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Assessment of Suitable Fixed Timed Artificial Insemination Protocols for Therapeutic Management of Post-Partum Anestrous Cow in the Foothill of Eastern Himalaya

Research Article

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ABSTRACT

Fixed timed artificial insemination (FTAI) is nowadays a very popular reproductive technology to handle anestrous problems during the postpartum period of dairy cattle. Therefore, the present study was designed to assess the suitable fixed timed AI protocols for therapeutic management of post-partum anestrous in cows. A total of ninety cows were selected in and around the Aizawl district of Mizoram, India which lies within the tropics between latitude 21˚ 56'N to 24˚ 31'N and longitude 92˚ 16'E to 93˚ 26'E. The experimental cows were examined per rectum to study the ovarian and genital status after two months of calving. They were randomly divided into three groups; A, B and C, comprising 30 animals in each group and were treated by three fixed timed AI protocols; G6G (PGF day-0; GnRH 2, 8 days; PGF 15, 16 days, GnRH 17 days; FTAI 18 days), select-synch and co-synch, respectively. Blood samples were collected to study the serum levels of glucose, total cholesterol, calcium, phosphorus, estrogen and progesterone on day 0 (before starting the protocol) and day 1 (day before AI). The serum level of glucose, total cholesterol, calcium, phosphorus, estrogen and progesterone significantly low in post-partum anestrous cows on day 0. But after post therapy, the level of glucose, total cholesterol, calcium, phosphorus, estrogen increased significantly except progesterone hormone on day before AI within each group. The conception rates of G6G, co-synch and select-synch protocols were 33.33%, 56.66% and 96.66%, respectively. In conclusion, the select-synch protocol was found to be the best in comparison to G6G and co-synch protocols ($P<0.05$) for therapeutic management of post-partum anoestrous in cows.

KEY WORDS artificial insemination, conception rate, co-synch, dairy cow, G6G, select-synch.

INTRODUCTION

Mizoram is a mountainous region and Aizawl is the capital of the state Mizoram, located north of the Tropic of Cancer in the northern part of Mizoram. It is located on a ridge of 1132 meters above sea level with the Tlawng river watercourse depression to its west and the Tuirial river watercourse depression to its east. The temperature ranges from 20-30 ˚C during the summer and 11-21 ˚C in the winter. As a hilly state with almost 80% forest coverage, Mizoram has only a small number of cattle populations mostly reared for meat and milk production. As per the 20th livestock census, the Government of India; Mizoram recorded 51767 total cattle population out of which exotic cattle is only 3704. For marginal farmers, dairy and piggery contribute as major earning sources. In the current scenario of India, the total cow milk production of Mizoram is 13639 tonnes and the availability of milk per capita per day was 33 g (GoM, 2014), which is very low if we consider the national average *i.e.* 132.4 million tonnes and 299 g, respectively (Basic Animal Husbandry and Fisheries Statistics, 2014). The profit of dairy farmers mainly depends on the sustainability of milk production which in turn depends on the sustained reproductive capacity of dairy animals. Reproductive performance of the herd is the imperative factor that determines the profitability of the dairy herds. Anestrous during the post-partum period of dairy cattle was recognized as a major problem by Short *et al.* (1990). Post-partum anestrous is the period where the animals are acyclic and do not exhibit behavioural signs of estrus after parturition (Wright and Malmo, 1992). As per the report of Bhat and Bhattacharyya (2012) and Boro *et al.* (2021); the main causes of anestrous were found as ovaro-bursal adhesion, cystic ovary (luteal cyst), silent estrus, pyometra, metritis, anovulation, subestrus and smooth ovary. The efficient reproductive performance of animals mainly depends on macro and micro minerals, energy level and hormonal status and its inequity causes various problems leading to inefficient reproductive performance of the dairy cows (Talukdar *et al.* 2016b). The extended calving interval may lead to poor reproductive efficiency and fertility. The profitability and sustainability of the farm mainly depend on regular the estrous cycle, conceiving at the optimal time and delivering a healthy calf each year (Hare *et al.* 2006; Talukdar *et al.* 2016b). Fixed timed artificial insemination (FTAI) is nowadays a very popular and cost-effective reproductive technology that increases the overall genetic merit of a herd as well as the profitability of a farm. Different protocols used for increasing the number of successful conceptions by artificial insemination (AI) without estrus detection are usually referred to as FTAI (Bo and Baruselli, 2014). By implementing the FTAI programmes in a herd, more numbers of cows can be impregnated at a predetermined time with a better conception rate (Bo *et al.* 2007). The estrus synchronization is achieved either by shortening the length of the luteal phase of the estrous cycle by administering prostaglandin $F_{2\alpha}$ or by lengthening the cycle by using exogenous progesterone or more potent progestogens (Abecia *et al.* 2012). Additional treatments can make the protocol more expensive for the marginal farmer (Whitley and Jackson, 2004). Choosing a suitable protocol mainly depends on cost, labour, time, animal handling, experience and other factors. The G6G pre-synchronization, Co-synch and Select-synch protocol was applied in a huge number of animals (Bello *et al.* 2006; Ribeiro *et al.* 2012; Ahmed *et al.* 2016). Ovsynch has some drawbacks such as premature estrus, reduced ovulation to first gonadotropin-releasing hormone (GnRH), and inconsistent conception rate (Kim *et al.* 2003; Ahmed *et al.* 2016). The G6G pre-synchronization method has the disadvantage concerning the length of the protocol period, labour and cost of each medicine. Estrus synchronization research is imperative to establish optimal doses and agents to use for favourable synchrony and fertility in each breed and introduce a simple, pragmatic and reliable protocol that can be adopted by the farmers. Zaituni *et al.* (2017) stated that co-synch protocol can induce the activity of ovarium, ovulation and conception rate of post-partum Simmental cows. The Select- synch protocol was recommended in the Algerian farms and Noui *et al.* (2020) stated that heat detection with this protocol was very easy with reduced the cost of heat synchronization, labour costs and the extra handling to daily estrus detection and AI. As per our knowledge, no comparative studies have been done in Mizoram. The main objective of the present study was to compare the efficacy of three different protocols of fixed-timed artificial insemination and to select the most efficient protocol for therapeutic management of post-partum anestrous in cows reared in the Foothill of Eastern Himalaya.

MATERIALS AND METHODS

Animals

A total of 90 (ninety) cows were selected in and around the Aizawl district of Mizoram *i.e.* Lungdai, Shiphir, Selesih, Ramthar, Leitan, Muthi and Bangkawn which lies within the tropics between latitude 21˚ 56'N to 24˚ 31'N and longitude 92˚ 16'E to 93˚ 26'E. The experimental cows were examined per rectum to study the ovarian and genital status after two months of calving. All cows have been kept in an intensive rearing system in where they received a total mixed rations formulated for lactating dairy cows producing 15 kg of 3.5% fat milk according to NRC (2001) guidelines and had free access to drinking water. Total mixed rations were fed twice daily (morning and evening) along with available green grasses and tree leaves. Major ingredients were silage (corn and alfalfa), grain (barley or corn), hay (alfalfa or grass), and mineral supplements. All the cows participating in this experiment were milked twice daily at approximately 8-h intervals and monitored daily for signs of diseases. If any health-related issues occurred, animals were moved to isolated pens and appropriate treatments were performed and the respective cows were removed from the rest of the study. The study was duly approved by the Institution Animal Ethics Committee, College of Veterinary Sciences and A.H., Central Agricultural

University, Aizawl, Mizoram, India (CVSC/CAU/IAEC/19-20/P-38). The experimental cows were divided into three groups *i.e.* A, B and C comprising 30 animals in each group and were treated by three fixed timed AI protocols *i.e.* G6G, Co-synch and Select-synch, respectively.

Blood sampling

Blood samples (10 mL) were collected on day 0 (before starting the protocol) and on day 1 (day before AI) in a vacuum clot activator vial containing no additives by jugular puncture with a sterile 18 gauge needle attached to a plastic syringe. Following standing at room temperature for 20 minutes, the clot activator vials were centrifuged at 3000 rpm (Weswox Serum Centrifuge, model WT-3BL, Haryana, India) for 10 minutes. Then obtained sera were kept at -20 ˚C until analysis. The serum levels of glucose, total cholesterol, calcium and phosphorus were analysed with a help of FUJIFILM autoanalyzer (DRI CHEM 4000i, FUJIFILM Corporation, Medical Systems Business Division, 26-30, Nishiazabu 2-Chome, Minato-Ku, Tokyo-1068620, Japan) and blood hormonal profile was analysed by using commercial ELISA kit (Competitive EIA (enzyme immunoassay) kit, LS-F39301, Colorimetric - 450nm, sensitivity 9.375 pg/mL, LSBio, 2041 4th Ave Suite 900, Seattle, WA 98121).

All the cows of the three groups were observed for the absence of external signs of estrus after AI and pregnancy were confirmed by rectal examination on day 60 of post-AI. The conception rate was calculated as a number of cows conceived after AI divided by the number of cows treated in each experimental group and multiplied by 100 and it was expressed in percentage.

Breeding management

Fixed timed AI protocol, G6G

In group A, post-partum anoestrous cows were treated with G6G protocol (Figure 1a) in which before starting of actual Ovsynch protocol (Pursley *et al.* 1995), the animal was pre-synched with prostaglandin and gonadotropin-releasing hormone (GnRH).

To pre-synch the animal, one dose of 10 µg GnRH injection intramuscularly (Gynarich injection, presentation 5 mL vials, Intas Pharmaceuticals Limited, Ahmedabad–380054, Gujarat, India) was given before 6 days of the first GnRH injection of Ovsynch protocol and a single $PGF_{2\alpha}$ injection 500 µg (Pragma injection, presentation 2 mL vials, Intas Pharmaceuticals Limited, Ahmedabad–380054, Gujarat, India) was given intramuscularly on day 8, before the first GnRH injection of Ovsynch protocol. Ovsynch protocol was followed after the pre-synchronization of the animals (Yalmaz *et al.* 2011).

Fixed timed AI protocol, Co-synch

In group B, the animals were treated with Co-synch protocol (Figure 1b) in which two GnRH injections were given intramuscularly at 7 days apart at a dose rate of 10 µg (total dose) and one injection of $PGF_{2\alpha}$ was given after 5 days of the first injection of GnRH *i.e.*, on 7th day at the dose of 500 µg intramuscularly. At the time of the second injection of GnRH (two days after prostaglandin injection), FTAI was performed.

Fixed timed AI protocol, Select-synch

In group C (Figure 1c), the estrus was induced by an injection of GnRH (100 µg) intramuscularly on day 2, after administration of prostaglandin, $PGF_{2\alpha}$ (500 µg) on day 0 (Geary, 1998). At the time of second injection of GnRH at the dose of 10 µg intramuscularly (48-72 hrs after prostaglandin injection), FTAI was performed.

Statistical analysis

The data obtained from the study were subjected to statistical analysis using a suitable formula for meaningful and accurate comparison and interpretation as per Snedecor and Cochran (1994). Analysis of variance of complete data was carried out by one-way ANOVA. Mean values of the blood biochemical and hormonal profiles were compared by 't-test' and the conception rate was compared by chi-square test.

RESULTS AND DISCUSSION

The serum level of glucose (P<0.01) and total cholesterol was low in post-partum anestrous cows on day 0 in all the groups. But after post therapy, the level of glucose (P<0.01), total cholesterol increased on day before AI within each group. There were no significant differences in glucose and total cholesterol value among the three treatment groups which are shown in Table 1. The glucose and cholesterol values in group B animals showed the lowest in comparison to group A and C. There were no significant differences in blood calcium and phosphorus level among the three groups which were shown in Table 1. Whereas, the serum level of calcium and phosphorus was significantly low in post-partum anestrous cows on day 0 in all the groups. But after post therapy, the level of calcium and phosphorus increased significantly in group B and C on day before AI except group A, the increased values were not significant. The level of serum estrogen in groups A, B and C were significantly (P<0.01) increased on day 1 but there were no significant differences between the groups (Table 1).

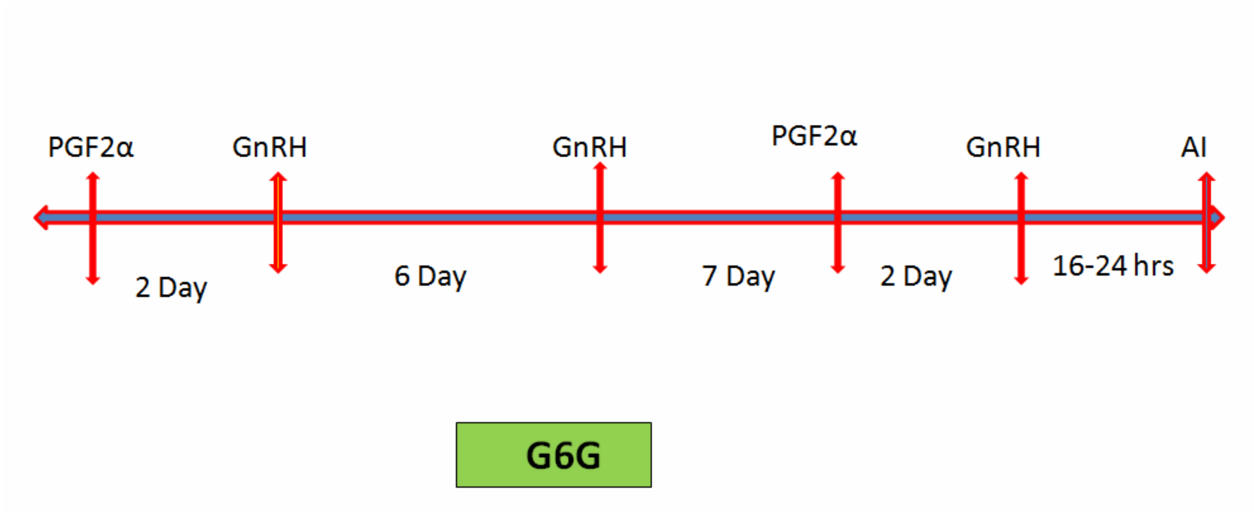

Figure 1a G6G protocol

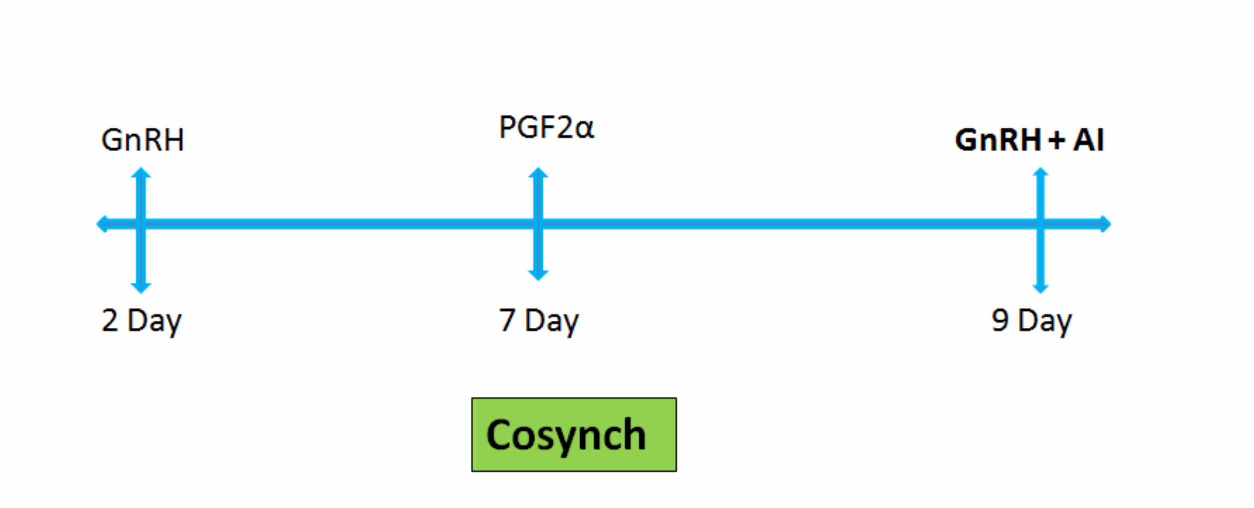

Figure 1b Co-synch protocol

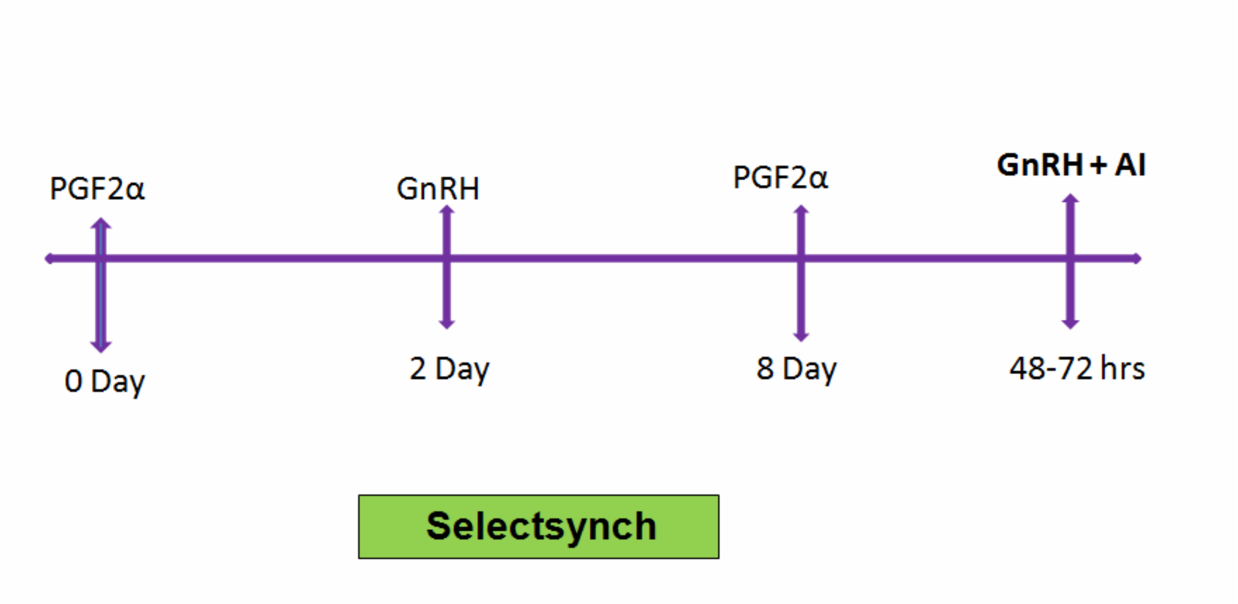

Figure 1c Select-synch protocol

Table 1 Level of blood biochemical and hormonal profiles in post-partum anestrous cow at day 0 (before treatment) and day 1 (day before artificial insemination)

Sl. No	Parameters	Days	Group A	Group B	Group C	F-value
1	Glucose (mg/dL)	0	41.10±3.79[a]	38.20±4.65[a]	41.20±4.29[a]	0.160[ns]
		1	71.90±4.43[b]	71.80±5.41[b]	84.70±6.24[b]	1.874[ns]
		T-value	7.274**	4.714**	4.906**	
2	Cholesterol (mg/dL)	0	133.40±18.08	130.00±10.24	133.80±11.90	0.023[ns]
		1	166.40±14.68	159.10±17.87	163.70±16.03	0.052[ns]
		T-value	1.284[ns]	1.401[ns]	1.621[ns]	
3	Calcium (mg/dL)	0	7.74±0.38	7.41±0.32[a]	7.64±0.43[a]	0.195[ns]
		1	8.98±0.39	9.75±0.50[b]	9.58±0.49[b]	0.878[ns]
		T-value	2.030[ns]	3.158*	3.184*	
4	Phosphorus (mg/dL)	0	5.90±0.45	6.27±0.38[a]	5.80±0.36[a]	0.373[ns]
		1	7.10±0.37	7.59±0.54[b]	7.20±0.35[b]	0.358[ns]
		T-value	1.620[ns]	2.494*	2.894*	
5	Estrogen (pg/mL)	0	4.58±0.68[a]	4.52±0.69[a]	4.55±0.66[a]	0.66[ns]
		1	7.69±0.32[b]	7.82±0.29[b]	8.37±0.50[b]	0.882[ns]
		T-value	4.171**	4.622**	4.729**	
6	Progesterone (ng/mL)	0	6.83±0.93[a]	6.78±0.84[a]	7.34±0.95[a]	0.113[ns]
		1	1.76±0.39[b]	1.48±0.26[b]	1.02±0.16[b]	0.880[ns]
		T-value	4.828**	6.811**	5.361**	

** (P<0.01) and * (P<0.05).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
NS: non significant.

On the other hand, the progesterone level in all the groups was significantly (P<0.01) decreased on day 1 but, there were no significant differences between the groups. The serum progesterone level ranged from 6.83 ± 0.93 to 7.34 ± 0.95 ng/mL before the treatment and the level was lower on the day before fixed timed AI in all the groups.

Conception rate

In the present study, the conception rate of G6G, Co-synch and Select- synch protocol were 33.33% (10/30), 56.66% (17/30) and 96.66% (29/30), respectively. The Select-synch protocol was found to be best in comparison to G6G and Co-synch protocols in response to conception rate (P<0.05) with a chi-square value of 0.049.

The lactation period in which the cows produce milk that lasts for 10 months is crucial for the reproductive performance of the individual. At the beginning of lactation, body weight would turn down, since most of the nutrients required for the formation of milk are taken from the cow's body. A steady descending trend is observed for high-yielding cows. This decline may result in increased time to the first insemination, poor exposition of estrus behaviour, increased days open, decreased success rate of artificial inseminations (AI), and high culling rates (Dobson *et al.* 2007; Lucy, 2019). It was reported that glucose was the main energy substrate in the bovine ovary, and a sufficient supply of glucose was necessary to sustain the ovarian activity (Talukdar *et al.* 2015; Talukdar and Talukdar, 2017). McClure (1968) also recorded that hypoglycemia during estrus might lead to a lack of energy in spermatozoa, ovum and embryo. Insufficient nutrient intake can reduce circulating glucose concentration (Talukdar and Talukdar, 2017). It supplies energy for life processes in the body of an animal and is the primary metabolic fuel used by the central nervous system (Talukdar *et al.* 2016a). In the present study, it was observed that in all the animals of three groups, the serum glucose level was significantly low on day 0 (before starting the protocol) which might be due to inadequate availability of utilizable glucose and then there was a decrease in GnRH leading to a decrease in LH release, and thus delayed estrus response (Hess *et al.* 2005). Earlier studies also stated that low levels of glucose can cause high levels of non-esterified fatty acids (NEFA) which have toxic effects on the follicle, the oocyte, embryo, and fetus and decrease the secretion of GnRH from the hypothalamus (Adewuyi *et al.* 2005). Decreased GnRH levels inhibit the synthesis of FSH and LH in the anterior pituitary and caused follicles does not to grow and does not elicit estrus (Oguike and Okocha, 2008; Hudaya *et al.* 2020). While the values were significantly increased after post therapy in all the groups, though between the groups, the values were not significant which might be the consequence of GnRH and its synthetic analogues which activates the hypothalamic neuronal glucosensing for maintaining peripheral metabolic homeostasis and relay metabolic information to the reproductive system (Roland and Moenter, 2011; Dina *et al.* 2020).

Cholesterol is a major component of blood plasma and an essential constituent of cell membranes. It is also a precursor for steroid hormones and plays important role in the production of bile acids, steroid hormones and vitamin D (Edfers-Lilja *et al.* 1980; Talukdar and Talukdar, 2017). In the present study, it was observed that in all the animals of three groups, the blood cholesterol level was low on day 0 (before starting the protocol). It was reported that the decrease in plasma cholesterol concentration led to a reduction of plasma concentrations of progesterone, which resulted in the suppression of luteal function and eventually delayed ovarian activity in dairy cows (Macial *et al.* 2001). There were no significant differences in cholesterol levels among the three groups. The variation in the level of serum cholesterol might be attributed to several phases like fertility status of the animal, stage of the estrous cycle (Jayachandran *et al.* 2013; Borpujari *et al.* 2018).

In the present study, it was observed that in all the animals of three groups, the serum calcium level was significantly low on day 0 (before starting the protocol). While the values were significantly increased after post therapy in all the groups except group A, the level was not significant. One of the main functions of calcium is to allow muscle contraction (Talukdar *et al.* 2016a). Uterine tonicity is adversely affected by the cow if circulating calcium is deficient and it may be experiencing prolonged uterine involution which may impair the subsequent fertility of the animal (Talukdar and Talukdar, 2017; Borpujari *et al.* 2019). There were significant differences in calcium levels between day 0 and day 1 within group B and C but not in group A.

The lower level of serum phosphorus was associated with impairment in reproduction, decreased fertility rate, decreased ovarian activity, anestrus, irregular estrous cycle, and increased occurrence of the cystic ovary in cattle (Bindari *et al.* 2013). Talukdar *et al.* (2016a) reported that mineral has been most commonly associated with decreased reproductive performance in dairy cows. Calcium: phosphorus ratio alteration was also found to affect ovarian function leading to reproductive disorders, inactive ovaries, and low conception rates. In the present study, it was observed that the ratio of calcium and phosphorus were 1.31: 1, 1.18: 1 and 1.31:1 on day 0 and 1.24:1, 1.28:1 and 1.33: 1 on day 1 in groups A, B and C, respectively. The ratio was slightly lower than the normal. It was increased after post therapy in group B and C. Significantly reductions in fertility and a higher number of services per conception had been reported by Morrow (1969) in phosphorus-deficient animals.

Hafez *et al.* (1987) reported that the level of serum estradiol 17β was higher during estrus and towards the proestrus and lower on day 8 of estrus. A high amount of estrogen, interestingly, has an opposite stimulatory effect on gonadotropins for which luteinizing hormone (LH) and follicle-stimulating hormone (FSH) begin to increase. As more estrogen is secreted, more LH receptors are made by the theca cells, inciting theca cells to create more androgen that may create estrogen downstream. This regeneration loop causes LH to spike sharply, which causes ovulation (Hafez *et al.* 1987). Following ovulation, LH stimulates the formation of the corpus luteum (CL). Estrogen has since been born to negative stimulatory levels when ovulation and thus serves to keep up the concentration of FSH and LH (Talukdar *et al.* 2013). In the present study, there was a significant increase in estrogen levels between day 0 and day 1 ($P<0.01$) within all three groups, but no significant differences among the three groups. Jena *et al.* (2016) recorded the same trend as in the present study. In the present investigation, an increased level of estrogen on the day before AI might be due to exogenous GnRH injection (Pursely *et al.* 1995). Low progesterone concentrations during an estrous cycle lead to a delayed stimulatory effect on uterine responsiveness to oxytocin during the $PGF_{2\alpha}$ secretion which might interfere with luteal maintenance by progesterone hormone during the early stages of pregnancy (Shaham–Albalancy *et al.* 2001). In the present investigation, the progesterone level gradually decreased from day 0 (before starting protocol) to day 1 (day before AI) in all three groups, which was also reported by Sathiamoorthy and Subramanian (2003) and El-Zarkouny (2010). This decreased level on day 1 might be due to the exogenous PGF2α injection, which caused luteolysis of the CL, and physiological declination of progesterone towards the basal level for the manifestation of estrus (Bhoraniya *et al.* 2012).

Out of the three protocols, the Select-synch protocol was found to be the best in comparison to G6G and Co-synch protocols in response to conception rate ($P<0.05$). Similarly, the highest response was also recorded by Esterman *et al.* (2016) and Caraba *et al.* (2018) in the Select-synch protocol. The increased level of glucose, cholesterol, calcium and phosphorus after treatment in Select-synch protocol enhanced follicular development and well synchrony of the protocol. The higher conception rate in Select-synch protocol is attributed to the GnRH priming, which sensitized the endocrine axis to obtain better follicular development and well synchrony between luteolysis, ovulation and fixed timed AI. It induces ovulation if a mature follicle is present at the time of administration by inducing the LH surge. The increased level of progesterone in the blood of all of the treated animals in the Select-synch protocol may be due starting of progesterone production from the preovulatory follicle (Smith *et al.* 1994) as anestrous animals usually record a significant decrease in progesterone level due to the absence of LH surge (Terzano *et al.* 2012). The inferior response observed in the G6G and Co-synch might be due to the presence of a small-sized less estrogenic dominant follicle (Brantmeier *et al.* 1987) and might be due to divergence in the stage of the estrous cycle at the time of commencement of the protocol (Vasconcelos *et al.* 1999). The higher level of serum progesterone at the time of AI might be reminiscent of incomplete luteal regression following the administration of $PGF_{2\alpha}$ which resulted in inferior response in terms of conception rate in G6G and Co-synch protocols of the present study (Kim *et al.* 2003).

CONCLUSION

An improvement has been noticed in terms of serum mineral and hormonal profiles along with conception in postpartum anoestrous cows while we used fixed timed AI protocols. Finally, concerning estrus response, fertility rate, labour costs, extra handling to daily estrus detection and the simplicity of the method, it can be concluded that the Select-synch protocol was found to be the best protocol for implementing the FTAI programmes as well as a therapeutic strategy for the treatment of post-partum anestrous in cows reared in the Foothill of Eastern Himalaya. Further studies on a larger sample will be recommended to determine the effects of the several factors on fertility to get a better result.

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