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Investigation of Subclinical Endometritis and Oviductal Occlusion in Holstein Repeat Breeders: Prevalence, Risks, and Therapeutic Interventions

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ABSTRACT

Because Repeat Breeder (RB) syndrome leads to considerable reproductive and financial losses in dairy farming, this research aimed to estimate how frequently subclinical endometritis (SE) and oviductal occlusion (OO) occur in RB cows, identify their risk factors, and explore possible treatments. Ninety-nine RB cows were examined using endometrial cytology to detect SE, diagnosed when polymorphonuclear neutrophils exceeded 5%. Oviduct patency was evaluated with the phenolsulfonphthalein (PSP) test. Body condition scores were recorded, and reproductive information—including parity, calving and insemination dates, number of artificial inseminations (AI), and postpartum illnesses—was collected from herd records. Cows positive for SE were assigned to one of three therapeutic groups: (a) non-steroidal anti-inflammatory drug (NSAID), (b) prostaglandin F2α (PGF2α), or (c) NSAID combined with PGF2α. Those diagnosed with OO received either (a) a therapeutic embryo or (b) AI. SE and OO were found in 22% and 3% of cows, respectively. Logistic regression revealed no significant associations between SE and any studied risk factor. Cows receiving NSAIDs, alone or combined with PGF2α, had higher conception rates at the next insemination ($p < 0.05$). Because OO was rare, statistical modeling was not performed for this condition. Overall, SE and OO do not appear to be major contributors to RB syndrome in this population. Nevertheless, NSAID therapy for SE may improve conception success when SE is confirmed as the main underlying issue.

Keywords: Dairy cows, Repeat breeder syndrome, Subclinical endometritis, Oviductal blockage

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Introduction

Intensive dairy systems now operate under growing global pressure, driven by market competition, volatile fuel and feed prices, and accelerating climate challenges. Under these circumstances, maintaining efficient reproduction is critical, as it directly influences farm profitability [1]. The fundamental aim of reproductive management is to achieve conception at an optimal interval following calving [2, 3].

During the past several decades, fertility rates in dairy cows have declined progressively—by roughly 0.5–1% per year worldwide [4]. Although recent years have shown slight recovery, likely due to refined management and genetic strategies, reproductive inefficiencies remain a major constraint [5, 6]. Among these issues, Repeat

Breeder (RB) syndrome stands out as a significant problem, characterized by extended calving-to-conception intervals, multiple inseminations per pregnancy, and an increased risk of involuntary culling, all of which reduce herd productivity [7].

RB syndrome is typically described as failure to conceive after three or more inseminations, despite the absence of evident reproductive tract abnormalities. Reported prevalence varies widely, from 10–25% in most regions [8–11], and may reach as high as 62% in tropical herds [12]. This global distribution suggests that RB syndrome remains a serious but potentially manageable fertility disorder [13].

Several predisposing factors have been proposed, including nutritional deficits [14], weak or irregular estrous behavior and inaccurate heat detection [15, 16], inefficient AI techniques [3], and hormonal disturbances [16]. Nonetheless, the role of uterine and oviductal pathology in RB syndrome has received comparatively little attention.

One uterine condition of interest is subclinical endometritis (SE)—an inflammatory disorder of the uterine lining that develops without visible clinical symptoms [17]. It is identified by the infiltration of polymorphonuclear neutrophils (PMN) within the endometrium and minimal to no uterine discharge [18]. The cytobrush sampling technique is a standard diagnostic method, with SE defined based on PMN percentage (%PMN). Reported diagnostic thresholds vary, from ≥5% to over 18% PMN [19]. SE has been repeatedly suggested as a contributing factor to RB syndrome [20], although available studies are limited and often yield conflicting prevalence results.

Additional factors related to infertility in repeat breeder cows

Beyond abnormalities in the uterine lining, oviductal disorders are another reproductive constraint that can severely compromise fertility in repeat breeder (RB) cows, as they frequently interfere with fertilization. The oviduct functions not merely as a passage connecting the ovary and uterus but also as an essential microenvironment that supports gamete maturation, fertilization, and the first stages of embryonic growth [21]. Therefore, its proper functioning is fundamental for reproductive success.

Determining how common these disorders are remains complex. Previous research has reported oviductal disease prevalence ranging from 36% to 89% among RB cattle [22], though the inconsistency likely arises from the different diagnostic tools used across studies. While ultrasound and rectal palpation can reveal major oviductal deformities, many obstructed oviducts present no visible abnormalities. For instance, Stephani de Souza *et al.* (2010) observed in slaughterhouse samples that 60.4% of oviducts later identified as occluded had a normal outward appearance [23].

A few in vivo diagnostic options exist, such as the phenolsulfonphthalein (PSP) test [24] and contrast-enhanced hysterosalpingosonography [25]. However, these procedures are time-consuming, costly, and technically demanding, which restricts their routine use. Consequently, most data on oviductal problems originate from post-mortem genital tracts, while live-animal trials remain limited and small-scale (n = 8–50) [24–26].

Because repeat breeder syndrome can result from several concurrent factors, treatment approaches must be tailored individually. When either subclinical endometritis (SE) or oviductal occlusion (OO) is confirmed, specific therapeutic actions can be applied. In the case of SE, inflammation can disrupt both the hypothalamic-pituitary-ovarian (HPO) axis and the uterine environment [27, 28]. Therefore, anti-inflammatory drugs—notably carprofen and meloxicam—have been explored as potential therapies with encouraging outcomes [29–31]. Conventional treatments, such as prostaglandins or antibiotics, have also been evaluated, though their efficacy has shown inconsistent results [28].

When oviductal blockage occurs, fertilization becomes impossible since sperm cells cannot reach the oocyte. Hence, the use of a therapeutic embryo transfer has been regarded as the most effective reproductive alternative [7].

Taking all these elements into account, the goals of this work were:

1. To estimate the prevalence and evaluate risk factors associated with subclinical endometritis and oviductal occlusion in Holstein repeat breeder cows, and
2. To propose a specific therapeutic plan suitable for managing these reproductive pathologies.

Materials and Methods

Animals

This investigation included 99 lactating Holstein cows identified as repeat breeders from four free-stall dairy herds located in Cantabria, northern Spain. The herd sizes ranged from 265 to 370 milking cows. Three herds used traditional milking parlors, milking cows two or three times daily, while the remaining herd used an automated milking system averaging 3.1 milkings per cow each day. Mean milk yield was approximately 12,128 kg per cow annually.

Cows were fed a total mixed ration (TMR) based on corn silage, grass silage, and alfalfa, supplemented with concentrates according to their individual energy needs. Weekly reproductive checks were carried out by an experienced veterinarian, and each herd followed a structured health program, which included uterine assessment at four weeks postpartum.

The voluntary waiting period (VWP) was 70 days for multiparous and 90 days for primiparous cows. Average days in milk (DIM) at sampling were 215.30 ± 77.20, ranging from 140 to 560 days. The first insemination after VWP was performed following heat detection in all herds. Multiparous cows reaching 80 DIM and primiparous cows at 100 days in lactation that had not been inseminated were subjected to an Ovsynch synchronization program. Cows that tested non-pregnant at diagnosis were resynchronized using the same protocol. Only one herd relied exclusively on heat detection, supported by automated activity sensors, for insemination timing.

Animal selection

Cows meeting the definition of repeat breeder were identified during routine herd examinations. Eligible cows were those with three or more unsuccessful artificial inseminations (range: 3–15; mean = 5.15) and confirmed non-pregnant. Each selected animal underwent a comprehensive reproductive evaluation, including external inspection, manual palpation, and transrectal ultrasonography with a 5.0 MHz linear-array transducer (ProVetScan SR-2C, New Veterinary Technologies, León, Spain).

Only cows free from clinical diseases—such as lameness, mastitis, metritis, or ovarian cysts—were retained for the study. For these individuals, endometrial cytology was carried out following Kasimanickam *et al*. (2004) to identify subclinical endometritis, while oviductal patency was examined through the PSP test described by Garrido *et al*. (2020) [24, 32]. Body condition scores (BCS) were visually assessed on a 1–5 scale [33].

All procedures were conducted during the luteal phase, minimizing the influence of elevated estradiol, which can lead to increased mucosal folds and intrauterine fluid accumulation, potentially causing false-positive diagnoses of oviductal occlusion.

Diagnosis of subclinical endometritis

For the assessment of subclinical endometritis, endometrial samples were obtained using a miniature cytobrush (20 mm length, 0.6 mm diameter) mounted inside an insemination rod (Quicklock 2000, Minitube Ibérica, Barcelona, Spain). Both components were inserted into an insemination sheath and encased in a protective sanitary cover (Chemise Sanitaire, IMV Technologies, L'Aigle, France). The apparatus was guided through the vagina until it reached the external cervical os. Once positioned, the sanitary sheath was perforated by the rod and advanced into the uterine cavity. The plunger was then pushed to expose the brush, which was rotated gently against the endometrial lining to collect the sample. Before removal, the brush was retracted to avoid cellular contamination from the cervix or vagina.

The collected material was immediately smeared onto a microscope slide, air-dried, and stained using Diff Quick (Quick Panoptic kit, Química Clínica Aplicada S.A., Tarragona, Spain). Slides were evaluated by a single trained observer under 400× magnification (B-192 microscope, Optika S.r.l., Ponteranica, Italy). A minimum of 150 nucleated cells (excluding erythrocytes) were counted per sample. Cytologies with ≥5% polymorphonuclear neutrophils (PMNs) were considered positive for subclinical endometritis (SE), whereas samples containing <5% PMNs were classified as non-inflamed [34].

Assessment of oviductal patency

The phenolsulfonphthalein (PSP) test was used to evaluate tubal patency, following the procedure described by Kothari *et al*. (1978) [35]. A solution was prepared daily by dissolving 0.3 g of phenol red and 4.2 g of sodium carbonate (Na_2CO_3) in 100 mL of distilled water with magnetic stirring. The pH was adjusted to 6.8–7.4, and the mixture filtered through a 0.54 µm sterile membrane (Millipore UK Ltd., Hertfordshire, UK).

For testing, cows were safely restrained, and the perineal region was cleansed thoroughly. Using rectal guidance, the cervix was stabilized, and a Foley catheter was inserted into one uterine horn, approximately 4–5 cm anterior to the uterine bifurcation. The balloon at the catheter tip was then inflated to fix it in place and prevent reflux of the dye. A total of 80 mL of the PSP solution was infused slowly via a syringe, which was then removed after clamping the catheter.

Urine was collected by catheterizing the bladder at intervals of 15, 25, and 45 minutes after infusion. The presence of a reddish coloration indicated a patent oviduct. If no dye was detected after the third sample (45 min), the oviduct was recorded as occluded. After collection, the air was released from the balloon, and the catheter was withdrawn.

Each cow underwent the test on two separate days, with a 24-hour interval between sessions. The right oviduct was examined on the first day and the left on the second. All evaluations were performed during the luteal phase to ensure hormonal consistency.

Data recording

Reproductive and management information was obtained from herd management software: DairyPlan C21 (GEA Group AG, Düsseldorf, Germany) for three farms and Lely Horizon (Lely Industries N.V., Maassluis, Netherlands) for one farm. Data included parity, calving and insemination dates, number of artificial inseminations (AIs), and the incidence of postpartum diseases categorized as metabolic, reproductive, or other disorders.

Diagnostic definitions were as follows:

- Ketosis: presence of urinary ketone bodies, reduced milk yield, and poor appetite.
- Displaced abomasum: accumulation of gas or liquid causing abomasal displacement within the abdominal cavity.
- Dystocia: calving difficulty requiring human assistance.
- Retained placenta: fetal membranes not expelled within 24 hours postpartum.
- Metritis: enlarged uterus with fetid reddish-brown discharge and signs of systemic illness within 21 days after calving.
- Clinical endometritis: uterine inflammation showing purulent/mucopurulent discharge 21 or more days postpartum, without systemic effects.
- Clinical mastitis: visible milk abnormalities.
- Subclinical mastitis: somatic cell count >200,000 cells/mL.

Treatment protocols

Cows identified as positive for SE were alternately assigned, according to detection order, to one of three therapeutic treatments:

a) Non-steroidal anti-inflammatory drug (NSAID): Carprofen 50 mg/mL (Rimadyl®), administered subcutaneously at 1 mL per 35 kg body weight.

b) Prostaglandin $F_2\alpha$ ($PGF_2\alpha$): Dinoprost 12.5 mg/mL (Dinolytic®), 2 mL intramuscularly per animal.

c) NSAID + $PGF_2\alpha$: both treatments given simultaneously.

Artificial insemination was performed at the first detected estrus post-treatment. Depending on the protocol, estrus was either natural (NSAID only) or induced ($PGF_2\alpha$ or NSAID + $PGF_2\alpha$). Each cow was inseminated once, and pregnancy diagnosis was carried out about 30 days later via transrectal ultrasonography.

Cows diagnosed with oviductal occlusion (OO) were also randomly assigned to one of two procedures:

a) Therapeutic embryo transfer: Only frozen in vivo–produced embryos preserved with 1.5 M ethylene glycol were used. Before transfer, the straw was exposed to air for 6 seconds and immersed in 25°C water for 30 seconds. The embryo transfer followed the method described by Yáñez *et al*. (2023) [36].

b) Artificial insemination: standard AI protocol performed.

Statistical procedures

Descriptive analyses were first carried out to determine the prevalence of SE and OO. To explore potential risk factors for SE, a binary logistic regression was applied, with all variables included in the model. Independent variables were season (winter, spring, summer, autumn), parity category (primiparous or multiparous), change in body condition score (≤0.5 or >0.5) between the dry and postpartum periods, and the presence of postpartum disorders (reproductive, metabolic, or other). The occurrence of SE served as the dependent variable.

The association between treatment type and conception rate was analyzed using Pearson's chi-square (χ^2) test. All statistical analyses were conducted with IBM SPSS Statistics 28.0 (IBM Corp., Armonk, NY, USA). Statistical significance was declared at $p \leq 0.05$.

Results and Discussion

Occurrence of subclinical endometritis and oviductal blockage

From the 99 repeat breeder cows assessed, 22 animals (22%) were diagnosed with SE. Additionally, 3 cows (3%) exhibited changes in oviductal permeability, each showing a one-sided obstruction.

Variables related to subclinical endometritis

The logistic regression model revealed that none of the evaluated factors had a statistically meaningful influence on the likelihood of SE **(Table 1)**. Owing to the minimal incidence of OO, this analysis could not be performed for that disorder.

Table 1. Binary logistic regression outcomes identifying possible contributors to subclinical endometritis in 99 repeat breeder Holstein cows.

Factor	Category	Prevalence % (n)	Odds Ratio	95% Confidence Interval	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	First calving	15.4 % (6/39)	0.43	0.14–1.31	0.135
	Multiple calvings	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 Issues	None	18.6 % (13/70)	0.50	0.17–1.45	0.204
	Present	31.0 % (9/29)	–	–	–
Postpartum Metabolic Issues	None	21.0 % (17/81)	0.65	0.18–2.36	0.512
	Present	27.8 % (5/18)	–	–	–
Other Postpartum Conditions	None	23.1 % (9/39)	1.19	0.43–3.31	0.741
	Present	21.7 % (13/60)	–	–	–

%: proportion of cows affected by SE; OR: odds ratio; 95% CI: 95% confidence interval; BCS: body condition score variation between the dry period and postpartum.

Influence of treatment on conception success

Regarding SE, notable differences were found among therapeutic protocols **(Table 2)**. Animals that received NSAIDs, whether individually or in conjunction with PGF2α, displayed significantly higher conception rates at the subsequent AI ($p < 0.05$). As with the risk factor analysis, the impact of therapy on conception in cows with OO could not be assessed because of the very few positive cases.

Table 2. Summary statistics for oviductal occlusion and Pearson's χ^2 test assessing the effect of three treatment approaches on conception rates in 22 repeat breeder Holstein cows diagnosed with SE.

Condition	Intervention	Pregnancy Rate (n)
SE∗ (n = 22)	Anti-inflammatory	14.3 % (1/7)b
	Prostaglandin F2α	0 % (0/7)a
	Anti-inflammatory + Prostaglandin F2α	62.5 % (5/8)b
OO (n = 3)	Artificial Insemination	0 % (0/1)

	Therapeutic Embryo Transfer	100 % (2/2)

∗$p < 0.05$. ab: Different letters within a column signify significant differences. SE: subclinical endometritis; OO: oviductal occlusion; NSAID: non-steroidal anti-inflammatory drug; PGF2α: prostaglandin F2α; AI: artificial insemination.

Frequency and contributing factors of subclinical endometritis

The frequency of SE in this investigation was 22%. Reported SE prevalence after calving varies considerably depending on sampling time, diagnostic cut-off for %PMN, and collection technique (cytobrush, lavage, or cytotape). Generally, between the third and seventh postpartum week, it ranges from 7% to 53% [28]. Data focusing on its prevalence at the first insemination remain limited [37–39]. Both large-scale studies—one analyzing 1625 AI samples from 873 cows and another including 1648 cows—reported comparable figures despite using slightly different PMN thresholds. Using a 3% limit, Diaz-Lundahl *et al*. (2021) found a 28% rate, while Pascottini *et al*. (2017a, 2017b) noted 27.8% with a 1% threshold [37–39]. The cytotape method was applied in both.

In repeat breeder cattle, prevalence rates differ broadly, from 12.7–16.6% [19, 40, 41] to 40.2–52.8% [42, 43]. These discrepancies reflect methodological variations, including sample recovery and diagnostic benchmarks, making it difficult to clearly define SE's real impact in RB cows.

No statistically significant associations were detected between SE and any examined variable, likely a consequence of the limited number of SE-positive animals. Even so, minor numerical trends appeared when comparing seasons, parity, and prior reproductive issues. In earlier research, cows suffering postpartum complications exhibited substantially higher SE incidence—40% in healthy individuals versus 80% in those with dystocia or uterine inflammation [43].

In general, SE after calving is associated with prior reproductive problems, metabolic imbalances, and housing or management factors influencing animal health [30]. Moreover, AI timing, parity, and days in milk (DIM) have been reported to affect cytological endometritis rates at first insemination [37–39]. In the current work, the season variable represented calving period rather than AI timing. The higher SE rate noted during warmer months might be connected to heat stress, which promotes immune suppression in postpartum cows, increasing their susceptibility to reproductive disorders and consequently to SE later in lactation.

Prevalence and influencing factors of oviductal occlusion

In the present research, the detected prevalence of OO was 3%, and all affected cows exhibited unilateral obstruction. This finding indicates that OO is unlikely to play a major role in the occurrence of RB syndrome, which contrasts with previous evidence [24]. In that study, 44% of examined cows showed some form of oviductal blockage—4% bilateral and 20% unilateral. Although both studies employed the same diagnostic technique, the earlier research involved 50 cows, roughly half the number included in this trial. Environmental and methodological conditions were nearly identical in both cases, suggesting that the observed disparity in prevalence might stem from random variation in animal selection or individual differences among examiners performing the PSP test. Nonetheless, both operators were trained professionals with proven experience. Another plausible explanation may involve differences in uterine disorder incidence, including variations in how early they were diagnosed or how effectively they were treated. Previous studies have proposed a relationship between uterine infections—such as metritis and endometritis—and subsequent oviductal alterations [44]. However, because Garrido *et al*. (2020) did not report the prevalence of uterine disease, this remains only hypothetical [24].

It is important to note that the PSP test is not the sole approach available for assessing oviductal permeability. Alternative diagnostic methods have been described by Kauffold *et al*. (2009) and Itoh *et al*. (2016), who used contrast-enhanced ultrasonography in cattle, and by Arnold and Love (2013), who evaluated mares through laparoscopy [25, 26, 45]. Despite small sample sizes (eight cows, five cows, and sixteen mares, respectively), each study contributed useful insights. In Kauffold *et al*. (2009), post-mortem examination confirmed the accuracy of the ultrasonographic assessment [25]: five patent oviducts were correctly identified, while two of the five oviducts considered occluded were structurally normal. They also observed that two blockages were caused by hydrosalpinx and one by inflammation. Meanwhile, Itoh *et al*. (2016) recorded only two cases of tubal obstruction [26]. This approach represents a practical alternative to the PSP test, although it also presents drawbacks—each exam requires roughly 20–30 minutes per cow and can cause minor complications such as rectal bleeding or poor image resolution.

Regarding laparoscopy, Arnold and Love (2013) reported 71.4% sensitivity and 85.7% specificity [45]. The predominant cause of occlusion was the presence of oviductal plugs, which varied in size—either partially filling the lumen or being smaller than its diameter. Despite its accuracy, the method involves invasive surgery and requires a uterine lavage 48 hours later, making it costly and impractical for routine dairy herd use.

Oviduct obstruction can result in infertility or sterility by interfering with gamete transport. Moreover, the oviduct provides a suitable microenvironment for gamete maturation, fertilization, and early embryonic growth [21]. Thus, any lesion that disturbs epithelial secretions can impair these processes even without complete blockage. Consequently, finding both oviducts patent in RB cows does not entirely eliminate the oviduct as a potential source of infertility [24].

Because the frequency of OO in this population was low, no reliable statistical model could be applied to identify its risk factors. Currently, there are no published studies addressing OO-associated risk factors in dairy cattle. However, some evidence suggests that salpingitis, often secondary to uterine infections such as metritis or endometritis [44], might lead to OO. Therefore, it can be speculated that risk factors commonly reported for uterine pathologies—such as dystocia, negative energy balance, or parity [46, 47]—could also predispose to oviductal blockage if untreated or improperly managed infections advance into chronic stages.

Influence of therapeutic strategy on conception rate

Although the number of SE-positive cows treated was relatively small due to its limited prevalence, the outcomes suggest that conception performance in RB cows could be improved through combined administration of an NSAID and PGF2α. Considerable information exists on this subject during the postpartum period; however, data specifically focused on RB cows remain scarce. Postpartum treatments using antibiotics and prostaglandins, either individually or in combination, have shown inconsistent outcomes [28]. Since SE is often associated with uterine inflammation rather than bacterial contamination, NSAID therapy has demonstrated beneficial effects [31].

In cases of unexplained infertility in RB cows, multiple interventions—such as hormonal therapy, uterine flushing with saline, antiseptic or antibiotic treatments, NSAIDs, and assisted reproduction—have been attempted [20]. Regarding NSAIDs, they may be applied alone or in combination with hormones. Amiridis *et al*. (2009) reported that administering meloxicam together with GnRH and progesterone enhanced conception rates to 35.7%, compared to 20.0% in cows treated only with GnRH and 17.8% in untreated controls [29]. When meloxicam was given alone (22.6%), no significant improvement was detected over other treatments.

These results correspond with the current findings, where carprofen treatment—whether used individually or with PGF2α—significantly boosted conception outcomes. Previous work has indicated that an early and intense influx of PMNs into the uterus post-calving is beneficial for uterine recovery [48]. However, PMN levels should subsequently decline; prolonged infiltration caused by immune dysregulation may damage the endometrial tissue and perpetuate inflammation [49]. According to these authors, SE arises from an inadequate metabolic adaptation and chronic inflammatory imbalance, which may begin before calving. Therefore, NSAID administration can modulate uterine inflammation, and when paired with optimal nutritional and management strategies, it supports recovery and enhances fertility.

With respect to OO, treatment effects on fertility could not be assessed because of the limited number of affected cows. Previous studies have indicated that embryo transfer, either using in vitro or in vivo produced embryos, may be a suitable reproductive alternative for cows suffering from bilateral occlusion [24]. If obstruction originates from reversible conditions such as salpingitis, successful pregnancy through AI remains possible. Nonetheless, this may necessitate multiple inseminations or repeated patency evaluations, which would inevitably increase economic losses related to RB syndrome.

Conclusion

Neither SE nor OO appears to be a principal contributor to RB syndrome in the studied cows. When SE was identified as the underlying cause, treatment with NSAIDs seemed to enhance conception rates. However, due to limited sample sizes, conflicting findings across studies, and the general scarcity of research on this topic, further large-scale investigations are required to confirm these results.

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