Turk. J. Vet. Anim. Sci.
2012; 36(6): 622-627

doi:10.3906/vet-1104-13

Research Article

The effect of intrauterine cephapirin treatment after insemination on conception rate in repeat breeder dairy cows subjected to the progesterone-based Ovsynch protocol

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Received: 20.04.2011 ● Accepted: 28.12.2011

Abstract: Subclinical endometritis contributes to repeat breeder syndrome in dairy cows. This study evaluated the effect of intrauterine cephapirin benzathine administration after timed artificial insemination (TAI) on the conception rate (CR) in repeat breeder dairy cows. To determine the antibiotic effects, all cows (n = 335) that had more than 3 services with no clinical abnormalities of the reproductive tract received the same combined synchronisation protocol: an ear implant containing progestagen using the Ovsynch protocol and a third gonadotropin-releasing hormone (GnRH) administration 7 days after TAI. Cows in the treatment group (TRT; n = 160) received intrauterine cephapirin 12 h after TAI, and cows in the control group (CON; n = 175) did not receive intrauterine antibiotics. The percentage of cows that responded to the first (67.5% in TRT, 70.9% in CON) and second (98.8% in TRT, 97.1% in CON) GnRH of Ovsynch was similar between the groups. In addition, the response to the third GnRH administration after TAI (88.1% in TRT, 83.9% in CON) did not differ between the groups. However, there was no effect of the cephapirin administration on CR on days 31 and 62 in the repeat breeder cows (43.8% in TRT and 44.0% in CON; 39.4% in TRT and 40.6% in CON, respectively). Thus, post-TAI intrauterine cephapirin administration was not found to be useful for the treatment of potential subclinical endometritis in repeat breeder dairy cows.

Key words: Repeat breeder, conception rate, endometritis, cephapirin, progesterone-based Ovsynch protocol

Introduction

Repeat breeder syndrome is a major economic loss in the dairy industry due to greater insemination costs, increased calving intervals, and increased culling rates (1). Cows that fail to conceive after more than 3 inseminations with fertile semen in the absence of detectable abnormalities are classified as repeat breeders (2). The causes of repeat breeding are multifactorial, but it is likely that subclinical endometritis is an important contributor to repeat breeder syndrome in bovine subfertility (3). Endometritis causes an abnormal

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uterine environment and subclinical endometritis results in repeat breeding. Pathogenic bacteria in the uterus cause inflammation, histological lesions of the endometrium, and a disrupted embryo survival (4). Therefore, an improvement of the intrauterine environment to enhance embryo survival represents a different therapeutic method for repeat breeding.

The treatment of bacterial endometritis with an intrauterine infusion of antibacterial agents and antibiotics before or after insemination results in varying degrees of success (5–8). Cephapirin, a first-generation cephalosporin antibiotic, is active against gram-positive organisms and anaerobic bacteria, but is less active against gram-negative organisms. Therefore, cephapirin is the rational antibiotic choice for intrauterine infusion (9,10). Kasimanickam et al. (7) reported that a single treatment with cephapirin significantly improved the reproductive performance of cows with subclinical endometritis. Veselinovic et al. (5) reported that a single application of cephapirin was sufficient to treat subclinical endometritis. Therefore, this study evaluated the effect of cephapirin benzathine (cephapirin) administration after artificial insemination (AI) on conception rate in repeat breeder dairy cows.

Materials and methods

Cows, housing, and management

This study used a commercial dairy herd with approximately 800 lactating dairy cows in the southern Marmara region of Bursa, Turkey. The herd was composed of two-thirds Holstein–Friesian and one-third Swedish Red cows. The lactating cows were housed in free stall facilities and grouped according to their milk production. All cows were milked 3 times per day and fed complete mixed rations based on Natural Research Council (NRC) recommendations (11). Mean 305-day milk production of the herd was 9.880 kg per cow. Daily milk yield was collected using the ALPRO™ system (DeLaval, Sweden). Average milk production for each cow was recorded for 7 days before and after AI. The body condition of all cows was scored using a 5-point scoring system in which 1 is thin and 5 is fat (12). All animal handling and procedures were approved by the Lalahan Livestock Central Research Institute Animal Care Committee.

Synchronisation protocol and treatment

A total of 335 cows (283 Holstein–Frisian and 52 Swedish Red cows) were used in this study. Cows that received more than 3 inseminations and had no clinical abnormalities of the reproductive tract were selected as repeat breeders in this study. All cows received the same timed artificial insemination (TAI) protocol, Ovsynch using progestagen, and the administration of gonadotropin-releasing hormone (GnRH) 7 days after TAI. Cows at random stages of the oestrous cycle received an ear implant containing progestagen (3 mg norgestomet, Crestar, İntervet, Turkey) and GnRH administration (10 µg buserelin acetate, Receptal, İntervet, Turkey) on day 0. Prostaglandin f2α ($PGF_{2\alpha}$) (500 µg cloprostenol, Estrumate, CEVA-DİF, Turkey) was administered at the time of implant removal on day 7, and the second GnRH injection was administered on day 9. The cows were artificially inseminated at a fixed time, 16–18 h after the second GnRH treatment, using frozen–thawed semen from bulls with previously proven fertility by farm veterinarians. All inseminated cows received a third GnRH administration (10 µg buserelin acetate, Receptal) 7 days after AI on day 17.

All inseminated cows were randomly divided into 2 groups. The cows in the treatment group (TRT) received 500 mg of intrauterine cephapirin benzathine (Metricure, İntervet, Turkey) 12 h after TAI; the cows in the control group (CON) did not receive any treatment after TAI. The experimental protocol is summarised in the Figure.

Ultrasonographic examinations

Cow ovaries were examined using an ultrasound machine (Honda HS 2000 equipped with a 7.5-MHz transducer, Honda, Japan) on the day of the first GnRH treatment and ear implant administration and 7 days later to determine the ovulation response to the first GnRH treatment. The response to the first GnRH treatment was characterised by the appearance of a new corpus luteum (CL) on the ovaries. The maximum follicular size was measured at the time of AI. Cows were examined 7 days after TAI to determine ovulation by the disappearance of the dominant follicle at the time of AI and the appearance of a new CL, and to measure follicle size before the third GnRH treatment. The response to the third GnRH administration was detected 7 days later (Figure).

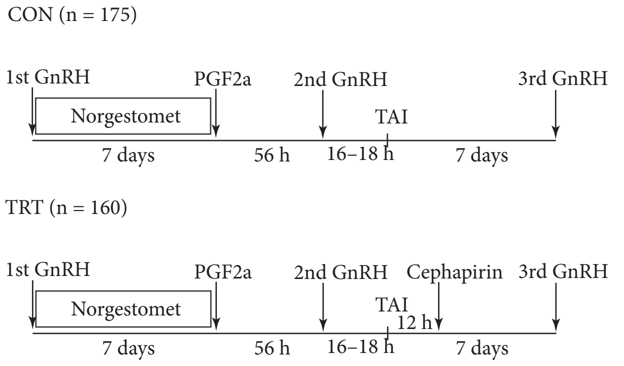

Figure. Summary of the experimental design showing the combined protocol and cephapirin benzathine (cephapirin) treatment in repeat breeder cows. The combined protocol was implemented to eliminate any possible hormonal problems in the repeat breeder cows. The protocol consisted of Ovsynch with an ear implant (3 mg norgestomet) for 7 days between GnRH and $PGF_{2\alpha}$, and GnRH treatment 7 days after insemination to enhance serum progesterone levels by producing accessory CL. Intrauterine cephapirin was used 12 h after insemination in the treatment group (TRT), and untreated cows served as a control (CON).

Pregnancy diagnosis was performed 31 days after insemination by the visualisation of a fluid-filled uterine horn with embryonic vesicles using ultrasonography. A pregnancy check for the presence of a foetus was performed 62 days after insemination. Pregnancy loss was recorded when the second pregnancy check was negative. The conception rate was calculated as the number of pregnant cows divided by the number of cows that received AI.

Statistical analyses

All statistical procedures were performed using SAS computational software (Release 9.2, SAS Institute, Cary, NC, USA). For the statistical analyses, the breed of lactating cows was coded as 1 (Holstein–Friesian) or 2 (Swedish Red). The ovulatory response to the first, second, and third GnRH administrations was coded as 0 (no) or 1 (yes). Presence of conception on days 31 and 62 after insemination was coded as 0 (not pregnant) or 1 (pregnant). Primiparous and multiparous cows were coded as 1 and 2, respectively.

The general linear model (GLM) procedure was performed to analyse and compare the following factors between groups: milk production, days in milk (DIM), body condition score (BCS), parity, number of services, and follicle size at the time of AI and at the third GnRH treatment. To determine the effect of the synchronisation protocol, parity and the response to the first and third GnRH treatments on follicle size at the time of AI were compared. A chi-square analysis using the PROC FREQ procedure was used to analyse the following factors: ovulatory response to the first, second, and third GnRH treatments; and CR at days 31 and 62. A logistic procedure was used to analyse the following factors: the effect of treatment, milk production, DIM, BCS, follicle size at the time of AI, response to the first and third GnRH treatments, and impact of breed on conception rate.

Results

The mean lactation number of cows was similar between groups (2.1 ± 0.07 in TRT and 2.2 ± 0.06 in CON). The mean DIM did not differ between the TRT and CON groups, at 260.3 ± 6.6 and 269.5 ± 6.4, respectively. BCS was similar between the TRT and CON groups, at 2.92 ± 0.01 and 3.01 ± 0.03, respectively. However, average milk production was different ($P = 0.03$) between the groups (31.4 ± 0.6 kg/day in TRT and 29.5 ± 0.6 kg/day in CON). The number of services was not different between the groups at 3.9 ± 0.1 in TRT and 4.1 ± 0.09 in CON.

The percentage of cows that ovulated in response to the first GnRH treatment did not differ between the TRT (67.5%, 108/160) and CON (70.9%, 124/175) groups (Table 1). The synchronisation rate (i.e. the response to the second GnRH treatment) was not different between the groups (98.8%, 158/160 in TRT and 97.1%, 170/175 in CON) (Table 1). The response to the third GnRH treatment was similar between the groups (89.2%, 141/158 in TRT and 82.9%, 141/170 in CON) (Table 1).

The maximum follicular size at the time of AI did not differ between the TRT (16.07 ± 0.18 mm) and CON (16.15 ± 0.18 mm) groups (Table 2). However, cows that ovulated in response to the first GnRH treatment of Ovsynch had smaller ($P = 0.01$) follicle sizes at the time of AI (15.8 ± 0.15 mm) than the nonresponsive cows (16.5 ± 0.23 mm). The presence of an accessory CL on days 31 and 62 in pregnancy examinations was similar between the groups (Table 1).

Table 1. Responses to the progesterone-based Ovsynch protocol in repeat breeder dairy cows.

	Treatment group (n = 160)	**Control group (n = 175)**
Response to first GnRH of Ovsynch	67.5% (108/160)	70.9% (124/175)
Response to second GnRH of Ovsynch (synchronisation rate)	98.8% (158/160)	97.1% (170/175)
Response to third GnRH (7 days after AI)	89.2% (141/158)	82.9% (141/170)
Pregnancy rate at 31 days	43.8% (70/160)	44.0% (77/175)
Pregnancy rate at 62 days	39.4% (63/160)	40.6% (71/175)
Embryonic loss	10.0% (7/70)	7.8% (6/77)
Presence of accessory CL at 31 days of pregnancy	38.2% (54/141)	40.4% (57/141)
Presence of accessory CL at 62 days of pregnancy	29.1% (41/141)	28.4% (40/141)

The effect of cephapirin on CR at 31 days was similar between the TRT (43.8%, 70/160) and CON (44.0%, 77/175) groups. At 62 days, there were no differences in CR between the TRT (39.4%, 63/160) and CON (40.6%, 71/175) groups (Table 1). Milk production; DIM; BCS; parity; breed; response to the first, second, and third GnRH administrations; follicle size at the time of AI; and post-AI cephapirin treatment had no effects on CR.

Discussion

Repeat breeder syndrome is a multifactorial condition, and there is no accurate method for diagnosing the cause in most individuals. Although there are many causative factors, hormonal imbalance and uterine infection are important aetiological factors in repeat breeder cows (2). In this study, the combined protocol of Ovsynch and progestagen plus GnRH administration 7 days after AI was used to eliminate the aetiological hormonal and management factors, such as anovulation, delayed ovulation, progesterone deficiency, and oestrus detection errors, in repeat breeder cows.

Recent studies have shown that the use of the Ovsynch protocol in normal lactating dairy cows produces an ovulatory response to the first GnRH treatment in 45%–95% of cows and a synchronisation rate between 75% and 90% (13,14). Stevenson et al. (15) reported an 85%–95% synchronisation rate after Ovsynch+CIDR. In our study, the first ovulatory response and synchronisation rate after a combined protocol of Ovsynch+progestagen in repeat breeder cows produced similar results as those in previous studies (16,17). In addition, earlier studies indicated a conception rate of 30%–45% after Ovsynch in normal dairy cows (13,17). In this study, the conception rate was 44.0% in the CON group and 43.8% in the TRT group. These results indicate that the combined protocol in this study improved fertility in repeat breeder dairy cows.

When the responses to this combined protocol were evaluated, the response to the first GnRH treatment, the synchronisation rate, the response to the third GnRH treatment, and the follicle size at the time of AI were similar between the TRT and the CON groups (Tables 1 and 2). There were no differences between the groups in terms of covariant factors such as DIM, BCS, the number of services, and parity. The similarity of the group responses to the combined protocol and covariant factors provides a clearer evaluation of the effect of post-AI cephapirin administration in repeat breeder cows.

The leading factors for repeat breeding are metritis and different degrees of endometritis due to the invasion of specific and nonspecific infectious agents in the uterus (18). Subclinical endometritis at AI might impair the uterine environment and disrupt the implantation and development of the

Table 2. Maximum follicle size at the time of TAI and the third GnRH treatment after TAI, and the difference in follicle size in repeat breeder dairy cows that responded to or did not respond to the first GnRH treatment.

	Treatment group	**Control group**
Follicle size at the time of AI	16.07 ± 0.18 mm	16.15 ± 0.18 mm
Follicle size at the time of the third GnRH	15.10 ± 0.20 mm	15.06 ± 0.21 mm
	Ovulatory response (+)	**Ovulatory response (–)**
Maximum follicle size	15.8 ± 0.1^{a} mm	16.5 ± 0.23^{b} mm

a and b superscripts indicate P = 0.01.

embryo (19). Therefore, this evaluation attempted to eliminate endometritis using several intrauterine antibiotic infusions during different periods in repeat breeder and normal cows. This effect has been the objective of several previous studies (5,7).

Although the use of post-AI intrauterine antibiotic administration is well known in the field, only a few scientific papers have evaluated the effect of post-insemination intrauterine antibiotic administration on endometritis (6,20,21). Consistent with our results, no beneficial effect of intrauterine antibiotic treatment was observed (6,20). Although Öztürkler et al. (21) did not mention the effect of intrauterine antibiotic treatment, they found that human chorionic gonadotropin (hCG) and intrauterine antibiotic administration after insemination improved fertility in repeat breeder cows.

Cephapirin was the rational antibiotic for intrauterine infusion because it has activity against gram-positive organisms and anaerobic bacteria with no withholding period for milk. In this study, we hypothesised that a single dose of post-AI intrauterine cephapirin would improve the CR in repeat breeder lactating cows due to the elimination of subclinical endometritis without any milk waste. Kasimanickam et al. (7) reported that a single treatment with cephapirin significantly improved the reproductive performance of cows with subclinical endometritis. Nevertheless, in the current study, cephapirin administration did not enhance CR in repeat breeder cows. Similarly, Ahmadi and Dehghan (8) reported a lack of cephapirin efficacy and proposed that uterine lavage plus $PGF_{2\alpha}$ administration, without cephapirin, may be preferable for the treatment of repeat breeder cows. Additional studies suggest that $PGF_{2\alpha}$ administration is more (22) or equally as (7,23) efficacious as intrauterine antibiotics for the improvement of reproductive performance in cows with endometritis.

Our results showed that post-AI intrauterine cephapirin administration did not enhance CR in repeat breeder dairy cows. Thus, intrauterine cephapirin administered after AI was not found to be useful for the treatment of potential subclinical endometritis in repeat breeder dairy cows.

Acknowledgements

The authors thank TARFAS Co. (Bursa, Turkey) for the use of their herd and facilities. Support was also provided by the Scientific and Technological Research Council of Turkey (TÜBİTAK) under Grant No. TOVAG 107O227.

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