Use of Carazolol at Pre-Synchronized Timed Artificial Insemination in Cows

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Abstract


Efficacy of the β-adrenergic receptor blocker (carazolol) at Timed Artificial Insemination (TAI) was investigated. Cows (n = 73) were pre-synchronized with two PGF2α injections given 14 d apart to initiate the Ovsynch protocol at early and middle luteal stages 14 d later, and received injections of GnRH and PGF2α seven d apart followed by GnRH 48 h later, and TAI 16 - 18 h later. Corpus luteum (CL) was detected via rectal palpation at the beginning of the Ovsynch protocol. Carazolol (Treatment I; n = 41) or physiologic saline (Treatment II; n = 32) were administered via jugular vein five min before TAI. Uterine tone was determined prior to infusion and at TAI via rectal palpation. Pregnancies were diagnosed with transrectal ultrasonography 40 ± 7 d after TAI. Uterine tone was 2.8 (1.4 - 5.3) times higher (P < 0.01) in Treatment I than that in Treatment II at TAI. Increase in uterine tone was affected by treatment × CL × parity (P < 0.05), and 66.7%, 75%, 52.6%, 100% and 16.7%, 33.3%, 25%, 28.6% in primiparous cows with CL and without CL, and in multiparous cows with CL and without CL in Treatments I and II, respectively. Pregnancy rates did not differ between treatments I (34.2%) and II (40.6%). In conclusion, no beneficial effect of carazolol administration prior to TAI was found except for the increased uterine tone.

Ovsynch, carazolol, uterine tone, pregnancy, cow

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embryonic loss (Dalton et al. 2001). Carazolol, a β-adrenergic receptor blocker, has an antagonistic effect on catecholamines. Increase in the uterine tone and PR were reported following carazolol administration prior to AI in cattle (Haas 1986; Hammerl and Rüsse 1990; Kirşan et al. 1998; Krebs 1988; Panowsky 1985). There is no study with regard to the use of carazolol in the Ovsynch protocol. Therefore, the objective of this experiment was to investigate the efficacy of carazolol administration prior to TAI.

Materials and Methods

This experiment was conducted on 73 lactating dairy cows (Brown Swiss, Simmental and their crossbreds with local breeds) in tied stall barn with access to pasture in the Kars District, Turkey. All animals were presynchronized with two PGF2α (Estrumate®; cloprostenol sodium, 526 mcg; i.m.) injections given 14 d apart. The objective of the pre-synchronization protocol was to target a high percentage of cows to initiate the Ovsynch protocol at the most favourable stages (d 7 to 12) of the oestrous cycle (Moreira et al. 2001). All cows enrolled to the Ovsynch protocol, and were palpated per rectum for the presence of CL 14 d after the second PGF2α injection of pre-synchronization, and received an injection of GnRH (Receptal®; 0.021 mg buserelin acetate, im). Seven d later PGF2α was injected followed by the ovulatory injection of GnRH 48 h later, and TAI 16 to 18 h later. Animals were assigned randomly to receive either carazolol (Suacron®, 2.5 mg; treatment I, n = 41) or physiologic saline (treatment II, n = 32) via jugular vein five min prior to TAI. Animals were palpated per rectum with ignorance of treatments to determine tonicity of the uterus immediately before injections and TAI. At each palpation, degrees of the uterine tones were evaluated using a 4-point scale (1 [no tone], 2 [slight tone], 3 [moderate tone] and 4 [extreme tone]). Afterwards, increases in uterine tones were calculated based on the differences of uterine tones at each palpation. For TAI, a single insemination dose possessing 30 - 50 × 10⁶/ml spermatozoa with at least 55% motile and 85% live spermatozoa was used. Body condition scores (BCS), using a 5-point scale (1 to 5) with intervals of 0.25, were recorded during the Ovsynch protocol (Edmonson et al. 1989). Pregnancies were diagnosed with transrectal ultrasonography at 40 ± 7 d after TAI.

PR following the first service and increased uterine tone were analyzed by the Logistic Regression-Stepwise Selection Procedure of SAS to determine independent variables among all variables and interactions. The mathematical model for PR included location, replicate (year), parity, treatment, day in milk (DIM) at the beginning of pre-synchronization, BCS, presence of CL, AI technician, sire, uterine tone at TAI, increase in uterine tone at TAI, and higher order interactions. The mathematical model for the increased uterine tone at TAI included location, replicate, parity, treatment, DIM, BCS, the presence of CL, AI technician, and higher order interactions. However, results for selected variables were reported as risk ratios to be more realistic as opposed to odds ratios following determination by stepwise procedure. The Proc Freq procedure of SAS was utilized to obtain frequency values and risk ratios. The Proc Univariate procedure of SAS was used to obtain median values of BCS and DIM.

For BCS, median value (2.75) was used as a cut point, and cows were classified as having poor (BCS ≤ 2.75) or optimal (BCS > 2.75) condition. For DIM, the median value (36) was used as a cut point, and cows were classified as early (DIM ≤ 36) or late (DIM > 36) postpartum.

Results

PR to the first service at 40 ± 7 d following TAI did not differ between treatment I (14/41; 34.2%) and treatment II (13/32; 40.6%; Fig. 1). Logistic regression-stepwise procedure indicated that interactions of presence of CL at the beginning of the Ovsynch protocol × BCS (P < 0.01), treatment × presence of CL at the beginning of the Ovsynch protocol × parity (P < 0.05) had an influence on PR. PR of cows having optimal BCS with CL, cows having poor BCS with CL, cows having optimal BCS without CL, cows having poor BCS without CL were 53.9% (14/26), 33.3% (9/27), 33.3% (3/9) and 9.1% (1/11), respectively. PR of primiparous cows with CL, primiparous cows without CL, multiparous cows with CL, multiparous cows without CL were 58.3% (7/12), 0% (0/4), 21.1% (4/19), 50% (3/6) and 66.7% (4/6), 0% (0/3), 50% (8/16) and 14.3% (1/7) in treatments I and II, respectively.

The distribution of cows with an increased uterine tone at TAI was significantly affected by treatment (P < 0.01) and interaction of treatment × presence of CL at the beginning of the Ovsynch protocol × parity (P < 0.05). Increase in the uterine tone at TAI was 2.8 (1.4 - 5.3) times higher in cows injected with carazolol (65.9%, 27/41) than those injected with physiologic saline prior to TAI (25%, 8/32; Fig. 1). Rates of the increased uterine tone
at TAI in primiparous cows with CL, primiparous cows without CL, multiparous cows with CL and multiparous cows without CL were 66.7% (8/12), 75% (3/4), 52.6% (10/19), 100% (6/6) and 16.7% (1/6), 33.3% (1/3), 25% (4/16), 28.6% (2/7) in treatments I and II, respectively (Fig. 2). Absence of palpable CL at the beginning of the Ovsynch protocol was 27.4% (20/73) in this study.

Discussion

Significant interaction of presence of CL at the beginning of the Ovsynch protocol × BCS indicates the importance of BCS and pre-synchronization to achieve higher PR in TAI protocol. In this matter, the highest PR in cows having optimal BCS with CL and the lowest PR in cows having poor BCS without CL indicates the effect of BCS and pre-synchronization on the efficacy of TAI protocol in the current study.

No effect of the uterine tone at TAI on PR in buffalo following the Ovsynch was reported (De Araujo Berber et al. 2002). Similarly, no beneficial effect of the uterine tone on PR was found in the present study (Fig. 1). Higher uterine tone at TAI in anoestrous or non-synchronized cows (based on palpable CL) might be attributable to newly recruited
ovulatory follicles escaped from luteinization by ovulatory injection of GnRH in the current study. In this regard, the higher uterine tone following carazolol administration in multiparous cows with no palpable CL at the beginning of the Ovsynch protocol could be due to higher rates of anoestrus or asynchrony. In contrast, cycling and synchronized cows might have a lower uterine tone due to luteinization of the ovulatory follicle leading to a decrease in confidence of AI technicians to the Ovsynch protocol (Fig. 2).

Numerically lower PR in carazolol treated group in this study is in disagreement with Kirşan et al. (1998) who reported higher PR following carazolol administration 3 - 5 min prior to AI following the detected oestrus in cattle; however, the time of AI relative to the onset of oestrus is not reported in that study. In this regard, PR to cows inseminated were lower for the Ovsynch program compared to conception rates of cows inseminated following the detected oestrus (Burke et al. 1996; Pursley et al. 1997; Stevenson et al. 1999). This lower PR to inseminated cows of the Ovsynch protocol compared to services made at detected oestrus could be due to insemination of all cows regardless of their cyclicity status and reproductive problems; whereas in the current study, tightly synchronized ovulation between 24 and 32 h following the Ovsynch protocol, and administration of carazolol immediately prior to TAI could probably result in numerically lower PR. A lower fertilization rate was reported following insemination of cows at the time of the onset of oestrus (Dalton et al. 2001). Probably, carazolol injection could increase the speed of spermatozoon transport to the fertilization site; thus, fertilization and embryo quality could be lower due to aged spermatozoa. Likewise, the administration of carazolol immediately prior to TAI could accelerate the transport of fertilized ovum in oviduct, and could cause disruption of synchrony between uterus and embryo because the effect of carazolol lasts up to 8 - 12 h. Gajewski et al. (2006a) reported that carazolol has an inhibitory effect on progesterone secretion by cultured granulosa cells in cow. Numerically lower PR in carazolol treated group in our study could also be attributed to lower progesterone production by granulosa cells leading to impairment of the early embryonic development in oviduct.

The interaction effect of treatment × presence of CL at the beginning of the Ovsynch protocol × parity indicates that PR is lower in carazolol administered cows than that in control cows among multiparous cows with palpable CL; whereas, PR are similar among primiparous cows with palpable CL between treatments. Although there are no adequate cows in each category to formulate an assumption, these results imply that among carazolol administered animals the decrease in PR is more severe in multiparous than in primiparous cows with palpable CL. These lower PR in multiparous cows with palpable CL treated with carazolol could be explained by more reproductive disorders in multiparous cows compared to primiparous cows.

Similarly to non-significant PR in carazolol treated group in the current study, no significant effect of carazolol administration 30 min prior to AI following a detected oestrus on ovulation and PR was reported other than a numerically lower rate of delayed ovulation (Bostedt et al. 1995). No effect of carazolol treatment was reported with regard to the number of follicles and CL, embryo quality and effectiveness of AI during superovulation protocol in heifers (Gajewski et al. 2006b). In a study investigating the efficacy of a blockade of the stress-induced adrenergic action around AI by carazolol administration 54 h after the second PGF$_{2 \alpha}$ injection given 11 d apart, it was found that the use of carazolol around the time of AI should be considered because carazolol administration following PGF$_{2 \alpha}$ induced oestrus postponed the onset of oestrous expression despite no significant effect on gonadal steroids and LH release in cows (Perez et al. 2005). Thus, numerically lower PR in carazolol treated group in the current study could not be attributed to the effect of carazolol on LH release.

The anoestrous rate based on the presence of palpable CL at the beginning of the Ovsynch
protocol was 27.4%, and it was acceptable in our region. It was reported that the sensitivity, specificity and positive predictive value of palpation per rectum for identifying mid-cyclic CL were 85%, 95.7% and 89.5%; respectively, with respect to plasma progesterone (P$_4$) concentrations (Ribadu et al. 1994). Similarly, detection of CL by palpation per rectum was verified by high concentrations of P$_4$ only in 82% of the cases (Ott et al. 1986). Moreover, the absence of CL could reflect the failure of pre-synchronization because a high percentage of cows should have been on d 7 to 12 of the oestrous cycle at the beginning of the Ovsynch protocol (Moreira et al. 2001).

In conclusion, no beneficial effects of the use of carazolol prior to TAI were found except for the increase in the uterine tone. Based on our results, we are unable to explain how other researchers achieved the increase in the conception rate following carazolol administration. Therefore, this experiment warrants further research to compare the effect of carazolol administration following natural and synchronized oestruses on the time of ovulation and fertility.

**Využití carazololu při synchronizaci ovulace k časované umělé inseminaci krav**

Byl zkoumán účinek β-adrenergijního blokátoru (carazolol) při synchronizaci ovulace k časované umělé inseminaci krav (TAI). Sedmdesát tři krav bylo ošetřeno dvěma injekcemi PGF$_{2α}$ s odstupem 14 dní (zahájení Ovsynch metody v počáteční a střední luteální fázi o 14 dní později). Dále byly kravám aplikovány GnrH a PGF$_{2α}$ s odstupem 7 dní a poté GnrH po 48 h a TAI byla provedena za 16 - 18 hod. Na počátku Ovsynch metody bylo rektálně detekováno corpus luteum (CL). Do v. jugularis byl 5 min před TAI aplikován carazolol (ošetření I; n = 41) nebo fyziologický roztok (ošetření II; n = 32). Před infuzí byl rektální palpací zkontrolován tonus dělohy. Březost byla diagnostikována transrektálním sonografickým vyšetřením 40 ± 7 dní po TAI. Tonus dělohy byl při TAI 2,8 (1,4 – 5,3) krát větší ($P < 0,01$) v rámci ošetření I ve srovnání s ošetřením II. Zvýšení tonu dělohy bylo ovlivněno ošetřením, CL a paritou ($P < 0,05$), a 66,7%, 75%, 52,6%, 100% a 16,7%, 33,3%, 25%, 28,6% u jalovic s CL a bez CL a u dojnic s CL a bez CL při ošetření I, respektive II. Počty březích krav se mezi ošetřenými I (34,2%) a II (40,6%) nelišily. Ze studie vyplývá, že aplikace carazololu před časovanou umělé inseminaci krav nemá pozitivní efekt, ale pouze zvyšuje tonus dělohy.

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