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Oestrus induction using insulin modified Co-synch protocol in post-partum anoestrus cows#

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Abstract

Efficacy of Co-synch and insulin modified Co-synch protocols for the treatment of post-partum anoestrus crossbred cows were studied. True anoestrus was confirmed by per-rectal examination conducted on day 50 and 60 (day 0) post-partum. Twenty-four cows were selected and divided in to two treatment and one control groups (n=8). Group I cows were treated with Co-synch protocol; group II (Insulin modified Co-synch protocol) cows in addition to Co-synch, insulin (0.2 IU/kg as SC) was also administered for three days on day 0, 1 and 2. Group III cows were the control group. Response to oestrus induction, time taken for the onset, duration and intensity of oestrus, physical changes in the reproductive tract and pregnancy rates were evaluated between the groups. Serum progesterone and insulin like growth factor-1(IGF-1) levels were estimated. Response to oestrus induction was 75 and 87.50 per cent for group I and II, respectively. Mean time taken for the onset and duration of oestrus were 41.41 ± 1.47 and 14.41 ± 0.43 h; 40.57 ± 2.09 and 18.92 ± 3.69 h for group I and II, respectively and for group III, only two cows showed oestrus on 68 and 76 day post-partum with average duration of 17.25 h. Significantly higher concentration of progesterone was observed in Group-I and Group-II than Group –III cows on day 7 of treatment and day 10 post AI. Serum concentration of IGF-1 on day 7 and 9 of treatment and on day 10 post AI in group II was significantly higher ($p < 0.01$) than group I and III. The pregnancy rate for the AI done after the protocol were 37.50 and 50 per cent from group I and group II, respectively. Present study recommends insulin modified co-synch protocol for the treatment of post-partum anoestrus.

Keywords: *Post-partum, anoestrus, cows, insulin, Co-synch, IGF-1*

Reproductive efficiency is one of the most important factors in determining the productivity and profitability of dairy farming. The calving interval is a crucial metric for evaluating the reproductive efficiency of cows and 12 months is considered as optimum period for which cows must achieve normal uterine involution and post-partum resumption of ovarian cyclicity and get inseminated within 60 days. Post-partum anoestrus accounts for the major cause of infertility. Various predisposing factors are involved in the aetiology of anoestrus, including nutritional, managemental, environmental, hormonal and changes in various biochemical parameters including IGF-1 and glucose (Fernandez *et al.,*

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2020; Kumar *et al*., 2014; Mwaanga and Janowski 2000). Insulin stimulated the synthesis of oestradiol *in vitro* by granulosa cells, and the insulin had a direct stimulatory effect on the aromatization of androgen to oestrogen. IGF-1 plays a significant role in follicular expansion and reproductive performance of cows. Ovarian cells synthesize IGF-I (granulosa cells) and IGF-II (theca cells). The IGF are synergistic with gonadotropins (LH and FSH) for steroidogenesis. The synergy involves an IGF-mediated up regulation of gonadotropin receptor expression and gonadotropin receptor second messenger systems. The gonadotropins increase ovarian IGF action by causing the synthesis of IGF-I and type I IGF receptors. Role of insulin in augmenting the reproductive potential of cattle is evident as it has high sequence similarity with IGF-1 (Butler *et al.,* 2004; Lucy, 2000).

Co-synch protocol is one of the oestrus induction protocols used for the management of post-partum anoestrus in dairy cows in which initial administration of gonadotropin releasing hormone (GnRH) induced follicular turnover, $PGF_2\alpha$ administration seven days later synchronised the oestrus, second dose of GnRH synchronised ovulation and insemination was done without oestrus detection. The incorporation of insulin in the oestrus induction protocols in buffaloes has been well documented (Gupta *et al.,* 2011; Ramoun *et al.,* 2007). However, reports available on insulin treatment in cattle for the same purpose is scanty. As the role of IGF-1 is highly related to the reproductive efficiency, this work aims to study the oestrus induction protocol with insulin and the estimation of progesterone and IGF-1 concentration.

Materials and methods

Fifty post-partum lactating crossbred dairy cows with a body condition score (BCS) of 2.5 to 3.5 (Smijisha, 2012) maintained at Kerala Veterinary and Animal Sciences University cattle farm, Pookode were screened for post-partum anoestrus. Rectal examination was performed on day 50 and 60 (day 0) post-partum to confirm anoestrus. Among them twenty-four anoestrus cows, without any palpable follicular or luteal structures were selected for the study as follow. In Group I (n=8), cows were subjected to Co-synch protocol, (10 µg busereline acetete on day 0, 500 µg closprostenol sodium on day 7, 10 µg busereline acetate and artificial insemination (AI) on day 9). Group II (n=8), had a modified Co-synch protocol (0.2 IU/kg insulin, subcutaneously on day 0, 1 and 2 along with Co-synch protocol). In group III (n=8), (control) the cows were observed for natural occurrence of oestrus signs until day 90 post-partum and AI was done on observed oestrus. Intensity of oestrus were assessed using score chart prepared by (Azeez, 2014).

Serum concentration of progesterone and IGF-1 were assessed day 50 post-partum and day 0 (day 60 post-partum), 7 (day 67 post-partum), 9 (day 69 post-partum) and day 10 post AI with competitive enzyme linked immunosorbent assay (ELISA) and sandwich ELISA, using commercial kits (Origin, India). Response to oestrus induction, onset, duration and intensity of oestrus were evaluated. Pregnancy diagnosis was carried out on day 50 post AI by rectal examination. First service and over all pregnancy rates were assessed.

The data obtained were analysed using SPSS version 24.0 statistical software package. The data regarding the time taken for onset and duration of oestrus in different groups were compared using one way ANOVA. Chi square test was used to the intensity of oestrus in different groups. Concentration of progesterone and IGF-1 obtained were analysed using one way and repeated measures ANOVA.

Results and discussion

Clinico-gynaecological observations

In the present study, it was observed that Co-synch protocol had 75 per cent responses. Meanwhile 87.5 per cent animals responded to modified Co-synch protocol with insulin. In group III, only 25 per cent animals showed spontaneous oestrus during the voluntary waiting period of 90 days (Table 1). Dhondiba (2012) treated post-partum anoestrus dairy cattle using Co-synch and modified Co-synch protocol with intramuscular administration of progesterone and recorded 88.88 and 94.44 per cent oestrus response respectively. Mishra *et al*. (2011) studied the efficacy of different hormonal protocols for the oestrus induction in post-partum anoestrus cows and observed an oestrus induction of 100 per cent (n=12) using Co-synch protocol.

The time taken for the onset of oestrus in group I ranged from 36.5 to 47 h, whereas in group II, the range was 30.5 to 45.5 h (Table 1). Pursley *et al*. (1997), using Ovsynch protocol in lactating dairy cows, reported that the oestrus onset following $PGF_{2\alpha}$ administration showed individual variability because of the differences in the developmental state of the preovulatory follicle at the moment of $PGF_{2\alpha}$ injection and there existed a considerable time interval between the regression of the corpus luteum and development of oestrus. Exogenous administration of insulin enhances diameter of dominant follicle as well as intrafollicular IGF-1and oestradiol concentration in cattle (Simpson *et al*., 1994). Reduced concentrations of insulin in blood were associated with nutritional anoestrus and the gluco-regulatory effects of insulin were compromised during anoestrus phase (Richards *et al*.,1989). Inadequate nutrient intake results in loss of weight and ultimately cease the ovarian activities which leads to extended post-partum anoestrus (Montiel and Ahuja, 2005). Reproductive performance and herd health parameters of dairy cattle have direct relationship with the BCS (Madushanka *et al*., 2016).

Table 1. Oestrus response, onset and duration of oestrus in post-partum anoestrus cows treated with different oestrus induction protocols (n=8)

Group	Oestrus response		Oestrus onset (h) (Mean ± SE)	Oestrus duration (h) (Mean ± SE)
	No.	%		
I	6	75.00	41.41 ± 1.47	14.41 ± 0.43
II	7	87.50	40.57 ± 2.09	18.92 ± 3.69
III#	2	25.00	-	17.25
			1.237ns t value (>0.05) (p value)	4.236* F value (<0.05) (p value)

ns - non-significant; * Significant at 0.05 level; **# -** not included in the statistical analysis of oestrus onset

Duration of oestrus in Group I varied from 13-16 h whereas in group 11 range was 14-23 h. In control group, only two animals showed oestrus with the duration of 15.5 and 19 h, (Table 1) and the values were observed to be significantly ($p<0.05$) different.

Intensity of oestrus was determined on the basis of observation regarding vulval oedema, hyperaemia of vestibular mucosa and tonicity of uterus. The cows were observed keenly and the intensity of oestrus were categorised as intense, intermediate and weak (Table 2). Statistically significant difference could not be observed for intensity changes of oestrus between group 1 and II.

Physiological changes in the genital tract with respect to vulval oedema, hyperaemia of vestibular mucous membrane and uterine tonicity in the treatment and control group cows were graded as high, medium and low and is depicted in table 3. According to Ammu e*t al. (*2012) among the cows treated under the Co-synch protocol, 50 per cent of animals had shown prominent oestrual signs, whereas 16.67 per cent animals exhibited moderate and weak oestrual signs. Addition of insulin had significant effect on the behavioural, physiological and gynaecological changes with regard to oestrus (Vijyakanth *et al*., 2020). Elevated oestradiol coupled with low concentration of progesterone causes profound behavioural and physiological changes in cattle as opined by Senger (2012).

Estimation of serum progesterone

The serum progesterone concentration (ng/mL) on day 50 post-partum, day 0 (60 post-partum) day 7 (67 post-partum), day 9 (69 post-partum) and day 10 post AI are presented in the table 4.

Table 2. Intensity of oestrus in post-partum anoestrus cows treated with two different oestrus induction protocols (n=8)

Groups	Animals exhibited oestrus		Intensity of oestrus						Chi square value (p value)
			Intense		Intermediate		weak		
	No.	%	No.	%	No.	%	No.	%	
I	6/8	75.00	3/6	50.00	3/6	50.00	0	0	4.362 (0.359) ns
II	7/8	87.50	4/7	57.14	2/7	28.58	1	14.28	
III	2/8	25.00	0	0	1/2	50.00	1/2	50.00	

ns - non-significant ($p > 0.05$)

Table 3. Grading of intensity of oestrus in post-partum anoestrus cows treated with different oestrus induction protocols

Groups	Intensity of oestrus	Vulval oedema (number and per cent)	Hyperaemia of vestibular mucosa (number and per cent)	Tonicity of uterus (number and per cent)
I (n=6)	High	3/6 (50.00)	2/6 (33.33)	3/6 (50.00)
	Medium	3/6 (50.00)	3/6 (50.00)	2/6 (33.33)
	Low	0	1/6 (16.67)	1/6 (16.67)
II (n=7)	High	3/7 (42.86)	4/7 (57.14)	4/7 (57.14)
	Medium	3/7 (42.86)	2/7 (28.58)	2/7 (28.58)
	Low	1/7 (14.28)	1/7 (14.28)	1/7 (14.28)
III (n=2)	High	0	0	0
	Medium	1/2 (50.00)	1/2 (50.00)	1/2 (50.00)
	Low	1/2 (50.00)	1/2 (50.00)	1/82 (50.00)

In group I cows, the progesterone concentration was significantly (p<0.01) higher on day 7 of treatment (day 67 post-partum) and day 10 post AI compared to other days of the study period. Similar trend was also noticed in group II cows. In group III (control) cows, no significant variation in progesterone concentration was obtained during different days of study.

While comparing different groups, significantly (p<0.01) higher progesterone concentration was observed in both treatment groups (group I and II) on day 7 of the treatment and day 10 post AI compared to group III cows and non-significant variation was only obtained on other days of study period.

Gupta *et al*. (2011) and Peter *et al. (*2009) observed that cattle exhibiting low P_4 levels (<1 ng/mL) in repeated examinations with an interval of 11 days were deemed to be in true anoestrus since their ovaries were non-functional, without any palpable structures on the ovary. This is in accordance with present study where repeated examinations on day 50 and 60 post-partum revealed no palpable structures in the ovaries and serum P_4 levels were estimated to be <1 ng/mL.

Higher values of progesterone on day 10 post AI could be correlated with the formation of CL. Co-synch and insulin modified Co-synch treatments were successful in inducing ovulation with the development of matured CL as evinced by the increased serum progesterone levels on day 10. This is in accordance with the observations of Ammu *et al.* (2012) and Azeez (2014), they stated that GnRH and prostaglandin-oriented oestrus induction protocols were successful in inducing ovulation in anoestrus cows followed by CL formation and elevated P_4 levels on day 10 post AI.

Estimation of serum IGF-1

Serum IGF-1 concentration (ng/mL) on day 50 post-partum, day 0 (day 60 post-partum) 7(day 67 post-partum), 9 (day 69 post-partum) and day 10 post AI are presented in the table 5. In group I cows, serum IGF-1 concentration on day 9 of treatment (day 69 post-partum) and day 10 post AI was significantly (p<0.01) higher than day 50 post-partum, day 0 and day 7 of treatment. However, no significant variation could be observed between day 9 of treatment and day 10 post AI. In group II, highest IGF-1 concentration was observed on day 9 of the

Table 4. Serum progesterone concentration (ng/mL) in post-partum anoestrus cows treated with different oestrus induction protocols (n=8)

Group	Day 50 post-partum (Mean ± SE)	Treatment days			Day 10 post AI (Mean ± SE)	F value (p value)
		Day 0/day 60post-partum (Mean±SE)	Day7/day67 post-partum (Mean ± SE)	Day 9/day 69 post-partum (Mean ± SE)		
I	0.60 ± 0.12^{cA}	0.69 ± 0.11^{cA}	1.89 ± 0.18^{bA}	0.86 ± 0.06^{cA}	2.80 ± 0.31^{aA}	27.775^{**} (0.0001)
II	0.68 ± 0.11^{cA}	0.75 ± 0.12^{cA}	2.37 ± 0.20^{bA}	0.83 ± 0.07^{cA}	3.16 ± 0.34^{aA}	34.274^{**} (0.0001)
III	0.53 ± 0.10^{aA}	0.52 ± 0.09^{aA}	0.58 ± 0.09^{aB}	0.65 ± 0.09^{aA}	0.58 ± 0.08^{aB}	0.307^{ns} (0.872)
F value (p value)	0.454^{ns} (0.641)	1.202^{ns} (0.320)	31.300^{**} (0.000)	2.586^{ns} (0.099)	26.653^{**} (0.0000)	

Means bearing different superscripts within a row differ significantly (p≤0.05) – (a, b, c)
Means bearing different superscripts within a column differ significantly (p≤0.05) – (A, B)
** Significant at 0.01 level; * Significant at 0.05 level; ns - non-significant

Table 5. Serum IGF1 concentration (ng/mL) in treatment and control groups (Mean ± SE, n=8)

Group	Day 50 post- partum (Mean±SE)	Treatment days			Day 10 post AI (Mean±SE)	F value (p value)
		Day0/day 60post-partum (Mean±SE)	Day7/day 67post-partum (Mean±SE)	Day 9/day 69post-partum (Mean±SE)		
I	25.60 ± 1.48^{bA}	26.05 ± 0.54^{bA}	31.90 ± 1.21^{bB}	46.27 ± 2.98^{aB}	39.90 ± 2.98^{aB}	23.912^{**} (0.0001)
II	23.44 ± 0.84^{cA}	26.72 ± 0.95^{cA}	61.78 ± 238^{abA}	70.25 ± 4.38^{aA}	52.62 ± 1.99^{bA}	72.290^{**} (0.0002)
III	23.06 ± 1.41^{aA}	23.10 ± 1.27^{aA}	23.82 ± 1.40^{aC}	27.49 ± 1.29^{aC}	28.23 ± 0.98^{aC}	3.859^{ns} (0.137)
F value (p value)	1.153^{ns} (0.335)	2.267^{ns} (0.128)	131.880^{**} (< 0.001)	46.453^{**} (< 0.001)	64.205^{**} (< 0.001)	

Means bearing different superscripts within a row differ significantly (p≤0.05) – (a, b)
Means bearing different superscripts within a column differ significantly (p≤0.05) – (A, B, C)
** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant

Table 6. Pregnancy rate in post-partum anoestrus cows treated with different oestrus induction protocols (n=8)

Pregnancy	Group I	Group II	Group III
	%	%	%
Following first AI	37.50	50.00	0
Following second AI	20.00	25.00	12.50
Following third AI	0	33.33	0
Overall	50.00	75.00	12.50

treatment (70.25 ± 4.38), and was significantly ($p<0.01$) higher than all days of study. In group III (control) cows, no significant variation in IGF-1 concentration was obtained during different days of study. While comparing different groups, significantly ($p<0.01$) higher IGF-1 values were observed in group II cows on day 7 and 9 of treatment and also on day 10 post AI. However, no significant difference could be observed on day 50 post-partum and day 0 of the treatment between the groups.

Shukla *et al.* (2005) reported insulin at induced oestrus was shown to provide a reasonable 50 per cent fertility rate, suggesting that insulin has a positive impact on ovarian function in anoestrus cattle. When insulin was used to induce oestrus, the conception rate was higher than in other treatment groups. GnRH injection will recruit new follicles. The insulin injection will increase small follicle growth, also there will be follicle growth to medium and large size, thereby increasing the steroidogenesis potential, which might cause the LH surge leading to ovulation and the changes related to oestrus (Butler *et al.,* 2004). The concentration of IGF-1 in group II, was higher in all the treatment days and day 10 post AI, which validated that the cows treated with insulin, had a considerable increase in the IGF-1, compared to the other groups, similar observations were made by (Vijyakanth *et al*., 2020).

Pregnancy rate

The first service pregnancy rate for group I, II and III were 37.50, 50 and 0 per cent, respectively. The overall pregnancy rate after AI performed in three consecutive cycles were 50, 75 and 12.5 per cent for group I, II and III, respectively (Table-6). Statistical difference could not be observed between the different groups.

Treatment expense, benefits and limitations

The treatment expenses with respect to cost of medicines for groups I, II and III were Rs 270/-, Rs 367/- and zero, respectively. Considering the value of pregnancy, treatment cost incurred could be justified. The precise loss lies in terms of the number of days taken to conceive, cost of extra feed, value of the milk loss, cost of additional labour, cost of further treatment, cost of the extra breeding, value of the lost calf and unplanned involuntary culling of animals as a result of post-partum anoestrum. Azeez (2014) suggested that oestrus induction protocols involving GnRH, $PGF_{2\alpha}$ and CIDR reduced days open in post-partum cows and improved the fertility, and further opined that these protocols are recommended for management of true anoestrus in crossbred cattle with good body condition score. Pawshe *et al*., (2013) observed that a post-partum cow with anoestrus beyond 60 days could cause an economic loss of Rs 193/day, considering mainly the approximate loss of feed cost, labour and loss through milk.

The present study was conducted in a population which is not a large one. More studies are needed in a larger population in various parts of the state. Gene expression studies and molecular analysis designed works need to be done on a larger section for getting more knowledge in the etiology and control of infertility.

Conclusion

Modified insulin Co-synch protocol yielded good oestrus response and conception rates and Co-synch alone yielded moderate results for the same. However, the results were statistically non- significant. But modified insulin protocol significantly increased serum progesterone and IGF-1 levels when compared with the Co-synch and control group. This increase in serum IGF-1 could be attributed to the exogenous administration of insulin which will increase the circulatory IGF-1, because of its structural similarity. So these IGF-1 which is a potent ovarian growth promoter will in turn increase the ovarian steroidogenesis, which might have been resulted in increased progesterone concentration.

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Conflict of interest

The authors declare that they have no conflict of interest.

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