

Influence of Progesterone Levels at AI and during Early Luteal Phase on Conception in Acyclic Sahiwal Cows Treated with Ovulation Synchronization Regimens

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ABSTRACT

The present experiment attempted to examine the influence of serum progesterone concentrations on conception rate in acyclic cows that were treated with three variants of ovsynch protocol. Postpartum (> 90 days) acyclic Sahiwal cows (n=18) were divided into three groups; each group consisted of six animals. Animals of group I were treated with modified ovsynch, group II animals with modified heat-synch and those of group III were treated with modified select-synch regimens. FTAI was performed in animals of group I and group II, whereas animals of group III were inseminated at detected estrus. Detection of conception was accomplished by ultrasonography on day 35 post-AI and confirmed later by examination per rectum between days 50-60 post-AI. Based on pregnancy diagnosis the animals were divided into conceived and non-conceived groups irrespective of treatment given to the animals. Blood samples were collected on the day of AI, days 10 and 20 post-AI for estimation of serum estradiol, insulin and progesterone levels using RIA kits. Results revealed that on the day of AI conceived animals had significantly higher serum estradiol concentrations (51.32±5.55 vs. 32.13±2.47 pg/mL, $p \leq 0.05$) and insulin concentrations (17.61±0.74 vs. 12.94±0.39 μ U/mL, $p < 0.01$) and lower serum progesterone levels (1.00±0.10 vs. 2.01±0.29 ng/mL, $p \leq 0.01$) than in non-conceived animals. Conceived animals also recorded significantly higher ($p \leq 0.01$) serum progesterone concentrations on day 10 (10.49±0.32 vs. 4.23±0.99 ng/mL) and day 20 (9.36±0.23 vs. 1.60±0.35 ng/mL) post-AI than in non-conceived animals. These observations highlighted two distinct prerequisites for successful conception; complete luteolysis before AI and strong luteal activity during early gestation.

Key words: Acyclic, Conception, Estradiol, Insulin, Sahiwal cows, Supra-basal progesterone.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.1.13

INTRODUCTION

Postpartum acyclicity is a prevalent reproductive challenge in indigenous dairy breeds including Sahiwal, resulting in prolonged calving intervals, delayed conception, reduced reproductive efficiency and consequent losses in milk productivity (Arshad and Santos, 2022). Different ovulation synchronization protocols aim to restore cyclicity and improve fertility; however, success of conception depends on achieving a precise metabolic and endocrine balance at insemination and following ovulation (Lucy, 2019). Insulin is a key metabolic hormone that regulates ovarian folliculogenesis, steroidogenesis, and oocyte competence (Lucy, 2019). Adequate insulin promotes estradiol secretion from dominant follicles, enhances LH responsiveness, and supports the peri-ovulatory surge required for ovulation (Maffi *et al.*, 2019). Furthermore, insulin interacts synergistically with IGF-I to improve granulosa and theca cell function, ensuring better follicular health and subsequent luteal activity (Butler, 2014). Thus, cows with higher insulin levels at AI are more likely to have a functional pre-ovulatory follicle, improved oocyte quality, and a receptive uterine environment for embryo development.

Progesterone (P_4) plays a pivotal role in regulating estrus cyclicity, ovulation and maintenance of pregnancy in cattle.

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How to cite this article: Paikra, D. K., Awasthi, M. K., Khan, J. R., Parveen, K., Paikra, D., & Sahu, S. (2026). Influence of Progesterone Levels at AI and during Early Luteal Phase on Conception in Acyclic Sahiwal Cows Treated with Ovulation Synchronization Regimens. *Ind J Vet Sci and Biotech*, 22(1), 68-71.

Source of support: Nil

Conflict of interest: None

Submitted 27/10/2025 **Accepted** 20/11/2025 **Published** 10/01/2026

Optimal fertility requires complete luteolysis before artificial insemination (AI) and adequate luteal function during the early luteal phase (Perry *et al.*, 2023). Supra-basal levels of progesterone (>1.5 ng/mL) at the time of AI are associated with incomplete luteolysis, reduced estradiol synthesis, impaired LH surge and consequently poor conception rates (Bisinotto *et al.*, 2015; Stevenson and Pulley, 2016). Conversely, elevated progesterone level during early luteal development supports embryo growth and uterine receptivity (Nyman *et al.*, 2018; Cortes-Vazquez *et al.*, 2022). Understanding the temporal relationship of progesterone concentrations around AI is therefore critical for improving reproductive efficiency in postpartum acyclic cows. In the perspective of above facts, present study investigated the effect of insulin, estradiol and progesterone levels at AI and effect of progesterone levels during early luteal phase on conception in postpartum acyclic Sahiwal cows, treated with three variants of ovsynch protocol.

MATERIALS AND METHODS

Present research work was undertaken in acyclic Sahiwal cows (n=18) with more than 90 days postpartum period. Animals had normal parturition and genitalia, and were maintained at Bull Mother Experimental Farm, Anjora, Durg, Chhattisgarh (India). Experimental animals were divided in three treatment groups (G), each group comprised of six animals. They were treated with modified ovsynch (G-I), modified heat-synch (G-II) and modified select-synch (G-III) protocols.

Briefly, the animals of G-I were treated with GnRH on day 0 with concurrent intra-vaginal placement of CIDR, followed by PMSG @ 300 IU on day 5 and PGF₂α on day 7. Second dose of GnRH was given on day 9 and FTAI was accomplished 16-22 h later. In G-II, the second dose of GnRH was replaced with estradiol benzoate @ 5 mg on day 9, and in G-III, the same protocol as in G-I was followed, but the second dose of GnRH was not given and AI was performed at detected estrus up to 72 h after PGF₂α treatment. Conception was

determined by ultrasound scanning on day 35 post-AI and pregnancy was confirmed by examination per rectum 50-60 days post-AI. Based on pregnancy diagnosis the animals were divided into the conceived (n=13) and non-conceived group (n=5), irrespective of treatment given to the animals. Blood samples were collected to harvest the serum from each experimental animal on the day of AI, days 10 and 20 post-AI. Serum insulin, estradiol (E₂) and progesterone (P₄) concentrations were determined by radio immuno-assay (RIA) kits procured from Beckman Coulter. The sensitivity, specificity and cross-reactivity were 0.49 µIU/mL, 55% and 100% for insulin; 10.41 pg/mL, 50% and 100% for estradiol and 0.04 ng/mL, 50% and 100% for progesterone kits, respectively.

Statistical differences in serum Insulin, E₂ and P₄ concentrations were compared between animals of conceived and non-conceived group using independent t-test (Snedecor and Cochran, 1994). Pearson correlation co-efficient “r” was calculated among serum insulin, P₄ and E₂ concentrations within conceived and non-conceived animals separately.

RESULTS AND DISCUSSION

Under current study, mean (±SE) serum P₄, Insulin and E₂ concentrations recorded in different groups of treated animals are presented in Table 1 and 2.

The perusal of Table 1 revealed that the conceived animals, irrespective of treatment groups, on the day of estrus and AI recorded significantly higher serum insulin (17.61±0.74 vs. 12.94±0.39 µIU/mL, p<0.01) and estradiol (51.32±5.55 vs. 32.13±2.47 pg/mL, p<0.05) concentrations and lower serum progesterone levels (1.00±0.10 vs. 2.01±0.29 ng/mL, p<0.01) than in non-conceived animals. Further, the conceived animals had significantly higher (p ≤ 0.01) serum progesterone concentrations on day 10 (10.49±0.32 vs. 4.23±0.99 ng/mL) and day 20 post-AI (9.36±0.23 vs. 1.60±0.35 ng/mL) than in non-conceived animals.

Table 1: Mean (±SE) serum insulin, estradiol and progesterone concentrations on the Day of AI, and progesterone concentrations during luteal phase in conceived and non-conceived cows across three treatment groups with overall observation

Groups	Animals status	No	Insulin (µIU/mL)	Estradiol (pg/mL)	Progesterone (ng/mL)		
			Day of AI	Day of AI	Day of AI	Day 10 post-AI	Day 20 post-AI
I	C	05	18.83±1.74	57.61±8.34	1.13±0.20	11.05±0.72	9.56±0.15
	NC	01	13.48±0.00	30.93±0.00	1.84±0.00	7.70±0.00	2.87±0.00
II	C	04	16.35±0.75	51.90±7.32	0.81±0.14	10.31±0.13	9.47±0.39
	NC	02	12.52±0.85	28.81±5.54	1.83±0.81	3.70±1.24	1.34±0.09
III	C	04	17.36±0.57	42.90±13.79	1.03±0.12	9.99±0.49	9.00±0.68
	NC	02	13.10±0.68	36.06±1.71	2.28±0.26	3.02±0.73	1.22±0.45
Overall	C	13	17.61±0.74	51.32±5.55	1.00±0.10	10.49±0.32	9.36±0.23
	NC	05	12.94±0.39	32.13±2.47	2.01±0.29	4.23±0.99	1.60±0.35
p value			0.002**	0.05*	0.001**	0.002*	0.001**

C=conceived, NC= non-conceived, * p<0.05, ** p<0.01

Adequate insulin concentration increases follicular responsiveness to LH and FSH, facilitating the development of a dominant follicle capable of ovulation, estradiol production and promotes a strong peri-ovulatory surge (Maffi *et al.*, 2019). This finding is in agreement with Singh *et al.* (2021) and Sukwan *et al.* (2023) who recorded higher insulin concentrations in conceived cows undergoing CIDR-based protocols. Thus, higher circulating insulin favours pre-ovulatory follicle function, oocyte quality, and uterine receptivity, thereby increasing possibility of conception. Likewise, elevated estradiol concentration at insemination reflects optimal follicular development and enhances estrus expression, induction of the LH surge, uterine receptivity and sperm transport, thereby improving conception rates (Bisinotto *et al.*, 2015; Perry *et al.*, 2023). Conversely, lower serum estradiol concentration in non-conceived animals suggested impaired folliculogenesis due to incomplete luteolysis or supra-basal progesterone levels on the day of AI.

These findings suggest that balanced metabolic and ovarian steroidogenesis, characterized by high insulin, estradiol and low progesterone at AI, is essential for successful conception in postpartum acyclic Sahiwal cows (Sukwan *et al.*, 2023). The findings of the current study clearly demonstrated distinct association between high insulin, estradiol and low progesterone concentration at the time of AI with an increased probability of conception, whereas low estradiol and supra-basal progesterone levels (>1.5 ng/mL) at AI were linked with reduced fertility and conception failure (Stevenson and Pulley, 2016).

In the present study, the correlation coefficient between serum P_4 and E_2 concentrations was low positive ($r = 0.19$) in conceived animals, and moderate negative ($r = -0.44$) in non-conceived cows. The correlation between serum insulin and E_2 concentrations was low and negative in both conceived ($r = -0.12$) and non-conceived animals ($r = -0.20$). Similarly, serum insulin and P_4 concentrations exhibited a weak positive correlation ($r = 0.16$) in conceived cows and a moderate positive correlation ($r = 0.39$) in non-conceived cows. These results indicated that a more balanced interaction between metabolic (insulin) and reproductive (P_4 and E_2) hormones supports follicular development, ovulation, and conception in conceived animals, while disrupted correlations in non-conceived animals reflected endocrine imbalance associated with reduced fertility (Lucy, 2019; Singh *et al.*, 2021).

The current findings of low P_4 concentrations at AI and high P_4 levels during the early luteal phase in conceived animals highlighted two critical but distinct prerequisites as complete luteolysis prior to insemination and strong luteal activity after breeding are mandatory for successful conception. Physiologically, low progesterone at AI is essential for expression of estrus, pre-ovulatory estradiol synthesis and induction of the LH surge (Wiltbank *et al.*, 2018). Present finding is in close agreement with the observations of Ammu *et al.* (2012) and Bhoraniya *et al.* (2012) who recorded similar pattern of mean plasma progesterone levels in conceived animals on the day of AI and day 20 post-AI in

anestrus Gir and Kankrej cows, respectively, which were under treatment with CIDR and Ov-synch protocols. Interestingly, Buhecha *et al.* (2015), Chaudhary *et al.* (2018), Niaz *et al.* (2022) and Manokaran *et al.* (2023) documented low progesterone levels at AI and high progesterone concentrations during early luteal phase for successful conception in dairy animals. Conversely, supra-basal progesterone reflects incomplete luteolysis or persistent luteal activity, which suppresses estradiol secretion, compromises follicular dominance, impairs oocyte maturation and creates a sub-optimal uterine environment (Nyman *et al.*, 2018; Cortes-Vazquez *et al.*, 2022). Previous studies also demonstrated that supra-basal progesterone level at AI reduces pregnancy per AI due to poor uterine receptivity and asynchronous endometrial development (Bisinotto *et al.*, 2015; Blavy *et al.*, 2018). Our results aligned with these findings, confirming the detrimental role of supra-basal progesterone in fertility at the time of AI.

The high serum P_4 concentrations during the early post-AI luteal phase in conceived animals are indicative of a well-formed corpus luteum and are strongly associated with embryo survival. Adequate post-ovulatory progesterone level promotes endometrial secretions, conceptus elongation and interferon- τ production needed for maternal recognition of pregnancy. Conversely, insufficient P_4 levels during days 5-20 post-AI is a well-documented risk factor for embryonic loss that reduces pregnancy per AI, whereas adequate P_4 after AI supports conceptus development (Lonergan and Sanchez, 2020). The efficacy of different variants of ovsynch protocol in terms of conception was severely influenced by supra-basal level of progesterone at the time of AI in the present study. To improve the conception rate in animals treated with variants of ovsynch regimens, a second AI may be attempted at an interval of 12-18 h after first AI in anticipation that progesterone concentration might be reduced below supra-basal level by the time when second AI would be performed. Another approach to enhance the conception rate might be use of progesterone receptor antagonists *viz.*, aglepristone and mifepristone in effective dose at the time of AI to mitigate the effect of supra-basal level of progesterone (Gogny and Fiéni, 2016).

CONCLUSION

From the results of present study, it may be concluded that adequate levels of insulin and estradiol and low level of progesterone on the day of AI and elevated P_4 concentration during the luteal phase resulted in successful conception, reflecting a favourable endocrine milieu for ovulation and embryo implantation and growth. In contrast, cows exhibiting elevated progesterone and inadequate estradiol and insulin levels on the day of AI experience poor follicular function and lower fertility. Supra-basal P_4 level at AI is detrimental and should be mitigated by ensuring complete luteolysis in synchronization protocols for improving conception rates in postpartum acyclic Sahiwal cows.



ACKNOWLEDGEMENT

Authors are grateful to the Director of Farm, BMEF, Anjora and authorities of Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Durg, Chhattisgarh, India for providing facilities to conduct this experiment.

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