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Case Report

Estrus quality, non-return rate and conception rate in the incidence of repeat breeding in dairy cow after 2%povidone iodine therapy combined with prostaglandin $F_{2\alpha}$ at KUD Tani Wilis Tulungagung

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ABSTRACT

Repeat breeding is a condition in which cows fail to conceive after being bred three or more times. This study aims to determine the quality of estrus, non-return rate (NRR) and conception rates obtained after cows were treated with 2% povidone iodine. The study involved 30 Holstein Friesian cows, each having calved three times and experiencing repeat breeding. Cows in estrus were treated with 10 mL of 2% povidone iodine intrauterinely, followed by an intramuscular injection of 2 mL (250 mg/head) of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Luteosyl) five days later. Estrus quality was observed by examining changes in vulvar mucosa color, mucus discharge, and vulvar temperature. Pregnancy diagnosis was made at 60 days. Results showed that 43.3% of cows had estrus quality score 3 (cows showed dark red vulvar mucosa, transparent mucus, temperature 38.9 ± 0.5 ºC), 23.3% had score 2 (cows showed reddish vulvar mucosa, some cloudy mucus, temperature 38.7 ± 1.0 ºC), and 33.3% had score 1 (cows showed pink vulvar mucosa, cloudy mucus, temperature 38.6 ± 0.5 ºC). Of the 30 cows, 20 had a non-return rate (NRR) of 66.7%. Based on these results, it can be concluded that 2% povidone iodine has a positive effect on estrus quality, NRR, and conception rates in dairy cows experiencing repeat breeding.

Keywords: conception rate, dairy cows, povidone iodine, quality of estrus, repeat breeding

INTRODUCTION

Repeat breeding referred to a condition in cows that failed to conceive despite undergoing artificial insemination (AI) or natural mating during three or more normal estrous cycles (Pérez-Marín and Quintela, 2023). This phenomenon adversely impacted the reproductive system of cows and overall milk productivity. Economically, repeat breeding led to resource wastage, encompassing costs related to insemination, medications, and the time expended in these processes. With each occurrence of repeat breeding, the potential for increased milk production diminished. The eradication of repeat breeding could optimize

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reproductive cycles, enhance insemination success rates, shorten intervals between calvings, and ultimately improve milk yield (Khair *et al*., 2018).

The incidence of repeat breeding could be attributed to various factors, among which the reproductive health of cows was paramount for successful conception. Reproductive infections, such as metritis, endometritis, and other reproductive tract infections, impeded the fertilization process and embryo implantation (Saraswat and Purohit, 2016). Research into the treatment of repeat breeding was essential for developing effective treatment and prevention protocols. Furthermore, heat detection was a critical aspect of dairy cattle reproduction. Insufficient understanding among farmers regarding heat detection contributed to repeat breeding, which, in turn, resulted in low conception rates in dairy cows (Villar *et al*., 2025).

At the time of the study, the standard protocol for managing repeat breeding cases at Tani Wilis village unit cooperative (koperasi unit desa, KUD) involved the use of 2% povidone-iodine; however, this treatment continued to yield a high incidence of repeat breeding. This persistent issue was likely attributed to delayed ovulation caused by the presence of residual corpus luteum (CL), which maintained elevated levels of progesterone in the circulatory system. Delayed ovulation resulted in spermatozoa failing to fertilize the ovum, preventing conception. This prolonged ovulation generated longer intervals between estrous cycles, extending from 24 to 48 hours, indicative of abnormal estrus quality (Båge *et al*., 2002). Observations made by inseminators at the KUD Tani Wilis showed that as of December 2021, repeat breeding cases remained high, around 19.7%. The high number of repeat breeding cases at KUD Tani Wilis caused losses to farmers. Consequently, this study aimed to investigate the efficacy of a combination therapy involving 2% povidone-iodine and $PGF_{2\alpha}$ for managing repeat breeding in dairy cows, focusing on the absence of return to estrus, estrus quality scores, and pregnancy rates.

MATERIALS AND METHODS

This research was conducted at KUD Tani Wilis, located in the Sendang district of Tulungagung regency, from March to May 2022. The sample used in this study comprised 30 dairy cows of the Holstein Friesian (HF) cross breed that experienced repeat breeding, which were treated with 2% povidone iodine followed by intramuscular injection of $PGF_{2\alpha}$ five days later.

The subjects of this study were female dairy cows diagnosed with repeat breeding. Repeat breeding was defined as female dairy cows that had been bred two or more times with estrous cycles ranging from 21 to 23 days. During the study period, all 30 dairy cows were provided with forage consisting of elephant grass (*Pennisetum purpureum*) and concentrate feed, which were supplied by KUD Tani Wilis, with water offered *ad libitum*. When the cows demonstrated estrus, they were treated with 10 mL of 2% povidone iodine administered intrauterine via a syringe. Subsequently, five days after the initial treatment, they received an intramuscular injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) Luteosyl at a dosage of 250 mg per cow or 2 mL per cow. The estrus quality scores were designed based on previous reports by Abidin *et al*. (2012), Irfan *et al*. (2017), and Kuswati *et al*. (2022) (Table 1).

Table 1 Vulva color, mucus and temperature scores (Abidin *et al*., 2012; Irfan *et al*., 2017; Kuswati *et al*., 2022).

score	vulva color	mucus characteristics	temperature (°C)
1	pale pink	mucus with lots of cloudy spots	38.6 ± 0.5
2	pink	mucus with a few cloudy spots	38.7 ± 1.0
3	reddish	mucus is abundant and transparent	38.9 ± 0.5

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Artificial insemination was performed by trained and certified inseminators using the same batch of frozen semen from HF bulls produced by the Singosari Artificial Insemination Center, Malang, Indonesia. Pregnancy diagnosis was performed 60 days after artificial insemination through rectal palpation by a veterinarian.

Data analysis

The data obtained on the quality of estrus, non-return to estrus and pregnancy rates were presented descriptively.

RESULTS

Table 2 Estrus quality score, return to estrus, reinsemination, and pregnancy diagnosis of repeat breeder cows treated with povidone iodine and $PGF_{2\alpha}$

sample no	estrus quality score	return to estrus and reinseminated	pregnancy diagnosis
1	1	yes	non-pregnant
2	3	no	pregnant
3	1	yes	non-pregnant
4	1	yes	non-pregnant
5	3	no	pregnant
6	2	no	pregnant
7	1	yes	non-pregnant
8	2	no	pregnant
9	3	no	pregnant
10	3	no	pregnant
11	2	no	pregnant
12	3	no	pregnant
13	1	yes	non-pregnant
14	1	yes	non-pregnant
15	3	no	pregnant
16	1	yes	non-pregnant
17	3	no	pregnant
18	3	no	pregnant
19	2	no	pregnant
20	3	no	pregnant
21	3	no	pregnant
22	2	no	pregnant
23	1	yes	non-pregnant
24	1	yes	non-pregnant
25	1	yes	non-pregnant
26	2	no	pregnant
27	3	no	pregnant
28	2	no	pregnant
29	3	no	pregnant
30	3	no	pregnant

Treatment of 30 repeat breeder cows with povidone iodine and $PGF_{2\alpha}$ resulted in estrus quality scores, return to estrus, reinsemination, and pregnancy diagnosis, as shown in Table 2. Grouping the repeat breeder cows based on estrus scores showed that all cows with an estrus quality score of 1 returned to estrus and thereby reinseminated, although they did not become pregnant. In contrast, all cows with estrus quality scores of 2 and 3 did not return to estrus, were not reinseminated, and all were pregnant (Table 3).

Table 3 The percentage of repeat breeder cows based on estrus score, non-return to estrus, reinsemination, and pregnancy rates

estrus score	number of cows	non-return rates	re-inseminated	pregnancy rates
1	10 (33.3%)	0% (0/30)	33.3% (10/30)	0% (0/30)
2	7 (23.3%)	23.3% (7/30)	0% (0/30)	23.3% (7/30)
3	13 (43.3%)	43.3% (13/30)	0% (0/30)	43.3% (13/30)
total	30 (100%)	66.7% (20/30)	33.3% (10/30)	66.7% (20/30)

DISCUSSION

Repeat breeding in dairy cows could have been caused by fertilization failure or early embryo death due to endocrine dysfunction, as well as infectious factors (Singh *et al*., 2017). One method for treating infections in the uterus of dairy cows was the use of the antiseptic povidone iodine. Povidone iodine is a complex of polyvinyl pyrrolidone and iodine that is water-soluble and possesses broad-spectrum

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antimicrobial properties, effective against gram-positive and gram-negative organisms, as well as fungi and viruses (Lepelletier *et al*., 2020). As a local antibacterial agent, povidone iodine was effective in killing bacteria and spores and was widely used as an antiseptic. This compound exhibited both bactericidal and bacteriostatic actions, meaning it could inhibit the growth of microorganisms and eliminate microorganisms present on or at the surface of body tissues (Meehan *et al*., 2024).

The administration of 2% povidone iodine intrauterine in dairy cows experiencing repeat breeding had been shown to increase the conception rate (CR). The increase in CR could have occurred through several mechanisms, such as the bactericidal effects of povidone iodine, adjustment of the pH of the reproductive tract prior to insemination, improved blood circulation within the uterus, and enhanced defense mechanisms of the reproductive system (Yoshida *et al*., 2020). Furthermore, povidone iodine gradually released iodine, which assisted in binding thyroxine globulin and enhanced the release of thyroxine hormone (Żarczyńska and Świerczyński, 2023), subsequently stimulating the hypothalamus and pituitary gland to release gonadotropin hormone-releasing hormone (GnRH), follicle stimulating hormone (FSH), and luteinizing hormone (LH) (Khar *et al*., 1980).

On the fifth day after the administration of povidone iodine, $PGF_{2\alpha}$ was given to address the active CL, which produced the hormone progesterone. $PGF_{2\alpha}$ assisted in the regression of the CL, with the expectation that signs of estrus would manifest two days later (López-Gatius, 2022). $PGF_{2\alpha}$ also played a role in ovulation, embryo implantation, and the maintenance of pregnancy. The administration of this hormone increased the responsiveness of the pituitary gland to produce GnRH, thereby accelerating the release of LH, which triggered ovulation (Mohammadi *et al*., 2019). The use of $PGF_{2\alpha}$ as a luteolytic agent was widely employed to induce estrus. $PGF_{2\alpha}$ lysed the CL, which was naturally released by the uterus in non-pregnant animals on days 16 to 18 of the cycle. The lysis of the CL induced by $PGF_{2\alpha}$ led to reduced blood flow to the CL and a decrease in progesterone levels, prompting the anterior pituitary to release FSH and LH, hormones responsible for follicle growth and maturation. These follicles ultimately produced estrogen, which manifested as signs of estrus (Martins *et al*., 2021).

A study was conducted involving 30 dairy cows that experienced repeat breeding, treated with 2% povidone iodine administered intrauterine at a dose of 0.2 mL, diluted with distilled water to a total volume of 10 mL. The results indicated that 2% povidone iodine enhanced the efficiency and performance of dairy cow reproduction through bactericidal effects, pH adjustment in the reproductive tract, and increased blood flow to the uterus, thereby stimulating follicular development (Yoshida *et al*., 2020).

The quality of estrus in dairy cows following therapy showed clear signs of heat, such as a reddened vulva, transparent mucus discharge, and an average body temperature of 38 ºC - 39 ºC. This was attributed to its ability to stimulate the production of prostaglandins in the uterus (Yoshiaki, 2020). Among the 20 dairy cows treated with 2% povidone iodine, their estrus quality showed significant improvement, with a pregnancy rate of 66.7% following artificial insemination and a corresponding non-return Rate (NRR) of 66.7%.

The failure to conceive in this case study was believed not to be due to improper timing of insemination or nutritional factors. Estrus detection was conducted by farmers with decades of experience in dairy cattle management. The frozen semen used also came from the same batch, and the insemination was performed by the same trained, certified, and experienced inseminator. Nutritional factors were ruled out, as abundant forage was available and farmers consistently provided concentrate in accordance with the guidelines of KUD Tani Wilis. In this case, 33.3% of dairy cows with an estrus score of 1 returned to heat after insemination but failed to conceive. It was

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suspected that there had been an excessive accumulation of pus, which may have prevented the povidone iodine from dissolving in the pus and adequately contacting the endometrium. It was recommended that, in such cases, irrigation of the uterine lumen should have been performed prior to administering intrauterine povidone iodine after achieving cleanliness (Mido *et al*., 2016).

CONCLUSIONS

In repeat breeding dairy cows, the administration of 2% povidone iodine and $PGF_{2\alpha}$ on day 5 positively affected the quality of estrus, non-return rate (NRR) and pregnancy rates.

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AUTHOR'S CONTRIBUTIONS

Amira Halimah Zahrorona (AHZ), Sri Pantja Madyawati (SPM), Sunaryo Hadi Warsito (SHW), Pudji Srianto (PS), Hermin Ratnani (HR), Sri Hidanah (SH).

AHZ, SPM, SHW: conceived the idea, designed the mainframe of this manuscript, acquisition, analysis and interpretation of data, and manuscript drafting. PS, HR, SH: critically read and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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