Research in Veterinary Science 183 (2025) 105511

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Evaluation of prevalence, risk factors, and therapeutic approach for subclinical endometritis and oviductal occlusion in repeat breeder Holstein cattle

Sofía L. Villar [a], Carlos C. Pérez-Marín [b], Jacobo Álvarez [a], Uxía Yáñez [a,*], Juan J. Becerra [a,c], Ana I. Peña [a], Pedro G. Herradón [a,c], Luis A. Quintela [a,c]

[a] Unit of Reproduction and Obstetrics, Department of Animal Pathology. Faculty of Veterinary Medicine, Universidade de Santiago de Compostela (USC), 27002 Lugo, Spain

[b] Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universidad de Córdoba, 14014 Córdoba, Spain

[c] Instituto de Biodiversidade Agraria e Desenvolvemento Rural (IBADER), USC, Lugo University Campus s/n, 27002 Lugo, Spain

ARTICLE INFO

Keywords:
Repeat breeder syndrome
Dairy cattle
Subclinical endometritis
Oviductal patency

ABSTRACT

Due to the productive and economic consequences of Repeat Breeder (RB) syndrome, the objectives of this study were to determine the prevalence and risk factors for subclinical endometritis (SE) and oviductal occlusion (OO) in RB cows, and to make a therapeutic approach for these pathologies. In 99 RB cows, endometrial cytologies were performed to assess the presence of SE (>5 % polymorphonuclear neutrophils), and the oviductal patency was checked using the phenolsulfonphthalein test. Body condition score was evaluated, and data from each animal were obtained from on-farm software (parity, calving date, artificial insemination (AI) date, number of AI, and occurrence of postpartum diseases). Cows positive to SE were assigned to one treatment protocol: a) Non-steroidal anti-inflammatory drug (NSAID), b) Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), c) NSAID+ $PGF_{2\alpha}$. Similarly, cows with OO were assigned to one treatment: a) Therapeutic embryo, b) Artificial insemination. The prevalence for SE and OO was 22 % and 3 %, respectively. Regarding SE, the logistic regression did not show any significant difference for the risk factors evaluated. Moreover, cows that were administered the NSAID, either alone or in combination with $PGF_{2\alpha}$, showed higher conception rates in the following AI ($p < 0.05$). Due to the low prevalence of OO, it was not possible to perform the logistic regression analysis. In conclusion, neither SE nor OO seem to be the main cause of RB syndrome in the cows under study. Additionally, when SE is diagnosed as the main cause of RB syndrome, treatment with NAISDs seems to increase conception rates.

1. Introduction

Nowadays, dairy cattle farming is developing under demanding production systems due to greater competitiveness in the market, amid an uncertain world economic situation characterized by high food and fuel prices, as well as the urgent climate emergency on our planet. In this context, it is important to maintain good reproductive efficiency as it is one of the main determinants of dairy farm profitability (LeBlanc, 2010).

The main objective of reproductive management on dairy farms is to get cows pregnant at an optimal interval after calving (Plaizier et al., 1997; Walsh et al., 2011). During the last decades, a progressive and worrying decline in fertility of dairy cows has been detected in many countries, estimating a decrease of about 0.5–1 % per year (López-Gatius, 2003). However, this decline, probably influenced by production-focused genetic selection, is being reversed in recent years, making reproductive problems more important (Powell, 2004; Norman et al., 2009).

In this way, the Repeat Breeder (RB) syndrome is one of the most important reproductive disorders in dairy cows. More days open, higher number of services per conception and high risk of early involuntary culling are important consequences of RB syndrome in dairy cattle, situations that significantly affect the profitability of the dairy industry

* Corresponding author.
E-mail addresses: sofialara.villar@rai.usc.es (S.L. Villar), pv2pemac@uco.es (C.C. Pérez-Marín), jacobo.alvarez.torres@rai.usc.es (J. Álvarez), uxia.yanez.ramil@usc.es (U. Yáñez), juanjose.becerra@usc.es (J.J. Becerra), ana.pena@usc.es (A.I. Peña), garcia.herradon@usc.es (P.G. Herradón), luisangel.quintela@usc.es (L.A. Quintela).

https://doi.org/10.1016/j.rvsc.2024.105511
Received 14 August 2024; Received in revised form 17 October 2024; Accepted 15 December 2024
Available online 16 December 2024

(Nowicki, 2021). This issue is defined as the lack of conception, after three or more artificial inseminations (AI), in absence of appreciable genital tract disease. The prevalence of RB cows in dairy cattle varies depending on the area, ranging from 10 to 25 % (Asaduzzaman et al., 2016; Deka et al., 2021; Garcia-Ispierto and López-Gatius, 2017; Gustafsson and Emanuelson, 2002), and even reaching up to 62 % in tropical conditions (Yusuf et al., 2012). Consequently, this worldwide point of view indicates that the occurrence of RB syndrome might potentially be reduced and managed (Pérez-Marín and Quintela, 2023).

Nutritional deficiencies (Talukdar et al., 2016), abnormal estrus behavior or inadequate estrus detection (Cummins et al., 2012; Sood et al., 2015), poor AI management (Walsh et al., 2011), and endocrine dysfunctions (Sood et al., 2015) have been considered potential causes of RB syndrome in dairy cattle. However, few studies have focused on uterine and oviductal health as a possible cause of RB syndrome. In this regard, one uterine disease that may impair fertility is subclinical endometritis (SE). It is defined as the inflammatory process of the endometrial lining of the uterus, without clinical signs of illness (Sheldon et al., 2006), and characterized by the presence of polymorphonuclear neutrophils (PMN) on the endometrium, and none or little exudate inside the uterus (Földi et al., 2006). The cytobrush technique has been established as a diagnostic method to detect SE, being the indicator the percentage of PMN (%PMN). Several thresholds have been used to define SE, ranging from ≥5 to greater than 18 % (Pothmann et al., 2015). This pathology has been repeatedly stated as one of the causes of the RB syndrome (Pérez-Marín et al., 2012). Nevertheless, the number of studies about the prevalence of SE in RB cows is scarce and the results have shown controversy about this matter.

In addition to an affected endometrium as a potential cause for infertility in RB cows, oviductal pathologies constitute another factor that may cause a reduction in fertility, as fertilization is usually impaired. The oviduct is not only a communication channel between the ovary and the uterus where the sperm and the oocyte converge, but it is also responsible for providing a suitable environment for gamete maturation, fertilization and early embryonic development (Killian, 2011). Therefore, its implication in fertility is undeniable. The problem arises when determining the prevalence of these pathologies. It has been reported that the prevalence of oviductal diseases ranges from 36 to 89 % in RB cattle (Ellington and Schläfer, 1993). Nevertheless, the techniques used to diagnose these pathologies vary among studies. Rectal palpation or ultrasonography might allow for the diagnosis of some alterations, especially those that cause serious changes in the oviduct. However, most times the occluded oviduct is normal macroscopically. Stephani de Souza et al. (2010), in a study performed with female genital tracts obtained in a slaughterhouse, determined that 60.4 % of the occluded oviducts had a normal appearance. There are some techniques to evaluate oviductal patency *in vivo*, like the phenolsulfonphthalein (PSP) test (Garrido et al., 2020) or the hysterosalpingo-contrast sonography (Kauffold et al., 2009). However, it is labor-intensive and expensive. Consequently, the prevalence of this pathology is mostly determined by using genital tracts from slaughterhouses, and the availability of *in vivo* studies is reduced and with a very low (n = 8–50) sample size (Garrido et al., 2020; Kauffold et al., 2009; Itoh et al., 2016).

Additionally, given the multifactorial origin of the RB syndrome, the therapeutic approach may differ between animals. In this way, as the diagnosis of SE and oviductal occlusion (OO) is possible, the administration of the proper therapeutic option is feasible. Regarding SE, the main issue is the inflammation and its consequences at the hypothalamus-pituitary-ovary axis and uterine level (Quintela et al., 2017, 2018). Therefore, one of the therapeutic approaches proposed in the literature, with positive preliminary results, is the use of anti-inflammatory drugs such as carprofen or meloxicam (Amiridis et al., 2009; Priest, 2013; Priest et al., 2013). On the other hand, traditional treatments like prostaglandin or antibiotic administration have also been studied, although the results were diverse (Quintela et al., 2018). Considering oviductal patency, the problem arises because of the impossibility of the sperm to reach the oocyte, making fertilization impossible. For this reason, the most effective solution would be the transfer of a therapeutic embryo (Nowicki, 2021).

Taking this into account, the objectives of the present study were (1) to determine the prevalence and risk factors for subclinical endometritis and oviductal occlusion in RB Holstein cows, and (2) to make a therapeutic approach for both pathologies.

2. Materials and methods

2.1. Animals

A total of 99 Holstein RB milking cows from 4 free-stall dairy herds located in the autonomous community of Cantabria (North of Spain) were included in the study. The size of the farms ranged from 265 to 370 milking cows. Three farms had a conventional milking parlor, and cows were milked 2–3 times a day. The other farm had an automatic milking system, with 3.1 milkings per cow per day. Overall, mean production was 12,128 kg/cow/year. Additionally, cows were fed a total mixed ration composed of corn silage, grass silage and alfalfa, and concentrate was supplemented according to their energy needs. Reproductive examinations were performed weekly by an experienced veterinarian, and all herds had an established health program that included the control of uterine status in the fourth week postpartum. The voluntary waiting period (VWP) was 70 and 90 days for multiparous and primiparous cows, respectively, in all farms. Additionally, the mean days in milk (DIM) were 215.30 ± 77.20 (140–560). Regarding the first AI after VWP, heat detection was performed in all farms. In general, multiparous cows that reach 80 DIM, and primiparous cows that reach 100 DEL and were not inseminated underwent an Ovsynch synchronization protocol, and so do cows diagnosed as non-pregnant at pregnancy check. Only one farm performed all AIs after heat detection, with the aid of activity monitoring devices.

2.2. Selection of animals

Repeat breeder cows were selected by the veterinarian during the routine reproductive examinations. First, cows with 3 or more AI (3–15, mean 5.15) that were not pregnant were selected. Thereafter, a physical examination was conducted. External visual inspection and palpation were performed, as well as transrectal ultrasound to evaluate the reproductive tract (5.0 MHz linear-array transducer, ProVetScan SR-2C, New Veterinary Technologies, León, Spain). Only animals without clinical signs (such as lameness, mastitis, endometritis, and ovarian cysts, among others) were included in the study. In these cows, endometrial cytologies were carried out to assess the presence of SE (Kasimanickam et al., 2004), and the PSP test was performed to check for oviductal patency (Garrido et al., 2020). Additionally, body condition score (BCS) was evaluated by visual observation, and animals were classified in a 1–5 scale (Edmonson et al., 1989). It should be noted that all animals were evaluated during the luteal phase to avoid the prominence of mucosal ridges and folds and fluid accumulation due to high estradiol concentrations, which could lead to OO misdiagnosis.

2.3. Diagnosis of subclinical endometritis

Endometrial cytologies were performed with a small cytobrush (length 20 mm and diameter 0.6 mm) placed inside an insemination rod (Quicklock 2000, Minitube Iberica, Barcelona, Spain). The rod and the cytobrush were protected by an insemination sheath and covered with a plastic sanitary sleeve (Chemise Sanitaire, IMV Technologies, L'Aigle, France). Thereafter, the rod was inserted through the vagina to the external cervical opening. Once inside the cervix, the sanitary sleeve was punctured by the insemination rod, which was then advanced into the uterine body. At this point, the plunger was pressed to externalize the cytobrush, which was rotated against the uterine wall to collect an endometrial sample. Before fully withdrawing the rod, the cytobrush was retracted back inside the rod to avoid contamination from cervical or vaginal cells.

After removing the cytobrush, the sample was extended on a glass slide by rotating the brush and was left to air-dry. The slides were stained using Diff Quick (Quick Panoptic kit, Quimica Clinica Aplicada S.A., Tarragona, Spain). Each slide was examined by the same individual with an optical microscope at 400× magnification (B-192, Optika S.r.l., Ponteranica, Italy). For a quantitative assessment of endometrial inflammation, at least 150 cells (excluding erythrocytes) were counted. Samples containing ≥5 % PMN were classified as positive for SE (Gilbert et al., 2005), while those with <5 % PMN were considered healthy.

2.4. Oviductal patency evaluation

To prepare the PSP solution to perform the evaluation of the oviductal patency (Kothari et al., 1978), 0.3 g phenol red and 4.2 g CO_3HNa were dissolved in 100 mL of distilled water using a magnetic stirrer. The pH was adjusted to 6.8–7.4 and the solution was filtered through a sterile 0.54 μm filter (Millipore UK Ltd., Hertfordshire, UK). Each experimental day, a fresh solution was prepared.

To perform the examination, cows were restrained, and the tail was pushed aside. The vulva and the perineal region were thoroughly cleaned. Thereafter, by rectal palpation, the cervix was hold and a sterile Foley catheter was introduced into one of the uterine horns up to ~4–5 cm cranial to the uterine bifurcation. Then, to affix it inside the horn and to prevent the backflow of the solution, the balloon was filled with air. Afterwards, 80 mL of the PSP solution were slowly introduced with a syringe attached to the catheter. Once all the solution was in, the catheter was clamped, and the syringe was removed. From this moment, up to 3 urine samples were collected by catheterization of the bladder. The first sample was collected 15 min after the PSP infusion; if the urine was dyed with red color, the oviduct was considered as patent. If the urine was not dyed, a second sample was collected 25 min after the PSP infusion. If there was still no dye in the urine, a third sample was collected 45 min after the PSP infusion. If this last sample was not dyed, the oviduct was considered to be occluded. Once urine collection was concluded, the air was removed from the balloon and the catheter was withdrawn.

Cows were examined on two different days, one per each oviduct, at least 24 h apart. The patency of the right oviduct was evaluated on the first day, and the patency of the left oviduct was evaluated on the second day. This technique was performed always during the luteal phase of the estrous cycle.

2.5. Data collection

Additional data were collected from on-farm software (DairyPlan C21, GEA Group AG, Düsseldorf, Germany) in three farms, and Lely Horizon (Lely Industries N.V., Maassluis, Netherlands) in one farm. Parity, calving date, AI date, number of AI, and occurrence of postpartum diseases, including metabolic (ketosis, displaced abomasum), reproductive (dystocia, dead born, twins, placental retention, metritis, clinical endometritis), and other pathologies (clinical and subclinical mastitis) were collected. The different diseases were defined as follows: ketosis, presence of ketone bodies in urine, decreased milk production and food intake; displaced abomasum, condition in which the abomasum becomes enlarged with fluid and/or gas with subsequent migration to the left or right and dorsally within the abdominal cavity; dystocia, difficult calving that required human intervention; placental retention, presence of the fetal membranes in the genital tract 24 h after calving; metritis, abnormally enlarged uterus and fetid watery red-brown uterine discharge associated with signs of systemic illness and fever, within 21 days postpartum; clinical endometritis, inflammatory process of the endometrial lining of the uterus, accompanied by a purulent or mucopurulent vaginal discharge, in the absence of systemic signs of illness, 21 days or more postpartum; clinical mastitis, macroscopic alterations of milk; and subclinical mastitis, somatic cell count >200,000 cell/mL.

2.6. Therapeutic approach

Cows diagnosed as positive to SE were assigned, in the same order as they were diagnosed, to one of the following treatment protocols:

a) Non-steroidal anti-inflammatory drug (NSAID): carprofen 50 mg/mL (Rimadyl®, 1 mL/35 Kg, subcutaneous administration)
b) Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$): dinoprost 12.5 mg/mL (Dinolytic®, 2 mL per cow, intramuscular administration)
c) NSAID+$PGF_{2\alpha}$: both treatments were administered at the same time.

AIs were performed following the first heat. Depending on the treatment, heat could be spontaneous (treatment with NSAID), or synchronized (treatment with $PGF_{2\alpha}$ or NSAID+$PGF_{2\alpha}$). Only one AI was performed, and pregnancy diagnosis was carried out ~30 days after by transrectal ultrasonography.

Additionally, cows with OO were assigned, in the same order as they were diagnosed, to one of the following treatments:

a) Therapeutic embryo: only frozen *in vivo* produced embryos with the cryopreservant Ethylene Glycol (1.5 M) were used. Before performing the direct transfer, the straw was air-exposed for 6 s and introduced in water at 25 °C for 30 s. The embryo transfer was performed as described elsewhere (Yáñez et al., 2023).
b) Artificial insemination: routine artificial insemination procedure was followed.

2.7. Statistical analysis

First, descriptive statistics were performed, and the prevalence of SE and OO was calculated. To evaluate the risk factors for SE in repeat breeder cows, a binary logistic regression was performed, forcing all variables into the model, and including season (winter, spring, summer, autumn), parity (primiparous, multiparous), change of BCS between the dry period and the postpartum (≤ 0.5, > 0.5) and postpartum pathologies (presence or absence, checking for reproductive, metabolic and other pathologies) as independent variables, and the occurrence of SE as dependent variable. Finally, to evaluate the effect of treatment on conception rates, a Pearson's χ^2 test was performed.

All analyses were conducted in IBM SPSS Statistics version 28.0 for Windows (IBM Corp., Armonk, NY, USA). Differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Prevalence of subclinical endometritis and oviductal occlusion

Out of the 99 repeat breeder cows included in the study, 22 (22 %) were positive for SE. Additionally, 3 (3 %) showed alterations of the oviductal patency, all presenting a unilateral occlusion.

3.2. Risk factors for subclinical endometritis

The results for the logistic regression did not show any statistically significant difference for any of the variables evaluated (Table 1). Due to the low prevalence of OO, it was not possible to perform the logistic regression analysis for this pathology.

3.3. Effect of therapeutic approach on conception rate

Regarding SE, statistically significant differences were observed between treatments, as is displayed in Table 2. Cows that were

Table 1
Results for the binary logistic regression analysis to determine the risk factors for subclinical endometritis in 99 repeat breeder Holstein cows.

Variable		% (n)	OR	95 % CI	P-value
Season	Winter	20,6 % (7/34)	1,33	0,27-6,54	0,723
	Spring	20,7 % (6/29)	1,70	0,34-8,31	0,515
	Summer	37,5 % (6/16)	3,93	0,70-22,01	0,119
	Autumn	15,0 % (3/20)	–	–	–
Parity	Primiparous	15,4 % (6/39)	0,43	0,14-1,31	0,135
	Multiparous	26,7 % (16/60)	–	–	–
BCS	≤0,5	20,4 % (10/49)	1,24	0,41-3,77	0,706
	>0,5	24,0 % 12/50)	–	–	–
Postpartum reproductive pathology	No	18,6 % (13/70)	0,50	0,17-1,45	0,204
	Yes	31,0 % (9/29)	–	–	–
Postpartum metabolic pathology	No	21,0 % (17/81)	0,65	0,18-2,36	0,512
	Yes	27,8 % (5/18)	–	–	–
Other postpartum pathologies	No	23,1 % (9/39)	1,19	0,43-3,31	0,741
	Yes	21,7 % (13/60)	–	–	–

%: percentage of cows positive for subclinical endometritis. OR: odds ratio. 95 % CI: 95 % confidence interval. BCS: change of BCS between the dry period and the postpartum.

Table 2
Descriptive statistics for oviductal occlusion and results for the Pearson's χ^2analysis to determine the effect of three different treatments on conception rates in 22 repeat breeder Holstein cows positive to subclinical endometritis.

Pathology	Treatment	Conception rate (n)
SE* ($n = 22$)	NSAID	14,3 % (1/7)[b]
	$PGF_{2\alpha}$	0 % (0/7)[a]
	NSAID+ $PGF_{2\alpha}$	62,5 % (5/8)[b]
OO ($n = 3$)	AI	0 % (0/1)
	Therapeutical embryo	100 % (2/2)

* $p < 0.05$. ab: Different letters within the same column indicate statistically significant differences. SE: subclinical endometritis. OO: oviductal occlusion. NSAID: treatment with non-steroidal anti-inflammatory drug. $PGF_{2\alpha}$: treatment with prostaglandin $F_{2\alpha}$. AI: artificial insemination.

administered the NSAID, either alone or in combination with $PGF_{2\alpha}$, showed higher conception rates in the following AI ($p < 0.05$). Similarly to risk factors, the statistical analysis for the effect of treatment on conception rate concerning OO was not possible due to the reduced prevalence of this pathology.

4. Discussion

4.1. Prevalence and risk factors for subclinical endometritis

The prevalence for SE observed in our study was 22 %. It should be noted that the prevalence of this pathology during the postpartum has been broadly studied, and different results were described depending on the day of the diagnosis, the threshold established for the %PMN, and even the technique used to collect samples (cytobrush, uterine lavage, cytotape). Taking this into account, the prevalence of SE between the third and the seventh week postpartum varies between 7 and 53 % (Quintela et al., 2018). However, at the time of the first AI, the number of studies is scarce (Diaz-Lundahl et al., 2021; Pascottini et al., 2017a, 2017b). The prevalence observed in these two studies, including a large sample size (1625 AI samples from 873 cows in the first case, and 1648 cows in the second case), was very similar, in spite of using slightly different cut-off points. Diaz-Lundahl et al. (2021), with a cut-off point of 3 %, observed a prevalence of 28 %; on the other hand, Pascottini et al. (2017a, 2017b) reported a prevalence of 27.8 % establishing a cut-off point of 1 %. In both studies, the technique used was the cytotape. Regarding RB cows, the prevalence of SE varies between 12.7 and 16.6 % (Bedewy and Rahawy, 2019; Pothmann et al., 2015; Wagener et al., 2017) and 40.2–52.8 % (Janowski et al., 2013; Salasel et al., 2010). However, different cut-off points and techniques were used among studies. Therefore, it is not yet possible to come to a clear conclusion about the importance of SE regarding RB cattle.

As for the risk factors for SE in RB cows, none of the variables included in the statistical analysis showed a significant effect, which might be probably due to the lower number of cases of SE. Although not significant, we observed numerical differences depending on season, parity and the presence of reproductive postpartum pathologies. In this way, statistically significant differences were observed in other studies between animals that had had reproductive pathologies during the postpartum (40 % in healthy cows and 80 % in animals that had suffered from dystocia or uterine inflammation, Salasel et al., 2010). Concerning postpartum cows, the occurrence of SE is related to previous reproductive pathologies, metabolic alterations and other factors involving the condition of the facilities and the physiology of the cow (Priest, 2013). Additionally, it has been reported that the month/season of AI, DIM and parity also significantly affect the occurrence of cytological endometritis in dairy cows at first AI (Diaz-Lundahl et al., 2021; Pascottini et al., 2017b). In our study, we included the variable season accounting for the calving time, not AI. However, the difference observed could be due to the heat stress during summer, which could favor immunosuppression during postpartum and the occurrence of postpartum pathologies, which in turn would also influence the presence of SE later in lactation.

4.2. Prevalence and risk factors for oviductal occlusion

The prevalence for OO observed in our study was 3 %, unilateral in all cases, suggesting that this pathology is not likely to be an important cause of RB syndrome, in contrast to what has been reported in previous studies (Garrido et al., 2020). These researchers found that 44 % of the animals explored had some degree of oviduct obstruction, with 4 % having bilateral occlusion and 20 % unilateral occlusion. It should be noted that the technique used in both studies was the same, although sample size in Garrido et al. (2020) was 50 cows, half of the sample size of the current study. The area conditions under which both experiments were conducted, and selection criteria were very similar. Thus, the different prevalence observed between studies could be due to the person who performed the PSP test or to the consequence of random selection of animals that met the inclusion criteria. However, it should be noted that both practitioners were experienced and had proper training to perform the PSP test. Another possibility could be the different prevalence of uterine diseases, including the precocity of the diagnosis and the effectiveness of the treatment. As mentioned below, oviductal issues have been linked to uterine diseases such as metritis or endometritis (Azawi, 2009). However, since the prevalence for uterine disease was not reported in the study conducted by Garrido et al. (2020), this option remains merely speculative.

Additionally, it should be noticed that the PSP test is not the only existing technique for diagnosing oviductal patency. For instance, Kauffold et al. (2009) and Itoh et al. (2016) tested tubal patency in dairy cattle using contrast sonography, and Arnold and Love (2013) used laparoscopic evaluation in mares. Although sample size was noticeably lower (eight cows, five cows, and sixteen mares, respectively), important findings were reported. As the animals used in the study conducted by Kauffold et al. (2009) were slaughtered the day after evaluation, they could confirm the veracity of the ultrasound evaluation. A total of five patent oviducts were diagnosed correctly, while two out of five oviducts diagnosed as occluded were morphologically intact. Additionally, these researchers observed that two oviducts were occluded due to hydrosalpinx and one due to inflammation. However, Itoh et al. (2016) only found two cases of tubal obstruction. This diagnostic technique seems a good alternative to the PSP test, although it also has some drawbacks. For example, ~20–30 min per cow are required to perform the ultrasound examination, which may lead to rectal bleeding and poor image quality.

As for laparoscopic evaluation, test sensitivity of 71.4 % and specificity of 85.7 % were reported (Arnold and Love, 2013). Additionally, the main cause of obstruction or occlusion observed in this study were oviductal plugs, either moderately sized (a mass that filled the lumen but not distend it) or small (a mass smaller in diameter than the lumen). However, this diagnostic technique not only requires surgical intervention, but also a uterine lavage 48 h after. Consequently, the cost of the procedure would be high and, therefore, unaffordable for most dairy farms.

Occluded oviducts may result in infertility or even sterility due to the disruption or obstruction of normal gamete transport. Additionally, the oviduct also offers the appropriate environmental conditions to facilitate gamete maturation, fertilization and early embryo development (Killian, 2011). Therefore, any pathological condition that disrupts the normal secretory function of the oviductal epithelium might impair fertilization or embryo development, even without causing tubal blockage. Consequently, the identification of bilateral oviduct patency in RB cows does not exclude the oviduct as a potential cause of infertility (Garrido et al., 2020).

Due to the low prevalence, it was not possible to perform a reliable statistical analysis to evaluate the risk factors for OO. To our knowledge, there are not any research articles about risk factors for OO in dairy cattle in the literature. Nevertheless, it has been suggested that OO could be a consequence of salpingitis, which could be associated with metritis or endometritis (Azawi, 2009). Therefore, it could be hypothesized that the risk factors widely described for these two reproductive pathologies, such as dystocia, negative energy balance or parity (Dubuc et al., 2010; Yáñez et al., 2022), could also lead to oviduct blockage if the disease is not properly diagnosed or treated and progresses to more serious conditions.

4.3. Effect of the therapeutic approach on conception rate

Although the number of animals treated for SE is reduced due to the relative low prevalence observed, our results suggest that it may be possible to improve conception rates in RB cows with the administration of a combination of an NSAID and $PGF_{2\alpha}$. There is plenty of information about this topic regarding the postpartum period. However, as for RB syndrome, little data is available in the literature. During postpartum, antibiotics and prostaglandins, either individually or combined, have been used to treat SE, although results are not conclusive (Quintela et al., 2018). Additionally, as in most SE cases there is not bacterial contamination in the uterus, but there is evidence of an inflammatory state, treatment with NSAIDs has been tested with positive results (Priest et al., 2013). In RB cows, when the main cause of infertility is unknown, multiple treatments were administered, including hormones, uterine lavages with saline solution, antiseptics or antibiotics, NSAIDs, and assisted reproduction techniques (Pérez-Marín et al., 2012). Concerning NSAIDs, they can be administered alone or in combination with different hormones. It has been reported that the combination of meloxicam, GnRH, and progesterone significantly improved conception rates in RB cows (35.7 %) compared to cows treated with GnRH alone (20.0 %) or non-treated (17.8 %) (Amiridis et al., 2009). In the same study, meloxicam administration alone (conception rate = 22.6 %) did not show significant differences with any of the other experimental groups.

This is in agreement with the results observed in the current study, as the administration of carprofen, either alone or in combination with $PGF_{2\alpha}$, significantly increased conception rates. It has been described that a strong and fast migration of PMN to the uterine lumen immediately after calving is linked to improved uterine health (Gilbert and Santos, 2016). Nevertheless, the amount of PMN inside the uterus should decrease after finalizing their duty. In this way, persistent recruitment of PMN to the uterus by malfunctioning immune cells can lead to damage of the endometrial layer and contribute to the development of disease (Pascottini et al., 2023). These researchers stated that SE reflects maladaptation to a metabolic challenge and dysregulation of inflammation, and that the metabolic stress and inflammation not only precedes uterine disease but also might be triggered in the prepartum period. Consequently, the administration of an NSAID would help controlling the immune response inside the uterus and, in addition to adequate management practices to avoid additional stress and ensure proper energy balance, would allow the animal to overcome this disease.

Regarding OO, the effect of treatment on conception rates could not be tested due to the low number of cases identified. It has been previously described that transfer of *in vitro* or *in vivo* produced embryos could be a good alternative for cows with bilateral OO (Garrido et al., 2020). Additionally, it should be noted that, if the cause of the occlusion is reversible, for example salpingitis, pregnancy following AI is still possible. However, this would require either multiple AI attempts or serial evaluations to check for oviductal patency, which would eventually increase the economic losses due to the RB syndrome.

5. Conclusions

Neither SE nor OO seem to be the main cause of RB syndrome in the cows under study. Additionally, in those cases in which SE is diagnosed as the main cause of RB syndrome, the therapeutic approach with NAISDs seemed to increase conception rates. However, the lack of studies on this matter, the great discrepancy among them, and the small number of animals included indicate the need for further research with a larger sample size.

Ethics approval

The experiment was conducted according to the European Union Legislation (2010/63/EU) and the Spanish Regulations for the protection of animals used for scientific purposes (RD 53/2013).

CRediT authorship contribution statement

Sofía L. Villar: Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization. **Carlos C. Pérez-Marín:** Writing – review & editing, Visualization, Validation, Resources, Investigation. **Jacobo Álvarez:** Writing – review & editing, Visualization, Methodology, Investigation. **Uxía Yáñez:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Juan J. Becerra:** Writing – review & editing, Visualization, Validation, Investigation. **Ana I. Peña:** Visualization, Investigation, Formal analysis, Data curation. **Pedro G. Herradón:** Writing – review & editing, Visualization, Validation, Investigation. **Luis A. Quintela:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

None of the authors have any conflict of interest to declare.

Acknowledgements

The authors wish to thank all farmers and veterinary surgeons for their collaboration in the study. Uxía Yáñez was funded by Xunta de

Galicia (Predoctoral Contract Ref. ED481A-2020/122).

Data availability

Data could be provided by the corresponding author under reasonable request.

References

Amiridis, G.S., Tsiligianni, Th., Dovolou, E., Rekkas, C., Vouzaras, D., Menegatos, I., 2009. Combined administration of gonadotropin-releasing hormone, progesterone, and meloxicam is an effective treatment for the repeat-breeder cow. Theriogenology 72, 542–548. https://doi.org/10.1016/j.theriogenology.2009.04.010.

Arnold, C.E., Love, C.C., 2013. Laparoscopic evaluation of oviductal patency in the standing mare. Theriogenology 79, 905–910. https://doi.org/10.1016/j.theriogenology.2012.12.004.

Asaduzzaman, K.M., Bhuiyan, M.M.U., Rahman, M.M., Bhattacharjee, J., 2016. Prevalence of repeat breeding syndrome and its effective treatment in cows at selected areas of Bangladesh. BJVM 14, 183–193.

Azawi, O.I., 2009. A study on the pathological lesions of oviducts of buffaloes diagnosed at postmortem. Vet. Res. Commun. 33, 77–85. https://doi.org/10.1007/s11259-008-9075-5.

Bedewy, R.B., Rahawy, M.A., 2019. Comparative study for detection of subclinical endometritis in local cows. Adv. Anim. Vet. Sci. 7. https://doi.org/10.17582/journal.aavs/2019/7.4.289.294.

Cummins, S.B., Lonergan, P., Evans, A.C.O., Butler, S.T., 2012. Genetic merit for fertility traits in Holstein cows: II. Ovarian follicular and corpus luteum dynamics, reproductive hormones, and estrus behavior. J. Dairy Sci. 95, 3698–3710. https://doi.org/10.3168/jds.2011-4976.

Deka, R.P., Magnusson, U., Grace, D., Randolph, T.F., Shome, R., Lindahl, J.F., 2021. Estimates of the economic cost caused by five major reproductive problems in dairy animals in Assam and Bihar, India. Animals 11, 3116. https://doi.org/10.3390/ani11113116.

Diaz-Lundahl, S., Garmo, R.T., Gillund, P., Klem, T.B., Waldmann, A., Krogenæs, A.K., 2021. Prevalence, risk factors, and effects on fertility of cytological endometritis at the time of insemination in Norwegian red cows. J. Dairy Sci. 104, 6961–6974. https://doi.org/10.3168/jds.2020-19211.

Dubuc, J., Duffield, T.F., Leslie, K.E., Walton, J.S., LeBlanc, S.J., 2010. Risk factors for postpartum uterine diseases in dairy cows. J. Dairy Sci. 93, 5764–5771. https://doi.org/10.3168/jds.2010-3429.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72, 68–78. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.

Ellington, J.E., Schläfer, D.H., 1993. Uterine tube disease in cattle. JAVMA 202, 450–454. https://doi.org/10.2460/javma.1993.202.03.450.

Földi, J., Kulcsár, M., Pécsi, A., Huyghe, B., de Sa, C., Lohuis, J.A.C.M., Cox, P., Huszenicza, Gy, 2006. Bacterial complications of postpartum uterine involution in cattle. Anim. Reprod. Sci. 96, 265–281. https://doi.org/10.1016/j.anireprosci.2006.08.006.

Garcia-Ispierto, I., López-Gatius, F., 2017. Progesterone supplementation in the early luteal phase after artificial insemination improves conception rates in high-producing dairy cows. Theriogenology 90, 20–24. https://doi.org/10.1016/j.theriogenology.2016.11.006.

Garrido, M.R., Peña, A.I., Herradon, P.G., Becerra, J.J., Sande, J., Quintela, L.A., 2020. Short communication: evaluation of tubal patency in repeat breeder Holstein cows. SJAR 17, e04SC02. https://doi.org/10.5424/sjar/2019174-15419.

Gilbert, R.O., Santos, N.R., 2016. Dynamics of postpartum endometrial cytology and bacteriology and their relationship to fertility in dairy cows. Theriogenology 85, 1367–1374. https://doi.org/10.1016/j.theriogenology.2015.10.045.

Gilbert, R.O., Shin, S.T., Guard, C.L., Erb, H.N., Frajblat, M., 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 64, 1879–1888. https://doi.org/10.1016/j.theriogenology.2005.04.022.

Gustafsson, H., Emanuelson, U., 2002. Characterisation of the repeat breeding syndrome in Swedish dairy cattle. Acta Vet. Scand. 43, 115–125.

Itoh, K., Endo, N., Kataoka, S.I., Tanaka, T., 2016. 0121 assessment of tubal patency by hysterosalpingo-contrast sonography in cow. J. Anim. Sci. 94, 56–57. https://doi.org/10.2527/jam2016-0121.

Janowski, T., Barański, W., Łukasik, K., Skarżyński, D., Rudowska, M., Zduńczyk, S., 2013. Prevalence of subclinical endometritis in repeat breeding cows and mRNA expression of tumor necrosis factor α and inducible nitric oxide synthase in the endometrium of repeat breeding cows with and without subclinical endometritis. Pol. J. Vet. Sci. 16, 693–699. https://doi.org/10.2478/pjvs-2013-0098.

Kasimanickam, R., Duffield, T.F., Foster, R.A., Gartley, C.J., Leslie, K.E., Walton, J.S., Johnson, W.H., 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 62, 9–23. https://doi.org/10.1016/j.theriogenology.2003.03.001.

Kauffold, J., Groeger, S., Bergmann, K., Wehrend, A., 2009. Use of contrast sonography to test for tubal patency in dairy cattle. J. Reprod. Dev. 55, 335–338. https://doi.org/10.1262/jrd.20168.

Killian, G., 2011. Evidence that oviduct secretions influence sperm function: a retrospective view for livestock1. J. Anim. Sci. 89, 1315–1322. https://doi.org/10.2527/jas.2010-3349.

Kothari, B., Renton, J., Munro, C., Macfarlane, J., 1978. Use of the phenolsulphonphthalein dye test for fallopian tube patency in cattle. Vet. Rec. 103, 229–232. https://doi.org/10.1136/vr.103.11.229.

LeBlanc, S., 2010. Monitoring metabolic health of dairy cattle in the transition period. J. Reprod. Dev. 56, S29–S35. https://doi.org/10.1262/jrd.1056S29.

López-Gatius, F., 2003. Is fertility declining in dairy cattle? Theriogenology 60, 89–99. https://doi.org/10.1016/S0093-691X(02)01359-6.

Norman, H.D., Wright, J.R., Hubbard, S.M., Miller, R.H., Hutchison, J.L., 2009. Reproductive status of Holstein and Jersey cows in the United States. J. Dairy Sci. 92, 3517–3528. https://doi.org/10.3168/jds.2008-1768.

Nowicki, A., 2021. Embryo transfer as an option to improve fertility in repeat breeder dairy cows. J. Vet. Res. 65, 231–237. https://doi.org/10.2478/jvetres-2021-0018.

Pascottini, O.B., Hostens, M., Sys, P., Vercauteren, P., Opsomer, G., 2017a. Cytological endometritis at artificial insemination in dairy cows: prevalence and effect on pregnancy outcome. J. Dairy Sci. 100, 588–597. https://doi.org/10.3168/jds.2016-11529.

Pascottini, O.B., Hostens, M., Sys, P., Vercauteren, P., Opsomer, G., 2017b. Risk factors associated with cytological endometritis diagnosed at artificial insemination in dairy cows. Theriogenology 92, 1–5. https://doi.org/10.1016/j.theriogenology.2017.01.004.

Pascottini, O.B., LeBlanc, S.J., Gnemi, G., Leroy, J.L.M.R., Opsomer, G., 2023. Genesis of clinical and subclinical endometritis in dairy cows. Reproduction 166, R15–R24. https://doi.org/10.1530/REP-22-0452.

Pérez-Marín, C.C., Quintela, L.A., 2023. Current insights in the repeat breeder cow syndrome. Animals 13, 2187. https://doi.org/10.3390/ani13132187.

Pérez-Marín, C.C., Molina-Moreno, L., Vizuete, C.G., 2012. Clinical approach to the repeat breeder cow syndrome. In: Pérez-Marín, C.C. (Ed.), A Bird's-Eye View of Veterinary Medicine. IntechOpen, Rijeka, pp. 337–362.

Plaizier, J.C.B., King, G.J., Dekkers, J.C.M., Lissemore, K., 1997. Estimation of economic values of indices for reproductive performance in dairy herds using computer simulation. J. Dairy Sci. 80, 2775–2783. https://doi.org/10.3168/jds.S0022-0302(97)76240-4.

Pothmann, H., Prunner, I., Wagener, K., Jaureguiberry, M., de la Sota, R.L., Erber, R., Aurich, C., Ehling-Schulz, M., Drillich, M., 2015. The prevalence of subclinical endometritis and intrauterine infections in repeat breeder cows. Theriogenology 83, 1249–1253. https://doi.org/10.1016/j.theriogenology.2015.01.013.

Powell, R., 2004. Genetic programs for dairy cattle in the United States. In: Proceedings of the International Conference on Dairy Cattle, July 14–16, Guadalajara, Mexico, pp. 123–132.

Priest, N., 2013. Effect of a Non-steroidal Anti-Inflammatory Drug on Subclinical Endometritis in Dairy Cows and the Identification of at-Risk Cows. Lincoln University.

Priest, N.V., McDougall, S., Burke, C.R., Roche, J.R., Mitchell, M., McLeod, K.L., Greenwood, S.L., Meier, S., 2013. The responsiveness of subclinical endometritis to a nonsteroidal antiinflammatory drug in pasture-grazed dairy cows. J. Dairy Sci. 96, 4323–4332. https://doi.org/10.3168/jds.2012-6266.

Quintela, L.A., Vigo, M., Becerra, J.J., Barrio, M., Peña, A.I., Herradón, P.G., 2017. Endometritis subclínica en ganado vacuno lechero: etiopatogenia y diagnóstico. ITEA 113, 250–266.

Quintela, L.A., Vigo, M., Becerra, J.J., Barrio, M., Herradón, P.G., Peña, A.I., 2018. Subclinical endometritis in dairy cattle. In: Payan-Carreira, R. (Ed.), New Insights into Theriogenology. IntechOpen, London, pp. 79–97.

Salasel, B., Mokhtari, A., Taktaz, T., 2010. Prevalence, risk factors for and impact of subclinical endometritis in repeat breeder dairy cows. Theriogenology 74, 1271–1278. https://doi.org/10.1016/j.theriogenology.2010.05.033.

Sheldon, I.M., Lewis, G.S., LeBlanc, S., Gilbert, R.O., 2006. Defining postpartum uterine disease in cattle. Theriogenology 65, 1516–1530. https://doi.org/10.1016/j.theriogenology.2005.08.021.

Sood, P., Zachut, M., Dube, H., Moallem, U., 2015. Behavioral and hormonal pattern of repeat breeder cows around estrus. Reproduction 149, 545–554. https://doi.org/10.1530/REP-14-0598.

Stephani de Souza, J., Costa-Alexandre, E., Quintela, L.A., Becerra, J.J., Cainzos, J., Rivas, F., Prieto, A., Herradón, P.G., 2010. Incidencia de alteraciones en el aparato reproductor de vacas sacrificadas en un matadero comercial de la Provincia de Lugo (Noroeste de España). ITEA 106, 100–114.

Talukdar, D.J., Talukdar, P., Ahmed, K., 2016. Minerals and its impact on fertility of livestock: a review. Agric. Rev. 37, 333–337. https://doi.org/10.18805/ag.v37i4.6464.

Wagener, K., Pothmann, H., Prunner, I., Peter, S., Erber, R., Aurich, C., Drillich, M., Gabler, C., 2017. Endometrial mRNA expression of selected pro-inflammatory factors and mucins in repeat breeder cows with and without subclinical endometritis. Theriogenology 90, 237–244. https://doi.org/10.1016/j.theriogenology.2016.12.013.

Walsh, S.W., Williams, E.J., Evans, A.C.O., 2011. A review of the causes of poor fertility in high milk producing dairy cows. Anim. Reprod. Sci. 123, 127–138. https://doi.org/10.1016/j.anireprosci.2010.12.001.

Yáñez, U., Herradón, P.G., Becerra, J.J., Peña, A.I., Quintela, L.A., 2022. Relationship between postpartum metabolic status and subclinical endometritis in dairy cattle. Animals 12, 242. https://doi.org/10.3390/ani12030242.

Yáñez, U., Barrio, M., Fernández, I., Becerra, J.J., Herradón, P.G., Peña, A.I., Quintela, L. A., 2023. Assessment of luteal function using rectal palpation, B-mode ultrasonography, and progesterone determination to improve recipient selection in embryo transfer programs. Animals 13, 2865. https://doi.org/10.3390/ani13182865.

Yusuf, M., Rahim, L., Asja, M.A., Wahyudi, A., 2012. The incidence of repeat breeding in dairy cows under tropical condition. Media Peternakan 35, 28–31. https://doi.org/10.5398/medpet.2012.35.1.28.