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EFFECT OF THIRD GNRH ADMINISTRATION SEVEN DAYS AFTER OVSYNCH PROTOCOL ON FERTILITY RATE IN REPEAT BREEDER COWS DURING SUMMER SEASON

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ABSTRACT
The aim of the study was to evaluate the effect of post artificial insemination GnRH administration on conception rate of repeat breeder cows in summer season. A total number of 30 pluriparous cows were equally divided into three experimental groups *viz*., Group I, II (Treatment groups) and Group III (Control group) during summer season (from April to September). The group I cows were treated with Ovsynch protocol (GnRH-PG-GnRH). The group II cows were treated with the Ovsynch protocol as Group I animals and additionally, all the cows were given 10 µg of GnRH intramuscularly 7 days after artificial insemination. The group III (control) cows were observed for estrus from the time of selection and artificial insemination was done during the observed estrus. In general, the conception was higher in Ovsynch and Ovsynch + post AI GnRH group than control group. Among the treatment groups, the Ovsynch + post AI GnRH (group II) recorded higher conception rate (70 %) than Ovsynch group (Group I) (40 %). The serum progesterone concentration was < 1 ng/ml during induced estrus. The mean serum P_4 concentrations on day 7 post AI in all the groups of HBS and LBS reached a maximum concentration of 3.94 ±0.07 ng/ml with a significant difference ($P \leq 0.05$) between pregnant and non-pregnant cows. On day 20 post AI, the pregnant cows had higher P_4 concentration than non-pregnant cows with a significant difference ($P \leq 0.05$) between them. The concentration was higher in group II than group I and III. The results of the study indicated that Ovsynch +post AI GnRH can be used to improve fertility rate in repeat breeder cows during summer seasons.

KEYWORDS: repeat breeder cow, Ovsynch, post AI GnRH, summer season.

INTRODUCTION

The causes of repeat breeding syndrome have been attributed to various factors like age, insemination time, artificial insemination (AI) technique, heat detection method, semen quality, genetics, nutrition, hormonal imbalance, summer stress, inadequate luteal function[1], delayed ovulation[2], and managemental errors[3], which results in either fertilization failure or early embryonic mortality[4]. Numerous studies have led to the conclusion that in female with normal fertility, the incidence of fertilization failure is approximately 10% and early embryonic death within three weeks following fertilization accounts for approximately 30% leading to a total early pregnancy loss of close to 40% during first 20 days post AI. In India, where summer heat stress is severe, high prolactin secretion has been identified as a factor contributing to acyclicity and poor fertility by lowering progesterone (P_4) secretion [5]. P_4 is involved in stimulation of variety of endometrial secretions necessary for successful development of early embryo[6]. Low level of P_4 concentration is correlated with failure of implantation and low fertility in cows [7]. Perusal of literature revealed that low interferon-τ production around day 16-18 lead to early embryo loss. The production of interferon-τ tends to be greater on day 18 when the production of P_4 has increased in response to the induction of accessory corpus luteum[8]. It is reported that administration of GnRH on day 5 or 11[9] and 11 to 14[10] after AI increased serum concentration of P_4 and caused tendency toward higher pregnancy rates.

Synchronization of ovulation that allows timed artificial insemination (TAI) has been widely used to improve reproductive performance in repeat breeder cows [11, 12]. However the response to first and second GnRH injection and subsequent conception rate was found to be inconsistent among previous studies[13, 14]. Hence adding the additional exogenous hormones like hCG, GnRH and estradiol can improve the outcome of Ovsynch[14]. Considering the above facts, the study was designed to find out the effect of administration of GnRH 7 days after AI on the fertility rate of crossbred cows during summer season.

MATERIALS AND METHODS

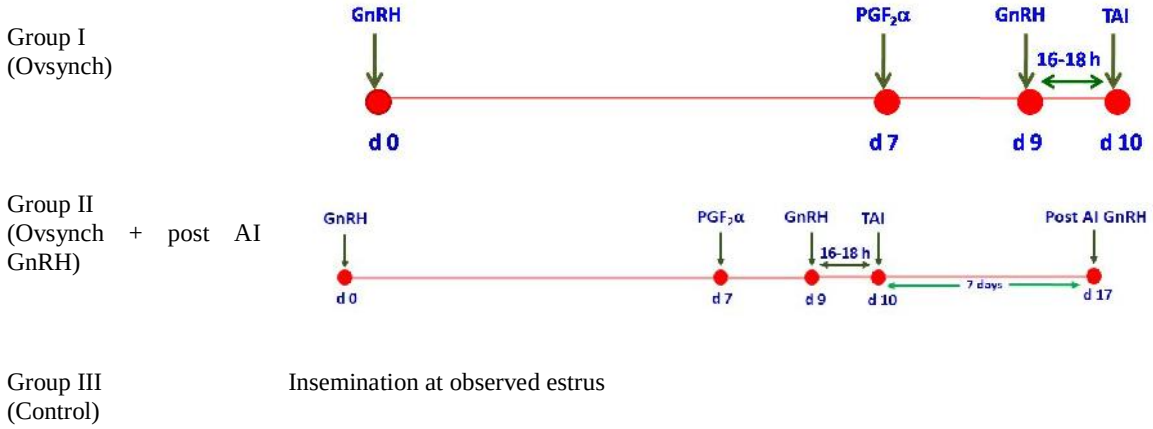
Selection of repeat breeder cows

A total number of 30 pluriparous, healthy crossbred cows which failed to conceive after three or more consecutive artificial inseminations with good quality semen were selected during estrus for the study. The selected cows were between 2nd and 5th parity. They were subjected to a thorough gynaeco-clinical examination at the time of selection and those cows with no palpable abnormalities in the genital tract and negative for white side test with

uterine discharge were utilized for the experiment. All the selected cows at random were equally divided into three experimental groups viz., Group I, II (Treatment groups) and Group III (Control group) during summer season (i.e., from April to September). The experiment was designed with 30 cows consisting of 10 cows in each group. All the cows were kept outdoors, fed with hay and concentrate twice daily and provided with water ad libitum.

Experimental design

The group I cows were treated with Ovsynch protocol as described by Pursley *et al*. (1995). The Ovsynch protocol consisted of intramuscular injections of 10 µg of GnRH (2.5 ml, Buserelin acetate, Ovulanta®, Vet Mankind, New Delhi, India) on the day of start of synchronization of ovulation (day 0), 25 mg of $PGF_2\alpha$ (5 ml, Dinoprost tromethamine, Lutalyse®, Pfizer India Ltd, Mumbai) seven days later (d 7) and another 10 µg of GnRH (2nd GnRH) 48 hours after the $PGF_2\alpha$ (d 9) and Timed Artificial Insemination (TAI) at 16-18 hours after the second GnRH injection (d 10). The group II cows were treated with the Ovsynch protocol as described for Group I animals. Additionally, all the cows were given 10 µg of GnRH intramuscularly 7 days after artificial insemination. The group III (control) cows were observed for estrus from the time of selection and artificial insemination was done during the observed estrus.



Collection of blood samples

Blood collection was done in groups I and II at the (i) time of selection of repeat breeder cows (ii) initiation of Ovsynch protocol (iii) time of $PGF_2\alpha$ injection (iv) TAI (v) 7 days following TAI and (vi) 20 days following AI. In group III animals, blood was collected at the (i) time of selection of animals (ii) AI (iii) 7 days following AI and (iv) 20 days following AI. The serum was separated and stored at -20°C until P_4 estimation by radioimmunoassay (RIA) technique.

Pregnancy diagnosis

All the inseminated cows were monitored regularly following TAI at induced estrus in groups I and II and at observed estrus in group III for non-returning of estrum. The cows which have not expressed heat signs after TAI were confirmed for pregnancy by rectal palpation and ultrasound scanning at 45 days post insemination. The conception rate was expressed in percentage.

Statistical analysis

The Completely Randomized Design (CRD) method and independent 'T' - test were followed for the experiment[15] and the data collected were analysed using SPSS® 20.0. software package. Post hoc analysis was done by Tukey's Honestly Significance Difference.

RESULTS

Effect of synchronization of ovulation on conception rate

In the present study, the percentage of first service, second service and overall conception rates observed in Group I (Ovsynch) cows were 20.00, 20.00 and 40.00, respectively during summer season. In group II (Ovsynch + post AI GnRH) cows, first service, second service and overall conception rates were 40.00, 30.00 and 70.00 % and in group III (control), 10.00, 10.00 and 20.00 % during summer season, respectively.

Serum progesterone concentration

The mean (±SE) serum P_4 levels before, during and after the different synchronization of ovulation protocol in repeat breeder cows during summer season are depicted in Table 1.

TABLE 1: Serum progesterone (p_4) levels before, during and after synchronization of ovulation in repeat breeder cows during summer season

S.No.	Treatment groups		P_4 (ng/ml)					
			At the time of selection	Day 0 (GnRH injection)	Day 7 ($PGF_2\alpha$ injection)	Day 10 (Timed AI)	7 days post AI	20 days post AI
1	Group I	P	0.47^{pa}±0.05	1.48^{qa}±0.13	2.55^{ra}±0.09	0.44^{pa}±0.05	3.15^{sa}±0.04	3.96^{sa}±0.12
		NP	0.73^{pb}±0.03	1.17^{qa}±0.10	1.68^{rb}±0.12	0.69^{pb}±0.02	2.54^{sb}±0.06	0.72^{pb}±0.04
		Overall	0.63^{p}±0.05	1.30^{q}±0.09	2.03^{r}±0.16	0.58^{p}±0.05	2.78^{s}±0.11	2.31^{s}±0.15
2	Group II	P	0.35^{pa}±0.06	1.75^{qa}±0.08	2.99^{ra}±0.16	0.35^{pa}±0.05	3.58^{sa}±0.13	4.53^{ta}±0.14
		NP	0.66^{pb}±0.08	1.33^{qb}±0.07	2.39^{ra}±0.11	0.58^{pb}±0.09	2.42^{rb}±0.30	0.62^{pb}±0.07
		Overall	0.44^{p}±0.07	1.63^{q}±0.09	2.81^{r} 0.15	0.42^{p}±0.05	3.23^{s}± 0.21	2.63^{r}± 0.14
3	Group III	P	0.42^{pa}±0.01	-	-	0.48^{pa}±0.05	2.99^{qa}±0.14	3.94^{ra}±0.13
		NP	0.59^{pb}±0.03			0.61^{pa}±0.04	2.22^{qb}±0.06	0.62^{pb}±0.04
		Overall	0.56^{p}±0.03			0.58^{p}±0.04	2.37^{q}±0.12	2.29^{q}±0.18

Means bearing different superscripts (p,q,r,s,t) among different days of blood collection within same row differ significantly (P≤0.05).

Means bearing different superscripts (a,b) between rows within a column differ significantly (P≤0.05).

The mean serum P_4 concentration during the various synchronization of ovulation protocol was higher in pregnant animals than non-pregnant animals during luteal phase of the cycle in repeat breeder cows treated during summer season. The repeat breeder cows were selected during the time of estrum and the mean serum P_4 concentrations was <1 ng/ml during selection in all the cows. In groups I and II, the mean serum P_4 levels ranged from 1.17 ±0.10 to 1.75 ±0.08 ng/ml on the day 0 of the experiment. It clearly showed that the first GnRH was given during the diestrum in these animals. In both the gropus, there was a drastic reduction ($P\leq0.05$) in the serum P_4 level on the day of induced estrum and the concentration was <1 ng/ml which indicated the prompt luteolysis following $PGF_2\alpha$ injection on day 7 of the trial. There was a significant difference ($P\geq0.05$) was observed in the progesterone concentration at the time of induced estrus in both group I and II. The mean serum P_4 concentrations on day 7 post AI was ranged from 2.42 ±0.30 to 3.58 ±0.13 ng/ml whereas on day 20 the concentration ranged between 0.62 ±0.07 and 4.53 ±0.14 ng/ml. In both the groups, there was a significant difference was observed between pregnant and non-pregnant cows on day 7 post AI. On day 20 post AI, the pregnant cows had higher P_4 concentration than non-pregnant cows with a significant difference ($P\leq0.05$) between them. The concentration was higher in group II than group I and III.

DISCUSSION

The results of the present study clearly indicated that Ovsynch programme increased the conception rates in repeat breeder cows. The conception rates of 30 to 38[16, 17], 47[18], 55[19], 60[20] and 62.50[21] were recorded in cows in different studies. The increased pregnancy rates in the repeat breeder cows of group I of this experiment might be related to the start of Ovsynch protocol during early to mid diestrus as indicated by serum progesterone profile in this study. Initiating the Ovsynch protocol during early to mid diestrus (day 5 to 12) produced greater pregnancy rates than when initiated on other days of the estrous cycle [22]. When the Ovsynch protocol was started between day 5 and 12 of the cycle, the pregnancy rates were greater because of greater incidences of ovulation after the first GnRH injection [23, 24]. El-Zarkouny *et al.* [25] stated that Ovsynch protocol increased the pregnancy rate by 10-12 % when implemented during favorable stage of the estrous cycle in cows. In this study, the administration of GnRH 7 days after Ovsynch in group II increased the conception rate considerably. Among the two treatment groups, group II had registered highest overall conception rate. It indicated that Ovsynch+post AI GnRH along with mineral mixture supplementation proved to be the best protocol to augment fertility during summer season in repeat breeder cows.

Progesterone concentrations following ovulation have been positively correlated to the volume of uterine secretions [26], conceptus development[26, 27], the embryos ability to secrete IFN-τ [28,29], embryo viability for subsequent survival [30], and perhaps most importantly conception rates [31, 32]. Progesterone has been proven to play a major role in embryo survival by stimulating variety of endometrial secretions that were necessary for successful development of the embryo in the uterine lumen[33]. Many researchers attempted to enhance the pregnancy percentage by administering GnRH after AI between 5 and 14 days in both normal cyclical and repeat breeder cows [6, 34, 35, 36, 37].

Gonadotropic hormones are known alternatives to progesterone application to increase endogenous progesterone and subsequent conception rate in normal cyclical and repeat breeder cows[34, 38]. GnRH or hCG or LH or their analogues have been used when the first wave dominant follicle was present to induce ovulation, accessory CL formation, increased progesterone secretion and improving the pregnancy rate [39, 40, 41]. Bulbul *et al.* [42] reported that GnRH injection at diestrus promoted formation of accessary CL by causing ovulation or luteinization (40 %) ovulation and 60 % luteinization) of the existing dominat follicle in the ovaries. Injection GnRH administration might have stimulated the transformation of small luteal cells to large luteal cells which had a higher basal secretion rate of P_4 [43]. Drew and Peters[44] reported 12 % higher pregnancy rate in cows treated with GnRH 12 days after AI. In a similar research, Ataman *et al.* [45] reported embryonic death rate was lower

in GnRH treated cows but there was no significant difference between embryonic deaths of GnRH and placebo treated groups. One of the most important reasons for embryonic death in these days is CL deficiency and insufficient P_4 secretion from the CL[46]. In the study, the group II had higher progesterone concentration on day 20 post AI than group I. This might be the reason for higher conception rate obtained in this group than group I.

Finally from the study, it was concluded that both Ovsynch and Ovsynch + post AI GnRH treatment can be used to enhance fertility in repeat breeder cows during summer season. The Ovsynch + post AI GnRH can give enhanced fertility rate than Ovsynch in repeat breeder cows during summer season. The administration of GnRH on day 7 after AI definitely improved the reproductive performance in dairy cows during warm seasons.

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