lleida (North East Spain) over a period of five years (2009-2014). During the study period, this farm was housing an average of 330 lactating Holstein cows with an average production of 11,100 kg of milk (3.6% Fat and 3.3% Protein) in 305 days by cow. Cows were housed in five straw-bedded free- stall barns and they were fed a total mixed ration consisting of corn silage, grass silage, and concentrates. Cows were milked three times a day (at 4 AM, 12 PM, and 8 PM). Each cow was sampled and analyzed for milk quality (fat, protein, and lactose concentration) and somatic cell count by the Central Laboratory for Milk Recording (ALLIC, Catalonia) once a month.

Breeding management was carried out by AI with Holstein semen and performed by a highly trained technician or veterinarians specialized in cattle reproduction. Cows were bred on observed estrus or diagnosed by a computerized pedometry system (Afimilk, Kibbutz Afikim, 1514800, Israel). Breeding programs based on the use of hormones were not applied and it was decided not to inseminate heifers and multiparious cows before 90 and 70 days post-partum, respectively. This decision was made considering historic data about reproductive performance in this particular farm.

Transrectal ultrasonography (Easi Scan, 4.5 MHz - 8.5 MHz, BCF Technology, Ltd. Scotland) was used to perform pregnancy diagnosis at 32-40 days post AI as well as to detect uterine and/or ovarian disorders such as clinical endometritis, pyometra, ovarian cysts and ovarian cyclicity failure**.** This reproductive control was carried out every Tuesday and Friday and also included post - partum examination (1-21 post-partum) and diagnosis and treatment of non-cycling or silent estrus cows. Non-cycling or silent estrus cows were treated with hormones. Various combinations of prostaglandins, gonadotropin-relising hormone GnRH and progesterone were used according to clinical criteria. In any case, these animals were excluded from the study to avoid any confounding factor in the data analysis. Finally, animals diagnosed and treated for other uterine and/or ovarian disorders outside the period of 1-21 days postpartum were excluded from the study to avoid any bias.

Clinical, reproduction, production and management data were recorded by the herd manager and the attending veterinarians using specific software (Afimilk, Kibbutz Afikim, 1514800, Israel). Lleida has a long “heat stress risk season” (from May to October,) that could affect the reproductive performance [15]. It is well known that heat stress can affect immunological status of the cows [16] and it could significantly affect the prevalence of uterine diseases and/or its severity. For the purposes of this study, the year was divided in four periods; 1 (1st January-31st March), 2 (1st April-30th June), 3 (1st July-30th September) and 4 (1st October-31st December).

Other factors that have been described as affecting the reproductive performance of cows are the origin of semen (bull factor) and the technicians that perform the AI. Both factors were excluded from the data analysis because the same number of cows were inseminated in each experimental group regarding the origin of semen and the technician.

2.2.- Definition of metritis case

All the cows underwent a clinical examination every 24 hours during the first 21 days post-partum. For each animal, the ruminal motility, breathing rate, consistency of the faeces, rectal temperature, milk production, pedometry readings, body weight (BW) and milk conductivity were recorded. Additionally, rectal palpation and observation of vaginal discharge were carried out every Tuesday and Friday from day 1 to day 21 postpartum, (Table 1).

Vaginal discharge (VD) was collected through rectal palpation massage to minimize not only contamination of the vagina and uterus but also microtrauma in the area. Quantity, color, proportion of pus, consistency and smell were evaluated [6]. All examinations were performed by the same veterinarian in order to minimize bias in the clinical record [17]. Cows that did not expel their fetal membranes (44 out of 1222) within 24 hours postpartum were diagnosed with retained fetal membranes (RFM). These animals were treated with one injection of 37.5 mg of sodium selenium and 1250 mg of α-Tocoferol acetate (Hipravit Selenio, HIPRA, Spain) and oral calcium (Bovicalk, Boehringer Ingelheim, Germany) every 12 hours until fetal membranes were expelled. In these

cows, rectal palpation was performed every 24h. If fever (>39,5ºC) was present, animals were treated with flunixin meglumine (Finadyne®, MSD Animal Health) at a dose of 2.2 mg/Kg BW/ 24 h for three days. Once fetal membranes were expelled (average of 48h postpartum), animals were classified according to Table 1. No attempt was made to manually remove the retained fetal membranes.

In this study, metritis (APM and CM) was diagnosed in animals that had an abnormally enlarged uterus and a purulent or a fetid red-brown watery uterine discharge detectable in the vagina within 21 days after parturition. The presence or absence of pyrexia (>39.5ºC) and other signs of systemic illness such as reduced milk yield, dullness, inappetence or anorexia, elevated heart rate, and apparent dehydration determined if animals were included in the APM group (present) or the CM group (absent) [6]. Cows treated with anti-inflammatory drugs, antimicrobial drugs or hormones for diseases not related with the study (e.g., acute mastitis, lameness, LDA, ketosis, other uterine or ovary disorders) were excluded from the trial.

2.3.- Experimental design

Cows were included in the study at day 1 after calving. Reproductive performance of healthy cows was used as reference (control group). Cows suffering from metritis were randomly allocated to one of two groups according to the last digit of their ear tag. Thus, cows with an odd number (1, 3, 5, 7, 9) were allocated to P group. In this group, cows only received parenteral amoxicillin by intramuscular route. Cows with an even number in their ear tag (0, 2, 4, 6, 8) were allocated to group P+I. In this group, cows received parenteral amoxicillin by intramuscular route plus an intrauterine infusion with oxytetracycline. Briefly, cows included in group P received a systemic antimicrobial treatment of amoxycillin trihidrate (Amoxoil Retard®, Laboratiorios SYVA SA) at 15 mg/kg BW every 48h by intramuscular route for three times. Injection was applied on the neck and the injection volume was always less than 20 mL on the same inoculation point. Cows included in

the P+I group received the same systemic treatment as group P plus 8000 mg of oxytetracycline dihidrate (Tenalina L.A.®, CEVA Santé Animal) (200 mg/ml) via intrauterine infusion every 72-96 hours twice. Intrauterine infusion was applied with a disposable uterine catheter (53.5 cm Bovivet, Kruuse, Denmark) and a single use 50 cm^3syringe. Cows that were diagnosed with metritis were considered cured when its classification was M0 or M1 (Table 1) after two regular rechecks. Cows allocated in any of the groups that showed fever were also treated with flunixin meglumine (Finadyne®, MSD Animal Health) at a dose of 2.2 mg/Kg BW/24 h for 3 days.

2.4.- Monitoring of antimicrobial susceptibility throughout the trial

Antimicrobial susceptibility (AS) for *Escherichia coli* (EC) and *Trueperella pyogenes* (TP) against amoxicillin and oxytetracycline was monitored every third month in this farm in, at least, four clinical cases. Briefly, AS tests were performed using the agar dilution method according to the Clinical Laboratory Standard Institute (CLSI) guideline M31-A3 in a commercial diagnostic laboratory. The threshold values to distinguish between susceptible and resistant microorganisms are detailed in Table 2.

2.5.- Statistical Analysis.

All statistical analyses were carried out using the SAS system V.9.1.3 (SAS institute Inc, Cary, NC, USA). For all analyses, the individual cow was used as the experimental unit and the data was grouped taking into account the parity of the cows: Heifers (first parturition) versus multiparous cows (two to six parturitions). The significance level (p) was set at 0.05 with statistical tendencies reported when $P<0.10$. The variables included in the statistical analyses were classified as nominal (pregnancy and delivery season), ordinal (parity) or continuous (days to conception and DIM). Shapiro Wilk´s and Levene tests were used to evaluate the normality of the distribution of the continuous variables and the homogeneity of variances, respectively. Statistical analyses were performed to test the association between the different experimental groups (control, P or P+I) with

the conception rate at first AI, days to conception and the proportion of non-pregnant cows with a DIM higher than 150 days. Contingency tables (Chi-square or Fischer exact tests) were used when the association between nominal and ordinal variables was assessed. To study the association between nominal or ordinal variables with the continuous non-normally distributed variables, the Wilcoxon test (with the U Mann-Whitney test to compare each pair of values) was used. To analyse the association between continuous normally distributed variables and nominal or ordinal variables, an ANOVA test (with Student's T-test to compare each pair of values) was used.

3.- Results.

3.1.- Description of metritis in the population

Metritis was diagnosed in 288 out of 1044 parturitions finally included in the study, (27.5% prevalence). In particular, metritis was diagnosed in 30.5% (118 out of 387) and 25.9% (170 out of 657) of parturitions resulting from heifers and multiparous cows, respectively. Cows scored as M2 (Table 1) were diagnosed with APM in 83 out of 288 cases of metritis (28.8% prevalence). These animals had an abnormally enlarged uterus, a purulent or a fetid red-brown watery uterine discharge detectable in the vagina, pyrexia (>39.5ºC) and/or systemic illness such as reduced milk yield, dullness, inappetence or anorexia, elevated heart rate, and apparent dehydration. Two-hundred and five out of 288 cows with uterine disease (71.2% prevalence) were diagnosed with CM (cows graded as M2 but without pyrexia (<39.5ºC) and/or systemic illness). Taking parity into account, 32.2% of heifers affected with metritis, suffered from APM (38 out of 118) and 67.8% from CM (80 out of 118). Within multiparous cows, 30.6% (52 out of 170) and 69.4% (118 out of 170) were diagnosed as APM and CM, respectively. Significant differences in the prevalence of each type of metritis between heifers and multiparous cows or within groups of treatment were not observed at the beginning of the trial (basal homogeneity). Thus, 27.9% (43 out of 154) and 72.2% (111 out of 154) of the animals were diagnosed as APM and CM, respectively in Group P and the prevalence of

APM was 29.8% (40 out of 134) and 70.2% of CM (94 out of 134), respectively for group P+I at the beginning of the trial.

3.2.- Reproductive performance in the population

Reproductive performance was not significantly affected by the number of parity, the season at first AI, the season at conception, the bull or the inseminator that carried out the AI, neither in healthy nor in metritis affected cows. Considering all the animals included in the study (healthy and metritis affected cows), the number of days at first insemination was not significantly different between heifers and multiparous cows but the number of days at conception was significantly higher in multiparous cows (129.5±60.3) than in heifers (118.6±53). In heifers, the conception rate at first insemination was significantly higher than in multiparous cows, whereas the percentage of non-pregnant animals at >150 DIM was significantly lower than in multiparous cows. For this reason, the analysis of the treatment efficacy was carried out separately for heifers and multiparous cows.

3.3.- Bacteriological examination

The isolates of EC and TP obtained were susceptible against amoxicillin and/or oxytetracycline throughout the trial, considering the threshold values detailed in Table 2.

3.4.- Effect of the two antibiotic treatments on the reproductive performance

In heifers with metritis, The P+I treatment was able to significantly reduce the number of days at first insemination (80.5±11.6 vs 91.9±24.2) and at conception (116.8±50.5 vs 151.5±67.7) in comparison with the P treatment. In the case of multiparous cows, this significant effect was only observed in the case of days at conception (140.8±62.7 vs 166.8±67.5) (Figure 2). In heifers, when the P+I group was compared to the control group, no significant differences were observed for any of these two parameters. However, in the case of multiparous cows, the number of days at conception for both P and P+I groups was significantly higher than in the control group

(120.6±55.5) (Figure 2). The P+I treatment was able to significantly increase the percentage of pregnant cows at first insemination for both heifers (47.5%) and multiparous cows (30.5%) in comparison with the P treatment (28.2% for heifers and 15.3% for multiparous) (Figure 3). The P+I treatment was able to significantly decrease the percentage of non-pregnant cows at >150 DIM when compared to the P treatment (18.3 % vs 42.4% for heifers and 38% vs 57.1% for multiparous cows, respectively). Finally, in heifers, no significant differences in the percentage of pregnant animals at first insemination or in the percentage of non-pregnant cows at >150 DIM were observed between the control group and the P+I group. However, in multiparous cows, both parameters for the P and P+I groups had significantly higher values than those observed in the control group (Figure 3).

4.- Discussion.

The main goal of this study was to assess the impact of two different treatments, parenteral only vs parenteral plus intrauterine infusion, for dairy cows affected with metritis by measuring their reproductive performance in the next gestation: days at first AI, conception rate at first AI and percentage of non pregnant animals at 150 DIM. These results were compared with the values observed for healthy cows as a reference. The authors did not consider the possibility to include a negative control group (affected with metritis and not treated with antimicrobials) due to welfare reasons and the increased risk of death or culling for these non-treated sick animals. Metritis decreases the reproductive performance of dairy cattle [3]. In this study, the control group (healthy cows) always showed better reproductive performance than metritis-affected cows. The results suggest that the combination of amoxicillin, administered by parenteral route, plus intrauterine infusion of oxytetracycline is more efficacious than the administration of parenteral amoxycillin alone for the treatment of metritis, when taking into account the reproductive performance in the next gestation as an end-point. This statement was true for both heifers and multiparous cows,

306 although the positive results were more consistently observed in heifers than in multiparous cows
307 for all the parameters evaluated.
308 In the literature, various techniques to diagnose metritis in cows can be found [6, 9]. In this study,
309 metritis (APM and CM) was described as an abnormally enlarged uterus and a fetid watery red-
310 brown or purulent uterine discharge showing or not pyrexia (>39.5ºC), with or without signs of
311 systemic illness within 21 days after parturition [6]. The authors decided to include both pyretic and
312 non pyretic animals since this is the way it has been previously described by other authors [4, 10].
313 The authors used a method with high sensitivity to diagnose metritis in cows, which avoids the risk
314 of underestimating metritis if only milk production or rectal temperature are considered as
315 diagnostic, but at the same time, trying to make it a reasonably fast, sensitive and economic
316 procedure for the farmer. The procedure takes some time to implement but once established as a
317 routine in the farm, the authors believe that it can be very useful to diagnose post-partum disease
318 and treat the animals during an early stage of illness, which improves the productive and
319 reproductive performance of the cows. It means that the animals included in this study were
320 diagnosed in a very early stage of the disease. This fact must be taken into account when the
321 treatment efficacy is compared with the results obtained by other authors.

322

323 In this study, the overall prevalence of metritis is close to 28%. This prevalence can vary
324 considerably between heifers and cows, between different farms and between studies. Thus, values
325 between 2.2% and 42.3% have been observed in previous studies [14, 18, 19]. For this reason, the
326 farm included in this trial could be considered representative of a high production dairy cattle
327 operation with a prevalence of metritis within reported values. Thus, the reproductive performance
328 of healty or metritis-affected cows was not affected by factors directly or indirectly associated with
329 management (season at first AI, season at conception, the bull and the inseminator that carried out
330 the AI). Taken together, these results suggest that the farm where the study was carried out was

appropriate to assess the impact of two different treatments for metritis in dairy cows by measuring their reproductive performance in the next pregnancy.

The bacteria most commonly isolated from metritis-affected dairy cows are well described in several studies; these include EC and TP [20, 21, 22]. *Fusobacterium necrophorum* and *Bacterioides spp* are also recognized as opportunistic bacteria that can cause metritis [23, 24]. In particular, it has been long suggested that uterine infections might depend on pathogenic synergism between EC, TP and bacteria from other groups. However, recent studies have suggested that the diversity of the uterine bacterial composition in dairy cows is likely to be even more complex than previously described by traditional culture-dependent methods [25]. Additionally, it is necessary to know the AS of the bacteria involved in clinical cases of bovine metritis in order to choose the most suitable antimicrobial. However, it is only feasible to determine this antimicrobial susceptibility in the isolated bacteria. In our case, the isolates of EC and TP obtained were susceptible against amoxicillin and/or oxytetracycline throughout the trial. Thus, the use of both antimicrobials in this farm did not seem to generate antimicrobial resistance for these bacteria during five years. Nevertheless, the diversity of the bacterial species involved in clinical cases of metritis makes absolutely compulsory to carry out clinical trials that measure clinical efficacy instead of carrying out only pharmacokinetic/pharmacodynamic studies that only take into account pharmacodynamic data from only the most commonly isolated bacteria (EC and TP) [26].

The use of antimicrobials is widely accepted to cope with infections in the genital tract, mainly in the case of metritis [27]. The rationale behind this therapeutic approach is to reduce the number of pathogens in the uterine cavity and consequently mitigate the associated inflammation, enhance the local immune defense, and facilitate the repair of the endometrium for a faster return to a normal uterine status. Unfortunately, there are few available data about the penetration of parenterally-

administered antibiotics into the cow genital tract. In humans, an endometrium to plasma concentration ratio of 0.6 and 0.4 for quinolones and a beta-lactamics, respectively, has been established [28, 29]. It is possible that a similar ratio could be achieved in cows. Intrauterine administration of antibiotics is expected to reach a higher antibiotic concentration in the endometrium compared to systemic administration and it is a common route to administer antimicrobials for metritis in cows and mares [30]. Thus, considering the administered dose of oxytetracycline (8000 mg) and the uterine volume post-partum (2000 mL) [31], an extraordinarily high oxytetracycline concentration in uterine lumen (4 mg/mL) was expected to be achieved. This concentration is notably higher than the established Minimum inhibitory Concentration for the 90% of the strains (MIC_9) for AP and EC in case of bovine metritis [32, 33, 34].

To the best of the authors' knowledge, no previous studies have explored the same combination of antimicrobials to treat cows diagnosed with early postpartum metritis. However, it has been described in the literature that the administration of beta-lactamics by parenteral route is able to improve the reproductive performance of metritis-affected cows [35]. The use of ceftiofur, administered intramuscularly, with or without a combination of cloxacillin plus ampicillin by intrauterine infusion, did not show differences in reproductive performance between groups in cows diagnosed with toxic puerperal metritis (TPM) [36]. In conclusion, there is a lot of variation in the treatment outcome of uterine disease using beta-lactamic antibiotics [13, 37, 38, 39, 40]. Similar studies have described a significant improvement in reproduction parameters applying an intrauterine infusion of tetracyclines in cows and heifers with CM [14] but other studies did not find significant differences in the treatment outcome between various combinations of intrauterine infusion of tetracyclines for the treatment of metritis [39], endometritis [12, 13] or retained fetal membranes [14, 42]. In our study, the combination of amoxicillin, by parenteral route, plus intrauterine infusion of oxytetracycline is more efficacious than using only the parenteral route. It must be highlighted that, in the current study, the dose of oxytetracyclines was higher and the

duration of administration was longer than the dosage regimen used in other studies [14, 39]. Globally, these results suggest that local treatment with antibiotics can be important to heal the genital tract and prepare it for the following pregnancy. The efficacy of intrauterine tetracycline might be related to other uncultured or unknown organisms that are sensitive to this antimicrobial and that are not easily isolated with standard microbiological techniques. Finally, this efficacy might also be related to non-antimicrobial effects of tetracyclines such as anti-inflammatory effects. In this regard, tetracyclines are potent inhibitors matrix metalloproteinases and inducible Nitric Oxide Synthase, which are known to play a role in the mediation and control of mucosal inflammation and immune response [43, 44].

In conclusion, based on reproductive performance as an end-point parameter, treatment of metritis with parenteral amoxicillin plus oxytetracycline via intrauterine route was superior in comparison with only the parenteral administration of amoxicillin.

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Table 1. Classification of cows according to the type of vaginal discharge during the post-partum period

CODE	TYPE OF VAGINAL DISCHARGE	DIAGNOSIS	TREATMENT
M0	No vaginal discharge.	Healthy	None
M1	Normal lochial secretion, odorless, viscous and translucid.	Healthy	None
M1S	Normal lochial secretion, odorless, viscous and reddish brown.	Healthy	None
M2	Purulent or fetid, watery and reddish-brown	Metritis	P: Parenteral Amoxycillin P+I: Parenteral Amoxicillin plus intrauterine infusion of Oxytetracycline.

Table 2.- Minimal inhibitory concentration (MIC) breakpoints for *Escherichia coli* and *Trueperella pyogenes* isolated from bovine metritis cases

			MIC breakpoint (㎍g/mL)			
		Escherichia coli			*Trueperella pyogenes*	
	S	I	R	S	I	R
Antimicrobial agent						
Amoxicillin*	<0.25	0.5	>1	<2	4	>8
Oxytetracycline**	<4	8	>16	<4	8	>16

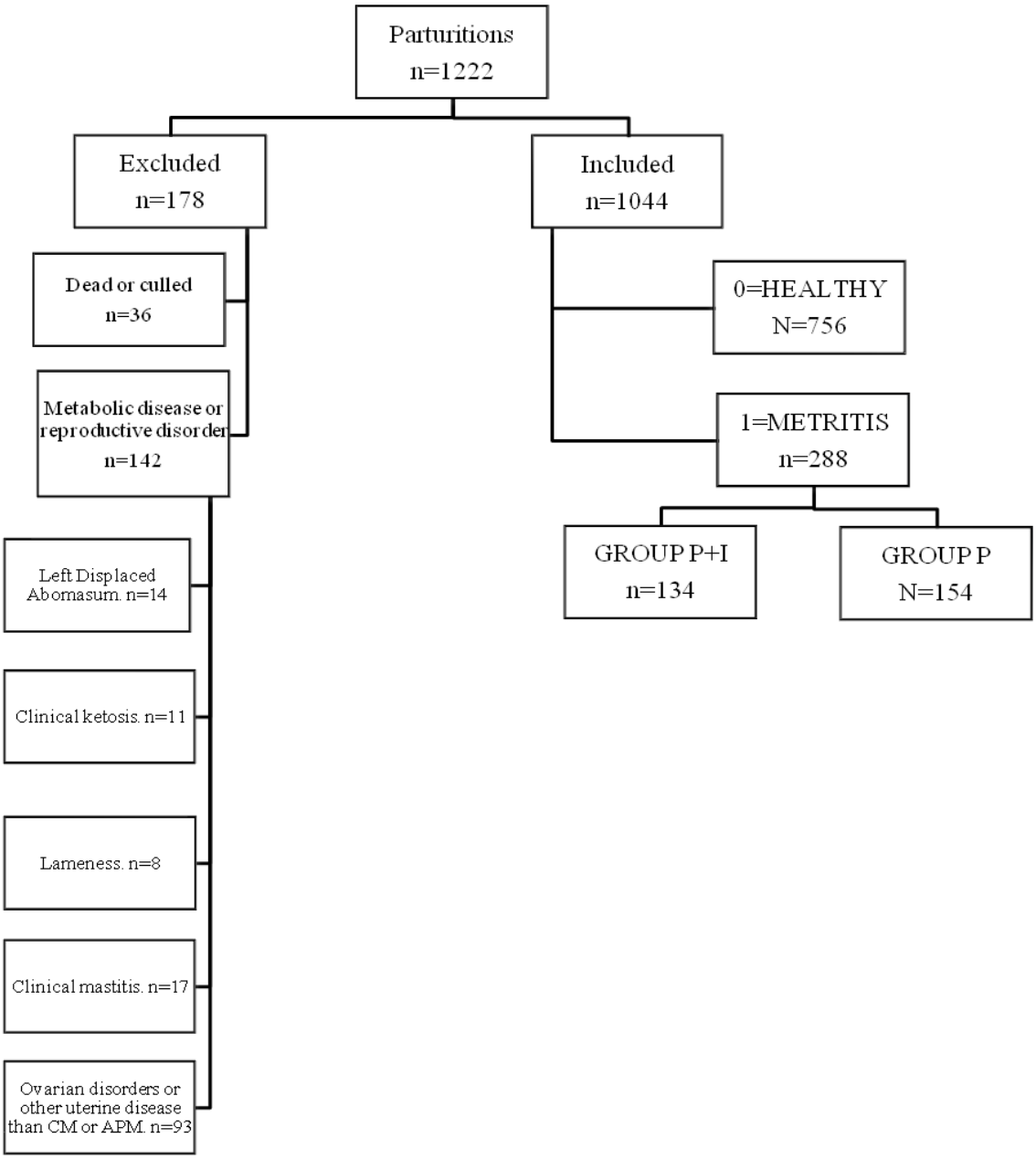
* According to the breakpoint established for similar pathogens in other veterinary species

** Derived from human data

S= Sensitive, I= Intermediate, R= Resistant

Figure

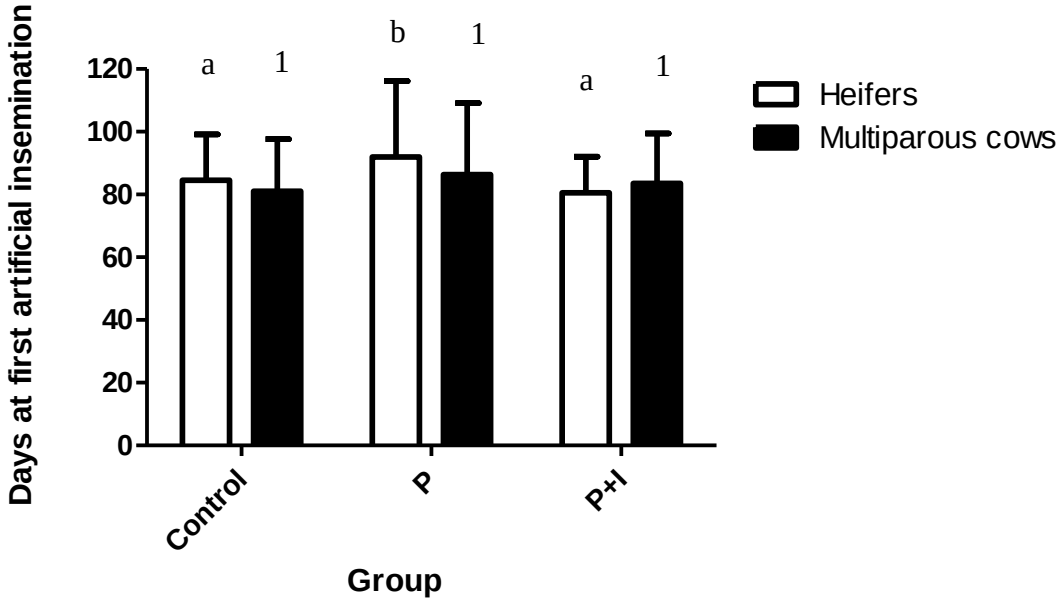
Figure 1.- Allocation of parturitions from animals discarded and enrolled in the study .



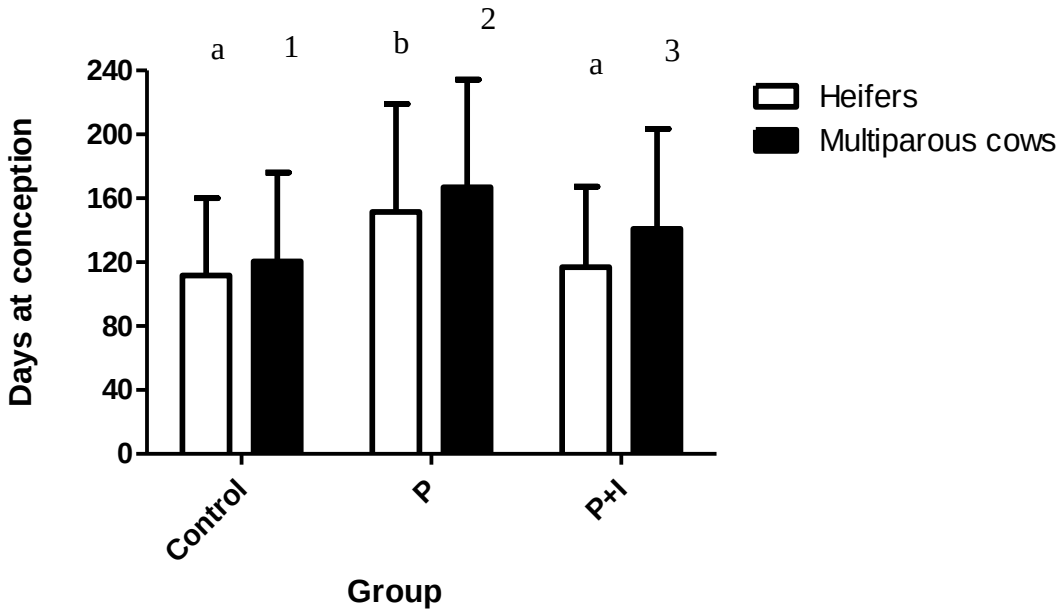
Figure

Figure 2.- Number of days at first artificial insemination (A) and at conception (B) in healthy cows (control) or in metritis affected animals treated with two different treaments (P or P+I). Groups connected with different letter or number means statistically significant differences between them ($p<0.05$).

A



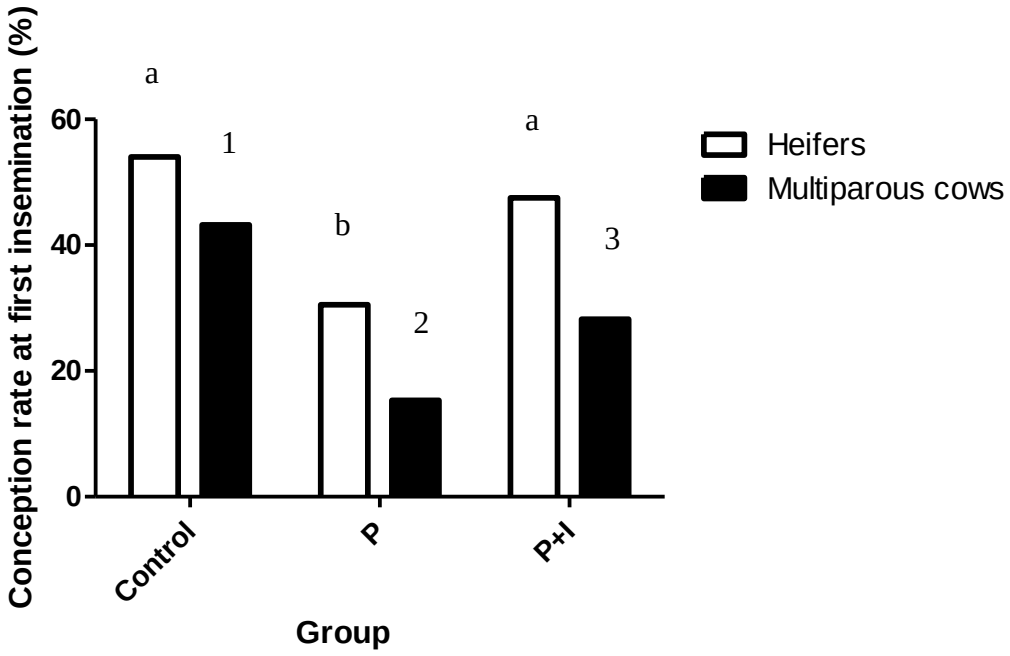
B



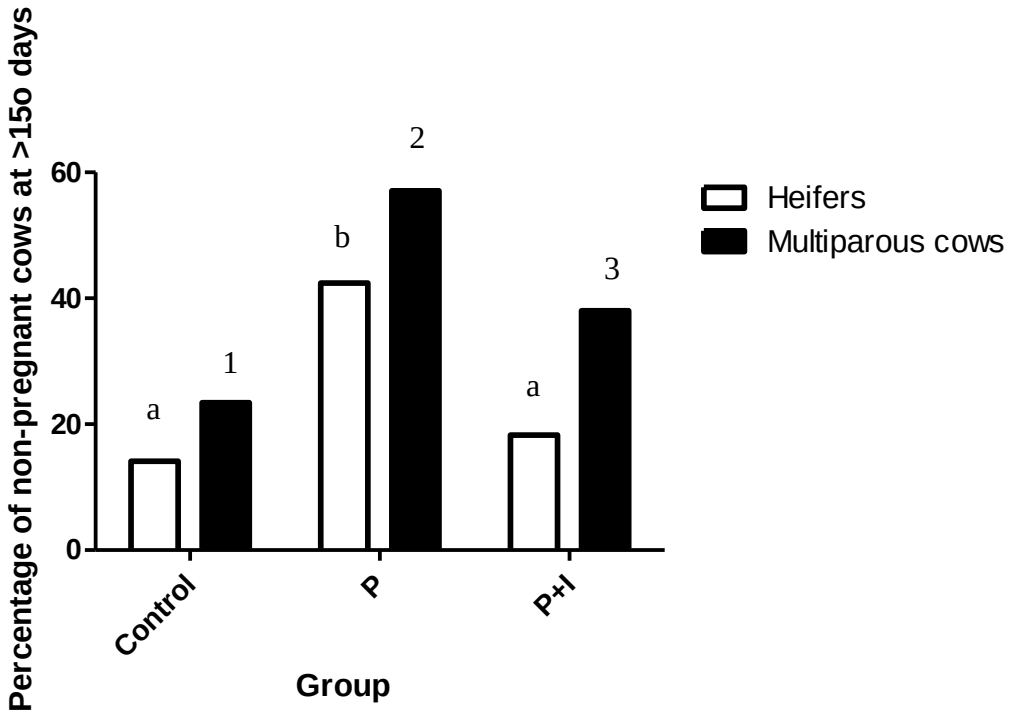
Figure

Figure 3.- Conception rate at first insemination (A) and percentage of non-pregnant cows at >150 days DIM (B) in healthy cows (control) or in metritis affected animals treated with two different treaments (P or P+I). Groups connected with different letter or number means statistically significant differences between them ($p<0.05$).

A



B



Revised Highlighted

Comparison of two treatment strategies for cows with metritis in high-risk lactating dairy cows

Ramon Armengol [a], Lorenzo Fraile [b,c*]

[a]Departament de Producció Animal, ETSEA, University de Lleida, Lleida, Spain. [b] Agrotecnio Center, Lleida, Spain.

* Corresponding author: Tel.: +34 973702814; fax: +34 973702874.

E-mail address: lorenzo.fraile@prodan.udl.cat

Abstract

Acute puerperal metritis (APM) and clinical metritis (CM) are uterine diseases frequently diagnosed in dairy cows. These diseases are responsible of important economic loss due to its effect not only on reproductive performance but also on milk production. The objective of this study was to assess the impact of two different treatments for metritis on dairy cows by measuring their reproductive performance in the next gestation. The endpoints to measure the reproductive performance included: the conception rate at first artificial insemination (AI), the number of days at conception and the proportion of non-pregnant cows at over 150 days after beginning milk production (>150 DIM). The study was carried out in a high production dairy cow farm located in Lleida (North East Spain). Recordings of 1044 parturitions of 747 Holstein cows were controlled in this farm from 2009 to 2014. Cows were diagnosed as suffering from metritis (APM or CM) if the following parameters were observed: an abnormally enlarged uterus, a fetid watery red-brown uterine discharge with (APM) or without (CM) fever (>39.5 ºC) and presence (APM) or absence (CM) of signs of systemic illness (decreased milk production, dullness or other signs of toxemia) within 21 days postpartum. Afterwards, cows suffering from metritis (APM or CM) were randomly assigned and balanced to two groups: 1) Animals receiving parenteral amoxicillin intramuscularly plus intrauterine infusion with oxytetracycline (P+I group); and 2) Animals receiving only parenteral amoxicillin intramuscularly (P group). Furthermore, reproductive performance of cows without metritis were used as reference (control group).

Metritis was diagnosed in 27.5 % of the total parturitions included in the study (288 out of 1044). In particular, metritis was diagnosed in 30.5% (118 out of 387) and 25.9% (170 out of 657) of parturitions from heifers and multiparous cows, respectively. Reproductive performance was not significantly affected by the parity, the season at first AI, the season at conception, the bull or the inseminator. The P+I treatment was able to significantly reduce the number of days at first insemination and at conception when compared to the P treatment in heifers. In multiparous cows, this significant effect was only observed for days at conception. Additionally, the P+I treatment was

able to significantly increase the percentage of pregnant animals at first insemination and decrease the percentage of non-pregnant cows at >150 DIM for both heifers and multiparous cows when compared to the P treatment.

Key words: Metritis, treatment strategies, reproductive performance, dairy cattle.

1.- Introduction

The reproductive performance of a cow after parturition is affected by many factors such as parity, the number of days without milk production, body weight loss, heat stress, season at first artificial insemination (AI), season at conception, bull used for the AI and the technician that carries out the AI [1]. Moreover, clinical conditions such as clinical ketosis, clinical hypocalcemia, and uterine diseases could negatively affect the future reproductive performance of the cow [2,3].

Metritis can cause important economic losses during the postpartum period due to poor reproductive performance, lower milk production and an increase in the culling rate in comparison with non-affected cows. The animals affected by metritis are unable to control the growth of aerobic and anaerobic bacteria in uterine lumen due to an abnormal post-partum uterine involution that may be triggered by immune, nutritional and management factors [4]. Metritis is characterized by an inflammation of the uterine tract with local and/or systemic implications for the cow [5]. Therefore, diagnosis and treatment of postpartum uterine diseases is common in dairy practice.

It is very important to clearly define the diagnostic criteria for metritis in cows. Thus, two types of metritis are usually observed under field conditions in the early postpartum period (1-21 days after parturition). On one hand, acute puerperal metritis (APM) is diagnosed in cows that have an abnormally enlarged uterus and a fetid, watery, reddish-brown uterine discharge, which is also associated with clinical signs of systemic disease (decreased milk yield, dullness or other signs of toxemia) and fever (>39.5ºC) within 21 days postpartum. On the other hand, clinical metritis (CM) is described in cows that have an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina within 21 days after parturition in the absence of clinical signs of systemic disease [6, 7, 8]. In any case, diagnosis of metritis is challenging for researchers and practitioners under field conditions [6, 7, 9] and the distinction between APM and CM is frequently not straightforward. Fortunately, the basis of the treatment is common for both types of metritis [10].

It has been described in the literature that the treatment for cows suffering from metritis during the early post partum period is important to minimize this risk of low milk production, increase

reproduction performance in the next gestation and reduce culling risk [7]. There are many treatment protocols for cows with metritis. These treatments include various combinations of antimicrobials, administered by intrauterine and/or parenteral routes, and/or hormones [7, 8]. Thus, beta-lactamic antibiotics (mainly amoxicillin and cephalosporins) are efficacious to treat and prevent metritis after their parenteral administration under field conditions [10, 11]. Tetracyclines administered by intrauterine route are extensively used in uterine disease, although their efficacy has been questioned in the literature [12, 13, 14].

The objective of this study was to assess the impact of two different treatments for metritis during early postpartum period (1-21 days) in dairy cows by measuring their reproductive performance in the next gestation. The endpoints to measure the reproductive performance included: the conception rate at first AI, the number of days at conception and the proportion of non-pregnant cows at >150 days in milk production (DIM).

2.- Materials and Methods

2.1- Animals and herd.

During a five-year period (January 2009- January 2014), a total of 1222 cow parturitions were evaluated for routine post-partum examination; of these, 414 corresponded to heifers and 808 to multiparous cows. Parturitions complicated by other diseases that could also affect the reproductive performance (clinical ketosis, left displaced abomasum (LDA), clinical mastitis, lameness, pneumonia, ovarian disorders such as ovarian cysts and other uterine disease such as endometritis and pyometra) were excluded from the study. Culled or dead cows from either group without full reproductive history were also excluded. Eventually, 1044 parturitions were finally included in the study, 387 corresponding to heifers and 657 to multiparous cows. The exact numbers of excluded cows for the various reasons exposed, as well as group allocation for enrolled cows are detailed in

126 Figure 1.

127 The study was conducted on a single high production commercial dairy farm in Lleida (North East
128 Spain) over a period of five years (2009-2014). During the study period, this farm was housing an
129 average of 330 lactating Holstein cows with an average production of 11,100 kg of milk (3.6% Fat
130 and 3.3% Protein) in 305 days by cow. Cows were housed in five straw-bedded free- stall barns and
131 they were fed a total mixed ration consisting of corn silage, grass silage, and concentrates. Cows
132 were milked three times a day (at 4 AM, 12 PM, and 8 PM). Each cow was sampled and analyzed
133 for milk quality (fat, protein, and lactose concentration) and somatic cell count by the Central
134 Laboratory for Milk Recording (ALLIC, Catalonia) once a month.
135 Breeding management was carried out by AI with Holstein semen and performed by a highly
136 trained technician or veterinarians specialized in cattle reproduction. Cows were bred on observed
137 estrus or diagnosed by a computerized pedometry system (Afimilk, Kibbutz Afikim, 1514800,
138 Israel). Breeding programs based on the use of hormones were not applied and it was decided not to
139 inseminate heifers and multiparious cows before 90 and 70 days post-partum, respectively. This
140 decision was made considering historic data about reproductive performance in this particular farm.
141 Transrectal ultrasonography (Easi Scan, 4.5 MHz - 8.5 MHz, BCF Technology, Ltd. Scotland) was
142 used to perform pregnancy diagnosis at 32-40 days post AI as well as to detect uterine and/or
143 ovarian disorders such as clinical endometritis, pyometra, ovarian cysts and ovarian cyclicity
144 failure**.** This reproductive control was carried out every Tuesday and Friday and also included post -
145 partum examination (1-21 post-partum) and diagnosis and treatment of non-cycling or silent estrus
146 cows. Non-cycling or silent estrus cows were treated with hormones. Various combinations of
147 prostaglandins, gonadotropin-relising hormone GnRH and progesterone were used according to
148 clinical criteria. In any case, these animals were excluded from the study to avoid any confounding
149 factor in the data analysis. Finally, animals diagnosed and treated for other uterine and/or ovarian
150 disorders outside the period of 1-21 days postpartum were excluded from the study to avoid any
151 bias.

Clinical, reproduction, production and management data were recorded by the herd manager and the attending veterinarians using specific software (Afimilk, Kibbutz Afikim, 1514800, Israel). Lleida has a long "heat stress risk season" (from May to October,) that could affect the reproductive performance [15]. It is well known that heat stress can affect immunological status of the cows [16] and it could significantly affect the prevalence of uterine diseases and/or its severity. For the purposes of this study, the year was divided in four periods; 1 (1st January-31st March), 2 (1st April-30th June), 3 (1st July-30th September) and 4 (1st October-31st December).

Other factors that have been described as affecting the reproductive performance of cows are the origin of semen (bull factor) and the technicians that perform the AI. Both factors were excluded from the data analysis because the same number of cows were inseminated in each experimental group regarding the origin of semen and the technician.

2.2.- Definition of metritis case

All the cows underwent a clinical examination every 24 hours during the first 21 days post-partum. For each animal, the ruminal motility, breathing rate, consistency of the faeces, rectal temperature, milk production, pedometry readings, body weight (BW) and milk conductivity were recorded. Additionally, rectal palpation and observation of vaginal discharge were carried out every Tuesday and Friday from day 1 to day 21 postpartum, (Table 1).

Vaginal discharge (VD) was collected through rectal palpation massage to minimize not only contamination of the vagina and uterus but also microtrauma in the area. Quantity, color, proportion of pus, consistency and smell were evaluated [6]. All examinations were performed by the same veterinarian in order to minimize bias in the clinical record [17]. Cows that did not expel their fetal membranes (44 out of 1222) within 24 hours postpartum were diagnosed with retained fetal membranes (RFM). These animals were treated with one injection of 37.5 mg of sodium selenium and 1250 mg of α-Tocoferol acetate (Hipravit Selenio, HIPRA, Spain) and oral calcium (Bovicalk, Boehringer Ingelheim, Germany) every 12 hours until fetal membranes were expelled. In these

cows, rectal palpation was performed every 24h. If fever (>39,5ºC) was present, animals were treated with flunixin meglumine (Finadyne®, MSD Animal Health) at a dose of 2.2 mg/Kg BW/ 24 h for three days. Once fetal membranes were expelled (average of 48h postpartum), animals were classified according to Table 1. No attempt was made to manually remove the retained fetal membranes.

In this study, metritis (APM and CM) was diagnosed in animals that had an abnormally enlarged uterus and a purulent or a fetid red-brown watery uterine discharge detectable in the vagina within 21 days after parturition. The presence or absence of pyrexia (>39.5ºC) and other signs of systemic illness such as reduced milk yield, dullness, inappetence or anorexia, elevated heart rate, and apparent dehydration determined if animals were included in the APM group (present) or the CM group (absent) [6]. Cows treated with anti-inflammatory drugs, antimicrobial drugs or hormones for diseases not related with the study (e.g., acute mastitis, lameness, LDA, ketosis, other uterine or ovary disorders) were excluded from the trial.

2.3.- Experimental design

Cows were included in the study at day 1 after calving. Reproductive performance of healthy cows was used as reference (control group). Cows suffering from metritis were randomly allocated to one of two groups according to the last digit of their ear tag. Thus, cows with an odd number (1, 3, 5, 7, 9) were allocated to P group. In this group, cows only received parenteral amoxicillin by intramuscular route. Cows with an even number in their ear tag (0, 2, 4, 6, 8) were allocated to group P+I. In this group, cows received parenteral amoxicillin by intramuscular route plus an intrauterine infusion with oxytetracycline. Briefly, cows included in group P received a systemic antimicrobial treatment of amoxycillin trihidrate (Amoxoil Retard®, Laboratiorios SYVA SA) at 15 mg/kg BW every 48h by intramuscular route for three times. Injection was applied on the neck and the injection volume was always less than 20 mL on the same inoculation point. Cows included in

the P+I group received the same systemic treatment as group P plus 8000 mg of oxytetracycline dihidrate (Tenalina L.A.®, CEVA Santé Animal) (200 mg/ml) via intrauterine infusion every 72-96 hours twice. Intrauterine infusion was applied with a disposable uterine catheter (53.5 cm Bovivet, Kruuse, Denmark) and a single use 50 cm^3syringe. Cows that were diagnosed with metritis were considered cured when its classification was M0 or M1 (Table 1) after two regular rechecks. Cows allocated in any of the groups that showed fever were also treated with flunixin meglumine (Finadyne®, MSD Animal Health) at a dose of 2.2 mg/Kg BW/24 h for 3 days.

2.4.- Monitoring of antimicrobial susceptibility throughout the trial

Antimicrobial susceptibility (AS) for *Escherichia coli* (EC) and *Trueperella pyogenes* (TP) against amoxicillin and oxytetracycline was monitored every third month in this farm in, at least, four clinical cases. Briefly, AS tests were performed using the agar dilution method according to the Clinical Laboratory Standard Institute (CLSI) guideline M31-A3 in a commercial diagnostic laboratory. The threshold values to distinguish between susceptible and resistant microorganisms are detailed in Table 2.

2.5.- Statistical Analysis.

All statistical analyses were carried out using the SAS system V.9.1.3 (SAS institute Inc, Cary, NC, USA). For all analyses, the individual cow was used as the experimental unit and the data was grouped taking into account the parity of the cows: Heifers (first parturition) versus multiparous cows (two to six parturitions). The significance level (p) was set at 0.05 with statistical tendencies reported when $P<0.10$. The variables included in the statistical analyses were classified as nominal (pregnancy and delivery season), ordinal (parity) or continuous (days to conception and DIM). Shapiro Wilk´s and Levene tests were used to evaluate the normality of the distribution of the continuous variables and the homogeneity of variances, respectively. Statistical analyses were performed to test the association between the different experimental groups (control, P or P+I) with

the conception rate at first AI, days to conception and the proportion of non-pregnant cows with a DIM higher than 150 days. Contingency tables (Chi-square or Fischer exact tests) were used when the association between nominal and ordinal variables was assessed. To study the association between nominal or ordinal variables with the continuous non-normally distributed variables, the Wilcoxon test (with the U Mann-Whitney test to compare each pair of values) was used. To analyse the association between continuous normally distributed variables and nominal or ordinal variables, an ANOVA test (with Student's T-test to compare each pair of values) was used.

3.- Results.

3.1.- Description of metritis in the population

Metritis was diagnosed in 288 out of 1044 parturitions finally included in the study, (27.5% prevalence). In particular, metritis was diagnosed in 30.5% (118 out of 387) and 25.9% (170 out of 657) of parturitions resulting from heifers and multiparous cows, respectively. Cows scored as M2 (Table 1) were diagnosed with APM in 83 out of 288 cases of metritis (28.8% prevalence). These animals had an abnormally enlarged uterus, a purulent or a fetid red-brown watery uterine discharge detectable in the vagina, pyrexia (>39.5ºC) and/or systemic illness such as reduced milk yield, dullness, inappetence or anorexia, elevated heart rate, and apparent dehydration. Two-hundred and five out of 288 cows with uterine disease (71.2% prevalence) were diagnosed with CM (cows graded as M2 but without pyrexia (<39.5ºC) and/or systemic illness). Taking parity into account, 32.2% of heifers affected with metritis, suffered from APM (38 out of 118) and 67.8% from CM (80 out of 118). Within multiparous cows, 30.6% (52 out of 170) and 69.4% (118 out of 170) were diagnosed as APM and CM, respectively. Significant differences in the prevalence of each type of metritis between heifers and multiparous cows or within groups of treatment were not observed at the beginning of the trial (basal homogeneity). Thus, 27.9% (43 out of 154) and 72.2% (111 out of 154) of the animals were diagnosed as APM and CM, respectively in Group P and the prevalence of

APM was 29.8% (40 out of 134) and 70.2% of CM (94 out of 134), respectively for group P+I at the beginning of the trial.

3.2.- Reproductive performance in the population

Reproductive performance was not significantly affected by the number of parity, the season at first AI, the season at conception, the bull or the inseminator that carried out the AI, neither in healthy nor in metritis affected cows. Considering all the animals included in the study (healthy and metritis affected cows), the number of days at first insemination was not significantly different between heifers and multiparous cows but the number of days at conception was significantly higher in multiparous cows (129.5±60.3) than in heifers (118.6±53). In heifers, the conception rate at first insemination was significantly higher than in multiparous cows, whereas the percentage of non-pregnant animals at >150 DIM was significantly lower than in multiparous cows. For this reason, the analysis of the treatment efficacy was carried out separately for heifers and multiparous cows.

3.3.- Bacteriological examination

The isolates of EC and TP obtained were susceptible against amoxicillin and/or oxytetracycline throughout the trial, considering the threshold values detailed in Table 2.

3.4.- Effect of the two antibiotic treatments on the reproductive performance

In heifers with metritis, The P+I treatment was able to significantly reduce the number of days at first insemination (80.5±11.6 vs 91.9±24.2) and at conception (116.8±50.5 vs 151.5±67.7) in comparison with the P treatment. In the case of multiparous cows, this significant effect was only observed in the case of days at conception (140.8±62.7 vs 166.8±67.5) (Figure 2). In heifers, when the P+I group was compared to the control group, no significant differences were observed for any of these two parameters. However, in the case of multiparous cows, the number of days at conception for both P and P+I groups was significantly higher than in the control group

(120.6±55.5) (Figure 2). The P+I treatment was able to significantly increase the percentage of pregnant cows at first insemination for both heifers (47.5%) and multiparous cows (30.5%) in comparison with the P treatment (28.2% for heifers and 15.3% for multiparous) (Figure 3). The P+I treatment was able to significantly decrease the percentage of non-pregnant cows at >150 DIM when compared to the P treatment (18.3 % vs 42.4% for heifers and 38% vs 57.1% for multiparous cows, respectively). Finally, in heifers, no significant differences in the percentage of pregnant animals at first insemination or in the percentage of non-pregnant cows at >150 DIM were observed between the control group and the P+I group. However, in multiparous cows, both parameters for the P and P+I groups had significantly higher values than those observed in the control group (Figure 3).

4.- Discussion.

The main goal of this study was to assess the impact of two different treatments, parenteral only vs parenteral plus intrauterine infusion, for dairy cows affected with metritis by measuring their reproductive performance in the next gestation: days at first AI, conception rate at first AI and percentage of non pregnant animals at 150 DIM. These results were compared with the values observed for healthy cows as a reference. The authors did not consider the possibility to include a negative control group (affected with metritis and not treated with antimicrobials) due to welfare reasons and the increased risk of death or culling for these non-treated sick animals. Metritis decreases the reproductive performance of dairy cattle [3]. In this study, the control group (healthy cows) always showed better reproductive performance than metritis-affected cows. The results suggest that the combination of amoxicillin, administered by parenteral route, plus intrauterine infusion of oxytetracycline is more efficacious than the administration of parenteral amoxycillin alone for the treatment of metritis, when taking into account the reproductive performance in the next gestation as an end-point. This statement was true for both heifers and multiparous cows,

although the positive results were more consistently observed in heifers than in multiparous cows for all the parameters evaluated.

In the literature, various techniques to diagnose metritis in cows can be found [6, 9]. In this study, metritis (APM and CM) was described as an abnormally enlarged uterus and a fetid watery red-brown or purulent uterine discharge showing or not pyrexia (>39.5ºC), with or without signs of systemic illness within 21 days after parturition [6]. The authors decided to include both pyretic and non pyretic animals since this is the way it has been previously described by other authors [4, 10]. The authors used a method with high sensitivity to diagnose metritis in cows, which avoids the risk of underestimating metritis if only milk production or rectal temperature are considered as diagnostic, but at the same time, trying to make it a reasonably fast, sensitive and economic procedure for the farmer. The procedure takes some time to implement but once established as a routine in the farm, the authors believe that it can be very useful to diagnose post-partum disease and treat the animals during an early stage of illness, which improves the productive and reproductive performance of the cows. It means that the animals included in this study were diagnosed in a very early stage of the disease. This fact must be taken into account when the treatment efficacy is compared with the results obtained by other authors.

In this study, the overall prevalence of metritis is close to 28%. This prevalence can vary considerably between heifers and cows, between different farms and between studies. Thus, values between 2.2% and 42.3% have been observed in previous studies [14, 18, 19]. For this reason, the farm included in this trial could be considered representative of a high production dairy cattle operation with a prevalence of metritis within reported values. Thus, the reproductive performance of healty or metritis-affected cows was not affected by factors directly or indirectly associated with management (season at first AI, season at conception, the bull and the inseminator that carried out the AI). Taken together, these results suggest that the farm where the study was carried out was

appropriate to assess the impact of two different treatments for metritis in dairy cows by measuring their reproductive performance in the next pregnancy.

The bacteria most commonly isolated from metritis-affected dairy cows are well described in several studies; these include EC and TP [20, 21, 22]. *Fusobacterium necrophorum* and *Bacterioides spp* are also recognized as opportunistic bacteria that can cause metritis [23, 24]. In particular, it has been long suggested that uterine infections might depend on pathogenic synergism between EC, TP and bacteria from other groups. However, recent studies have suggested that the diversity of the uterine bacterial composition in dairy cows is likely to be even more complex than previously described by traditional culture-dependent methods [25]. Additionally, it is necessary to know the AS of the bacteria involved in clinical cases of bovine metritis in order to choose the most suitable antimicrobial. However, it is only feasible to determine this antimicrobial susceptibility in the isolated bacteria. In our case, the isolates of EC and TP obtained were susceptible against amoxicillin and/or oxytetracycline throughout the trial. Thus, the use of both antimicrobials in this farm did not seem to generate antimicrobial resistance for these bacteria during five years. Nevertheless, the diversity of the bacterial species involved in clinical cases of metritis makes absolutely compulsory to carry out clinical trials that measure clinical efficacy instead of carrying out only pharmacokinetic/pharmacodynamic studies that only take into account pharmacodynamic data from only the most commonly isolated bacteria (EC and TP) [26].

The use of antimicrobials is widely accepted to cope with infections in the genital tract, mainly in the case of metritis [27]. The rationale behind this therapeutic approach is to reduce the number of pathogens in the uterine cavity and consequently mitigate the associated inflammation, enhance the local immune defense, and facilitate the repair of the endometrium for a faster return to a normal uterine status. Unfortunately, there are few available data about the penetration of parenterally-

administered antibiotics into the cow genital tract. In humans, an endometrium to plasma concentration ratio of 0.6 and 0.4 for quinolones and a beta-lactamics, respectively, has been established [28, 29]. It is possible that a similar ratio could be achieved in cows. Intrauterine administration of antibiotics is expected to reach a higher antibiotic concentration in the endometrium compared to systemic administration and it is a common route to administer antimicrobials for metritis in cows and mares [30]. Thus, considering the administered dose of oxytetracycline (8000 mg) and the uterine volume post-partum (2000 mL) [31], an extraordinarily high oxytetracycline concentration in uterine lumen (4 mg/mL) was expected to be achieved. This concentration is notably higher than the established Minimum inhibitory Concentration for the 90% of the strains (MIC_9) for AP and EC in case of bovine metritis [32, 33, 34].

To the best of the authors' knowledge, no previous studies have explored the same combination of antimicrobials to treat cows diagnosed with early postpartum metritis. However, it has been described in the literature that the administration of beta-lactamics by parenteral route is able to improve the reproductive performance of metritis-affected cows [35]. The use of ceftiofur, administered intramuscularly, with or without a combination of cloxacillin plus ampicillin by intrauterine infusion, did not show differences in reproductive performance between groups in cows diagnosed with toxic puerperal metritis (TPM) [36]. In conclusion, there is a lot of variation in the treatment outcome of uterine disease using beta-lactamic antibiotics [13, 37, 38, 39, 40]. Similar studies have described a significant improvement in reproduction parameters applying an intrauterine infusion of tetracyclines in cows and heifers with CM [14] but other studies did not find significant differences in the treatment outcome between various combinations of intrauterine infusion of tetracyclines for the treatment of metritis [39], endometritis [12, 13] or retained fetal membranes [14, 42]. In our study, the combination of amoxicillin, by parenteral route, plus intrauterine infusion of oxytetracycline is more efficacious than using only the parenteral route. It must be highlighted that, in the current study, the dose of oxytetracyclines was higher and the

duration of administration was longer than the dosage regimen used in other studies [14, 39]. Globally, these results suggest that local treatment with antibiotics can be important to heal the genital tract and prepare it for the following pregnancy. The efficacy of intrauterine tetracycline might be related to other uncultured or unknown organisms that are sensitive to this antimicrobial and that are not easily isolated with standard microbiological techniques. Finally, this efficacy might also be related to non-antimicrobial effects of tetracyclines such as anti-inflammatory effects. In this regard, tetracyclines are potent inhibitors matrix metalloproteinases and inducible Nitric Oxide Synthase, which are known to play a role in the mediation and control of mucosal inflammation and immune response [43, 44].

In conclusion, based on reproductive performance as an end-point parameter, treatment of metritis with parenteral amoxicillin plus oxytetracycline via intrauterine route was superior in comparison with only the parenteral administration of amoxicillin.

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Table 1. Classification of cows according to the type of vaginal discharge during the post-partum period

CODE	TYPE OF VAGINAL DISCHARGE	DIAGNOSIS	TREATMENT
M0	No vaginal discharge.	Healthy	None
M1	Normal lochial secretion, odorless, viscous and translucid.	Healthy	None
M1S	Normal lochial secretion, odorless, viscous and reddish brown.	Healthy	None
M2	Purulent or fetid, watery and reddish-brown	Metritis	P: Parenteral Amoxycillin P+I: Parenteral Amoxicillin plus intrauterine infusion of Oxytetracycline.

Table 2.- Minimal inhibitory concentration (MIC) breakpoints for *Escherichia coli* and *Trueperella pyogenes* isolated from bovine metritis cases

			MIC breakpoint (µg/mL)			
		Escherichia coli			*Trueperella pyogenes*	
	S	I	R	S	I	R
Antimicrobial agent						
Amoxicillin*	<0.25	0.5	>1	<2	4	>8
Oxytetracycline**	<4	8	>16	<4	8	>16

* According to the breakpoint established for similar pathogens in other veterinary species

** Derived from human data

S= Sensitive, I= Intermediate, R= Resistant

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534 Figure 1.- Allocation of parturitions from animals discarded and enrolled in the study .

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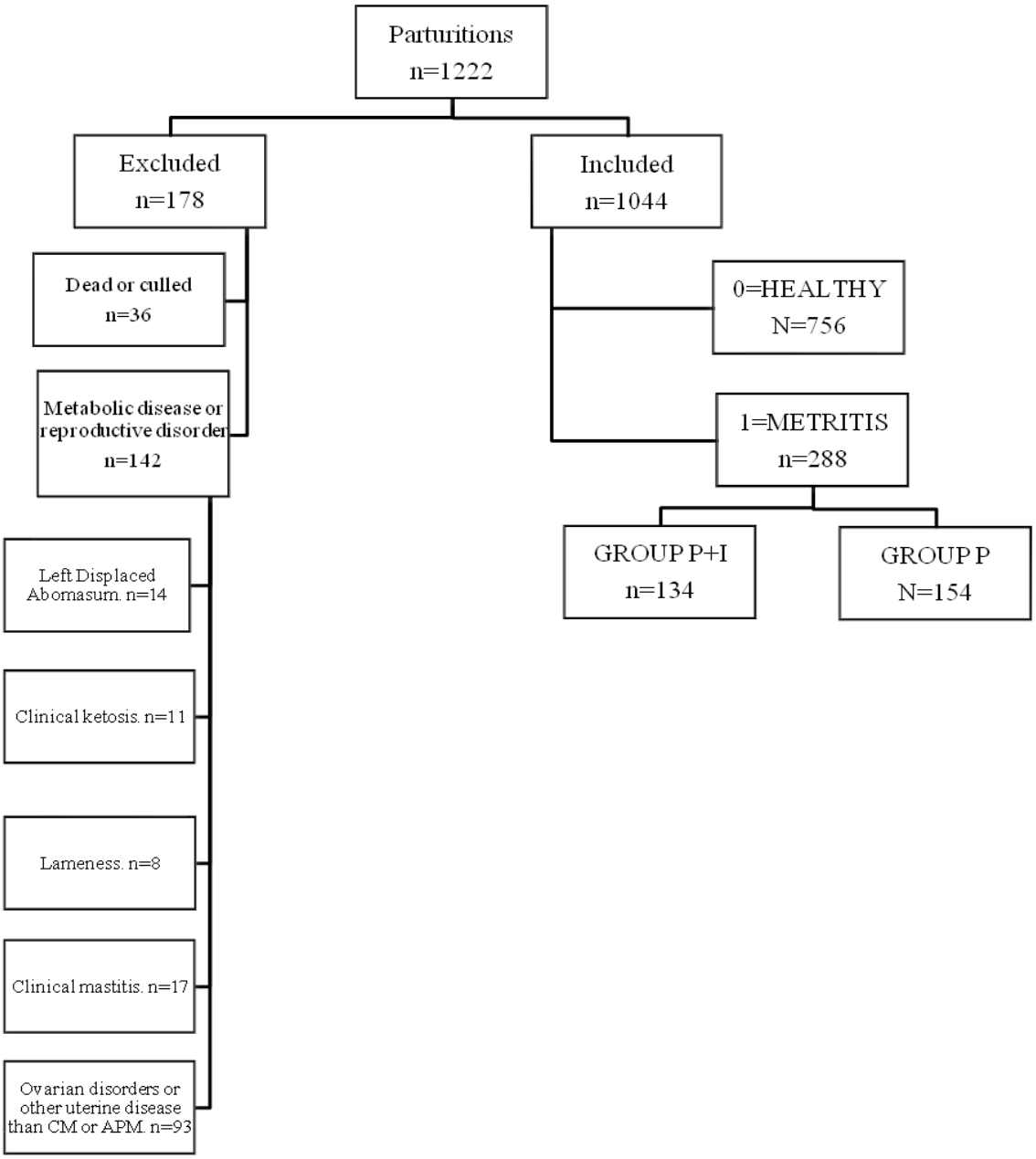

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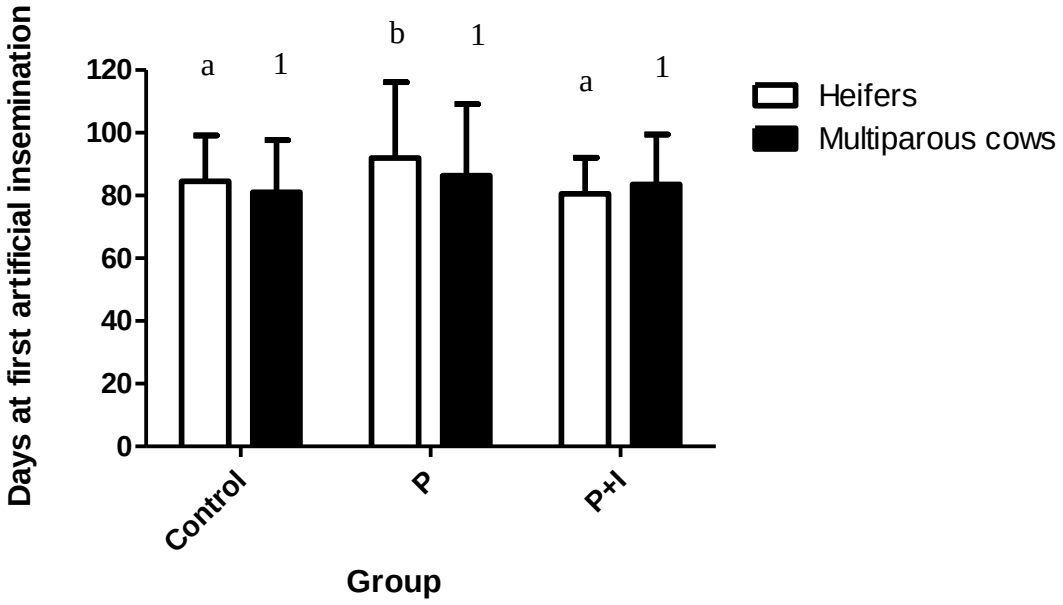
Figure 2.- Number of days at first artificial insemination (A) and at conception (B) in healthy cows (control) or in metritis affected animals treated with two different treaments (P or P+I). Groups connected with different letter or number means statistically significant differences between them ($p<0.05$).

A



B

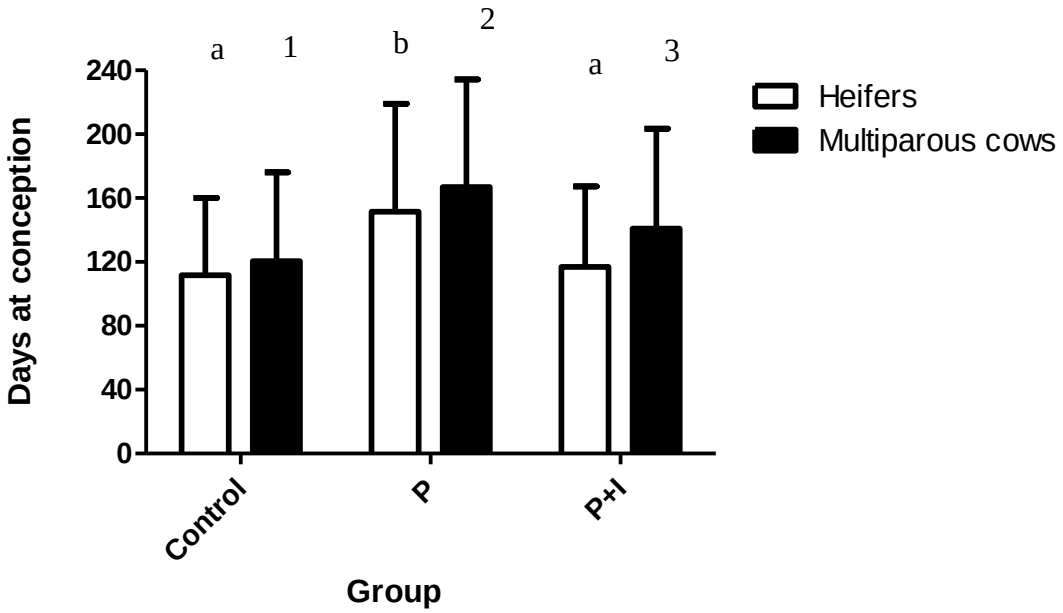
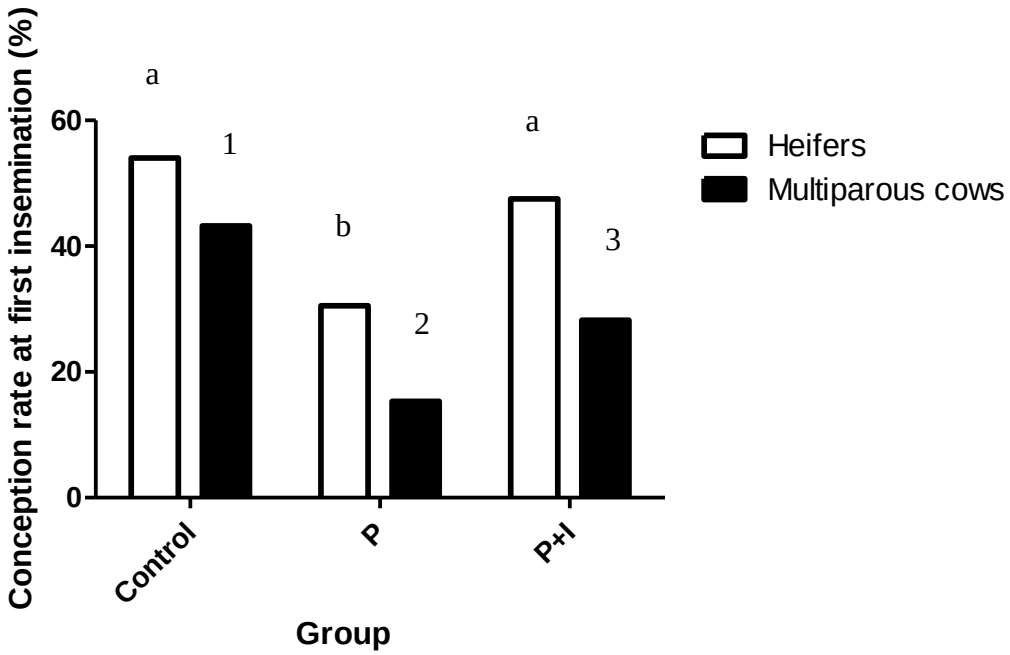
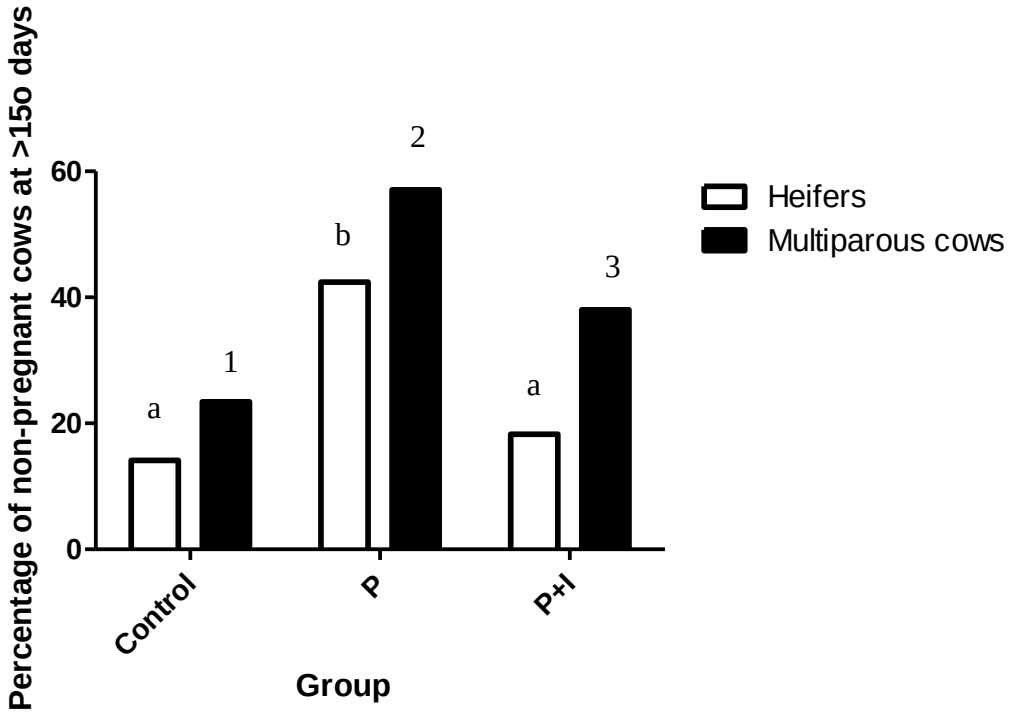


Figure 3.- Conception rate at first insemination (A) and percentage of non-pregnant cows at >150 days DIM (B) in healthy cows (control) or in metritis affected animals treated with two different treaments (P or P+I). Groups connected with different letter or number means statistically significant differences between them ($p<0.05$).

A



B



Highlights

The objective of this study was to assess the impact of two different treatments for metritis on dairy cows by measuring its reproductive performance in the next gestation.

Cows suffering metritis were randomly assigned to two groups: 1) P+I group: Animals receiving parenteral amoxicillin by intramuscular route plus intrauterine lavage with oxytetracicline; 2) P group: Animals receiving only parenteral amoxicillin by intramuscular route. Furthermore, reproductive performance of non-suffering metritis cows (healthy ones) were used as reference values (control group).

The endpoints to measure its reproductive performance were the conception rate at first artificial insemination (AI), the number of days at conception and the proportion of non-pregnant cows at more than 150 days of beginning milk production (>150 days DIM).

The P+I treatment was able to reduce significantly ($p<0.05$) the number of days at first insemination and at conception in comparison with the P treatment in heifers. In the case of multiparous cows, this significant effect was only observed in the case of days at conception.

The P+I treatment was able to increase and decrease significantly ($p<0.05$) the percentage of pregnant animals at first insemination and the percentage of non-pregnant cows at >150 days DIM, respectively for heifers and multiparous cows in comparison with the P treatment.