SYNCHRONIZATION OF OVULATION USING CIDR FOR OVARIAN CYSTS IN CATTLE

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ABSTRACT

The objective of this study was to document ovarian and endocrine responses associated with the treatment of ovarian cysts in dairy cattle, using controlled internal drug release (CIDR) regimens. Eight cattle of group-A were administered GnRH analogue (20µg Buserelin) on day 0, $PGF_{2\alpha}$ (500µg Cloprostenol) on day 7 and 2nd GnRH (20µg Buserelin) on day 9. In addition, CIDR (1.38g progesterone) was inserted on day 0 till day 7. Eight cows of group-B received $PGF_{2\alpha}$ (500µg Cloprostenol) on day 0, concurrent with the intravaginal placement of CIDR for 15 days followed by GnRH analogue (20µg Buserelin) on day 17. Cattle were inseminated (AI) at 72 hrs after CIDR removal. In group-A, following day 0 treatment, complete luteinization of cystic structure as well as of large follicle occurred in all cattle ($p<0.05$). In response to day 7 $PGF_{2\alpha}$, all the cattle ($p<0.05$) exhibited a decrease ($p<0.05$) in plasma progesterone as well as regression ($p<0.05$) of ovarian cyst / luteinized follicle on day 10. In group-B, day 0 treatment, resulted in considerable decrease ($p<0.05$) in the diameter of cystic structure and the cyst was no longer detectable by day 15. In both groups, all the cattle ($p<0.05$) exhibited synchronous ovulation between 72-96 hrs after CIDR removal (or 24-48 h after GnRH injection). Moreover, luteal profile on day 6 post-AI as well as conception rate (62.5%) was similar in both the groups. These data suggested that both the CIDR-based timed AI protocols were equally successful for the treatment of ovarian cyst and recruitment of a follicle for synchronous ovulation in dairy cattle with ovarian cysts.

Key words: Cattle, CIDR, Conception rate, GnRH, Ovarian cyst

INTRODUCTION

Cystic ovary is characterized by the presence of anovulatory follicle-like structure (>20 mm) on the ovaries that persists for at least 10 days. Ovarian cyst with an incidence between 6-19% is a major clinical condition affecting fertility viz., calving to conception interval of dairy cattle and is thus responsible for economic loss in the dairy industry (Peter, 2004). A physiologic mechanism responsible for the development of an ovarian cyst is lack of release or inappropriate release of gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) at the time of estrus (Gümen and Wiltbank, 2002). Administration of GnRH induces luteinization of ovarian cyst or other follicles followed by their spontaneous or induced regression (Douthwaite and Dobson, 2000). Others suggested that a large proportion of cystic cattle (25-39%) treated with GnRH did not respond due to absence of GnRH responsive ovulatory follicles (Tebble *et al.*, 2001). Recent research indicates that ovsynch protocol-induced synchronized ovulation followed by timed artificial insemination (TAI) lead to 25% conception rate in cattle with ovarian cysts (Bartolome *et al.*, 2000). In our study, treatment of cystic cattle with GnRH or hCG and $PGF_{2\alpha}$ lead to 43% conception rate (Singh *et al.*, 2010). However, a progesterone-releasing device (controlled internal drug releases, CIDR) regimen yielded 52% conception rate in dairy cattle with ovarian cysts (Kim *et al.*, 2006). The objective of this study was to assess whether CIDR-based TAI protocols can be used as an efficient tool for the treatment of ovarian cysts in lactating crossbred dairy cattle.

MATERIALS AND METHODS

The study was conducted on sixteen lactating crossbred dairy cattle (Age: 3-6 years, BCS: 3-4, >120 days in milk) maintained at private dairy farms. Cattle were kept in loose housing system and were fed chaffed green fodder, wheat straw, concentrates, mineral mixture and ad libitum drinking water. Confirmation of ovarian

cyst in the selected cattle was done by ultrasonography of reproductive tract and plasma progesterone analysis at two time points, separated by 10 days prior to the application of hormone protocols.

Protocol: Cystic cattle were divided into two groups; Group-A (n=8) received 20µg GnRH analogue (Buserelin Acetate, Receptal® VET, Intervet India Private Ltd., Pune, India) on day 0, followed by 500µg Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Cloprostenol sodium; VetmateTM, Vetcare, Bangalore) on day 7 and a 2nd GnRH (20µg Buserelin) on day 9. In addition, CIDR device (1.38g progesterone, Pfizer) was inserted (intra-vaginal) on day 0 till day 7. All cattle were subjected to artificial insemination (AI) at 24 hrs after 2nd GnRH (Day 10) without estrus detection using frozen-thawed semen. Group-B (n=8) received 500µg $PGF_{2\alpha}$ (Cloprostenol) on day 0, concurrent with the intravaginal placement of a CIDR for 15 days. At the end of the 15-day treatment, CIDR device was removed and all the cattle received a 20µg GnRH analogue (Buserelin) on day 17. About 24 h later (day 18), cattle were inseminated as in group-A. In both groups, ovarian ultrasonography was carried out at each treatment, at AI, about 24 h after AI (to confirm ovulation), and also at 6th day post-AI (to confirm the presence/ diameter of CL). During ultrasonography, ovarian cysts were recognized by their hypoechogenisity. Ovulation was verified based upon the subsequent emergence of a CL on a site previously occupied by follicle (Ghuman *et al.*, 2010). Pregnancy diagnosis was done 90 days post-AI.

Blood sample: Blood samples were collected at the time of each treatment, at AI, about 24 h after AI, and also at day 6 post-AI from the jugular vein in a heparinized vial. Plasma was separated immediately after blood collection and frozen at -20°C until analysis. Plasma progesterone was assayed with the 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.

Statistical analysis: Statistical analysis (Chi-square test and two sample Student's t-test) was performed using MINITAB release 13.2 statistical software.

RESULTS AND DISCUSSION

In all the cattle, the presence of a persistent partially luteinized cystic structure on either of the ovaries was confirmed by their ovarian and endocrine profile on day -10 (Cyst diameter: 24.48±0.75 mm, Plasma progesterone: 1.65±0.23 ng/ml) and day 0 (Cyst diameter: 25.31±0.72 mm, Plasma progesterone: 1.42±0.23 ng/ml, $p>0.05$). The continuous presence of at least one large follicular structure (20mm) for 8-10 days with visible signs of luteinization and plasma progesterone >1 ng/ml in the absence of a detectable CL has been designated as a luteinized cyst in dairy cattle (Ambrose *et al.*, 2004).

Subsequent to the CIDR regimens in both groups, no differences ($p>0.05$) were observed between the number of cattle responding to either of the treatments. In group-A, following day 0 treatment, complete luteinization of cystic structure occurred in all the cattle ($p<0.05$) and the luteinized cystic structure resembled a large CL with a central cavity on day 7. Additionally, in response to day 0 treatment, none of the cattle ovulated a follicle, whereas, luteinization of a large follicle was observed in all cattle ($p<0.05$) on day 7. However, the observed absence of alteration in ovarian cyst diameter subsequent to 7 day CIDR treatment ($p>0.05$) was in contrast to previous observations (Ambrose *et al.*, 2004). On day 7, the presence of considerable amounts of plasma progesterone (1.83±0.49 ng/ml) could be due to contributions from CIDR as well as luteinized cyst and luteinized follicle. All the cattle responded ($p<0.05$) to $PGF_{2\alpha}$ treatment on day 7 as determined by the decrease ($p<0.05$) in plasma progesterone on day 9 (0.43±0.12 ng/ml). Similarly, in previous studies, plasma progesterone declined to basal concentrations within 2-3 days after $PGF_{2\alpha}$ treatment in buffaloes (Dadarwal *et al.*, 2009). The diameter of luteinized follicle also decreased ($p<0.05$) from 10.08±2.52 mm at $PGF_{2\alpha}$ treatment to 3.13±1.01 mm on day 10. Furthermore, cyst diameter on day 10 was remnant (7.25±1.03 mm) compared to day 7 (25.51±0.97 mm). Nevertheless, all the cattle ($p<0.05$) of group-A had a large follicle (15.38±1.61 mm) on the day of CIDR removal (day 7) that ovulated between 72-96 h after CIDR removal as in a previous study (Todoroki *et al.*, 2001). Based on our findings, the potential importance of completing the full ovsynch protocol in the treatment of ovarian cyst can be emphasized. This study revealed that treatment with GnRH about 48 hrs after CIDR withdrawal has the ability to induce ovulation of a newly recruited follicle in cystic cattle that had failed to ovulate spontaneously after CIDR withdrawal in a previous instance (Ambrose *et al.*, 2004).

In group-B, following day 0 treatment, there was a considerable decrease in the diameter of cystic

structure and the cyst was not detectable by day 15 in all the cattle ($p<0.05$). These observations differed from previous findings after a 12 day progesterone implant treatment of ovarian cysts (Douthwaite and Dobson, 2000). Moreover, the large follicle observed on day 0 (10.38±0.86 mm) also regressed by day 15. On day 15, the presence of considerable amounts of plasma progesterone (1.73±0.25 ng/ml) could be due to contributions from CIDR as plasma progesterone reached basal concentrations (0.21±0.07 ng/ml, $p<0.05$) about 72 hrs subsequent to CIDR removal as reported in a previous study (Singh *et al.*, 2009). The presence of large follicle on the day of CIDR removal (13.74±0.95 mm) yielded a synchronous ovulation between 72-96 hrs after CIDR removal in all the cattle ($p<0.05$). These observations also confirmed that remnant cystic structures observed on the day of AI in group-A had no impact on ovulation because the mean diameter of ovulatory follicle on the day of AI (group-A: 17.13±1.01 mm, group-B: 14.75±0.75 mm), synchrony of ovulation following CIDR removal and the proportion of cattle ovulating did not differ ($p>0.05$) between both the groups.

On day 6 post-AI, the luteal profile was similar ($p>0.05$) between group-A (CL: 11.13±2.61 mm, Plasma progesterone: 1.21±0.12 ng/ml) and group-B (CL: 10.88±1.04 mm, Plasma progesterone: 0.88±0.13 ng/ml). Moreover, the conception rate was similar in both the treatment protocols (62.5%, $p>0.05$). Nevertheless, the conception rate observed in this study is higher than the conception rate (41-43%) observed in previous studies that used only GnRH-based protocols for the treatment of cystic cattle (Ambrose *et al.*, 2004, Singh *et al.*, 2010). Moreover, the diameter of preovulatory follicle and plasma progesterone concentrations on the day of AI was suggested to be associated with subsequent conception rate (Duchens *et al.*, 1996). In cattle of both the groups that subsequently conceived or failed to conceive, there was no difference ($p>0.05$) in the diameter of ovulatory follicle on the day of AI. However, plasma progesterone on the day of AI was higher in non-conceiving cattle of group-A (0.59±0.21 ng/ml, $p<0.05$) and group-B (0.35±0.15 ng/ml) in comparison to their conceiving counterparts (0.10±0.07 and 0.09±0.05 ng/ml, respectively). Suprabasal plasma progesterone (0.3ng/ml) around the period of LH surge is well known to have adverse impact on the parameters of LH surge (Duchens *et al.*, 1996). Furthermore, poor luteal profile during early embryonic development may cause pregnancy failure and thereby reduce the pregnancy rate (Lucy, 2001). In both groups, the luteal profile of conceiving counterparts by day 6 post-AI was slightly better in comparison to their non-conceiving counterparts (CL: group-A, 13.80±0.97 versus 6.67±6.67 mm; group-B, 12.80±0.73 versus 7.67±0.33 mm, Plasma progesterone: group-A, 1.25±0.12 versus 1.15±0.30 ng/ml; group-B, 1.11±0.10 versus 0.49±0.10 ng/ml).

The study suggested that use of CIDR-based protocols are highly effective therapeutic strategy for establishing pregnancies in crossbred dairy cattle diagnosed with ovarian cysts. Both the protocols were100% successful for the treatment of ovarian cyst and 100% of the treated cattle recruited an ovulatory follicle. Furthermore, synchronous ovulation between 72-96 hrs after the CIDR removal occurred in all cattle. Conception rates to first AI were similar (62.5%) for cystic cattle receiving either of the treatments.

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