Research Article

Efficacy of Different Synchronization Protocols on Generation of Fertile Estrus, Hormonal Profile, and Conception Rate in *Bos indicus* Achai Cows

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Abstract | This study evaluated the efficiency of two different estrus synchronization protocols in Achai breed of cattle. Twelve lactating Achai cows were divided into two equal groups and subjected to Presynch Ovsynch (POP) or Modified 7-day Select Synch (MSS) protocol. In POP protocol animals were administered with PGF_2-α injections on day -38, -24 and -3. GnRH-1 was given on day -10 and 0, along with timed artificial insemination after 16 hrs. Animals in MSS group were administered with a controlled internal drug release (CIDR) insert on day -7 with simultaneous use of prostaglandin 2-alpha (PGF_2-α), GnRH-1 at day -5, removal of CIDR at day 0, concurrent with two doses of PGF_2-α at 8 hr. interval, GnRH-2 on day 3, followed by TAI after 16 hrs. Results showed higher estrogen (E_2) and lower progesterone (P_4) concentrations for MSS than POP group. Cows with higher E_2 and lower P_4 levels at estrus got conceived ($P<0.05$). Highest level of E_2 was recorded in pregnant cows in POP group in comparison with MSS. The P_4 concentration of pregnant cows in MSS was lowest at the time of insemination ($P<0.05$). Induction of fertile estrous was maximum in POP group than MSS, but conception rate in cows receiving TAI was significantly higher in MSS than POP group (66.66% vs 33.33%;). Efficiency of MSS protocol was virtually double than POP for estrus synchronization and conception in Achai cattle.

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Keywords | Estrus synchronization, Estrogen, Progesterone, Timed AI, Achai cattle

Introduction

In Pakistan Red Sindhi and Sahiwal are valuable milk breeds of global recognition, due to their distinctive characteristics. In addition to these well-known breeds of cattle, there is a large number of non-expressive and crossbred cattle in Pakistan. Among them, Achai, is a short stature, reddish brown coated breed of Khyber

Pakhtunkhwa, Pakistan, famous for coping with extreme environmental influences (Kenyanjui and Sheikh Ali, 2009). It is a dual purpose breed and can be used both for dairy and draught purposes (Saleem *et al*., 2013).

Among various factors, the short and weak estrus expressions (Nebel *et al*., 2000), failure of estrus detection (Cavestany *et al*., 2003), prolong postpartum anestrus (Cavestany and Galina, 2001) and low rate of conception (Wiltbank *et al*., 2006) are the basic issues affecting reproductive efficiency of cows. Heat detection is primarily a management problem. Considerable skill is needed to detect estrus from visible signs (Fesseha and Degu, 2020). The factors responsible for these problems may be heat stress, floor conditions, genetic manipulation of dairy cows for high production, negative energy balance (NEB), delayed ovarian rebounce, and gait problems (Garbarino *et al*., 2004; Lopes *et al*., 2004). The primary intension behind the use of estrus synchronization protocol is to pass up heat detection and perform timed artificial insemination (Komar *et al*., 2001). Estrus synchronization and proper resynchronization process is a significant component of better reproductive management in dairy industry (Shahzad *et al*., 2019). Estrous synchronization will result in reduced calving season, facilitation of AI, and higher uniformity in calves (Larson *et al*., 2006). Estrous synchronization controls follicular dynamics, precise onset of estrus, maximizing pre-ovulatory estradiol concentrations, and ensuring the ovulation of a competent ovum, following estrus or the GnRH-induced ovulation (Bridges *et al*., 2008). Therefore, successful breeding program is dependent upon selection of right and practically applicable synchronization protocol (Bihon and Assefa, 2021).

A previous study (Elzarkouny *et al*., 2004) reported higher fertility in cattle receiving presynch earlier than Ovsynch (48.8%) than cattle not treated with presynch protocol (37.5%). Another study revealed that level of progesterone (P_4) concentration can manipulate the sequence of follicular waves (Johnson *et al*., 1996). The Achai breed of cattle inhabits in the hilly and mountainous regions of the northern Pakistan, having little access to veterinary and insemination services. Timed-AI (TAI) might be a solution for this problem that may be more convenient with the application of synchronization of the scattered herd in remote areas. Evaluation of synchronization protocols can be accomplished by different methods like conception rate, total pregnancy rate, and estrous detection (Odde and Holland, 2021). Therefore, the current study was undertaken to determine the changes in hormonal profile (estrogen and progesterone), associated estrus behavior, and conception rates using Presynch Ovsynch (38-days) and Modified 7-days Select Synch + CIDR + Timed A.I synchronization protocols in Achai cows.

Materials and Methods

Study area and animals

This experiment was conducted in Khyber Pakhtunkhwa, District Charsada, Government dairy farm Harichand. A total of 12 lactating and cyclic Achai cows were equally divided into 2 group i.e. Presynch Ovsynch (POP) and modified 7 days select synch (MSS) for estrous synchronization. Two protocols were adopted for estrus synchronization as given under Table 1. All the experimental animals were having approximately same age, body weight, body condition score, stage of lactation, and were reared under same managemental conditions. Briefly, average age of experimental animals was in between 6 and ½ year, they were in 3rd to 4th parity and were in early lactation (first 3 months). Body condition score was recorded as 2.8 to 3.0 keeping in view the 5 point BCS. Average weight of individual Acahi cow was 250 ± 6.12 Kg. Animals were kept together in a herd and reared in a similar lot/ pasture during synchronization protocols i.e. stall feeding at the rate of 3% dry matter (DM) from forages and concentrate, as well as free grazing in pasture.

Table 1: *Concentration of progesterone (ng/ml) and estrogen (pg/ml) in Achai cows in two different protocols at the time of AI.*

Parameters	POP	MSS	P-value
Progesterone (ng/ml)	9.17 [a]	1.42[a]	0.0003
Estrogen (pg/ml)	68.88 [b]	80.28 [b]	0.007

**Means with different superscripts within the same row are different significantly at P<0.05.*

Presynch-ovsynch (POP group)

Animals assigned to POP group were treated with Presynch-Ovsynch synchronization protocol (Figure 1) and timed artificial insemination (TAI, n=6). Cloprostenol (synthetic analogue of $PGF_2\alpha$) was administered at the rate of 0.075 mg, intramuscular (IM) i.e. injection Dalmazin 2ml (Fatro-

Pharmaceutical veterinary industry, Bologna, Italy) on Days -38 and -24. It was followed by Ovsynch protocol, comprising GnRH analogue (25 µg lecirelin acetate) in the form of injection Dalmarelin 2ml, IM (Fatro-Pharmaceutical veterinary industry, Bologna, Italy) on Day-10. The $PGF_2\alpha$ analogue was repeated on Day-3. The animals were examined for heat detection, if found positive for heat signs within 72 h after last $PGF_2\alpha$ administration were artificially inseminated at detected estrus (AIDE). Cows showing no estrus signs were readministered with GnRH analogue at 72 h interval, and TAI performed after 16 hr (Figure 2).

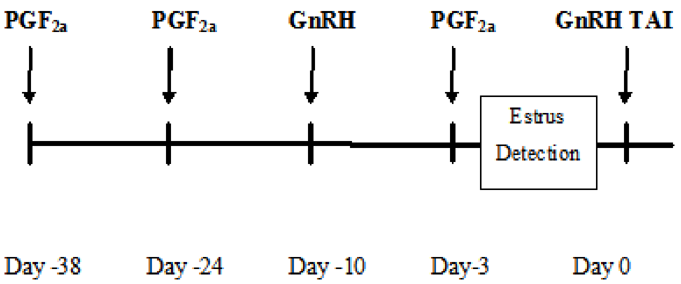

Figure 1: *Design for Presynch-Ovsynch synchronization protocol with artificial insemination at detected estrus (AIDE).*

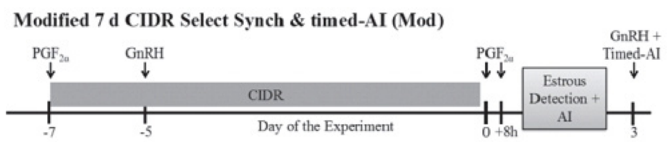

Figure 2: *Modified 7-d Select synch + CIDR and TAI (Timed artificial insemination) protocol.*

Modified 7-days select synch + CIDR (MSS-Group)

In MSS group (Modified 7 days Select Synch + CIDR), CIDR (Cattle insert, Pfizer, progesterone 1.38 g) was placed in vagina of the selected cows with the help of CIDR applicator and retained for 7 days. It was considered day -7. On d -5, the cows received GnRH and removal of CIDR inserts from cows on d 0 followed by administration of PGF_2-α analogue. PGF_2-α analogue 2nd dose was given to the cows after 8 hrs. of removal of CIDR inserts. The treated cows were inspected for standing estrus twice a day for 72 h, following CIDR insert removal. AI was performed in cows with standing heat according to am-pm rule. Cows with no estrus response within 72 h following CIDR insert removal received an injection of GnRH analogue and a TAI after 16hr. Cows found positive for heat signs within 72 h got AI first, followed by those receiving TAI.

Blood samples collection and assays

Samples of blood were collected through jugular venipuncture early in the morning (7:00 am) from POP group on days -10, -9, -7, -3, -2, -1, 0, and at AI. Similarly, samples of blood were collected from MSS group on day -7, -5, -4, and on day 0, 1, and 3, and same timing, followed by TAI. Samples were kept in blood collection tubes without anticoagulant (Bio-Vac, EStars-Pakistan) and shifted to laboratory shortly after collection (within 2hr) in ice packs. Samples were centrifuged at 1500 rpm for 20 min to get serum and stored at lower temperature of -20°C till analysis for E2 and P_4. Serum samples were assayed for P4 and E2 using commercially available ELISA Kits (MonoBind Inc. Lake Forest, CA USA; BioCheck, Inc. Foster city, CA 94404 U.S.A) for progesterone and estrogen, respectively.

Estrus response, TAI, and conception rate

Estrous response was considered if the cows expressed heat signs/ behaviour by 72 h after last PGF analogue injection (in POP group) and CIDR insert removal (in MSS group). The rate of conception was determined by dividing the number of cows showing estrous behaviour within 72h following CIDR insert removal or from last PGF analogue injection (in POP group) that got AI and found pregnant, by sum of the cows showing heat signs within 72h and inseminated. While rate of conception for TAI was the number of animals receiving TAI and considered as conceived divided by sum of animals that were TAI. Overall AI rate of pregnancy was the percentage of treated cows that conceived to artificial insemination (AI). Pregnancy diagnosis for the services was determined on day 60 post-insemination by palpating the genital tract by trans-rectal examination.

Statistical analysis

Analysis of data was accomplished with statistical analysis software, Statistics 8.1. heat response, TAI, interval to estrus, rate of conception, and overall rate of pregnancy with AI were determined by descriptive analysis. Effect of factors were declared significant at $P<0.05$ using least significant test (LST).

Results and Discussion

Hormonal profile's correlation with pregnancy

The E2 concentration varied significantly between synchronization protocols (($P<0.007$) during estrus (Table 1). Highest concentration of E2 was observed for MSS as compared to POP group. Similarly, the P4 varied significantly ($P< 0.001$) between the two groups at the time of AI i.e. highest in POP than MSS group. The pregnancy significantly affected the E2 level (*P*<0.05) in experimental cows (Table 2). Pregnant cows at the time of AI showed higher level of E2 compered to non-pregnant cows. Lower level of P4 was observed in pregnant cows at the time of AI than non-pregnant cows (Table 2). The results revealed that Achai cows having lower level of P4 and higher level of E2 at the time of estrus become pregnant, and vice versa.

Table 2: *Concentration of progesterone (ng/ml) and estrogen (pg/ml) of pregnant and non-pregnant cows detected at the time of AI.*

Parameters	Pregnant	Non-pregnant	P-value
Progesterone (ng/ml)	4.72 [a]	8.65[a]	0.032
Estrogen (pg/ml)	76.48[b]	26.16[b]	0.005

**Means with different superscripts within the same row, are different significantly at P<0.05.*

Table 3: *Least significance test for concentration of progesterone and estrogen in pregnant and non-pregnant cows in two different protocols at the time of AI.*

Groups	Pregnancy	Progesterone (ng/ml)	Estrogen (pg/ml)
POP	Pregnant	4.30[b]	46.28[b]
	Non-pregnant	9.60[b]	31.25[b]
MSS	Pregnant	1.13[a]	21.07[a]
	Non-pregnant	1.70[a]	16.70[a]
P-value		0.026	0.003

**Means with different superscript within the column are different significantly at P<0.05*

Interrelationship of protocol, pregnancy, and hormones

The E2 level was significantly higher (*P*<0.003) in pregnant cattle in POP followed by pregnant cows in MSS group. The non-pregnant Achai cattle in both the protocols showed lower concentrations of E2 (Table 3). The P4 concentration of pregnant cows in MSS protocol was lowest (*P*< 0.05) at the time of insemination followed by pregnant cows in POP protocol than cows found non-pregnant (Table 3).

Interrelationship of estrus response, conception rate, and protocol

The estrus induction percentage was higher in the POP than MSS (*P*<0.05) group as shown in Table 4. Cows treated with POP showed estrus after 60 hours of last PGF2α injection, while the cows in MSS protocol exhibited estrus after 48 hours of last PGF2α injection (*P*<0.05). The rate of conception with TAI was found higher (*P*<0.05) in MSS compared to POP group. The overall pregnancy rate and TAI conception rate were found higher in MSS than POP group, indicating better efficiency of MSS protocol in estrus synchronization.

Table 4: *Descriptive analysis for different studied parameters in Achai cows.*

Groups	No. of animals	Estrus response %	Interval to estrus, h	TAI C. R%	AI pregnancy rate %
POP	6	33.33	60	25.00	33.33
MSS	6	16.66	48	60.00	66.66
P-value		0.032	0.044	0.021	0.045

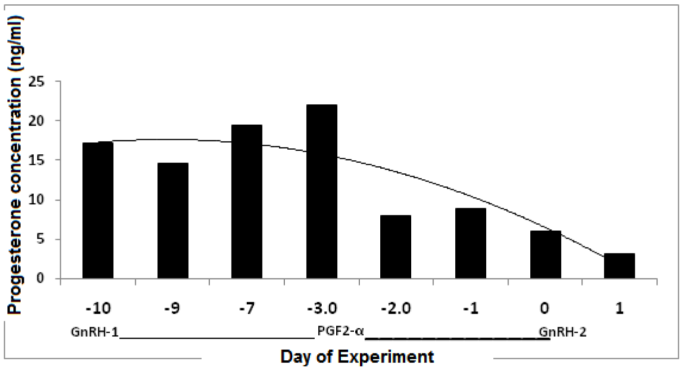

Figure 3: *Changing pattern of progesterone concentrations in Presynch-Ovsynch protocol in Achai Cows ($y = -0.430x^2 + 1.650x + 15.98$, $R^2 = 0.726$).*

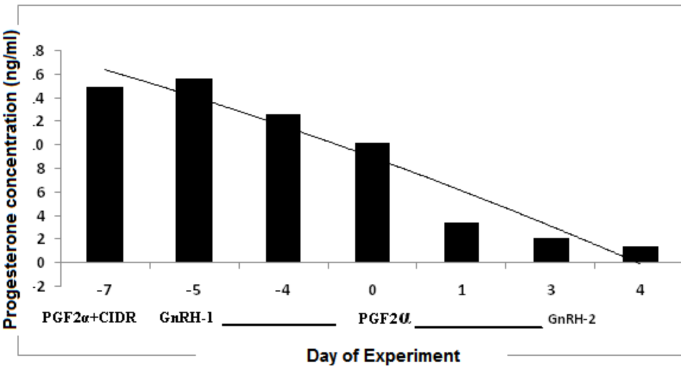

Figure 4: *Changing pattern of progesterone concentrations in Mod-7dSS protocol in Achai Cows ($y = -0.087x^2 - 2.037x + 18.54$, $R^2 = 0.923$).*

Modulating pattern of hormonal profile

The changing pattern of P4 concentration in POP and MSS group is shown in Figures 3 and 4, respectively. The P4 level was higher in POP group following GnRH administration and remained higher until PGF^{2-}α administration, while it showed a decreasing tendency throughout the MSS protocol. The CIDR released progesterone slowly and gradually into the blood so the level of progesterone was maintained at the low level consistently throughout the CIDR insertion period even after the luteolytic dose of PGF2α. The E2 concentration throughout the POP protocol is shown in Figure 5. It is evident from the figure that the E2 concentration was lower during the early injection of GnRH and then shows a gradual increase after the PGF_2-α injection. In MSS protocol (Figure 6) the E2 level stayed higher at the time of 2nd GnRH administration which may lead to the surge level of LH and release of ovum.

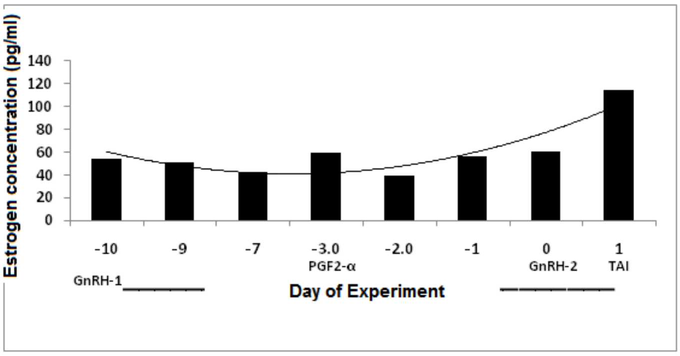

Figure 5: *Changing pattern of estrogen concentrations in Presynch-Ovsynch treated in Achai Cows ($y = 3.009x^2 - 21.28x + 78.84$, $R^2 = 0.766$).*

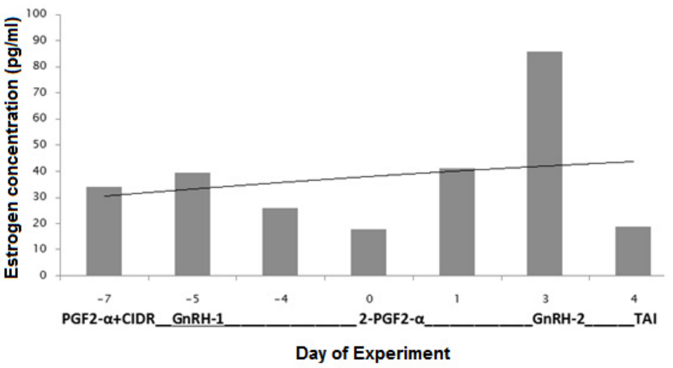

Figure 6: *Changing pattern of Estrogen concentrations in Mod-7dSS treated Achai Cows ($y = 0.100x^2 + 3.006x + 27.63$, $R^2 = 0.042$).*

Estrus incidence

The cows were not detected in heat prior to days -3 and 0 in POP as well as MSS protocols, respectively. However, previous reaearch (Geary *et al*., 2000; Dejarnette *et al*., 2001a) reported an incidence of estrus as 8–10% and 20–25% before day-3 and day 0 in both protocols, respectively. As reported earlier (Dejarnette *et al*., 2001b) before PGF_2-α estrus commonly appear prematurely that resulting in additional estrus detection. This problem was successfully overcome by giving exogenous P4 device (CIDR) in MSS protocol in this study. More estrous response was noticed for POP (33.33%) than for MSS protocol (16.66%) after 72 hours of the last PGF_2-α injection. The poor estrus response may be due to failure to detect estrus, weak estrus behavior, and high incidences of silent heat as reported by Galina *et al.* (1996). The average time interval of behavioral estrus was 60 hours for POP protocol where 48 hours for MSS protocol from last PGF_2-α injections.

Conception/ pregnancy rates

Cows inseminated with TAI in MSS group had greater (60%) pregnancy rate than the POP group (25%) which was significantly lowered than reported by Souza *et al.* (2008). Overall pregnancy occurrence determined 60 days' post AI between the POP and MSS groups was significantly (P<0.05) different (33.33% vs. 66.66%, respectively). Similarly, Souza *et al.* (2008) reported a similar rate of pregnancy for multiparous cows by comparing POP with double Ovsynch (39.3% vs. 37.5) respectively. The overall Pregnancy rate obtained in POP protocol was in accordance with the results of (Chebel *et al*., 2010) where they documented a pregnancy rate of 35.1% and 33.6%, respectively. Results of the current study pertaining to conception rates for POP protocol were contrary with (Chebel *et al*., 2010; Moreira *et al*., 2000) where they documented the pregnancy rate 40-50% in POP protocol. Recently Pacala *et al.* (2010) obtained a pregnancy rate of 56.5% by using CIDR protocol. Earlier studies (Lucy *et al*., 2001; Stock and Fortune, 1993) reported 57% and 61.5% increased conception rates in cattle by CIDR and MAP (Methylacetoxyprogesterone) sponges respectively. Moreover, Eski *et al.* (2021) reported higher fertility parameters and antioxidant defense system in hair goats with combined treatment of PGF2α, GnRH, and intravaginal progesterone. An earlier study (Shahid *et al*., 2021) reported that protocols of Ovsynch in cows, and CO-Synch+CIDR in heifers may result in better CR/TAI and therefore can be used to enhance the genetic improvement through AI and improve the reproductive parameters.

The increased synchronized conception rate in MSS treatment may be due to second injection of PGF_2-αat 8 hr. interval after the first one which induced complete and rapid luteolysis. Stevenson *et al.* (2008) found that sudden elevated level of PGF_2α results in immediate lysis of corpus luteum (CL) and a declined level of P4 accompanied with degeneration and death of luteal cells. While incomplete lysis of CL may result in weak heat response, which will lead to reduced rates of synchronized pregnancy. A significant increase of 9% in pregnancy rate have been reported with the addition of a second PGF_2-α injection, which enhanced PGF_2-α induced luteolysis, positively affecting rates of TAI pregnancy (Bridges *et al*., 2008). A potential explanation for reduced conception rates in POP group in our study might be due to extended gap between GnRH and PGF_2-α administration and thus the potential for an increased duration of follicular dominance before ovulation as reported by Atkins *et al*. (2008). Exogenous progesterone prolong effect on follicles dominance negatively affect pregnancy rates than those follicles ovulation which are under normal duration of dominance. When a follicle remains dominant for greater than 5 d after emergence, a reduction in fertility has been documented (Stock and Fortune, 1993). Similarly, decreasing the treatment duration with CIDR from 7 to 5days in postpartum cattle (*Bos taurus*) resulted in higher pregnancy rates with TAI in Co-Synch protocol (Bridges *et al*., 2008). The researches attributed this higher fertility rate to a smaller period of dominance of follicles with induction of ovulation after the removal of CIDR. So the increased rate of conception in MSS group may be caused by reduction in the period of follicles before ovulation. Another study reported improved embryonic quality with follicle of short dominance interval that lead to increased pregnancy rates (Austin *et al*., 1999). However greater than 66% conception rate in our MSS group revealed that the potential negative effects of extended LH exposure were minimal as evidenced by Sparks *et al*. (2012).

Hormones' concentration

The resultant peripheral concentrations of P4 in MSS were less than that in POP group during the synchronization. It may be due to the luteolytic dose of PGF2α at the start of protocol in MSS group, that potentially minimized the negative effect of high P4 concentrations on LH secretion during follicular development, in accordance with the findings reported by Galina *et al*. (1996). Devices having progesterone have the potential to maintain LH pulsatility and inhibit the surge release of LH, and may create endocrine environment for the development of persistent follicles (Risley, 2008). Increasing evidence suggested that the reduced concentration of P4 during follicular growth for a finite period may enhance ovulatory follicular diameter, enhance oocytes development potential and higher rate of pregnancy to AI as documented in various studies (Dias *et al*., 2009; Martins *et al*., 2014). So in the current experiment, it was proposed that proper progesterone insert administration for 7 days suppress dominant follicle's development and estrus thus enhancing response of dominant follicle to GnRH-2 injection. It was reported by Singh *et al*. (2019) that in repeat breeder cows Ovsynch protocol resulted in significantly higher conception rates. Progesterone regulates the pulsatile release of (LH) that has significant role in the development and growth of pre-ovulatory follicles. Circulatory concentration of progesterone in early luteal phase just after ovulation, is mandatory for embryo survival (Morris and Diskin, 2008). A potential approach to reduce progesterone concentration during follicular development within a CIDR-based estrous synchronization protocol, is to deliver a luteolytic dose of PGF2α (PGF) at the beginning of the protocol.

There was increase in concentration of circulating P4 within 24 h after CIDR insertion in MSS group compared with cows treated with GnRH-1 of the POP protocol. The concentration P4 in MSS at the time of AI was significantly less compared to POP group which may have resulted higher pulse frequency of LH and mean LH concentrations that may have promoted follicular growth and increased (P< 0.007) E2 production. Following PGF_2-α injection there was rapid increase in circulating E2 concentrations for CIDR and POP protocols. Lower concentration of P4 resulted in enhanced pulse frequency and lower pulse amplitude of LH, and higher pulse frequency of LH may have stimulated the secretion of estradiol by follicles (Stegner *et al*., 2004). Oocyte competence mainly depends upon the steroidogenic ability of follicle than its diameter during GnRH-induced ovulation (Machatkova *et al*., 2004).

Association of pregnancy with hormones

There was a significant association of pregnancy with P4 and E2 concentrations at the TAI. The P4 concentration varied significantly (P< 0.032) among cows. The pregnant cattle showed lower P4 concentration at the time of AI than non-pregnant cows. Schoonmaker *et al*. (2003) reported that low P4 concentrations may enhance the overgrowth of follicle with dominance, which further increase the function of CL and may be associated with higher fertility and ovulation of competent oocyte (Perry, 2003). Similarly, E2 level in non-pregnant and pregnant cows varied significantly (P< 0.005) in the experiment. The E2 concentration was higher in pregnant Achai cows at the time AI, than non-pregnant cattle. In line with our findings, greater pre-ovulatory E2 concentrations was linked to improved likelihood of successful pregnancy establishment (Lopes *et al*., 2007). The greater conception rates reported for CIDR-based treatments may be at least partially attributable to higher pre-ovulatory concentrations of E2 (Herlihy *et al*., 2011). Decrease in pre-ovulatory concentration of E2 might negatively affect the transport of spermatozoa in the female reproductive tract, and give rise to a suboptimal oviductal/uterine environment, and may impair oocyte fertilization (Hawk, 1983).

Conclusions and Recommendations

It is concluded that P4 insert administration strategically for 7 days suppressed estrus and dominant follicle's development, thus improved response of dominant follicle to GnRH-2 injection. The pregnancy rate was higher in MSS than POP protocol. The TAI results were comparatively better qualifying this protocol for synchronization of herd at farm level in the remote regions with little access to veterinary services.

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Novelty Statement

The indigenous breed of cattle (Achai) showed promising results regarding MSS synchronization protocol and TAI.

Author's Contribution

SMK, HK, YM: Conceptualization, methodology.
HAU, FR, AG: Software, writing-original draft preparation.
MTT: Visualization and investigation.

Conflict of interest
The authors have declared no conflict of interest.

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