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Effects of Hormonal Regimens on Ovarian Follicular Activity, Induction of Estrus, Ovulation and Conception Rate in Postpartum Acyclic Sahiwal Cows

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ABSTRACT

The study was conducted on postpartum (≥ 90 days) acyclic Sahiwal cows (n= 24), which were randomly divided into three treatment (n=18) and a control group (n=06). Animals of all three treatment groups were treated with GnRH @ 10 µg i/m on day 0 with intra-vaginal placement of CIDR* containing 1.38 g progesterone for 7 days. Animals were treated with i/m injection of PMSG@ 300 IU on day 5, which was followed by administration of $PGF_2\alpha$ (Cloprostenol) @ 500µg i/m on day 7. Animals of Group-I were treated with second dose of GnRH on day 9, while animals of Group-II were administered with Estradiol Benzoate (EB) @ 5 mg i/m. No treatment was given to animals of Group-III on day 9 and animals were observed for sign of estrus and A.I. was carried out at detected estrus up to 72 hrs. Fixed time artificial insemination (FTAI) was carried out at 16-22 hrs after 2nd dose of GnRH in Group-I and EB in Group-II. No treatment was given to animals of control group. Secondary signs of estrus were recorded in all animals of treatment groups. Mean follicular diameters (mm) on day of A.I. were 16.85±1.1, 15.2±0.88 and 14.22±0.65 with ovulation rate of 100%, 83% and 66.6% in animals of Group-I, II and III, which subsequently recorded conception rate of 83.3%, 66.6% and 66.6% respectively. A significant difference ($P \leq 0.05$) in mean follicular diameter was observed between animals of Group I and Group III. The highest conception rate was recorded in animals of Group I, which exhibited the largest mean follicular diameter and the highest ovulation rate.

Keywords: Sahiwal Cow, Acyclic, CIDR, Estrus Induction.

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INTRODUCTION

High reproductive performance is a critical determinant of maximum production and satisfactory economic returns and estrus induction plays a role in achieving optimal fertility in cows (Bo *et al*., 2018). However, like many other dairy breeds, infertility remains a significant challenge in fully exploiting their production potential. True anestrus (acyclicity) is the condition in which both the ovaries are small, smooth, inactive with the absence of any palpable structure and although there is follicular development, none of the ovarian follicles becomes mature enough to ovulate (Naikoo *et al*., 2021). This phenomenon can be attributed to various factors such as limited energy intake, lower body reserves, endocrine dysfunction and postpartum reproductive disorders etc. Different hormonal regimens such as gonadotropins, prostaglandins, progesterone-based protocols and their combination have a good therapeutic value to enhance reproductive efficacy in acyclic animals with proper nutritional status (Savalia *et al*., 2014). CIDR treatment with smooth inactive ovary in cows has been used with variable success rate. With application of CIDR, resultant increased circulatory concentration of progesterone (P_4) exerts negative feedback on hypothalamus and anterior pituitary. Following withdrawal of CIDR on day 7 after insertion, the rapid drop in circulatory concentration of P_4 promotes the release of GnRH as the negative feedback of P_4 is abolished, followed by FSH and LH release with subsequent resumption of ovarian cyclicity (Elbehiry, 2023). Since use of CIDR resulted in variable success rate among acyclic dairy cows, CIDR was used with various combinations of reproductive hormones to evaluate the effective combination to induce estrus and ovulation in acyclic Sahiwal cows in the present study.

MATERIALS AND METHODS

Present experiment was conducted using acyclic Sahiwal cows (n= 24) of more than 90 days postpartum with history of normal parturition and normal genital tract based on gynaeco-clinical examination prior to start of experiment. All the animals were maintained at Bull Mother Experimental Farm, Anjora, Durg (Chhattisgarh), India under similar feeding and management practices. The experimental cows were randomly divided into three treatments and a control group, each group comprised of 6 animals.

Each experimental animal including that of control group was dewormed with a broad-spectrum anthelmintic (Niaz *et al*., 2022) and provided with a mineral mixture 15 days before and 15 days after the initiation of treatment (Behera *et al*., 2012). During entire experimental period each animal was also provided with 1kg additional concentrate ration. A total of 40 postpartum (≥ 90 days) acyclic Sahiwal cows were screened through gynaeco-clinical examination twice at 10 days apart to determine the presence or absence of a corpus luteum (CL). An animal was declared acyclic when the CL was found absent on both examinations either on day – 10 or on day 0 and total 24 animals were selected for present trial.

Experimental Design

Group-I (Modified Ovsynch): The animals of this group were treated with GnRH (Inj. Receptal @ 10 µg) i/m on day 0 with concurrent intra-vaginal placement of CIDR® containing 1.38 g of P_4 for 7 days. Single i/m injection of 300 IU of Pregnat Mare Serum Gonadotrophin (PMSG) was administered on day 5 of initiation of treatment. On day 7, $PGF_2\alpha$ (Estrumate) @ 500 µg was administered i/m followed by second injection of GnRH (Inj. Receptal @ 10 µg) on day 9. Fixed time artificial insemination (FTAI) was carried out at 16-22 hrs of second GnRH treatment.

Group-II (Modified Heat-synch): The animals of this group were treated similarly to that of Group-I, except that Estradiol Benzoate @ 5 mg was administered i/m in place of second dose of GnRH on day 9. FTAI was carried out similar to that of Group-I.

Group-III (Modified Select-synch): The similar regimen was followed for animals of this group to that of Group-I except that second dose of GnRH was omitted**.** Animals were observed for signs of estrus through visual observation thrice a day at morning, mid-day and evening for half an hr. Artificial insemination was carried out at detected estrus up to 72 hrs of $PGF_2\alpha$ treatment. In absence of estrus signs up to 72 hrs, FTAI was planned along with second dose of GnRH (Inj. Receptal @ 10 µg).

No treatment was given to animals of control group and heat detection was carried out in similar manner to that of Select-synch group. Ovaries of each animal of treatment groups were scanned on days 0, 2, 4, 7, 9 till ovulation/ atresia using 7.5 MHz trans-rectal probe to record the diameter of the largest follicle. Ovulatory response was determined through ultrasound scanning of ovary based on detection of sudden disappearance of the pre-ovulatory follicle on succeeding day of examination. Pregnancy was detected by ultrasound scanning using 7.5 MHz trans-rectal probe on day 35-40, which was later confirmed through examination per rectum between days 55 and 60 post-insemination. The means of dominant follicle of the treatment groups were analyzed by independent t-test as per the procedure outlined by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The present study evaluated the efficacy of hormonal regimens for estrus induction, ovulation and conception rates in postpartum acyclic Sahiwal cows. All animal showed signs of standing estrus before 72 hrs and second dose of GnRH was not administered in animals of Select-synch group, while one animal of control group displayed signs of standing estrus. These animals were bred with A.I. using AM-PM rule, while all the animals of Ovsynch and Heat-synch groups displayed secondary signs of estrus at the time of FTAI.

Table 1: Effects of hormonal regimens on follicular development, estrus induction, ovulatory response and conception rate in postpartum acyclic Sahiwal cows

Groups	No. of Animal	Mean follicular diameter (mm)		Estrus induction (%)	Ovulatory response (%)	Conception Rate (%)
		Day 0	Day 10/A. I.			
I	06	4.98±0.59	16.85±1.1[a]	100	100	83.3
II	06	4.60±0.43	15.20±0.88	100	83.3	66.6
III	06	4.78±0.23	14.22±0.65[b]	100	66.6	66.6
IV	06	4.44±0.35	-	16.6	-	-

(Superscript a, b indicates significantly difference (P ≤ 0.05) between treatment groups)

Animals of all treatment groups exhibited 100% estrus induction rate, whereas only one animal (16.6%) displayed signs of estrus in control group on day 27 after deworming and feeding of extra concentrate ration with mineral mixture. Similar estrus induction responses were reported by Virmani *et al.* (2013), Buhecha *et al.* (2015), Dhami *et al.* (2015), Ahmed *et al.* (2016), Deshmukh *et al.* (2017) and Prajapati *et al.* (2019). Meanwhile, Ramalakshmi *et al.* (2021) observed estrus responses of 75%, 100% and 87.5% in acyclic crossbred cows under the Ovsynch, Ovsynch + PRID and PRID + PG protocols, respectively. However, Naikoo *et al.* (2016) reported a lower estrus induction response in Kankrej cows.

The mean largest follicular diameter (mm) on the day AI was recorded as 16.85±1.1, 15.2±0.88 and 14.22±0.65 in animals of modified Ovsynch, heat-synch and modified select-synch group, respectively. Patil *et al.* (2016) reported a mean follicular diameter of 16.12 ± 0.56 mm, while Hassan *et al.* (2021) recorded 14.7 ± 0.7 mm in cyclic Sahiwal cows treated with Ovsynch protocol. Similarly, Singh *et al.* (2021) reported mean follicular diameters of 10.3±0.61 mm, 11.4±0.48 mm, and 10.2±0.42 mm in the Ovsynch, Ovsynch + CIDR, and Estradiol Benzoate groups, respectively, in Sahiwal cows. A larger dominant follicle on day 10 favoured ovulation and pregnancy establishment in Sahiwal cows (Singh *et al.*, 2021). Atkins *et al.* (2010) also reported that follicular diameter significantly influenced ovulation and conception rates in cows.

The ovulation rates in the modified Ovsynch, modified Heat-synch and modified Select-synch groups were recorded as 100%, 83%, and 66.6%, respectively. A similar ovulatory response was observed by Khade (2010), who reported 100% ovulation in Gir heifers treated with the Ovsynch + CIDR protocol. Singh *et al.* (2021) also recorded 100% ovulation in Sahiwal cows treated with the Ovsynch + CIDR protocol, whereas a 60% ovulation rate was recorded in both Estradiol-based and Ovsynch protocols. On the contrary lower ovulation rate (66.6%) was recorded by Vijyakanth *et al.* (2021) in postpartum anestrus cow that were treated with Ovsynch protocol.

The conception rates were recorded as 83.3%, 66.6%, and 66.6%, respectively, in animals of Group-I, II and III (Table 1). The highest conception rate (83.3%) was recorded in Group I (modified Ovsynch), which approximates to the findings of Dhami *et al.* (2015) and Maravi *et al.* (2024). A higher conception rate was also reported by Ansari *et al.* (2008) and Muneer *et al.* (2009), whereas a lower conception rate was recorded by Ramakrishnan (2011) and Bhoraniya *et al.* (2012). The higher conception rate in Group I may be attributed to the second dose of GnRH, which enhances pulsatile LH secretion, leading to a higher ovulatory response. Additionally, the development of a large luteal structure secreting higher progesterone levels may have prevented early embryonic loss, resulting in a higher conception rate (Singh *et al.*, 2021). On the contrary lower conception rate (33.3%) was recorded by Vijyakanth *et al.* (2021) in Heat-synch protocol. In contrast, the Heat-Synch and Select-Synch groups had comparatively smaller dominant follicles than the Ovsynch group and Estradiol Benzoate had a lower ovulatory response than GnRH (Vijayakanth *et al.*, 2021).

The administration of GnRH on Day 0 stimulates uniform follicular growth, preparing the ovaries for subsequent hormonal interventions. CIDR steadily releases progesterone over seven days, mimicking the luteal phase and promoting regression of any existing ovarian follicle,

while synchronizing a new follicular wave. The inclusion of PMSG in Ovsynch protocol is used in an attempt to improve follicular development and ovulation rates specially in acyclic postpartum cows. Treatment with PMSG aids in restoring hypothalamic-pituitary-gonadal axis function, which is already stimulated by progesterone treatment, ultimately inducing behavioral estrus through enhanced ovarian stimulation (Nergiz and Saribay, 2018). On Day 7, administration of PG triggers the regression of an existing corpus luteum (CL), whether spontaneously formed or induced by the first GnRH injection. This regression allows the dominant follicle of a new follicular wave to continue developing. A second dose of GnRH injection and Estradiol Benzoate were administered 48 hours after PG, inducing an LH surge that triggers ovulation within approximately 30 hours. Cows were then inseminated at a fixed time typically 16–22 hours after the second dose of GnRH and Estradiol Benzoate injection (Pursley *et al.*, 1994). This protocol is commonly known as fixed-time artificial insemination (FTAI).

Fig. 1: Dominant follicle on day of A.I.

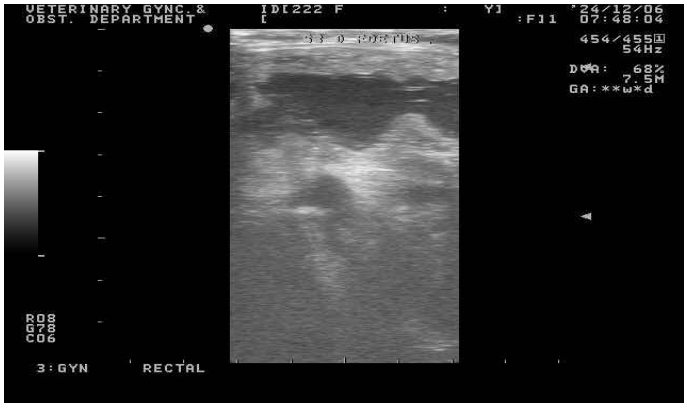

Fig. 2: 35 days old embryo.

CONCLUSIONS

Among three hormonal regimens used in present study, modified Ovsynch protocol was found most effective in inducing largest ovarian follicle, higher ovulation rate with higher conception rate than other two protocols. Modified Heat-synch protocol being cost effective had higher intensity of secondary signs of estrus, moderate mean follicular diameter, ovulation and conception, while Select-synch protocol exhibited comparatively smaller ovarian follicle but this protocol had similar ovulation and conception rate to that of Heat-synch protocol. By using these hormonal protocols with good herd management practices, significantly enhanced reproductive performance of cows may be achieved.

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CONFLICT OF INTEREST

None

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