INITIATING OVSYNCH ON DAY 6 POST ESTRUS ONSET ± POST AI EARLY LUTEAL PHASE GnRH TREATMENT TO IMPROVE OVARIAN AND FERTILITY RESPONSE IN REPEAT BREEDING CROSSBRED CATTLE

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ABSTRACT

The study was designed to evaluate the success of ovsynch protocol by initiating the protocol on day 6 after estrus onset with an additional GnRH treatment during post insemination early luteal phase in repeat breeder crossbred cattle. Twenty four repeat breeder cattle were allocated into four groups, Gp I, control; Gp II, GnRH treatment on day 6 post estrus onset; Gp III, Ovsynch treatment initiated on day 6 post estrus onset; Gp IV, same as in Gp III with an additional GnRH on day 6 after 2nd GnRH of ovsynch. The conception rate for gp I, II, III and IV was 0.0, 16.7, 50.0 and 50.0%, respectively. In group II and IV, serum progesterone was high ($p<0.01$) on day 12 post AI in comparison to Gp I and III, respectively. In conclusion, circulating progesterone was better with an additional GnRH subsequent to ovsynch protocol and the use of ovsynch with or without an additional GnRH treatment during post-AI luteal phase can improve fertility response in repeat breeder crossbred cattle.

Key words: Cattle, Conception Rate, Ovsynch, Progesterone, Repeat breeding

INTRODUCTION

Several methods of synchronizing estrus and ovulation were used to improve the conception rate in repeat breeder cattle with a limited success (Keskin *et al.,* 2010). The protocols like ovsynch have inconsistent results with respect to premature estrus, reduced ovulation rate to first GnRH and variable conception rate (Kim *et al.,* 2003 and Vasconcelos *et al.,* 1999). In fact, fertility following ovsynch is high when the animals ovulate in response to first GnRH of the protocol. Ultrasonography studies opined that synchronization of follicular wave emergence can be achieved by administering GnRH on day 5 or 6 of estrous cycle resulting in ovulation of first wave dominant follicle with formation of accessory corpus luteum in cycling crossbred cattle (Satheshkumar *et al*. 2008 and 2012). In fact, GnRH treatment on day 4-6 after artificial insemination (AI) was tried to improve conception rate by enhancing circulating progesterone through accessory corpus luteum formation during luteal phase in repeat breeder cattle (Stevenson *et al*., 1993). Therefore, the present study aimed to improve the fertility response and progesterone profile by initiating ovsynch on day 6 post estrus onset with or without early luteal phase GnRH treatment in repeat breeding crossbred cattle.

MATERIALS AND METHODS

Apparently healthy, crossbred cattle (n=24) with the history of repeat breeding were subjected to thorough gynaeco-clinical examination twice at ten days interval. These cattle were confirmed as cyclic as they were having a corpus luteum (CL) with no palpable abnormalities of the genital tract. The control and GnRH group cattle (n=6 each, Gp I & II, respectively) were observed for the signs of estrus and AI was performed during estrus by AM/PM rule. In addition, cattle of GnRH group were administered

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a GnRH analogue (10µg buserelin acetate, i.m.) on day 6 post-estrus onset. The remaining cattle were included in ovsynch and ovsynch plus early luteal phase GnRH group (n=6 each, Gp III & IV, respectively). In these two groups, ovsynch protocol was started on day 6 of estrous cycle after detection of estrus onset and was considered as day 0 for these groups. Ovsynch protocol consisted on hormonal administrations (d0 and d9, 10µg buserelin acetate; d7, 500µg $PGF_{2\alpha}$ analogue, cloprostenol sodium) followed by timed AI (TAI) at 16-18 h after second GnRH injection (d10). In addition, cattle of ovsynch plus early luteal phase GnRH group were administered a GnRH analogue (10µg buserelin acetate, i.m.) on day 6 after 2nd GnRH of ovsynch protocol. Conception rate was confirmed per rectally on day 45 after AI.

Blood samples were collected at the time of AI and on day 12 post AI in group I and II, and on days 0 (GnRH), 7 ($PGF_{2\alpha}$), 10 (TAI) and day 12 post TAI in group III and IV. Serum samples were stored at -80°C till analysis. Serum progesterone was analyzed by progesterone ELISA kit (Enzo Life Sciences®). The assay sensitivity was 8.57 pg/ml. The data was analyzed by using software SPSS® version 17.

RESULTS AND DISCUSSION

At the start of ovsynch protocol (d0), the elevated concentrations of serum progesterone (Gp III, 1.42±0.11 ng/ml; Gp IV, 1.88±0.31 ng/ml) could be due to the presence of a functional corpus luteum as ovsynch was started during early luteal phase. Subsequently, compared to day 0 of the protocol, serum progesterone increased further on day 7 (Gp III, 3.65±0.10 ng/ml; Gp IV, 3.73±0.08, $p<0.05$) of the protocol. However, due to the lack of ultrasonographic investigations, it remains unknown that this increase in serum progesterone following GnRH administration on the day 0 of protocol was due to the maturation of primary CL or due to the formation of accessory CL. Nevertheless, the basal levels of serum progesterone at AI/TAI were indicative of complete luteolysis of the CL (Table).

On day 12 post-AI, serum progesterone in group II and IV was respectively higher from the concentrations in group I and III (Table). This could be the impact of improved functionality of primary CL or due to the formation of accessory CL following GnRH administration during the early luteal phase. Usually, an increase in CL number and total CL tissue area is likely responsible for the increased serum progesterone concentration (Kerbler *et al*., 1997). An aim of the study was to evaluate the impact of initiation of ovsynch on day 6 of estrous cycle, which could have lead to emergence of a new follicular wave. Thus, the presence of a dominant follicle at the time of second GnRH of ovsynch protocol might have resulted in greater effectiveness and formation of CL, which secreted and maintained higher progesterone concentration during critical period of luteal phase.

Nevertheless, elevated concentrations of circulating progesterone are required for conceptus development in repeat breeding crossbred cattle (Savalia *et al.,* 2014). Therefore, in ovsynch-based groups, the conception rate was high ($p<0.05$) in comparison to others (Table). Similar results were reported earlier (Vijayarajan *et al.,* 2009). However, the finding that conception rate in group II was

Table: Conception rate (CR) and serum progesterone in repeat breeding crossbred cattle cows following hormonal treatments. EO, estrus onset, early luteal phase indicates d6 post-EO.

Group (n=6 each)	**Progesterone** (ng/ml)		**CR (%)**
	AI/TAI	**d12 post-AI**	
I, Control	0.25±0.02*	3.17±0.29*[a]	0.0
II, GnRH during early luteal phase	0.35±0.02*	4.69±0.53*[b]	16.7[a]
III, Ovsynch (started on d6 post-EO)	0.46±0.03*	4.13±0.09*[c]	50.0[b]
IV, Ovsynch (started on d6 post-EO) + early luteal phase GnRH	0.36±0.01*	5.48±0.40*[d]	50.0[b]

[a vs b, c vs d]$p<0.05$, *$p<0.05$, between column

low despite the presence of higher luteal phase progesterone suggested that other factors like fertilization failure or early embryonic death may be involved in these repeat breeder crossbred cattle. These factors might have been taken care of in ovsynch-based group (Gp III and IV), thus leading to higher conception rate (Table). Furthermore, following ovsynch protocol, additional GnRH treatment during early luteal phase (Gp IV) had no beneficial impact on conception rate, however, this requires to be validated by including a more number of animals in further studies.

In conclusion, use of ovsynch protocol with or without an addition GnRH treatment during post-AI luteal phase can improve fertility response in repeat breeder crossbred cattle. Ovarian response in terms of circulating progesterone concentration was better with an additional GnRH subsequent to ovsynch protocol but its impact on additional increase in conception rate remains to be validated further.

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