Spanish Journal of Agricultural Research
17 (4), e04SC02, 6 pages (2019)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2019174-15419
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

SHORT COMMUNICATION

OPEN ACCESS

Evaluation of tubal patency in repeat breeder Holstein cows

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Abstract

Aim of study: To evaluate tubal patency in repeat breeder Holstein cows with the purpose of estimating the frequency of oviduct obstruction as a cause of the repeat breeding syndrome.

Area of study: Lugo (NW Spain).

Material and methods: In 50 repeat breeding cows, a solution of the dye phenol-sulphon-phthalein (PSP) was instilled into one uterine horn with the aid of a balloon catheter. Urine was collected 15, 25 and 45 min post-PSP infusion. Urine of a reddish color was used as indicator of tubal patency, as the PSP had passed across the oviduct into the abdominal cavity and was eliminated in urine. The other oviduct was evaluated on a different day.

Main results: Of the 50 cows tested, 44% had some degree of oviduct obstruction: 4% had bilateral oviduct occlusion, 20% unilateral oviduct occlusion, 16% bilateral oviduct stenosis and 4% unilateral oviduct stenosis. No significant relationship could be established between tubal impermeability and postpartum reproductive or metabolic diseases.

Research highlights: Tubal stenosis or occlusions were frequent and they are likely to contribute to the repeat breeding syndrome in dairy cows; thus, tubal patency should be routinely evaluated in repeat breeders. The PSP test is easy to perform, well tolerated by animals, and allows evaluating both oviducts separately.

Additional keywords: dairy cows; infertility; repeat breeding; oviduct impermeability; phenol-sulphon-phthalein.

Abbreviations used: AI (artificial insemination); IVF (*in vitro* fertilization); PSP (phenol-sulphon-phthalein); RBS (repeat breeding syndrome).

Authors' contributions: All authors have equally contributed to the study.

Citation: Garrido, MR; Peña, AI; Herradon, PG; Becerra, JJ; Sande, J; Quintela, LA (2019). Short communication: Evaluation of tubal patency in repeat breeder Holstein cows. Spanish Journal of Agricultural Research, Volume 17, Issue 4, e04SC02. https://doi.org/10.5424/sjar/2019174-15419

Received: 05 Jul 2019. **Accepted:** 27 Nov 2019.

Funding agencies/Institutions	Project / Grant
Xunta de Galicia, Spain: Programme for consolidating and structuring competitive research groups	GRC2015/003
Xunta de Galicia, Spain: Project 'RUMIGAL: Rede de estudo multidisciplinar dos ruminantes en Galicia	R2014/005

Competing interests: The authors have declared that no competing interests exist.

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Introduction

Cows that failed to conceive after a number of services, generally three or more, and that regular return to estrus at normal intervals, without detectable anomalies of the genital tract, have been described as repeat breeders (Zemjanis, 1980; Parkinson, 2019). Causes of the repeat breeder syndrome (RBS) are multiple and can be extrinsic or intrinsic to the cow. Among extrinsic causes, poor heat detection (Heuwieser *et al.*, 1997; Pursley *et al.*, 1998), low semen quality or unsuitable insemination techniques (Perez-Marin *et al.*, 2012) are some of the most recognized ones. Intrinsic causes of RBS may include anatomical defects, genital tract infections (Moss *et al.*, 2002), endocrine disturbances (Gustafsson *et al.*, 1986; Bage *et al.*, 2002; López-Gatius *et al.*, 2004), anovulation (Kimura *et al.*, 1987), early embryo mortality (Bage *et al.*, 2002; Villarroel *et al.*, 2004), etc. When extrinsic causes, obvious pathological lesions and genital tract infections can be excluded, the three main causes of RBS are believed to be ovulatory abnormalities, chronic endometrial damage, and luteal deficiency (Parkinson, 2019).

Oviduct pathologies are known to cause infertility as they usually prevent or hinder fertilization. However, these conditions often remain undiagnosed because the oviduct is difficult to evaluate in living cows. Although much research has been devoted to unraveling the roles of the oviduct in mammalian fertilization (Killian, 2011; Hunter, 2012), from a clinical perspective the bovine oviduct has been somewhat neglected; thus, the importance of tubal affections within the etiology of the RBS is still not known (Kauffold *et al.*, 2009).

Oviduct pathologies have been mainly diagnosed in abattoir studies (Kessy & Noakes, 1985; Stephani *et al.*, 2010; Shivhare *et al.*, 2012). Acquired alterations such as salpingitis, hydrosalpinx, pyosalpinx, adhesions or occlusions were the most frequently reported in the cited studies.

Clinical diagnosis by rectal palpation and/or trans-rectal ultrasonography is possible when the oviduct is affected by severe lesions causing it to be thickened, swollen or adhered. However, mild lesions are difficult to identify and in many cases occluded oviducts are macroscopically normal (Kessy & Noakes, 1985). Stephani *et al.* (2010) evaluated tubal patency in 150 reproductive tracts from slaughtered dairy cows and found that 21% of the tracts had occluded oviducts, with bilateral blockage in 50% of cases. They found a total of 48 occluded oviducts of which 29 (60.4%) had normal macroscopic appearance. In a previous study in buffaloes, Khasatiya *et al.* (1998) had also observed that about half of the occluded oviducts did not show macroscopic lesions and that approximately half of the oviducts with macroscopic lesions were patent. It seems therefore important to evaluate the patency of the oviducts in repeat breeder cows; otherwise they could be empirically treated and repeatedly inseminated without success before being culled.

The common approach to evaluate the patency of the oviduct in live animals is the Phenol-sulphon-phthalein (PSP) test (Purohit, 2014). This method consists of infusing PSP into one uterine horn. A Foley catheter is inserted through the uterus and the balloon is inflated on the end of the horn before the dye is infused. If the oviduct is normal, the dye readily pass through the oviduct, reaches the abdominal cavity, and is eliminated in urine. Bladder is catheterized 15-45 min later and urine turns to reddish if the oviduct is normal. After 4 h, the test can be repeated in the other oviduct (Perez-Marin *et al.*, 2012).

Instead of PSP, Kauffold *et al.* (2009) used an echo-contrast medium instilled into the uterus, and its exit into the abdominal cavity was imaged by trans-rectal ultrasound. They used this method to evaluate tubal patency in 8 repeat breeder cows, but only in 5 animals the two oviducts could be examined. In two cows the procedure was stopped because of rectal bleeding and in one the image had insufficient quality. Out of the 10 oviducts evaluated, 5 were diagnosed as occluded, but 2 of them were found patent at postmortem examination. This method, although still little investigated, seems to have some drawbacks such as a prolonged time of rectal palpation, what may induce bleeding before imaging the two oviducts, insufficient image quality in some animals, what may cause misdiagnosis, or the high cost of the contrast medium (Kauffold *et al.*, 2009).

The aim of the present study was to determine the frequency of tubal occlusion in 50 repeat breeder Holstein cows using the PSP test.

Material and methods

The present study was carried out in accordance with European Union legislation (OJ, 2010) as transposed into Spanish law (BOE, 2013).

Animals

The study was performed using 50 Holstein cows belonging to 5 dairy farms in the province of Lugo (NW Spain). All cows had received more than 3 inseminations (AI) at regular intervals of 18-24 days, were not pregnant, had normal general health status and, by rectal palpation, no detectable anomalies of the genital tract. Based on these findings, the cows were classified as repeat breeder cows (Zemjanis, 1980). Cows were of parity 1 to 5 (mean: 2.42±1.09), had received an average of 5.64±2.15 AIs (min: 3; max: 13) and milk production was from 14.20 to 51.00 kg/cow/day (average: 27.87 kg/cow/day).

Farms size averaged 466 animals (min: 335; max: 575) with a mean of 256 producing cows (min: 190; max: 310) and 210 heifers (min: 145; max: 265). The five farms were free stall without access to pasture, milked twice a day and all had conventional milking parlor. For this study, 8-13 repeat breeder cows were recruited from each farm.

Data collection

For all the cows used, the following data relative to the last calving and postpartum period were collected: calving date, occurrence of dystocia, twin delivery or stillbirths, existence of postpartum ovarian or uterine alterations (retained placenta, metritis, endometritis, ovarian cysts), metabolic diseases (abomasal displacement, ketosis), mastitis, lameness, average milk yield, number of AIs so far, and date of the last AI. Such information was obtained from farm registries or

provided by the veterinarians responsible for reproductive control, since the farms were enrolled in reproduction programs with periodic visits every 15 or 30 days.

Evaluation of oviductal patency: phenol-sulphonphthalein (PSP) test

A PSP solution was prepared as follows (Kothari *et al.*, 1978): 0.3 g phenol red and 4.2 g CO_3HNa were dissolved in 100 mL dH_2O with the aid of 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). A fresh solution was prepared for each experimental day.

Cows were restrained with the tail tied on one side, and the vulva and perineum areas were thoroughly cleaned. By rectal palpation, the cervix was grasped and a sterile Foley catheter passed through the cervix and uterine body into one of the horns, up to 4-5 cm cranial to the uterine bifurcation. Then, the balloon was filled with air to keep the catheter fixed inside the uterine horn and prevent backflow of the solution. The PSP solution (80 mL) was instilled with a syringe attached to the catheter and when instillation was complete, the lumen of the catheter was clamped and the syringe removed.

Up to 3 urine samples were collected by bladder catheterization. The first sample was drained 15 min post-PSP infusion; if the dye appeared in urine, the oviduct was considered to be patent. If the dye did not appear in urine, a second sample was collected at 25 min post-PSP infusion. If still there was not dye in urine, a third sample was collected at 45 min post-PSP infusion. If the third sample did not contain any dye, the oviduct was considered to be blocked. When urine collection had finished, the catheter was unclamped and withdrawn.

Cows were tested in 2 different days, at least 24 h apart. Patency of the right oviduct was evaluated on the first day and that of the left one on the second day. Cows were always tested during the luteal phase of the estrous cycle.

Statistical analysis

Descriptive statistics and frequency distribution analysis were used to describe the oviduct patency of cows. Relationship between oviduct blockage and history of calving problems or postpartum pathologies was analyzed by Chi square test using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Two of the 50 cows tested (4%) were diagnosed as having complete bilateral oviduct occlusion and 10/50 (20%) as having unilateral oviduct occlusion. In these cases, the PSP dye was not present at all in the urine samples collected 45 min post-intrauterine infusion. However, some of the positive urine samples were dyed a very dim red, almost imperceptible, indicating that the oviduct was not completely blocked but it could have stenosis. Pooling both, occluded and stenotic oviducts as oviductal blockage, bilateral blockage rose to 20% (10/50) and unilateral blockage to 24% (12/50), whereas only 56% (28/50) of the cows had both oviducts patent.

For most of the cows tested, positive samples were obtained at 25-45 min post-intrauterine infusion of the PSP dye. The accumulated frequencies of positive responses at 15, 25 and 45 min urine collections, for the two oviducts, are shown in Fig. 1. Percentages of right and left patent partially obstructed or completely blocked oviducts are presented in Fig. 2. There was not significant relationship between oviduct impermeability and difficult calving (dystocia, twins and stillbirths) or postpartum reproductive or metabolic diseases.

The present results showed that tubal pathology is likely to be an important cause of repeat breeding in dairy cows. To the best of our knowledge, this is the first time that tubal patency was investigated in a relatively high number of repeat breeder dairy cows. Of the 50 cows evaluated in this study, 22 had some degree of oviduct obstruction, indicating that it is very important to evaluate tubal patency in cows described with this syndrome.

Kessy & Noakes (1985) found that occluded oviducts were macroscopically normal but the lumen was obstructed because the mucosa had been invaded by proliferative connective tissue with extensive cellular infiltration. Such findings suggest that oviduct occlusion occurs as a consequence of salpingitis, which in turn may be associated to metritis or endometritis (Azawi, 2009). In the present study, a relationship between oviduct blockage and previous uterine inflammatory processes could not be established, probably due to the small number of affected animals included in the present study.

Tubal occlusion results in infertility or, if bilateral, in sterility because the normal transport of gametes is prevented or hindered; hence the importance of identifying repeat breeder cows suffering this affection. However, the mammalian oviduct not only serves as a conduit for the transport of gametes and embryos, but also creates an environment that facilitates final gamete maturation, fertilization, and early embryo development (Killian, 2011). Any pathological condition that may alter the normal secretory function of the oviductal epithelium may prevent fertilization or embryo development, without inducing tubal occlusion. Thus, a diagnosis of bilateral tubal patency in a repeat

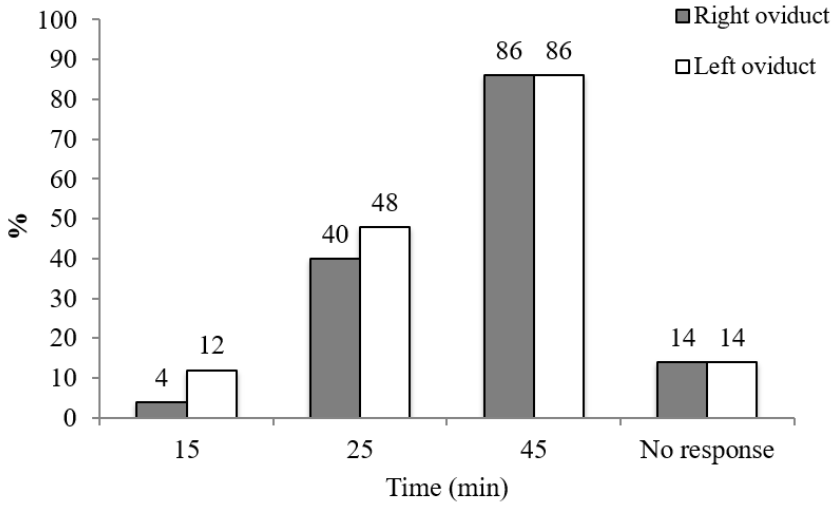

Figure 1. Cumulative percentage of positive responses at the 15, 25 and 45 min urine collections, for the two oviducts.

breeder cow does not exclude the oviduct as the cause of infertility. In fact, 3 of the cows that in the present study were diagnosed with bilateral tubal patency, and that did not conceive after 1 or 2 further inseminations, got finally pregnant by transfer of *in vitro* fertilization (IVF) produced embryos, which could be compatible with these cows having nonfunctional oviducts. In contrast, one cow diagnosed with bilateral oviduct stenosis conceived four months later at the first insemination post-PSP test, suggesting that the condition that caused oviduct stenosis and infertility in this cow was reversible. Nevertheless, the majority of the cows diagnosed in this study with tubal occlusion or stenosis were slaughtered later on, but postmortem confirmation of the diagnosis was not possible due to the difficulty of identifying an individual genital tract in the abattoir chain.

In the present study tubal patency was always evaluated during the luteal phase of the estrous cycle. This was because during the follicular phase the muscular tone of the oviduct increases, and the mucosa becomes highly edematous due to the vascular congestion elicited by high estradiol concentrations. This in turn causes mucosal ridges and folds to become prominent, reducing the patency of the oviduct, particularly in the region of the isthmus. In addition, the secretory activity of non-ciliated cells reaches a maximum close to the time of ovulation, which results in fluid accumulation occurring during the follicular phase (Hunter, 2012). Therefore, oviduct evaluation during estrous could lead to false diagnoses of tubal occlusion.

In conclusion, tubal pathology very likely contributes to the repeat breeding syndrome in dairy cows. In the

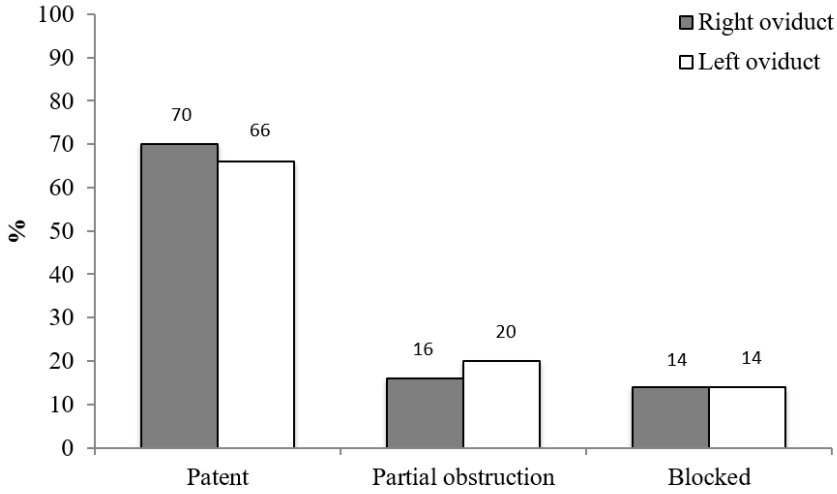

Figure 2. Percentages of right and left patent, partially obstructed, or completely occluded oviducts.

present study, 44% repeat breeder cows had unilateral or bilateral oviduct stenosis or occlusion. Further studies involving a higher number of animals are needed for better estimating the incidence of this problem within the repeat breeding syndrome. The classical PSP test is easy to perform and safe and well tolerated by cows, and can be performed at the same time as endometrial cytology so that cows with subclinical endometritis and/or tubal occlusion or stenosis can be identified.

Acknowledgements

We wish to thank the veterinary surgeons and farmers who cooperated in the study.

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