RESEARCH ARTICLE

Efficacy of Ovsynch and Ovsynch Plus Protocol for Improvement of Fertility in Postpartum Sahiwal Cows

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Abstract

The present study was conducted to investigate the efficacy of Ovsynch and Ovsynch plus protocol in postpartum (day 60) Sahiwal cows (n=18). Animals were randomly divided into three equal groups, *viz.,* Ovsynch group, Ovsynch plus group and Control group. Animals of group I (n = 6) were treated with traditional Ovsynch protocol. The animals (n = 6) of group II were treated with Ovsynch plus protocol which consisted of an initial intramuscular injection of eCG (Folligon) @ 250 IU on day 60 postpartum followed 3 days later by GPG (Ovsynch) protocol. In group-III Control, no treatment was given to animals (n = 6). Treated animals were inseminated at a fixed time between 14 and 20 hrs after second GnRH injection, irrespective of estrus detection. Blood samples were collected from each animal on days 50 and 60 postpartum to determine the status of cyclicity in animals based on serum concentrations of progesterone (P_4). A third blood sample was collected on the day of prostaglandin treatment to determine the response of first GnRH injection. Four animals each were cyclic, and two were acyclic in both treatment groups. Four animals each responded to first GnRH treatment in both treatment groups.

Similarly, two animals each got conceived giving conception rate of 50% (2/4) in each treatment. In the control group, one out of 6 animals got conceived yielding 16.66 % conception rate (1/6) during the study period. It may be thus concluded that Ovsynch and Ovsynch plus protocol may be used during the early postpartum period to improve the reproductive efficiency in postpartum Sahiwal cows.

Keywords: Conception, Ovsynch, Ovsynch plus, Postpartum, Progesterone, Sahiwal cows.

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Introduction

High-producing dairy cows invariably remain acyclic for an extended period leading to financial losses to dairy farmers. Optimum fertility in postpartum cows can be achieved by a holistic approach including improved management, nutrition, and application of advanced reproductive technology. Reproductive efficiency of postpartum cows may be improved by inducing early cyclicity through Ovsynch programme, which is one of the most classical and widely known systems of cattle breeding for reproductive management (Twagiramungu *et al*., 1995; Pursley *et al.,* 1997). Analyses of ovarian response in various studies revealed that the animal with the presence of a large follicle at the initiation of Ovsynch treatment responds well to this treatment (Pursley *et al*., 1995). A new protocol "Ovsynch Plus" has been devised using PMSG/eCG 2–3 days before the start of Ovsynch protocol (Sharma *et al*., 2004) in dairy buffaloes to ensure the presence of a large follicle at the time of first GnRH treatment to improve the response of an animal to ovsynch treatment. In this perspective, the present experiment was carried out to investigate the efficacy of Ovsynch and Ovsynch plus protocol in postpartum Sahiwal cows.

Materials and methods

The present investigation was conducted on 18 suckled postpartum (day 60) Sahiwal cows, maintained at Bull Mother Farm of Veterinary College at Anjora, Durg. All these animals had normal calving and subsequent normal genital health as assessed by gynaeco-clinical examination. The selection of experimental animals was strictly based on the absence of postpartum genital infection. The animals were selected randomly and divided equally into three groups, viz., Ovsynch group, Ovsynch plus group, and Control group. The ovsynch protocol consisted of an initial intramuscular injection of GnRH, i.e., Buserelin acetate @ 10 µg on day 60 postpartum, 8 days later PGF2α (Inj. Cloprostinol @ 500 µg) was injected intramuscularly. Two days later (day 10) animals received the second injection of GnRH. Animals of Ovsynch plus group were treated with an initial intramuscular injection of PMSG/eCG (Folligon) @ 250 IU

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i/m on day 60 postpartum, which was followed 3 days later by above Ovsynch protocol. All the cows were inseminated at a fixed time (FTAI) of 14 to 20 hours after second GnRH injection irrespective of estrus detection in both the groups. In the control group, no treatment was given to animals (n = 6), and AI was carried out at detected estrus.

Blood samples were collected from the animals of group I on days 50, 60 and 68 (just before PG inj.) postpartum; however for animals of Ovsynch plus group third blood sample was collected on day 71 instead of day 68 postpartum. Serum progesterone (P4) concentration was determined using BARC Radio Immuno Assay Kits (Kubasic *et al*., 1984) to assess the status of cyclicity in experimental animals. Animals showing serum P4 level ≥ 1 ng/mL on day 50 and/or 60 postpartum were classified as cyclic and those with <1 ng/mL P4 were classified as acyclic (Cartmill *et al*., 2001). The response of treated animals to first GnRH injection was assessed based on serum P4 concentration (≥ 1 ng /mL) on the day of prostaglandin treatment. Pregnancy diagnosis was carried out through examination per rectum between days 45 and 60 after artificial insemination in animals of treatment and control groups.

Results and discussion

On day 50 postpartum, three animals recorded ≥ 1 ng/mL of serum P_4 and remaining 15 animals had < 1 ng/ml of serum P_4 concentration. However, by day 60 postpartum, 8 animals recorded serum P_4 concentration ≥ 1 ng/mL supporting the fact that with the increasing postpartum intervals ovarian cyclical activity is increased. This finding approximated with the observation of Naidu *et al*. (2006).

In Ovsynch group, four animals were cyclic, and 2 were acyclic. The corresponding figures for the animals of Ovsynch plus group were 4 and 2, respectively, while those of the control group were 2 and 4 animals, respectively (Table 1). Among the animals of Ovsynch group, 3 cyclic and one acyclic animals responded to treatment based on serum P_4 concentration on day 68 postpartum. Two cyclic animals, which responded to treatment, got conceived after FTAI, whereas 2 responding animals did not conceive after FTAI.

In Ovsynch plus group, 2 cyclic and 2 acyclic animals responded with serum P_4 concentration ≥1 ng/mL on day 71 postpartum, while two cyclic animals did not respond to this treatment. One cyclic and one acyclic responded cow got conceived after FTAI. In control group, one cyclic animal got conceived during the experimental period, whereas one cyclic animal did not express signs of estrus.

The Ovsynch protocol has been devised that synchronizes both follicular development and regression of CL in cycling dairy cows (Thatcher *et al*., 1989). The GnRH luteinizes or ovulates the mature follicle and initiates the recruitment and selection of a new dominant follicle 7-8 days later. The injection of PGF2α initiates the regression of spontaneous CL or a potential CL induced by GnRH or both. A strategic second injection of GnRH is administered 48 hrs after the injection of PGF2α. This does of GnRH induces a surge of LH causing ovulation of newly formed dominant follicle within 30 hrs of injection (Pursley *et al*., 1997). Analyses of ovarian response in various studies revealed that the animal with the presence of a large follicle at the initiation of Ovsynch treatment responds well to this treatment (Twagiramungu *et al*., 1992; Pursley *et al*., 1995). In an attempt to ensure the presence of a large ovarian follicle at the time of first GnRH injection a new protocol "Ovsynch Plus" has been devised for dairy buffaloes (Sharma *et al*., 2004). In this protocol, an injection of PMSG is administered 3 days before first GnRH injection of Ovsynch treatment to support ovarian follicular development so that at least one large follicle develops at 72 hours to respond first GnRH injection with either ovulation or luteinization. The resulting luteal structure is subjected to luteolysis by PGF2α given 7 or 8 days later. Further administration of GnRH after 48 hrs of PGF2α injection ensures synchronous ovulation of preovulatory follicle allowing fixed-time artificial insemination in treated animals. In the present study, 250 IU of PMSG was administered in Sahiwal cows in an attempt to induce growth and development of a large follicle. A lower dose of PMSG was used in the present study than used in previous studies in buffaloes (Sharma *et al*., 2004) in an attempt to prevent the development of persistent large follicle.

Results of the present study demonstrated that 66.6% of animals (8/12) of both the treatment group responded to first GnRH treatment. Among these eight animals, five animals were cyclic, and three animals were acyclic. The present observation corroborates with the finding of Vasconcelos *et al*. (1999) who reported that 64% of cows ovulated to first GnRH injection and response varied significantly (p <0.01) by

Table 1: Cyclic status before initiation of treatment, response to treatment and conception in experimental animals

Treatment group	*No. of animal*	*Cyclic status**		*Response to treatment*		*No. of animal conceived*	
		Cyclic	*Acyclic*	*Cyclic*	*Acyclic*	*Cyclic*	*Acyclic*
Ovsynch	06	04	02	03	01	02	-
Ovsynch plus	06	04	02	02	02	01	01
Control	06	02	04	–	–	01	–

* Progesterone level >1.0 ng/mL either on day 50 and/or 60 postpartum

stage of the estrous cycle and follicular development. Similarly, Murugavel *et al*. (2003) reported that first GnRH treatment was effective in 60% of animals in postpartum cows.

One and two cyclic animals in Ovsynch and Ovsynch plus group, respectively, did not respond to first GnRH treatment in the present study. Vasconcelos *et al*. (1999) reported that first GnRH injection is more effective between day 5 and 13 of estrus cycle compared to the animals that received GnRH during early (day 1 to 4) or late (day 14 to 21) stage of the cycle (Ambrose *et al*., 2000). Animals were treated at an unknown stage of follicular development in the present study, and it may be possible that these three cyclic animals might have been treated with first GnRH injection either at the early stage or at the later stage of the estrus cycle. It may be unrealistic to expect normal CL formation and functional performance when the small follicle is luteinized under the influence of GnRH (Macmillan *et al*., 2003). The synchronization of ovulation and conception rates in bovines are higher when there is a presence of both a corpus luteum and a mature dominant follicle on the day of GnRH injection (Twagiramungu *et al*., 1992).

Among animals of Ovsynch plus group, two animals were acyclic, and both responded to a physiological dose of FSH (PMSG). These two animals probably developed a sizeable ovarian follicle under the influence of exogenous FSH that underwent ovulation after first GnRH treatment. Out of these two animals, one animal got conceived after FTAI. The present finding confirms the fact that inclusion of physiological dose of FSH in Ovsynch protocol helps to respond to first GnRH injection in postpartum acyclic animals. This finding also further confirms the fact that presence of a large ovarian follicle is a prerequisite factor for better response and conception with Ovsynch protocol, although the importance of higher serum P4 concentration on the day of prostaglandin treatment cannot be underestimated.

The cyclic status of an animal before the initiation of treatment influenced the response to treatment as three cyclic animals of Ovsynch group, and two cyclic animals of Ovsynch plus group responded to treatment. Interestingly, among two cyclic animals of control group one animal expressed estrus and got conceived after AI. Sirosis and Fortune (1990) concluded that the serum P4 concentration appears to be a determining factor as to whether a dominant follicle continues to grow and develop into a persistent dominant follicle at the time of GnRH treatment.

Three and two animals in the Ovsynch group and Ovsynch plus group, respectively, had serum P_4 concentration ≥1.0 ng/mL, both at first GnRH treatment as well as on the day of prostaglandin treatment. Out of these five animals, three animals conceived after FTAI. The present finding supports the fact that the presence of a functional corpus luteum is mandatory to respond to Ovsynch and Ovsynch plus treatment.

Four animals each responded to first GnRH treatment in Ovsynch and Ovsynch plus group. Similarly, two animals each got conceived from both the group giving a conception rate of 50% (2/4) in each treatment group. In the control group, one out of six animals got conceived yielding 16.66 % of conception rate. The present finding approximates well with the observation reported by Ambrose *et al*. (2000), Cordoba and Fricke (2001), Ali *et al*. (2012) and Sneha *et al*. (2018) who documented conception rate ranging from 40 to 60% after Ovsynch treatment. However lower conception rate of 20% has been reported by Prajapati *et al*. (2018) for Ovsynch and Ovsynch plus protocol in crossbred cows under field conditions. One of two acyclic animals of Ovsynch plus group got conceived after FTAI. Cordoba and Fricke (2001) reported a 30% conception rate in anovular cows. From the present study, it may be concluded that both Ovsynch and Ovsynch protocol may be used during the early postpartum period to improve the reproductive efficiency in postpartum Sahiwal cows. The inclusion of PMSG before first GnRH treatment may enhance the response of acyclic animals to Ovsynch protocol.

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