



A CIDR-based AI protocol establishes pregnancy in repeat-breeder dairy cattle

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ABSTRACT

The objectives of this study were to document ovarian and endocrine responses as well as pregnancy establishment following the treatment of repeat-breeder crossbred dairy cattle with a progesterone-releasing controlled internal drug release (CIDR) -based artificial insemination (AI) protocol. Cattle (25) received prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 500 μ g) on day 0, concurrent with the intravaginal placement of CIDR (1.38 g progesterone) for 15 days, followed by gonadotropin releasing hormone (GnRH) analogue (20 μ g) administration at the spontaneous onset of estrus (day 17, 18 or 19). About 10 h after GnRH, AI was carried out in all the cattle. On day 0, a corpus luteum (CL, 13.7 $\pm$ 1.4 mm) and a large follicle (11.1 $\pm$ 0.6 mm) were detectable in all the cattle. On day 15, the former was regressed ($P<0.05$), whereas the latter disappeared completely in all the animals. Estrus detection was carried out daily subsequent to CIDR removal. All the cattle exhibited onset of estrus between 48–96 h after CIDR removal. The preovulatory follicle observed at the time of AI (15.0 $\pm$ 0.5 mm) ovulated in all the cattle ($P<0.05$) between 72–120 h after CIDR removal (or 24–48 h after GnRH). First service conception rate was appreciably greater (52%, $P<0.05$) in comparison to 0% conception rate of all the cattle during their previous AIs (4.2 $\pm$ 0.2). Retrospective analysis suggested that cattle failing to conceive had persistently higher ($P<0.05$) luteal activity on the day of AI compared to their conceiving counterparts. In conclusion, a CIDR-based ($PGF_{2\alpha}$ +CIDR-GnRH) based AI protocol is highly successful for inducing ovulation in the repeat-breeder (due to endocrine dysfunctions) cattle, and resulted in an acceptable conception rate.

Key words: Cattle, CIDR, GnRH, Pregnancy, Repeat-breeder

Repeat-breeder cattle are defined as sub-fertile animals which fail to conceive with at least three services and have no anatomical or infectious irregularities. Huge economic losses are encountered due to high incidence (20–39%) of repeat-breeding (Nanda and Singh 2008). Causes of repeat-breeding include estrous detection errors, endocrine dysfunction, ovulatory defects, poor fertilization rates and/or early embryonic loss (Nanda and Singh 2008). Some of these problems associated with repeat-breeders can be eliminated by the use of hormonal protocols designed to induce timely follicular development, ovulation and acceptable pregnancy rates (Rodrigues *et al.* 2010). Artificial insemination (AI) subsequent to the treatment of repeat-breeder dairy cattle with a progesterone releasing device (controlled internal drug release, CIDR) is an effective technique to achieve high pregnancy rate (52%) through a

fine regulation of folliculogenesis (Kim *et al.* 2006). Others have suggested that treatment with gonadotropin releasing hormone (GnRH) after CIDR removal is necessary to induce ovulation of a large follicle that occasionally fails to ovulate on its own subsequent to CIDR removal (Ambrose *et al.* 2004). Thus, the aim of present study was to assess the efficacy of a CIDR-based AI protocol for pregnancy establishment in repeat-breeder dairy cattle.

MATERIALS AND METHODS

The present study was conducted on 25 repeat-breeder crossbred dairy cattle (age, 3–6 years; parity: 1–3; BCS, 3–5) reared at private dairy farms of Punjab state. The enrolled repeat-breeder cattle had normal estrous cycles but had failed to conceive after repeated AIs (4.2 $\pm$ 0.2). Before the start of present study, the average duration of estrus exhibited by these cattle was 59.0 $\pm$ 2.6 h and there was no gross abnormality in the estrual cervico-vaginal mucus and the reproductive tract.

Protocol: All the cattle received 500 μ g prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on day 0, concurrent with the intravaginal placement of a CIDR (1.38 g progesterone) for 15 days. At the end of 15 day treatment period, the CIDR device was removed.

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Thereafter, estrus detection was carried out daily (three times a day at regular intervals) through vasectomised (teaser) bull parading, cervico-vaginal discharge, standing estrus and/or other secondary estrus signs. Animals exhibiting onset of estrus (day 17, 18 or 19) immediately received a 20µg GnRH analogue (buserelin acetate). About 12 h after GnRH, cattle were inseminated using frozen-thawed semen from bulls of proven fertility. Inseminations were carried out by one person after properly checking the microscopic picture of semen.

Transrectal ovarian ultrasonography and blood sampling: Ultrasonography and jugular vein blood sampling was carried out on day 0 and 15 and at the time of AI. In addition, ultrasonography was conducted about 24 h after AI to confirm ovulation. Ovaries were systematically examined and images were recorded on a diagram of ovary by carefully sketching the size and relative location of largest follicle and visible corpus luteum (CL) (Ghuman *et al.* 2010). Ovulation was verified based upon the subsequent emergence of a corpus haemorrhagicum on the site previously occupied by a disappeared largest follicle (Ghuman *et al.* 2010). In heparinized vials, blood was sampled from the jugular vein; plasma was separated immediately and was frozen at -20°C until analysis.

Fertility response: First service conception rate was noted by transrectal diagnosis of pregnancy on day 90 post-AI.

Hormone analysis: Plasma progesterone was assayed with solid-phase radioimmunoassay using a progesterone antibody (Ghuman *et al.* 2009). Sensitivity of the assay was 0.1 ng/ml; intra- assay and inter-assay variation coefficients were 6.6% and 8.9%, respectively.

Statistical analysis: Chi-square (χ^2) test was employed for the number of cattle that responded to various treatments of the protocol and conceived subsequently. Two sample Student's t-test was employed to compare different parameters of cattle that conceived or failed to conceive.

RESULTS AND DISCUSSION

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 13.70 ± 1.40 mm diameter as well as luteal phase plasma progesterone (1.82 ± 0.16 ng/ml) was recorded in all the cattle (Fig. 1).

Following $\text{PGF}_{2\alpha}$ plus CIDR treatment on day 0, there was a considerable decrease in the diameter of CL (3.2 ± 1.1 mm) on day 15 in all the cattle ($P < 0.05$, Fig. 1). This could be due to the fact that, in the present study, CIDR treatment was continued for 15 days and a luteolytic dose of $\text{PGF}_{2\alpha}$ was also given at the time of CIDR insertion. A prolonged treatment with progesterone-releasing device lowers pulsatile LH secretion leading to atresia of luteal structures as well as large follicles (Kim *et al.* 2006). In fact, large follicles observed on day 0 (11.1 ± 0.6 mm) also disappeared by day 15 (Fig. 1).

On the day of CIDR removal (day 15), the presence of

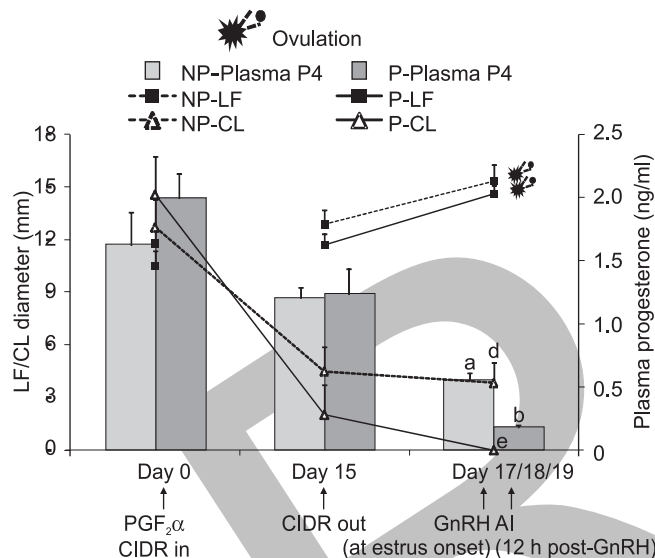


Fig. 1. Details of various variables during the treatment of repeat-breeder crossbred cattle (n=25) with a CIDR-based artificial insemination (AI) protocol. Data has been presented as data of cattle that conceived (n=13) or failed to conceive (n=12) subsequently. avs b, b vs c, d vs e, f vs g $P < 0.05$. CIDR, Controlled internal drug release; CL, corpus luteum; GnRH, gonadotropin releasing hormone; LF, large follicle; NP, non-pregnant; P, pregnant; P₄, plasma progesterone; $\text{PGF}_{2\alpha}$, prostaglandin $\text{F}_{2\alpha}$.

considerable amounts of plasma progesterone (1.22 ± 0.11 ng/ml) could be due to contributions from CIDR as plasma progesterone reached basal concentrations (0.36 ± 0.05 ng/ml, $P < 0.05$) subsequent to CIDR withdrawal (Fig. 1). In a previous study, progesterone-based intravaginal implants were shown to maintain luteal phase plasma progesterone for a period of 15 days in true anestrus buffaloes (Singh *et al.* 2009).

On the day of CIDR removal, a large follicle (12.30 ± 0.50 mm) was observed in all the cattle ($P < 0.05$, Fig. 1). This could be due to the fact that, in the present study, prolonged-CIDR treatment-induced atresia of the follicular structures might have lead to emergence of a new synchronous follicular wave after an increase in follicle stimulating hormone (Kim *et al.* 2006). In fact, CIDR induces emergence of a synchronous follicular wave with the same pattern as observed in cattle having normal estrous cycles (Kim *et al.* 2006).

All the repeat-breeder cattle exhibited estrus between 48–96 h after CIDR removal. Prolonged-treatment with a progesterone device increases the ability of the hypothalamo-pituitary gonadal axis to generate estrus / LH surge in response to an increase in endogenous estradiol (Todoroki and Kaneko 2006).

The large follicle observed in all cattle on the day of CIDR removal continued to grow ($P < 0.05$) till the time of AI (15.0 ± 0.5 mm, Fig. 1). Furthermore, this follicle ovulated between 24–48 h after GnRH in 100% cattle (Fig. 1), as

reported in a previous instance that utilized similar protocol (Kim *et al.* 2006). Based on these findings, the potential importance of administering GnRH can be emphasized. This protocol (PGF_{2α}+CIDR-GnRH) revealed that treatment with GnRH at the onset of estrus after CIDR withdrawal has the ability to induce ovulation of a newly recruited follicle that may fail to ovulate spontaneously after the removal of CIDR (Ambrose *et al.* 2004).

All the repeat-breeder cattle used in present study had failed to conceive during at least three previous inseminations (4.2±0.2), however, subsequent to PGF_{2α}+CIDR-GnRH-AI protocol, an appreciably high conception rate (52%, P<0.05) was recorded. This could be the impact of fine regulation of plasma progesterone profile during pre-conception period. It is well known that the priming of reproductive system with adequate amount of circulating progesterone during the pre-conception period is favorable for the better development of ovulatory follicles that will yield a better developed CL and hence successful conception subsequently (Folman *et al.* 1990). Similar to the present results, in normal breeding cattle, the pregnancy rate was 65% when progesterone was added to the ovsynch protocol (Kim *et al.* 2005). The beneficial impact of progesterone-based protocol can also be assessed from a study in which only 21% conception rate was observed following the application of ovsynch protocol in repeat-breeder dairy cattle (Kasimanickam *et al.* 2005). Between repeat-breeder cattle that subsequently conceived or failed to conceive, there was no difference (P>0.05) in the ovulatory follicle diameter on the day of CIDR removal (11.7±0.6 versus 12.9±0.8 mm, respectively) or on the day of AI (14.6±0.4 versus 15.3±0.9 mm, respectively, Fig. 1). Recent reports have suggested that the diameter of preovulatory follicle may (Lynch *et al.* 2010) or may not (Colazo *et al.* 2009) have a role in pregnancy rate in dairy cattle subjected to estrus synchronization protocols.

However, repeat-breeder cattle that failed to conceive had active (P<0.05) luteal profile (plasma progesterone: 0.55±0.1 ng/ml, CL diameter: 3.8±1.2 mm) at the time of AI in comparison to their conceiving counterparts (plasma progesterone, 0.18±0.0 ng/ml; CL diameter, 0.0±0.0 mm; Fig. 1). Suprabasal plasma progesterone (≥0.3ng/ml) around the period of LH surge is known to interfere with nuclear and cytoplasmic maturation of the oocytes by affecting LH surge parameters, and hence, subsequent embryo development (Duchens *et al.* 1996).

The history of repeat-breeder cattle enrolled suggested that these were having prolonged duration of estrus period (59.0±2.6 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 repeat conception failure in cattle used (Bage *et al.* 2002). However, the conception rates reported in this study indicate that some of these endocrine dysfunctions might have been eliminated as timely ovulation was observed subsequent to AI. Thus, PGF_{2α}+CIDR-GnRH protocol followed by AI is an effective treatment regimen for repeat-breeder dairy cattle especially with prolonged duration of estrus.

Use of a CIDR (PGF_{2α}+CIDR-GnRH) -based AI protocol is a highly effective therapeutic strategy for establishing pregnancies in repeat-breeder (due to endocrine dysfunctions) crossbred dairy cattle. This protocol yields timely follicular development, induces ovulation between 24–48 h following GnRH and appreciably high first-service conception rate (52%) in repeat-breeder cattle with endocrine dysfunctions.

REFERENCES

- Ambrose D J, Schmitt E J P, Lopes F L, Mattos R C and Thatcher W W. 2004. Ovarian and endocrine responses associated with the treatment of cystic ovarian follicles in dairy cows with gonadotropin releasing hormone and prostaglandin F_{2α}, with or without exogenous progesterone. *Canadian Veterinary Journal* 45: 931–37.
- Bage R, Gustafsson H, Larsson B, Forsberg M, Rodriguez-Martinez H. 2002. Repeat breeding in dairy heifers: follicular dynamics and estrous cycle characteristics in relation to sexual hormone patterns. *Theriogenology* 57: 2257–69.
- Colazo M G, Gordon M B, Rajamahendran R, Mapletoft R J and Ambrose D J. 2009. Pregnancy rates to timed artificial insemination in dairy cows treated with gonadotropin-releasing hormone or porcine luteinizing hormone. *Theriogenology* 72: 262–70.
- Dadarwal D, Singh Jagir, Honparkhe M, Cheede G S and Kang R S. 2005. Investigations on repeat breeding crossbred cattle with history of prolonged estrus. *Indian Journal of Animal Science* 75: 922–24.
- Duchens M, Rodríguez-Martínez H, Forsberg M and Gustafsson H. 1996. Ultrastructure of bovine ovarian follicles induced to extended growth by perioestrous suprabasal progesterone levels. *Acta Veterinaria Scandinavica* 37: 239–50.
- Folman, Y., M. Kaim, Z. Herz, and M. Rosenberg. 1990. Comparison of methods for the synchronization of estrous cycles in dairy cows. 2. Effects of progesterone and parity on conception. *Journal of Dairy Science* 73: 2817–25.
- Ghuman S P S, Dadarwal D, Honparkhe M, Singh J and Dhaliwal G S. 2009. Production of polyclonal antiserum against progesterone for application in radioimmunoassay. *Indian Veterinary Journal* 86: 909–11.
- Ghuman S P S, Singh J, Honparkhe M, Dadarwal D, Dhaliwal G S and Jain A K. 2010. Induction of ovulation of ovulatory size nonovulatory follicles and initiation of ovarian cyclicity in summer anoestrous buffalo heifers (*Bubalus bubalis*) using melatonin implants *Reproduction in Domestic Animals* 45: 600–607.
- Kasimanickam R, Cornwell J M and Nebel R L. 2005. Fertility following fixed-time AI or insemination at observed estrus in Ovsynch and Heatsynch programs in lactating dairy cows.

- Theriogenology* **63**: 2550–59.
- Kim I, Suh G, Kim U and Kang H. 2006. A CIDR-based timed AI protocol can be effectively used for dairy cows with follicular cysts. *Animal Reproduction Science* **95**: 206–13.
- Kim U H, Suh G H, Nam H W, Kang H G and Kim I H. 2005. Follicular wave emergence, luteal function and synchrony of ovulation following GnRH or estradiol benzoate in a CIDR-treated, lactating Holstein cows. *Theriogenology* **63**: 260–68.
- Lynch C O, Kenny D A, Childs S and Diskin M G. 2010. The relationship between periovulatory endocrine and follicular activity on corpus luteum size, function, and subsequent embryo survival. *Theriogenology* **73**: 190–98.
- Nanda A S and Jagir Singh. 2008. Factors responsible for increased calving intervals in cross-bred cows in India. *Proceedings of XXV World Buiatrics Congress*. Budapest, Hungary. pp. 100–07.
- Rodrigues C A, Teixeira A A, Ferreira R M, Ayres H, Mancilha R F, Souza A H and Baruselli P S. 2010. Effect of fixed-time embryo transfer on reproductive efficiency in high-producing repeat-breeder Holstein cows. *Animal Reproduction Science* **118**: 110–17.
- Singh J, Ghuman S P S, Honparkhe M and Singh N. 2009. Investigations on dominant follicle development, estrus response, ovulation time and fertility in PRID-treated anestrus buffalo heifers. *Indian Journal of Animal Sciences* **79**: 773–77.
- Todoroki J and Kaneko H. 2006. Formation of follicular cysts in cattle and therapeutic effects of controlled internal drug release. *Journal of Reproduction and Development* **52**: 1–11.