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Impact of initiating a $PGF_{2\alpha}$-GnRH fixed-time AI protocol at the late luteal phase on reproductive performance of repeat-breeder crossbred 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 prostaglandin $F_{2\alpha}$-gonadotropin releasing hormone ($PGF_{2\alpha}$-GnRH) fixed-time artificial insemination (AI) protocol at the late luteal phase. Cattle (15) were administered $PGF_{2\alpha}$ (500 µg cloprostenol) on day 16 of the estrous cycle, followed by GnRH analogue (20 µg buserelin) 5 days later. About 24 h after GnRH, AI was carried out in all the cattle. Transrectal ovarian ultrasonography was carried out at the time of $PGF_{2\alpha}$ administration, at AI and on days 5, 16 and 21 post-AI. Plasma progesterone was assayed with solid-phase radioimmunoassay. All the cattle responded to treatment with a luteolytic dose of $PGF_{2\alpha}$. On the day of AI, large follicles of 15.94±0.60 (12.7–22.0) mm diameter were observed in all the cattle. The presence of active luteal profile on day 5 post-AI confirmed the occurrence of ovulation in all the cattle. Post-treatment first service conception rate was appreciable (66.7%) in cattle with history of repeat breeding. Repeat-breeder cattle failing to conceive after hormonal treatment had suprabasal plasma progesterone on the day of AI in comparison to their non-conceiving counterparts. In conclusion, initiation of $PGF_{2\alpha}$-GnRH protocol based fixed-time AI protocol at the late luteal phase is highly successful for inducing ovulation in all the repeat-breeder cattle, and yielded an appreciably high conception rate.

Key words: Cattle, Conception rate, GnRH, $PGF_{2\alpha}$, Repeat-breeder

Repeat-breeding with incidence between 20–39% is emerging as a major cause behind the poor reproductive performance of dairy cattle (Nanda and Singh 2008). Repeat-breeder cattle are sub-fertile animals without any anatomical or infectious irregularity, and that fail to conceive with at least 3 services (Kim *et al*. 2007). Some of the causes of repeat-breeding include estrus detection errors, endocrine dysfunction, ovulatory defects, poor fertilization rates and/or early embryonic loss (Nanda and Singh 2008). In fact, about 30–40% repeat-breeder crossbred cattle display prolonged estrus (Dadarwal *et al*. 2005). Out of these, about 70% recorded suprabasal progesterone at estrus, thus, leading to delayed ovulation of 76% follicles due to improper luteinizing hormone (LH) surge (Bage *et al.* 2002). The occurrence of prolonged estrus and presence of suprabasal progesterone at estrus is a phenomenon that occurs repeatedly in consecutive cycles, and leads to repeated conception failure in dairy cattle (Nanda and Singh 2008). One therapeutic strategy to overcome this problem associated with repeat-breeders is the use of a hormonal protocol designed to stimulate complete luteolysis of corpus luteum (CL) followed by fixed-time artificial insemination (AI) after induced ovulation. Therefore, the aim of present study was to assess the efficacy of initiating a prostaglandin $F_{2\alpha}$-gonadotropin releasing hormone ($PGF_{2\alpha}$-GnRH) fixed-time AI protocol at the late luteal phase on ovarian and endocrine responses as well as pregnancy establishment in repeat-breeder crossbred dairy cattle.

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MATERIALS AND METHODS

The present study was conducted on 15 lactating repeat-breeder crossbred cattle (15, age: 3–6 years, parity: 1–4, BCS: 3.82±0.17, milk production: 26.53±2.38 l/day) reared at private dairy farms of Punjab state. The enrolled repeat-breeder cattle had normal estrous cycles but had failed to conceive during at least 3 previous services. Before the start of the present study, all these cattle exhibited prolonged duration of estrus (73.60±4.36 h) and there was no abnormality in the estrual cervico-vaginal mucus. Moreover, all the animals had no gross abnormality of the reproductive tract as revealed by rectal palpation and transrectal ultrasonography. Cattle were kept in loose housing system

and were fed chaffed green fodder, wheat straw, concentrates, mineral mixture and *ad lib.* drinking water.

Protocol: Estrus detection in all the cattle was carried out daily (3 times a day at regular intervals) through vasectomised (teaser) bull parading, cervico-vaginal discharge, standing estrus and/or other secondary estrus signs. On day 16, after the observed spontaneous estrus (late luteal phase), all the cattle were administered 500 µg $PGF_{2\alpha}$ (cloprostenol sodium), followed 5 days later by the administration of 20 µg GnRH analogue (buserelin acetate). Cattle were subjected to fixed-time AI around 24 h after GnRH (day 0) without estrus detection. Inseminations using frozen-thawed semen from bulls of proven fertility were done by the same person after properly checking microscopic quality of semen.

Ultrasonography: Transrectal ovarian ultrasonography was carried out at the time of $PGF_{2\alpha}$ administration, at AI and on days 5, 16 and 21 post-AI. Ovarian ultrasonography was carried out with a battery operated B-mode ultrasound scanner equipped with inbuilt interchangeable 5/7.5 MHz linear-array rectal transducer. Ovaries were systematically examined and images were recorded on a diagram of ovary by carefully sketching the size and relative location of large follicle and visible corpus luteum (CL) (Ghuman *et al.* 2010). Optimal scan images were frozen and the size of large follicle and CL was determined by measuring their diameter at the widest poles. Ovulation was confirmed by the appearance of a CL at the site previously occupied by disappeared large follicle (Ghuman *et al.* 2010).

Blood sampling: Jugular vein blood samples were collected daily starting from the day of $PGF_{2\alpha}$ treatment till the day of AI and thereafter on days 5, 16 and 21 post AI. Plasma was separated immediately and was frozen at –20°C until analysis.

Fertility response: First service conception rate was noted by diagnosing pregnancy on day 90 post AI by transrectal ultrasonography of genital tract.

Hormone analysis: Plasma progesterone was assayed with solid-phase radioimmunoassay (Kamboj and Prakash 1993), using a progesterone antibody raised in our laboratory (Ghuman *et al*. 2009). Sensitivity of the assay was 0.1 ng/ml; intra- and inter-assay variation coefficients were 6.6 and 8.9%, respectively.

Statistical analysis: Chi-square (χ^2) test (Dytham 1999) was employed for the number of cattle that responded to various treatments of the protocol and conceived subsequently. Two sample Student's t-test (Dytham 1999) was used to compare different parameters of cattle that conceived or failed to conceive. Statistical procedures were performed using MINITAB release 13.2 statistical software.

RESULTS AND DISCUSSION

As the cattle enrolled in present study were exhibiting regular estrous cycles, therefore, on the day of beginning of protocol (day 16 of estrous cycle, late luteal phase), a CL of 23.27±0.76 mm diameter as well as luteal phase plasma progesterone (1.85±0.16 ng/ml) was recorded in all the cattle. Following $PGF_{2\alpha}$ injection, there was abrupt decline ($P<0.05$) in plasma progesterone within 24 h and continued thereafter ($P<0.05$) to reach 0.26±0.05 ng/ml on the day of AI. Moreover, a regressed CL (3.96±1.19 mm, $P<0.05$) was also found on day of AI. This decline suggested the response of all the repeat-breeder cattle ($P<0.05$) to a luteolytic dose of $PGF_{2\alpha}$ administered during the late luteal phase of estrous cycle. The observed response was parallel to that occurring after $PGF_{2\alpha}$-induced luteolysis (Dadarwal *et al.* 2009).

On the day of $PGF_{2\alpha}$ administration, the mean diameter of large follicle was 11.09±1.98 mm in the cattle of present study. However, none of these follicles might have ovulated after $PGF_{2\alpha}$ treatment as a mature CL was not observed 6 days later (day of AI) in either of the cattle. This suggested that on day 16 of estrous cycle, known as the period of emergence of last follicular wave during a cycle, all these large nonovulatory follicles might be in late static phase and hence not able to ovulate and regressed (Manik *et al.* 2002, Ghuman *et al.* 2008). On the other hand, $PGF_{2\alpha}$-induced luteolysis might have lead to synchronous emergence of a follicular wave. This can be suggested by the observation that on the day of AI, large follicles of 15.94±2.28 mm diameter were observed in all the cattle ($P<0.05$).

Kaim *et al*. (2003) showed that when GnRH is administered at the onset of estrus, the GnRH-induced LH peak coincides with the spontaneous LH peak, and the resulting merged peak is higher than either of the LH peaks, thus leading to ovulation. The occurrence of ovulation in all the cattle ($P<0.05$) was confirmed by the presence of active luteal profile on day 5 post-AI (plasma progesterone: 1.01±0.07 ng/ml, CL: 15.31±1.18 mm). In fact, the rationale behind the use of GnRH 24 h before AI was to decrease the variation in interval to ovulation following $PGF_{2\alpha}$-induced luteolysis (De Rensis and Lopez-Gatius 2007). In normal breeding cattle, the usual duration of spontaneous LH surge is ~10 h and the interval from the peak of LH surge to ovulation is 25.00±0.4 h (Kaim *et al*. 2003). Moreover, GnRH-treatment induces LH surge within 2 h (Kaim *et al*. 2003). Therefore, as none of the cattle had ovulated at the time of AI, it can be suggested that at the time of GnRH (24 h before AI) administration all these cattle might have been around the period of spontaneous LH surge.

All the repeat-breeder cattle enrolled had failed to conceive during at least 3 previous inseminations, however, subsequent to the fixed-time AI protocol, an appreciable conception rate (66.7%) was recorded. The observed conception rate is higher compared to previous results (43%) in which GnRH at estrus was used for the treatment of repeat-breeder dairy cattle (Mee *et al*. 1993).

Analysis of data of repeat-breeder cattle that subsequently conceived or failed to conceive suggested that there was no difference ($P>0.05$) in the diameter of ovulatory follicle on

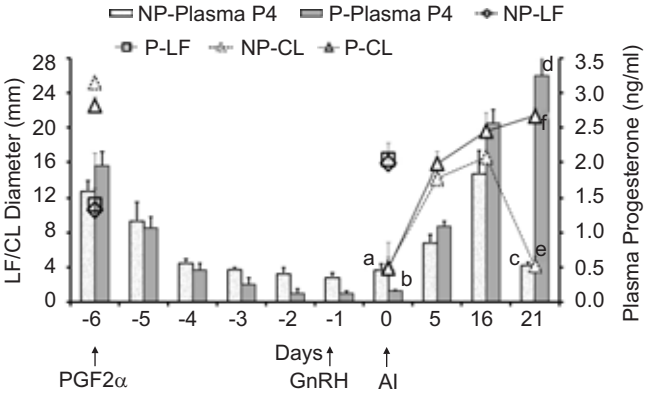

Fig. 1. Variables after initiating a prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-gonadotropin releasing hormone (GnRH) fixed-time artificial insemination (AI) protocol at the late luteal phase of repeat-breeder crossbred cattle (n=15). Data of cattle has been grouped as conceived (n=10) or failed to conceive (n=5) after treatment. a vs b, c vs d, e vs f P<0.05. CL, corpus luteum; LF, largest follicle; NP, non-pregnant; P, pregnant; P4, progesterone.

the day of AI (Fig. 1). The recording of similar diameter of ovulatory follicle in conceiving or non-conceiving counterparts was in contrast to previous studies (Lynch *et al*. 2010). In fact, the diameter of preovulatory follicle and plasma progesterone concentrations on the day of AI were associated with subsequent conception rate (Duchens *et al.* 1996, Colazo *et al.* 2009). In present study, on the day of AI, plasma progesterone was higher (P<0.05) in non-conceiving cattle in comparison to cattle that conceived subsequently (0.46±0.09 vs 0.16±0.02 ng/ml, respectively, Fig. 1). It can be emphasized that the repeat-breeder cattle enrolled in our study had prolonged duration of estrus (73.60±4.36 h) that is mainly due to release of suprabasal progesterone (>0.35ng/ml) at estrus from the previous cycle CL (Bage *et al.* 2002). Thus, fixed-time AI protocol used in this study was successful to eliminate the occurrence of suprabasal plasma progesterone at AI in repeat-breeder cattle that conceived subsequently.

However, the source of suprabasal plasma progesterone in the cattle that failed to conceive could be other than previous cycle CL as the diameter of regressed CL at AI was similar (P>0.05) in cattle that conceived or not (Fig. 1). Factors such as lactational and nutritional stress are also related to occurrence of suprabasal progesterone, prolonged estrus and poor conception rate of dairy cattle (Nanda and Singh 2008). However, in present study, there was no difference (P>0.05) in per day lactation yield, body condition score, as well as pre-treatment estrus duration of repeat-breeder cattle that conceived (lactation: 24.30±2.63 l/day, BCS: 3.88±0.23, pre-treatment estrus duration: 67.20±3.20 h) or failed to conceive (lactation: 31.00±4.59 l/day, BCS: 3.70±0.20, pre-treatment estrus duration: 86.40±9.60 h).

Several factors may be involved for conception failure due to the presence of suprabasal progesterone at AI such as compromised development of oocyte due to exposure to small LH surge, and suboptimal luteal profile which is unable to support embryo development (Bage *et al.* 2002). In the present study, there was no difference (P>0.05) in luteal profile (plasma progesterone and CL diameter) on day 5 and day 16 post AI in cattle that conceived or failed to conceive, however, the luteal profile was poor but in the latter in comparison to the former (Fig. 1).

In brief, application of $PGF_{2\alpha}$-GnRH protocol during the late luteal phase of estrous cycle followed by fixed-time AI protocol is a highly effective therapeutic strategy for establishing pregnancies in repeat-breeder crossbred dairy cattle. This protocol yields induces ovulation and achieves an appreciable first-service conception rate (66.7%) in repeat-breeder cattle.

Initiating a $PGF_{2\alpha}$-GnRH fixed-time AI protocol at the late luteal phase is an optimal therapeutic strategy for better optimization of ovarian and reproductive events viz. induction of luteolysis, ovulation and conception rate in repeat-breeder crossbred dairy cattle.

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