SYNCHRONIZATION OF OVULATION IN POSTPARTUM SAHIWAL COWS*

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ABSTRACT

Present investigation was conducted to evaluate two different Ovsynch protocols for synchronization of ovulation in postpartum Sahiwal cows. Experimental animals (n=12) were randomly divided in to 2 groups; each group consisted of 6 animals. Animals of group I were treated with traditional Ovsynch protocol, while animals of group II were treated with a modified Ovsynch protocol where first GnRH injection was replaced with hCG. Treated animals were inseminated at fixed time of 14 to 20 hrs after second GnRH injection without estrus detection. Blood sample were collected from the experimental animals on days -10, 0, 8 and 16 of Ovsynch protocol (day 60 postpartum = day 0 of Ovsynch protocol) to estimate serum progesterone concentrations. Animals were classified as cyclic (n=8, P_4 e" 1ng/ml) and acyclic (n=4, P_4 < 1ng/ml) based on serum progesterone concentration on days -10 and 0. Response to treatment was assessed in treated animals on the basis of progesterone concentration on day 8 (PG treatment); 83.3% (5/6) animals responded to first GnRH treatment in group I and 100% (6/6) animals responded to hCG treatment in group II. Two cyclic animals each from both the treatment groups got conceived to fixed time AI giving conception rate of 40% (2/5) in group I and 33.3% (2/6) in group II. Three cyclic and 4 acyclic animals did not conceive after responding to treatment, while one cyclic animal did not respond to first GnRH treatment. No any beneficial effect of replacing first GnRH injection with hCG could be obtained in ovulation synchronization and conception rate with Ovsynch protocol in Sahiwal cows during early postpartum period.

Keywords: Cows, Postpartum, Sychronization, Ovulation, Ovsynch

INTRODUCTION

Optimum fertility in postpartum cows can be achieved by a holistic approach including improved management, nutrition and application of advanced reproductive technology such as Ovsynch protocol. Ovsynch is one of the most classical and widely known systems of breeding for reproductive management in postpartum cows (Pursley *et al.*, 1997). The protocol consists of two injections of GnRH analogue separated by a single administration of $PGF_{2\alpha}$. The ovulation is precisely synchronized in dairy cows and usually occurs approximately 26-32 hrs after second GnRH injection. Thus, a timed insemination at 14-20 hours after second injection of GnRH results in a high probability of successful conception (Peters *et al.*, 1999). Traditionally, in most of the ovulation synchronization schemes GnRH is used to control follicular development and induce ovulation of a dominant follicle. However, recent research has shown that hCG is more effective than GnRH for causing ovulation (Stevenson *et al.*,

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2007). The present investigation was therefore, designed to test the hypothesis that by replacement of first GnRH injection with hCG is more effective than GnRH for causing ovulation in Ovsynch protocol during early postpartum period in Sahiwal cows.

MATERIALS AND METHODS

A total of 12 suckled postpartum Sahiwal cows maintained at Bull Mother Experimental Farm, College of Veterinary Science and Animal Husbandry, Anjora, Durg in healthy state from first to sixth lactation were utilized for the present study. All the animals had normal calving and subsequent normal genital health as assessed by gynaeco-clinical examination and they were apparently free from genital infection. The animals were randomly divided in to two groups viz. Ovsynch group I and Ovsynch group II, each group having equal number of animals. Animals in group I were treated with initial intramuscular injection of 10 µg Buserelin acetate, a synthetic anlogue of GnRH (Receptal 2.5 ml) on day 60 postpartum (Day 0 of Ovsynch protocol), 8 days later 500 µg Cloprostenol, a synthetic anlogue of $PGF_{2\alpha}$ (Inj. Estropur 2 ml) was injected intramuscularly. Two days later (day 10 of Ovsynch protocol) animals received second injection of 10 µg Buserelin acetate (GnRH). All the cows were inseminated at fixed time (FTAI) of 14 to 20 hrs after second GnRH injection without estrus detection. Similar Ovsynch protocol was followed in animals of group II except first GnRH injection was replaced with 1500 I.U. of hCG (Chorulon). Blood samples were collected from experimental animals on days -10, 0, 8 and 16 of Ovsynch protocol for estimation of serum progesterone concentration. Progesterone concentration was determined using Radio Immuno Assay Kits from BRIT, BARC, Mumbai. Animals showing serum progesterone level > 1.0 ng / ml either on day 10 or 0 were classified as cyclic and animals with concentration of serum progesterone < 1.0 ng / ml on day -10 and 0 were classified as acyclic (Cartmill *et al.*, 2001). Animals were considered as responders to first GnRH or hCG treatment if they had serum progesterone level > 1.0 ng / ml on day 8 (Day 68 postpartum) of Ovsynch protocol. Similarly, animals were considered as responders to second GnRH treatment if they had serum progesterone level > 1.0 ng / ml on day 16 (Day 76 postpartum) of Ovsynch protocol. Pregnancy diagnosis was carried out through examination per rectum between day 50 and 60 after artificial insemination in animals of both groups. Differences of significance in variables were determined with the help of independent 't' test between two groups using SPSS computer programme version 10.0. Difference of significance in ovulation response between animals of two groups was determined using Chi-square test. The conception rate was defined as the proportion of animals that got conceived from those that responded to treatment.

RESULTS AND DISCUSSION

Four animals each were cyclic and two each were acyclic before initiation of Ovsynch protocol in group I as well as in group II. Such equal distribution of cyclic and acyclic animals occurred spontaneously. Acyclic animals recorded serum progesterone concentration as 0.38 ± 0.04 (0.34-0.50) ng/ml and 0.61 ± 0.14 (0.28-0.90) ng/ml on days -10 and 0, respectively. Three cyclic and 2 acyclic animals (83.3%, 5/6) responded to first GnRH treatment as they recorded serum plasma concentration > 1.0 ng/ml on day 8 of Ovsynch protocol in group I. One cyclic animal did not respond to GnRH treatment. Two responding cyclic animals got conceived after FTAI, whereas, two acyclic and one cyclic animal, which responded to treatment, did not conceive after FTAI. All 6 animals responded to treatment in Ovsynch group II (100%, 6/6) as they recorded serum progesterone concentration > 1.0 ng/ml on day 8 of Ovsynch protocol. Two responding cyclic animals got conceived after FTAI, whereas two cyclic and two acyclic animals did not conceive after FTAI. Non-significant difference was recorded in ovulation response between animals of group I and II.

The present observation corroborates with the finding of Vasconcelos *et al.* (1999) who reported that 64 per cent of cows ovulated to first GnRH injection and response varied significantly ($P<0.01$) by stage of estrous cycle. Similarly, Murugavel *et al.* (2003) reported that first GnRH treatment was effective in 60

per cent of animals in postpartum cows. Two acyclic animals responded to GnRH treatment in present study suggesting that probably GnRH injection might have rescued the dominant follicle from atresia thereby resulted to final stage of growth reaching preovulatory size and finally ovulated. The first GnRH injection is more effective between day 5 and 13 of estrous cycle compared to the animals that received GnRH during early (day 1 to 4) or late (day14 to 21) stage of cycle (Ambrose *et al.*, 2000). Animals were treated at unknown stage of the ovarian cycle in the present study, and it may be possible that non-responding cyclic animal in group I might have been treated with first GnRH injection either at the early or at the later stage of the estrous cycle.

All six animals of group II responded to hCG treatment and mean serum P_4 concentration was non-significantly greater in animals of group II than in animals of group I on day 8 (3.22 ± 0.58 vs. 2.62 ± 0.65 ng/ml) in the present study. Progesterone concentrations are greater in hCG-treated dairy cows (Santos *et al.*, 2001). This could be due to hCG with its longer half-life, providing a longer period of LH-like stimulation to the follicles and developing corpora lutea, and furthermore, hCG as opposed to GnRH, has direct gonadotrophic effects on the ovary rather than indirect effects involving the release of endogenous gonadotrophins. The use of hCG has other advantages over GnRH as it reduces the occurrence of spontaneous estrus among Ovsynch-treated animals, which is desirable with timed insemination (Geary *et al.*, 2001).

Cyclic status of animal before the initiation of treatment influenced the response to treatment as 2 cyclic animals each in group I and II conceived after Ovsynch treatment with FTAI. Three animals in Ovsynch group I and four animals in Ovsynch group II had serum progesterone concentration > 1.0 ng/ml, both at the initiation of treatment on day 0 as well as on the day of prostaglandin treatment. It is noteworthy that irrespective of type of group, 4 responding cyclic animals got conceived and 3 responding cyclic animals did not conceive after FTAI. Responding and conceived animals recorded significantly higher ($P<0.01$) serum progesterone concentration than responding and non-conceived animals on day 8 and day 16 of Ovsynch protocol. Cyclic animals have more probability to respond to Ovsynch treatment and to conceive compared to acyclic animals as positive effect of Ovsynch treatment has been reported in cyclic cows based on plasma progesterone concentration (Klindworth *et al.*, 2001).

It may be unrealistic to expect normal CL formation and functional performance when a small follicle is luteinized under influence of GnRH or hCG (Macmillan *et al.*, 2003). The small dominant follicle may have limited receptors and may contain fewer granulosa cells than a normal LH dependant dominant follicle. Even when ovulation has been induced, the resultant CL is less likely to be functional. The resultant smaller CL sustained lower luteal concentration of progesterone (Burke *et al.*, 2001). Low circulating progesterone after breeding has been reported to impact embryo development and maternal recognition of pregnancy (Mann *et al.*, 1999). This seems to be possible reason for conception failure in 7 responding animals treated with Ovsynch treatment in present study. All these animals recorded serum progesterone concentration < 1.0 ng / ml, 6 days after FTAI. These results demonstrated that sustained proper luteal concentration of progesterone is required for establishment of pregnancy.

Two animals each from both group I and II got conceived giving conception rate of 40% (2/5) in Ovsynch group I and 33.33% (2/6) in Ovsynch group II. Present finding approximates well with previous reports on conception rate ranging from 29 to 60% after Ovsynch treatment (Pursley *et al.*, 1998; Cordoba and Fricke, 2001). However, many workers (Schmitt *et al.*, 1996; Klindworth *et al.*, 2001) reported lower conception rate in dairy cows after Ovsynch treatment.

From the present study, it may be concluded that injection of hCG had no beneficial effect over GnRH injection in ovulation synchronization in postpartum Sahiwal cows. More studies are needed to examine the effect of hCG on ovulation synchronization and conception rate involving a large number of animals.

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