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Relationship of plasma progesterone levels following controlled internal drug release (CIDR) insertion and conception in repeat breeder cows

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Abstract
A total of ninety apparently healthy crossbred cows which failed to conceive in three or more inseminations with good quality semen and with a BCS of 2.5 to 3.5 and in their first to fourth parity were selected under field conditions to study the effect of controlled breeding using a progesterone device on fertility in repeat breeder cows which were brought to the Teaching Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli and infertility camps at nearby villages of Tirunelveli during the period of October 2016 to April 2017. The repeat breeder cows were randomly and equally assigned to one of the three groups. viz. control (Group I; n=30) CIDR (Group II; n=30) and CIDR + post AI CIDR (Group III; n=30). The pregnancy verification was carried out in all the animals by rectal palpation after 60 to 90 days post insemination. The animals were conceived due to increasing the levels of plasma progesterone in relation to Controlled Internal Drug Release (CIDR) insertion on day 10 (10.23±0.11 ng/ml) and $PGF_2\alpha$ in combination with post insemination CIDR therapy (9.92±0.19 ng/ml) on 12 day or alone in repeat breeder cows under field conditions. Hence, the insertion of CIDR and post insemination CIDR led to increase in the plasma progesterone concentration and thereby improves the conception rate in repeat breeder cows.

Keywords: CIDR, conception, repeat breeder, economic loss, plasma progesterone

1. Introduction
The high incidence of Repeat breeding is one of the most common problems faced by the farmers and veterinarians in India and it causes huge economic loss to the farmers. Repeat breeding was associated with estrus detection errors, endocrine dysfunctions, ovulation defects, uterine infections and poor gamete quality and all these conditions led to poor fertilization rate and/or early embryonic deaths in dairy cows [1]. A higher incidence of repeat breeding was associated with inadequate estrus detection [2] resulting in errors in timing of insemination in relation to the onset of standing estrus or insemination of cows not in estrus. The early embryonic death was considered to be the main cause of pregnancy failure in cows [3]. Insufficient luteal progesterone had been implicated as a cause for abnormal embryonic development and early embryonic losses [4]. A luteal insufficiency and lower progesterone concentration were known to be causing embryonic mortality and thereby lowering the pregnancy rates [5, 6]. Thus the main cause for repeat breeding could be attributed to hormonal asynchrony around estrus [7]. As there were apparently several reasons for repeat breeder syndrome, no single treatment was likely to alleviate the condition in every herd or animal. Controlled breeding using progestagens such as Progesterone Releasing Intravaginal Device (PRID), Controlled Internal Drug Release Device (CIDR) or Norgestomet ear implants had been found to be effective in achieving good fertility in normally cycling dairy cattle [8]. The reproductive performance of dairy cattle was often limited by many factors like failure to ovulate, silent estrus along with poor detection and improper timing of artificial insemination and luteal insufficiency. In repeat breeding cows, controlled breeding using a progesterone releasing device (CIDR) was found to be an effective tool to achieve high pregnancy rate through a fine regulation of folliculogenesis [9]. Therefore having these in mind the present study was undertaken in the field conditions to assess the efficacy of CIDR protocol with fixed time insemination and with post insemination CIDR therapy in repeat breeder cows for their better conception.

2. Materials and Methods

The repeat breeder cows brought to the Teaching Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli and infertility camps at nearby villages of Tirunelveli during the period of October 2016 to April 2017 were used. Ninety apparently healthy crossbred cows which failed to conceive in three or more consecutive inseminations with good quality semen and with Body Condition Score (BCS) of 2.5 to 3.5 were selected and randomly assigned to one of the three groups viz. control (Group I; n=30) CIDR (Group II; n=30) and CIDR + post AI CIDR (Group III; n=30). All the selected animals were observed for one complete estrous cycle before starting the experiment.

The cows in control group were inseminated twice at 24 h interval at the observed estrus. In the CIDR group, each cow received an intravaginal insert of CIDR from day 10 to day 19 of observed estrus and an intramuscular injection of prostaglandin $F_2\alpha$ 500 µg on day 18. All the cows were inseminated at 48 and 72 h after removal of CIDR. In the CIDR + post AI CIDR group each cow received CIDR, $PGF_2\alpha$ and insemination similar to CIDR group cows and in addition a second CIDR was inserted on day 5 post insemination and removed on day 13. The pregnancy verification was carried out in all the animals by rectal palpation after 60 to 90 days post insemination.

2.1 Blood collection

Blood samples were collected randomly from ten animals in each group. Whole blood was collected from jugular vein in EDTA vacutainers on days 5 and 7 after insemination in group I. In group II blood samples were collected on days 0 and 2 of CIDR insertion (day 10 and 12) and in group III blood samples were collected on day 0 and 2 of second CIDR insertion (day 5 and 7 after AI)

2.2 Separation of plasma

Plasma was separated by centrifuging blood at 3000 rpm for 10 minutes. The plasma samples were stored at - 20°C until progesterone assay.

2.3 Progesterone assay

Progesterone levels were estimated in the collected blood samples using a solid phase enzyme immunoassay kit [10]. The kit manufactured by XEMA Co., Ltd., (The 9-ya Parkovaya street, 48, 105043, Moscow) Russia, had an assay sensitivity of 0.25nmol/l or 0.08 ng/ml and a cross reaction of <1.0 per cent with other steroid hormones.

2.4 Statistical Analysis

All the collected data were analyzed statistically by the method described by Snedecor and Cochran (1989) [11]. BCS and plasma progesterone concentration were analyzed using independent 't' test. Duration of estrus was analyzed using two way ANOVA. Conception rate, intensity of estrus, lactation number and post calving interval on conception rate were analyzed using chi-square test.

3. Results and Discussion

Repeat breeding syndrome was responsible for long service period; long inter calving interval, low milk and calf production resulting in greater economic loss to the dairy industries. It was one of the major gynaecological problems affecting reproductive efficiency and economy of milk production in dairy animals. Major causes of repeat breeding include estrus detection errors, endocrine dysfunction, ovulatory defects, poor fertilization rates and early embryonic loss [7]. The cause of repeat breeding might originate either during early stages of follicular maturation and / or during pre-ovulatory period [12]. In present study we hypotheses that the combination of controlled breeding and progesterone supplementation with a CIDR device beginning on day 5 after insemination would result in better pregnancy rates when compared to control animals.

The conception rate in repeat breeder cows in untreated control group was 20 per cent. The conception rate following controlled breeding with CIDR

(group II) was significantly (P<0.05) higher at 43.33 per cent than the control group. The conception rate in repeat breeder cows following controlled breeding with CIDR and post-insemination treatment with CIDR was 63.33 per cent which was higher than both groups I (P<0.01) and II (P>0.05) (Table 1). The significantly higher conception rate recorded in this study following controlled breeding in repeat breeder cows could be due to the impact of fine regulation of plasma progesterone profile during preconception period [13] and priming of reproductive system with adequate amount of circulating progesterone during preconception period which was favorable for the better development of ovulatory follicles that would yield a better developed CL [14]. The higher conception in repeat breeder cows supplemented with progesterone post AI, might be due to improved uterine environment for embryo survival and development [15] Endometrial secretions, essential for stimulating and mediating the changes in conceptus growth and differentiation throughout early pregnancy was directed by the steroid environment generated by the ovary [16]. Higher progesterone concentration and a lower ratio of estradiol to progesterone on days 3 and 6 were very conducive for the growth of embryo [17]. Increase progesterone concentrations during early stages of pregnancy were associated with an advancement of embryonic development leading to an increased level of interferon – τ production and an associated increase pregnancy rate [18]. The results of this study corroborated to the previous state above in that the progesterone supplementation during the crucial post AI period improves the conception rate.

In the present study, the mean plasma progesterone levels on day 5, 7 and 10 of the cycle in repeat breeder cows increased significantly (P<0.01) as days progressed (Table 2). In group II, following CIDR insertion on day 10, the mean plasma progesterone level increased significantly to 9.97 ± 0.29 on day 12 from 8.80 ± 0.31 ng / ml on day 10. Similarly in group III, following CIDR insertion on day 5, the mean plasma progesterone level increased significantly to 9.65 ± 0.26 on day 7 from 6.83 ± 0.37 ng/ml on day 5. The mean plasma progesterone level on day 7 in CIDR treated cows was significantly (P<0.01) higher than that of untreated cows on the same day. The mean plasma progesterone levels did not vary between the cows that conceived and those failed to conceive (Table.3). The progesterone level during the days 10 to 18 after ovulation was similar in cycling cows and in inseminated cows, whether the latter were pregnant or not [19]. No difference in progesterone levels of conceived and non-conceived buffaloes following CIDR treatment [20]. However, the exogenous progesterone administration through intravaginal device during early luteal phase post AI increased blood concentration of progesterone and conception rate [21]. The conception rate in cows following induced estrus was positively correlated with plasma concentration of progesterone during the days preceding the luteolysis [22].

Increased levels of plasma progesterone were observed in repeat breeder cows on different days of early diestrus (Fig 1). This was in accordance with various studies as stated that, when repeat breeder dairy cows received a CIDR between day 5 and 19 after insemination the pregnancy rate was 46.6 per cent when compared to 37 in control [5]. Insertion of CIDR from day 4 to 9 post estrus increased concentrations of milk progesterone in supplemented cows by 4.78 ng/ml between day 4 and 4.5 in comparison with a 0.55 ng/ml increase in control cows [23].

Table 1: Conception rate in repeat breeder cows following Controlled breeding with Controlled Internal Drug Release (CIDR) and post insemination treatment with CIDR.

Groups	Number of cows in group	Conceived		Failed to conceive		χ^2 value	
		number	%	Number	%		
Control	30	6	20.00	24	80.00	3.774*	11.58**
Controlled breeding with CIDR	30	13	43.33	17	56.66		
Controlled breeding with CIDR + post AI CIDR	30	19	63.33	11	36.66		

*significant at (P<0.05); ** significant at (P<0.01)

Table 2: Effect of Controlled Internal Drug Release (CIDR) insertion during early or mid diestrus on plasma progesterone levels in repeat breeder cows.

Sl. No.	Plasma progesterone levels (ng/ml)			
	CIDR inserted on day 5 post AI		CIDR inserted on Day 10	
	Day 5	Day 7	Day 10	Day 12
1	7.70	9.33	7.50	10.45
2	5.90	8.24	9.88	10.05
3	6.83	8.37	7.47	10.14
4	6.87	9.56	8.48	10.1
5	7.16	10.07	9.24	9.75
6	7.26	9.84	8.67	8.81
7	4.19	9.65	8.31	8.71
8	6.27	10.4	9.93	10.4
9	8.34	10.88	8.25	9.37
10	7.83	10.2	10.35	11.99
Mean ± SE	6.83 ± 0.37	9.65 ± 0.26	8.80 ± 0.31	9.97 ± 0.29
t-value	4.348**		2.68*	

** Significant at (P<0.01); *significant at (P<0.05) level

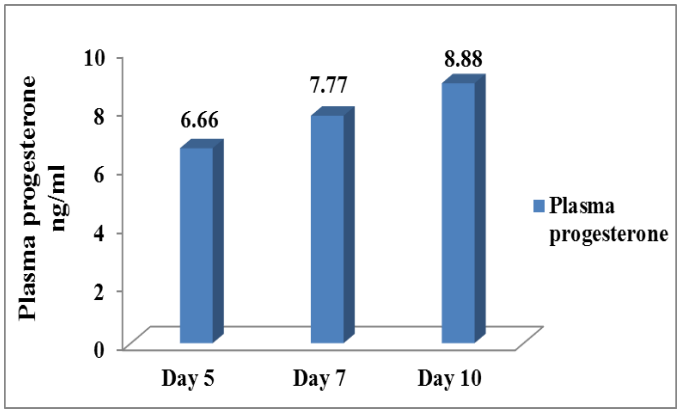

Fig 1: Plasma progesterone levels in repeat breeder cows on different days of early diestrus.

Table 3: Levels of plasma progesterone (ng/ml) in repeat breeder cows in relation to Controlled Internal Drug Release (CIDR) insertion and conception.

Group	Conception Status	No CIDR insertion		CIDR insertion on day 5 post AI		CIDR insertion on day 10	
		Day 5	Day 7	Day 5	Day 7	Day 10	Day 12
Control	Conceived	5.81±0.19	8.21±0.05	-	-	-	-
	Failed to conceive	6.87±0.22	7.55±0.38	-	-	-	-
Controlled breeding with CIDR	Conceived	-	-	-	-	7.82±0.33	10.23±0.11
	Failed to conceive	-	-	-	-	9.05±0.31	9.87±0.42
Controlled breeding with CIDR + post AI CIDR	Conceived	-	-	6.12±0.67	9.92±0.19	-	-
	Failed to conceive	-	-	7.13±0.35	9.48±0.42	-	-
t-value		3.65*	1.25	1.56	0.94	3.07*	0.82

*significant at (P<0.05)

4. Conclusion

It could be concluded from the present study that controlled breeding using CIDR and $PGF_2\alpha$ in combination with post insemination CIDR therapy or alone led to increase the plasma progesterone concentration and thereby improves the conception rate in repeat breeder cows under field conditions. This procedure of controlled breeding using CIDR could be easily adaptable and vide effectively/economically used to alleviate the repeat breeder in cows.

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