Indian Journal of Animal Sciences **81** (3): 257–59, March 2011

Ovsynch plus CIDR-based fixed-time AI protocol as a therapeutic strategy in repeat-breeder crossbred dairy cattle

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Received: 6 August 2010; Accepted: 22 December 2010

Key words: Cattle, CIDR, Conception rate, Ovsynch, Repeat-breeder

Repeat-breeding in dairy cattle is associated with estrous detection errors, endocrine dysfunctions, ovulatory defects and hence, poor fertilization rates. An approach to overcome these problems is the use of controlled internal drug release (CIDR)-based fixed-time breeding protocol (Kim *et al*. 2007). However, a newly recruited follicle may sometimes fail to ovulate spontaneously after CIDR withdrawal (Ambrose *et al*. 2004). On the other hand, Ovsynch, a timed-artificial insemination (AI) protocol may yield 21% conception rate in repeat-breeders (Kasimanickam *et al*. 2005). Hence, to overcome the shortcomings encountered when CIDR or Ovsynch were used alone, present study assessed the efficacy of Ovsynch plus CIDR-based fixed-time AI protocol for improving the conception rate in repeat-breeder dairy cattle.

A study was conducted on 18 lactating repeat-breeder crossbred cattle (age, 3–6 years; parity, 2.06±0.30; BCS, 3.36±0.16; milk production, 19.06±2.7 litre/day) reared at private dairy farms. The enrolled repeat-breeder cattle had normal estrous cycles but had failed to conceive after repeated AIs (4.06±0.29). Before the start of present study, the average duration of estrus exhibited by these cattle was 59.33±2.99 h and there was no abnormality in the estrual cervico-vaginal mucus. All the animals had no gross abnormality of the reproductive tract as revealed by rectal palpation and transrectal ultrasonography.

All the cattle were subjected to ovsynch plus CIDR program. In brief, ovsynch involved administration (i.m.) of 20µg GnRH analogue on day 0, followed by 500 µg of prostagland in $F_{2\alpha}$ ($PGF_{2\alpha}$, Cloprostenol sodium) on day 7, and a second GnRH treatment (20 µg) on day 9. In addition, each of these cattle was inserted, intra-vaginally, a CIDR (1.38 g progesterone) device from day 0 till day 7. Animals were subjected to fixed-time AI at 24 h after second GnRH (day 10) without estrus detection.

Transrectal ovarian ultrasonography and blood sampling were carried out at the time of each treatment and at AI. In addition, ultrasonography was conducted about 24 h after AI to confirm ovulation. Blood was sampled from the jugular vein; plasma was separated immediately and was frozen at –20°C until analysis. First service conception rate was noted by diagnosing pregnancy on day 90 subsequent to second GnRH. Plasma progesterone was assayed with solid-phase radioimmunoassay using a progesterone antibody raised in our laboratory (Ghuman *et al*. 2009). Sensitivity of the assay was 0.1 ng/ml; intra-assay and inter-assay variation coefficients were 6.5% and 9.0%, respectively.

Chi-square test (Dytham 1999) compared the number of cattle that responded to various treatments of the protocol and conceived subsequently. Two-sample Student's *t*-test (Dytham 1999) compared different parameters of cattle that conceived or failed to conceive.

As the cattle enrolled in present study were exhibiting regular estrous cycles, therefore, on the day of beginning of protocol (day 0), a CL of 10.94±1.53 mm diameter as well as luteal phase plasma progesterone (2.01±0.2 ng/ml) was recorded in all the cattle. On same day, a large follicle of 10.73±0.7 mm diameter was also detected. Within 24 h of initiation of treatment, this follicle ovulated in all the cattle ($P<0.05$) and that is in agreement with our previous findings (Ghuman *et al*. 2010b).

On the day of CIDR removal (day 7), the presence of considerable amounts of plasma progesterone (2.20±0.30 ng/ml) could be due to contributions from CIDR as well as from the CL (11.50±1.61 mm) that had appeared concomitant to first GnRH-induced ovulation. In a previous study, progesterone-based intravaginal implants maintained luteal phase plasma progesterone for 15 days in true anestrous buffaloes (Singh *et al.* 2009).

All the cattle responded ($P<0.05$) to a luteolytic dose of

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$PGF_{2\alpha}$ administered on day 7 as revealed by the decrease (P<0.05) in plasma progesterone (0.60±0.10 ng/ml) and CL diameter (3.39±0.88 mm) on day 9. Similarly, in previous studies, plasma progesterone declined to basal concentrations within 2 days after $PGF_{2\alpha}$ treatment of mature CL (Dadarwal *et al*. 2009) or CIDR removal (Ghuman *et al.* 2010a).

In a previous study, a GnRH plus CIDR treatment induced the synchronous emergence of a follicular wave (Kim *et al.* 2006). In fact, injection of GnRH induces release of LH from the pituitary, leading to ovulation and emergence of new follicle wave 1–2 days later (Ghuman *et al*. 2010b). Thus, on day 7, a large follicle (10.39±0.60 mm) was observed in all the cattle (P<0.05), that continued to grow (P<0.05) till the day of second GnRH (14.17±0.57 mm) and AI (15.44±0.6 mm). Furthermore, ovulation occurred between 24 and 48 h after second GnRH and was synchronized in 100% cattle, as reported in a previous instance (Kim *et al.* 2006). Based on our findings, the potential importance of completing the full ovsynch protocol can be emphasized. This study revealed that treatment with GnRH about 48 h after CIDR withdrawal has the ability to induce ovulation of a newly recruited follicle that sometimes fail to ovulate spontaneously after CIDR withdrawal (Ambrose *et al.* 2004).

All the repeat-breeder cattle used in this study had failed to conceive during at least 3 previous inseminations, however; subsequent to the current fixed-time AI protocol, an appreciably high conception rate (61%, P<0.05) was recorded. The observed conception rate was higher compared to previous results (21%) in which Ovsynch protocol was used alone for the treatment of repeat-breeder dairy cattle (Kasimanickam *et al*. 2005).

Repeat-breeder cattle that subsequently conceived or failed to conceive indicated that there was no difference (P<0.05) in plasma progesterone or the diameter of ovulatory follicle on the day of AI. The recording of similar diameter of ovulatory follicle in conceiving or non-conceiving counterparts was in contrast to previous studies (Lynch *et al*. 2010). Further, no difference (P>0.05) was observed in per day lactation yield, body condition score, as well as pre-treatment estrus duration of repeat-breeder cattle that conceived or not.

The history of repeat-breeder cattle enrolled suggested that these were having prolonged duration of estrus (59.33±2.99 h). In fact, about 40% repeat breeder crossbred cattle display prolonged estrus (37–60 h vs 24–36 h; Dadarwal *et al*. 2005). Out of these, about 70% record suprabasal progesterone (≥ 0.35ng/ml) at estrus, thus, leading to delayed ovulation of 76% follicles due to improper LH surge (Bage *et al.* 2002). The occurrence of prolonged estrus and presence of suprabasal progesterone at estrus is a phenomenon which occurs repeatedly in consecutive cycles, and thus might have lead to repeated conception failure in cattle used. Several factors may be involved for conception failure: (a) unsuccessful fertilization due to poor association between time of AI and ovulation, (b) compromised development of oocyte due to exposure to small LH surge, and (c) suboptimal luteal profile which is unable to support embryo development (Bage *et al*. 2002). However, the conception rates reported in this study indicate that these endocrine dysfunctions might have been eliminated as synchronized ovulation was observed subsequent to fixed-time AI. Thus, the present protocol is an effective treatment regimen for repeat-breeder dairy cattle especially with prolonged duration of estrus.

SUMMARY

Cattle (18) were administered GnRH analogue (20 µg buserelin) on day 0 and day 9, and $PGF_{2\alpha}$ (500 µg cloprostenol) on day 7. A controlled internal drug release (CIDR, 1.38 g progesterone) device was inserted from day 0 till day 7. On day 10, AI was carried out in all the cattle. In response to day 0 GnRH, all the cattle ovulated within 24 h with subsequent development of corpus luteum (CL). All the cattle responded to $PGF_{2\alpha}$ on day 7. All the cattle exhibited synchronous ovulation between 72–96 h after CIDR and removal on day 7 (or 24 and 48 h after day 9 GnRH). First service conception rate was appreciably higher (61%) than 0% conception rate of all the cattle during their previous AIs (4.06±0.29). It is inferred that Ovsynch plus CIDR-based fixed-time AI protocol is highly successful for inducing synchronized ovulation in repeat-breeder cattle, and yields an appreciably high conception rate.

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