

## Repeat breeder syndrome in dairy cows: influence of breed and age on its prevalence and the success of a hormone therapy\*

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**Abstract:** The aim of this study was to establish the prevalence of repeat breeder syndrome (RBS) in different breeds and reproductive ages of cattle in central Croatia and to assess the effectiveness of hormone therapy as a treatment option. A total of 1088 dairy cows were observed, 218 heifers and 870 multiparous cows. After the third unsuccessful artificial insemination (n = 164) and a thorough clinical and gynecological examination, the cows were tested for sexually transmitted diseases. An RBS diagnosis was based on failure to conceive, negative laboratory tests for sexually transmitted diseases, and the absence of other reproductive disorders. The highest prevalence of RBS was found in crossbreed cows, followed by the Holstein-Friesian, Red Holstein, and Simmental breeds. RBS was not detected in cows of the Swiss Brown breed. The prevalence of RBS was significantly higher in heifers in comparison to multiparous cows. Cows diagnosed with RBS were administered hormone therapy based on the Double Ovsynch protocol with applications of gonadotropin-releasing hormone (GnRH) and a prostaglandin analogue (cloprostenol). This hormone treatment resulted in a higher conception rate in all treated animals and significantly impacted the culling rate due to the subfertility.

**Key words:** Repeat breeder syndrome, cattle, Croatia, prevalence, treatment

### Introduction

The term repeat breeder or repeat breeder syndrome (RBS) was created to describe cows that failed to conceive after 3 or 4 inseminations as defined by Parkinson et al. (1). Repeat breeder females return to service repeatedly after being bred with a fertile male. According to Parkinson et al. (1), these cows exhibit normal signs of estrus every 18 to 24 days but require more than 3 services to become pregnant. According to Allen (2), some of these cases may be associated with early embryonic deaths, since

most of the embryonic losses in cows occur much earlier in pregnancy than previously believed. This theory is supported by Ayalon (3) and Maurer and Echterkamp (4), who reported that repeat breeders show a significant level of embryonic deaths. Ayalon (3) reported that RBS is a major source of economic loss in dairy herds in North America and its prevalence ranges from 10% to 18% between different states. Maurer and Echterkamp (4) also reported a higher prevalence of RBS in heifers (15.1%) than in multiparous females (8.3%) for beef cattle.

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According to Espey (5), ovulation is initiated by the increase of the luteinizing hormone (LH), which results in the rupture of the follicle and release of an egg. Brackett (6) reported that after ovulation there is a short period during which the egg can be fertilized. Since the period between the first signs of estrus and ovulation occurs at 72 h or later, the cows that ovulate later should be inseminated daily until rectal detection of ovulation is confirmed. This phenomenon is known as a delayed ovulation and is most prevalent in Holstein-Friesian and crossbred cows in Croatia, as reported by Zobel et al. (7). Delayed ovulation should not be confused with repeat breeder syndrome (RBS). These cows, if not inseminated daily until ovulation, can also return to service repeatedly after artificial insemination while showing normal signs of estrus every 18 to 24 days. Due to this very important consideration, we speculate that certain percentages of cows in previous reports were misplaced in the RBS group instead of being diagnosed with delayed ovulation.

Several different strategies for RBS treatment have been reported. Ahmadi and Deghan (8) studied the effect of uterine lavage and a prostaglandin  $F_{2a}$  ( $PGF_{2a}$ ) application (with and without antibiotic treatment) on the conception rate in dairy cows with RBS. The study included 33 repeat breeder cows free of significant detectable disorders associated with the reproductive tract. The results showed that uterine lavage with a  $PGF_{2a}$  application and without an antibiotic application, may be a preferable treatment method for cows. However, further studies are required to confirm these findings.

A gonadotropin-releasing hormone (GnRH) application significantly increases the conception rate in repeat breeders, as reported by Stevenson et al. (9) and Morgan and Lean (10). On the other hand, research conducted by Singh et al. (11) suggested that frequent insemination (every 6 h) can increase the conception rate in cases of RBS. However, no significant results were found by double (repeated) insemination of cows within 12 or 18 h without an application of GnRH, as reported by Stevenson et al. (9). Therefore, GnRH must be considered in the hormone treatment for RBS.

Treatment options for RBS in dairy cows have been reviewed by Dawson (12) and Levine (13) and except for the administration of GnRH at the time of insemination, as was conducted by Morgan and Lean (10), responses to other treatment options have been generally poor. Strategies may be used to optimize the time of insemination, including an intravaginal progesterone releasing device through a controlled internal drug release (CIDR) program, as shown by Day et al. (14) and the Ovsynch protocol described by Pursley et al. (15).

To our knowledge, there were no studies on the prevalence of RBS in Croatian cattle and its treatment, especially using a Double Ovsynch protocol. The aim of this study was to establish the prevalence of RBS in cows in central Croatia and to evaluate the efficacy of the Double Ovsynch protocol as a treatment option for this condition.

## Materials and methods

### Animals

Out of a total of 1088 cows included in this research, 218 were heifers and 870 were cows; 598 were of the Simmental breed (54.96%), 298 were of the Holstein Friesian breed (27.39%), 98 (9%) were of the Red Holstein breed, 28 (2.57%) were of the Swiss Brown breed, and 66 (6.10%) were crossbreeds. The research was conducted over a 3 year period (October 2005 to November 2008) in the central region of Croatia, characterized by a moderate continental climate. All animals were housed in 89 commercial dairy farms with  $65 \pm 20$  cows per farm, and with an average of  $6550 \pm 390$  kg/year milk production per cow. The average age of observed cows was  $5.3 \pm 3.1$  years. Cows were milked twice daily (morning-evening milking schedule) and during the warm period of the year (March until November) were released to the pasture during the day. Starting from November until March, cows were in stables with open air access for a few hours daily. Animals were fed hay ad libitum and a concentrated ration consisting of oat, corn meal, soybean, barley, and mineral supplements. Grass silage, corn silage, and propylene glycol were added to the rations according to age, stage of lactation, or

milk yield. Starting in June, fresh alfalfa and clover were added. Cows were fed twice daily (morning and evening). Before milking, hay was given ad libitum and after the milking, concentrates and silage were provided. During the day, cows had hay freely available on the pasture and in the stable. The average milk yield of Simmental, Holstein-Friesian, Red Holstein, Swiss Brown and crossbred cows from all observed farms was  $6200 \pm 160$  kg/year,  $6800 \pm 140$  kg/year,  $6700 \pm 190$  kg/year,  $6400 \pm 120$  kg/year, and  $6120 \pm 130$  kg/year, respectively.

### Experimental design

A total sample pool of 1088 cows/heifers was used in this study. The onset of estrus was recorded based on the data provided by owners. A clinical examination was performed by practitioners from the veterinary practice VETMED, who had experience with cattle reproduction. After a manual rectal examination, the cows were examined by ultrasound using a linear rectal probe 7.5 MHZ (Draminski, PROFIL, Poland) and based on these findings, inseminated daily until conformation of ovulation. Out of 1088 cows, 816 (75%) ovulated within 24 h after the onset of heat. In 174 cows (16%), ovulation occurred between 24 and 48 h, and in 98 cows (9%), ovulation occurred between 72 and 96 h after the first signs of estrus. This resulted in some of the cows being inseminated daily for 3 consecutive days (98 or 9%), and some of them (174 or 16%) for 2 consecutive days. Only cows with regular estrus and a confirmed ovulation were included in this research. Based on the clinical examination and gynecological criteria, 47 cows were diagnosed with reproductive disorders that included cystic ovarian disease (COD), pyometra/endometritis, and anovulatory cycles and were excluded from the research.

A thorough physical and gynecological examination (rectal and ultrasound) was repeated for 164 of the cows (15.07% of the total population sample,  $n = 1088$ ), that did not conceive after the third insemination. Blood samples and vaginal swabs were collected in order to test for sexually transmitted diseases (STDs). All of the laboratory tests were performed at the Croatian Veterinary Institute in Zagreb, Croatia, and were negative. Since there were no abnormalities of the reproductive organs, the

cows were free of STDs and were cycling in regular intervals within 18 to 23 days, they were diagnosed with RBS. These cows were further treated for this condition with a hormonal treatment based on a Double Ovsynch protocol, as described by Souza et al. (16), for the treatment of the cows suffering from subfertility. According to that protocol, during the fifth estrus, and after finding a dominant Graafian follicle on 1 of the ovaries, 0.05 mg of gonadorelin was administered by intramuscular injection (IM). After 7 days, 0.05 mg of cloprostenol was given IM and 3 days after that, the dominant Graafian follicle was found in each cow. On that day, an additional 0.05 mg of gonadorelin was administered. The same application of cloprostenol (0.05 mg) was administered again 7 days later and a rectal examination was performed 2 days after that in order to confirm onset of estrus. Cows were inseminated on the same day, followed by another application of 0.05 mg gonadorelin IM and the next day insemination was repeated if ovulation did not occur. The following day all the cows were rectally examined in order to confirm ovulation. If ovulation did not occur at that time, cows were inseminated daily until ovulation was achieved.

### Artificial insemination

Artificial insemination (AI) was performed using thawed frozen semen. Semen was thawed in water at a temperature between 30 °C and 37 °C for 30 s. Cows were inseminated into the uterine body. The period between thawing and insemination was 15 min. Pregnancies were confirmed by ultrasound 35 to 45 days after the insemination.

### Statistical analysis

Collected data were analyzed using the Statistica 8 program (StatSoft, Tulsa, USA) at the Veterinary Faculty in Zagreb, Croatia. The Kolmogorov-Smirnov test was used in order to determine the range distribution. Since distribution was not normal, further analyses were performed by 2-sided sign test for 2 independent groups. The groups were: (1) cows versus heifers regarding the prevalence of RBS, (2) breed versus prevalence of RBS, and (3) prevalence of RBS versus culled cows (therapy success). All data were calculated with statistical significance (P) and correlation index (CI) expressed in the results.

## Results

The results are presented in Tables 1 and 2 and Figures 1 and 2.

The total population sample of cows/heifers involved in the study was 1088. As is presented in Table 1, a total of 347 (31.89%) cows failed to conceive after the first round of AI and had to be rebred; this group included 240 cows (69.16%) and 107 heifers (30.84%). A total of 283 cows/heifers (26.01%) failed to conceive after the second insemination. After the

third insemination, estrus reoccurred in 211 cows/heifers (19.39%).

From a total of 211 cows/heifers that failed to conceive after the third AI, 164 (15.07%) were diagnosed with RBS. These cows were used for the epidemiological analysis and later for the treatment study. Based on the clinical examination and gynecological criteria, the remaining 47 cows/heifers were diagnosed with COD, metritis/endometritis, and anovulatory cycles, and were excluded from the research following the exclusion criteria.

Table 1. Breed distribution of cows with repeat breeder syndrome diagnosis, number of artificial inseminations, and the success of the hormonal treatment (expressed as conception and culling rate).

Breed	Simmental	Holstein Friesian	Red Holstein	Swiss Brown	Crossbreeds
Total sample (a)	598	298	98	28	66
CnC after 1st AI (b)	163	68	58	8	50
b × 100/a	27.25%	22.82%	59.18%	28.571%	75.76%
CnC after 2nd AI (c)	128	57	55	2	41
c × 100/a	21.40%	19.13%	56.12%	7.14%	62.12%
CnC after 3rd AI (d)	109	45	19	0	38
d × 100/a	18.23%	15.10%	19.38%	0.00%	57.57%
<b>Number of RBS (e)</b>	83 <sup>a</sup>	38 <sup>a</sup>	15 <sup>a</sup>	0	28 <sup>a</sup>
<b>RBS% (e × 100/a)</b>	13.88% <sup>a</sup>	38.77% <sup>a</sup>	15.30% <sup>a</sup>	0.00% <sup>a</sup>	42.42% <sup>a</sup>
Conceived after 1st AI posttreatment (f)	78 <sup>a</sup>	24 <sup>a</sup>	11 <sup>a</sup>		18 <sup>a</sup>
Conception rate (f × 100/e)	93.97% <sup>a</sup>	63.16% <sup>a</sup>	73.33% <sup>a</sup>		64.28% <sup>a</sup>
Conceived after 2nd AI posttreatment (d)	5 <sup>a</sup>	8 <sup>a</sup>	2 <sup>a</sup>		9 <sup>a</sup>
Conception rate (d × 100/e)	6.02% <sup>a</sup>	21.05% <sup>a</sup>	13.33% <sup>a</sup>		50.00% <sup>a</sup>
Culled cows	0	3 <sup>a</sup>	0	0	5 <sup>a</sup>

CnC: cows that did not conceive after artificial insemination (AI), <sup>a</sup>P < 0.05.

Table 2. Protocol for the Double Ovsynch protocol hormone therapy for repeat breeder syndrome.

Days	1-----7-----10-----17-----19-20-21				
<b>Hormone</b>	GnRH	PGF <sub>2α</sub>	GnRH	PGF <sub>2α</sub>	AI + GnRH

GnRH: Gonadotropin-releasing hormone;  
 PGF<sub>2α</sub>: Prostaglandin hormone analogue;  
 AI: artificial insemination.

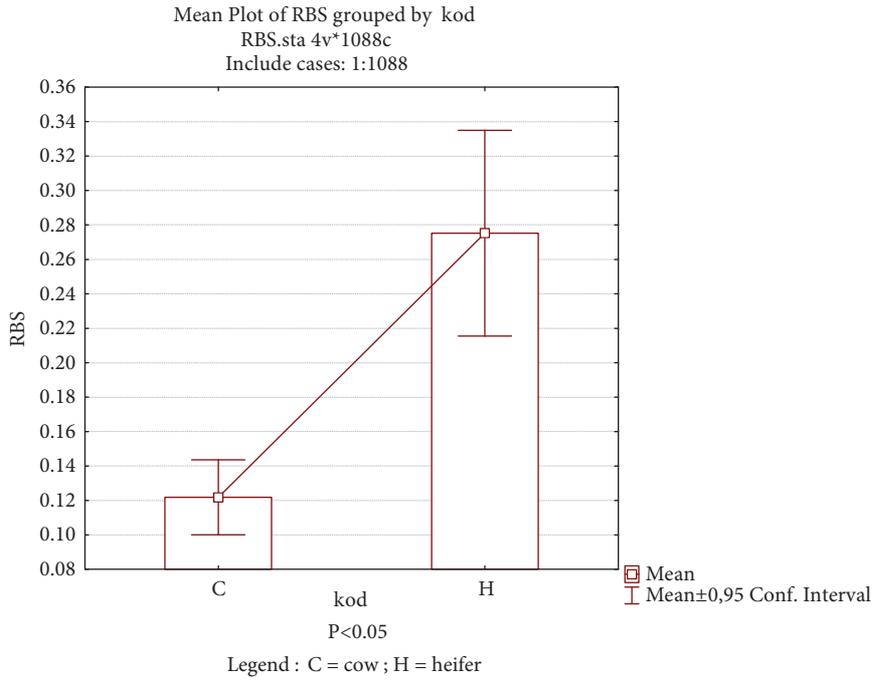


Figure 1. Age prevalence of the repeat breeder syndrome.

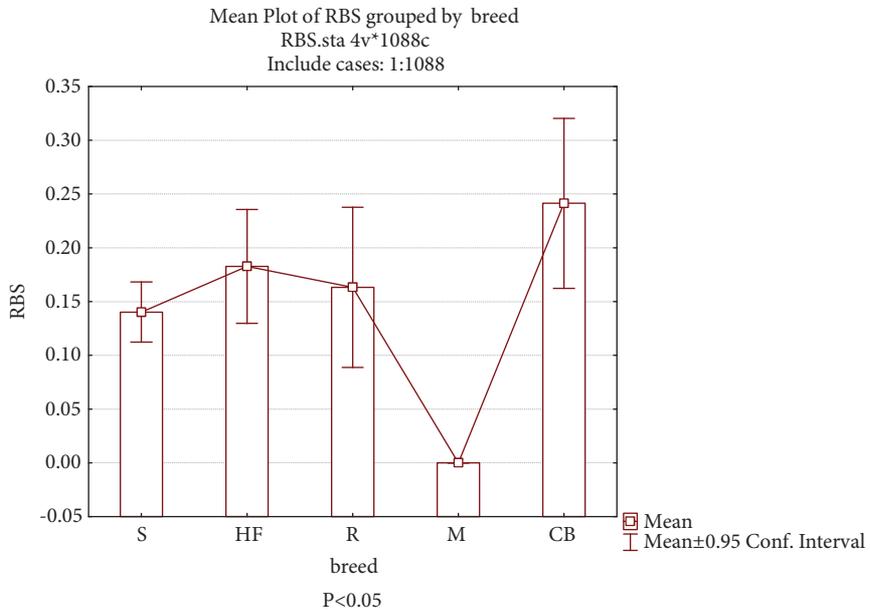


Figure 2. Breed prevalence of repeat breeder syndrome in the population (n = 1088).

S: Simmental breed, HF: Holstein-Friesian, CB: Crossbreed; M: Swiss Brown breed; RBS: number of animals diagnosed with repeat breeder syndrome.

Considering the reproductive age, RBS was diagnosed in 106 multiparous cows (106/870 or 12.18%), and in 58 of the 218 heifers (26.61%). RBS

was found to be 2 times more frequent in heifers than in multiparous cows, and the difference is statistically significant at  $P < 0.05$  and  $CI \pm 0.95$ .

Considering the breed, RBS was the most frequent in crossbreeds (28/66 or 42.42%;  $P < 0.05$ ), followed by the Holstein-Friesian cows (38/298 or 38.7%;  $P < 0.05$ ). It was less frequently observed in the Red Holstein (15/98 or 15.30%;  $P < 0.05$ ) and Simmental cows (83/598 or 13.88%;  $P < 0.05$ ). Although represented by a small sample size, none of the Swiss Brown cows were diagnosed with RBS. The differences in the prevalence of RBS in these breeds are statistically significant at  $P < 0.05$  and  $CI \pm 0.95$  for all breeds.

The hormone treatment selected as the treatment option for RBS was based on the Double Ovsynch protocol and involved administration of 2 hormones, gonadorelin and cloprostenol, as described earlier. The results are presented in Table 2.

Out of 164 treated cows/heifers, 131 cows (79.88%) conceived after the first insemination posttreatment, 24 cows/heifers (14.63%) conceived after the second insemination, 1 cow died during treatment, and 8 were finally culled (4.88%) due to infertility/subfertility as is presented in Table 1. The success level of the RBS treatment was statistically significant ( $P < 0.05$  and  $CI \pm 0.95$ ) in all breeds, regardless of the reproductive age (cow vs. heifer).

## Discussion

Repeat breeder syndrome in cattle has gained importance in recent decades as a significant cause of subfertility and economic loss in the dairy industry, as was reported by Ayalon (3). In this study, by definition, all cows with the RBS diagnosis had normal sexual cycles and were returning to estrus within 18 to 24 days, but failed to conceive, which is in accordance with the findings of Allen (2). It is possible that some of the causes involve early embryonic deaths as was reported by Ayalon (3) and Maurer and Echterkamp (4). All of these cows were also confirmed negative for STDs or other abnormalities in the reproductive organs as was suggested by Ahmadi and Dehghan (8).

To our knowledge, there are no accessible data about the prevalence of RBS in different breeds of cattle. Our data show that, in central Croatia, the greatest prevalence of RBS is in crossbreeds, followed by the Holstein-Friesian breed. It was rare in the Red Holstein and Simmental cows, while no RBS was

diagnosed in cows of the Swiss Brown breed. The effect of breed on the RBS prevalence is statistically significant. The reported data for the prevalence of RBS in cattle in the United States of America varied from 10% to 18%, according to Ayalon (3). In the present study, the prevalence of RBS varies from 0.00% to 42.42%, depending on the breed. This could be associated with the effects of crossbreeding or the inadequate adaptation of the imported, more sensitive, Holstein-Friesian breed to our climate, housing, and management. These cows may have inherently lower fertility, as reported by Lucy (17), and Harris and Kolver (18), or it can be associated with energy deficits, as reported by Butler (19), and/or abnormal ovarian activity, as reported by Lamming and Royal (20). The higher milk yield (average of 300 kg/year more than in crossbreeds), and a possible genetic predisposition of the Holstein-Friesian breed for RBS, could be taken into consideration for a higher prevalence of RBS in that breed.

Low incidence of RBS in the Simmental breed is most probably the result of the smaller milk yield, genetic resistance of the breed, and a good adaptation to our continental climate and management system. Since the Swiss Brown breed of cows were negative for RBS in our study, we can speculate that the smaller milk yield in those cows in our region (Croatia) and the breed vigor may be associated with this finding, although a small sample size for those cows needs to be taken into the consideration.

Regarding the reproductive age, we found the prevalence of RBS to be 2 times higher for heifers versus multiparous cows, which is in concordance with the findings of Maurer and Echterkamp (4).

When it comes to treatment options for subfertility in cattle in general, and RBS being one of its causes, the physiology of the estrous cycle and various endogenous (hormonal) and exogenous components have to be taken into the consideration. Several different GnRH preparations are available on the market. Gonadorelin (Cystorelin®, Factel®) is a decapeptide from the hypothalamus that stimulates secretion of FSH and LH, according to Adams (21). Prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) administration results in luteolysis in cattle, and it is typically followed by estrus 2-5 days postinjection, as stated by Adams (21). In this study we used  $PGF$  analogue cloprostenol

combined with GnRH as medical treatment option for RBS. This is based on reports by Stevenson et al. (9), stating that GnRH significantly increases conception rate in RBS cows. Although Ahmadi and Dehghan (8) used PGF<sub>2α</sub> and uterine lavage in combination, we did not consider it necessary since all the RBS cows were negative for uterine or vaginal infections (endometritis and STDs). We conclude that hormone therapy based on a Double Ovsynch protocol can be used successfully as a treatment option for RBS, to result in a significant increase in the conception rate in the affected cows that are negative for sexually transmitted diseases and endometritis.

Furthermore, according to the recommendations of Morgan and Lean (10) for frequent repeated inseminations in order to increase conception rate in RBS cattle, we performed daily inseminations as part of the treatment for RBS cows until Graafian follicle rupture was detected by rectal palpation.

This study shows that the appropriate selection of hormone therapy in cows with RBS coupled with repeated inseminations and a thorough rectal examination for evidence of ovulation can significantly improve the conception rate in the affected cattle and consequently reduce the culling rate in the dairy industry due to subfertility.

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