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Evaluation of kisspeptin and ovsynch protocol in the treatment of postpartum anoestrus cattle with respect to intensity and ovulation rate

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Abstract
Reproductive performance and timing for AI is depend on intensity of oestrus. Irregularity in the manifestation of oestrous behaviour can complicates the reproductive efficiency. In the present study fifty postpartum anestrous crossbred cows were selected based on history, clinico-gynaecological examination and ultrasonography. The animals were divided into four treatment groups and treated with ovsynch, ovsynch fortified with minerals and bypass fat, kisspeptin and kisspeptin fortified with minerals and bypass fat, respectively. Most of the animals responding to treatment showed intermediate oestrus behavior in all the treatment protocols. However, Kisspeptin with or without fortification lead to delayed ovulation and prolonged oestrus with a dose rate of 1.3mcg/kg in the treatment of postpartum anestrous.

Keywords: Kisspeptin, oestrus intensity, ovsynch, postpartum anestrous, ultrasonography

Introduction

Livestock sector is an important component of the agriculture of Indian economy. This sector has been an important source of livelihood for small and marginal farmers. The country is world's largest milk producer and consumer of dairy products consuming 100 percent of its own milk. Inspite of being largest producer and consumer of dairy products but the average milk production per animal is less. Perusal of available literature showed that the major reproductive disorders which leads to decrease the productivity and reproductive ability of farm animals were anestrous and repeat breeding and their incidence, were recorded as high as 51.12 and 33.71 percent, respectively (Dixit *et al*. 2020) [9]. Parallely the government is aiming to reorient agriculture sector by focusing on income generation (Singh, 2018) [21]. Therefore government has set a target for doubling farmer's income by the year 2022.

Normally during early lactation, animal milk production is greater but feed intake is low. This combination creates negative energy balance (NEB) and the animals start to use body reserves to overcome the energy deficit. Production of reproductive hormone depends on the energy status of animals. Negative energy balance leads to decreasegrowth, reproduction, production and fetal growth. Thus NEB (negative energy balance) will result in decreased secretion of reproductive hormones resulted in delayed post-partum oestrus and ovulation (Butler *et al*. 2003) [3]. Minerals have a beneficial role in endocrine system and play an important role in resumption of follicular growth (Smith and Akinbamijo, 2000) [23]. Nutritional supplement with bypass fat (BPF) and mineral mixture (MM) might have showed a beneficial effect on postpartum ovarian activity by increasing the number of ovarian follicles and enhancing follicular growth (Colazo *et al*. 2009 and Dixit *et al*. 2020) [8, 9].

Considering the Gravity and economic importance of the problems attempts were made to address prolonged post-partum anestrous in dairy cattle with various protocol *viz*., Ovsynch, Ovsynch plus, Double synch, Estra-Doublesynch, CIDR, Ovsynch + CIDR, Heatsynch, Single PG injection, Double PG injection, Co-synch have been developed. Studies for better reproductive health management of herd was conducted by several authors and found variation in result (range 16-100 percent in oestrus response).

Therefore, studies on fortification need of the oestrus induction protocols seem to be one of the areas to be addressed.
In recent years, new concepts for early onset of puberty, induction of oestrus with the use of kisspeptin has been reported. (Smith *et al*. 2005; Caraty *et al*. 2007) [22, 4]. Kisspeptin is a neuropeptide which is encoded by KiSS1 gene and its cognate. KiSS1, the gene encoding kisspeptin, is expressed in specific areas of the hypothalamus (preoptic area and arcuate nucleus) that are critical for secretion of gonadal steroid hormone (estradiol and progesterone) which control reproduction (Pielecka-Fortuna *et al*. 2008) [16]. It has been reported that kisspeptin stimulates GnRH neurons in brain and generate pulsatile release of GnRH, in turns causes release of LH and FSH. Thus kisspeptin act as secretagogue of GnRH (Mondal *et al*. 2018) [13]. Keeping the above mention facts, the present research work was planned to address a common field oriented problem with the objective of comparison of Kisspeptin and ovsynch protocol in determine the intensity of oestrus and Ovulation rate.

Materials and methods

The present study was conducted in and around Guwahati city of Assam during the period of one year from May 2018 to June 2019 to study the effect of Ovsynch and Kisspeptin in induction of oestrus in postpartum anestrous cows. Thirty postpartum anestrous crossbred cows showing no sign of oestrus for more than 90 days with smooth ovaries on rectal palpation and confirmed with ultrasonography were utilized for the present study. The experimental cows were divided into two treatment groups and one control group comprising 10 cows in each group and treated with Ovsynch and Kisspeptin protocol, respectively.Ten animals receiving no treatment and served as control.

Ovsynch Protocol: The cow in this group were subjected to Ovsynch protocol were injected with 20 mcg of GnRH analogue (PREGULATE™ Buserlin injection, Virbac Animal Health India Private Limited, Maharastra, India) intravenously on day 0 followed by 500µg of cloprostenol sodium (PREGOVA™ INJECTION-Cloprostenol Sodium, Virbac Animal Health India Private Limited, Maharastra, India) intramuscularly on day 7 and 20mcg of GnRH intravenously on day 9. Fixed time AI was performed 16-24 hrs after 2nd GnRH injection.

Ovsynch + MM + BPF Protocol: Animals in this group were supplemented with mineral mixture 50 g/cow /day + bypass fat 150 g/cow /day for 21 days along with concentrate feed and phosphorus injection 10 ml intramuscular at alternate days for three occasions followed by Ovsynch protocol

Kisspeptin Protocol: The cows were injected with Kisspeptin protocol as described by Mondal *et al*. (2018) [13]. Kisspeptin (Metastin 45-54 calbiochem cat: 445888 U.S.A.) @1.3µg/kg body weight (Khan *et al*.2016) intravenously on Day 0 and followed by intramuscular injection of Cloprostenol sodium (PREGOVA™ INJECTION-Cloprostenol Sodium, Virbac Animal Health India Private Limited, Maharastra, India) 500 µg on Day 7 and again Kisspeptin injection on Day 9 of treatment. Fixed time AI was performed 16-24 hrs after second Kisspeptin injection.

Kisspeptin + MM +BPF Protocol: Animals in this group were supplemented with mineral mixture 50 g/cow /day + bypass fat 150g/cow /day for 21 days along with concentrate feed and phosphorus injection 10 ml intramuscular at alternate days for three occasions followed by Kisspeptin protocol.

Control: The animals did not receive any feed supplementation or treatment for induction of oestrus was served as control.

Detection of ovulation by USG through rectal palpation: Transrectal palpation was performed to detect the status of ovulation as described by Paul and Prakash (2005) [15]. Ovulation was confirmed by the change of ovarian surface from turgid to flaccid. All the experimental animals were scanned for detection of ovulation (Image 1, 2 and 3) by ultrasonography and presence of corpus luteum (Image 4) following treatment with different oestrus induction protocol was recorded using real-time B mode ultrasound machine (M-SONOSITE, FUJIFILM inc Bothell, WA 98021-3904 USA) with a 5–7.5 MHz linear array transducer designed for per-rectal examination in all the experimental animals.

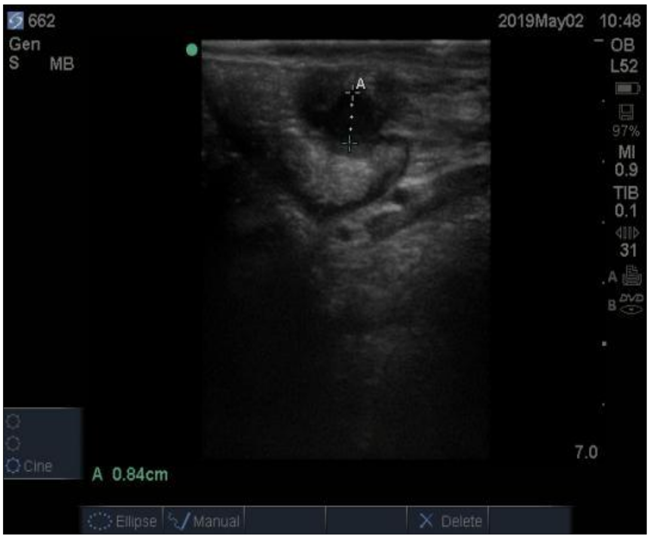

Image 1: USG of ovary shows presence of follicle of 0.84 diameter (Day 0)

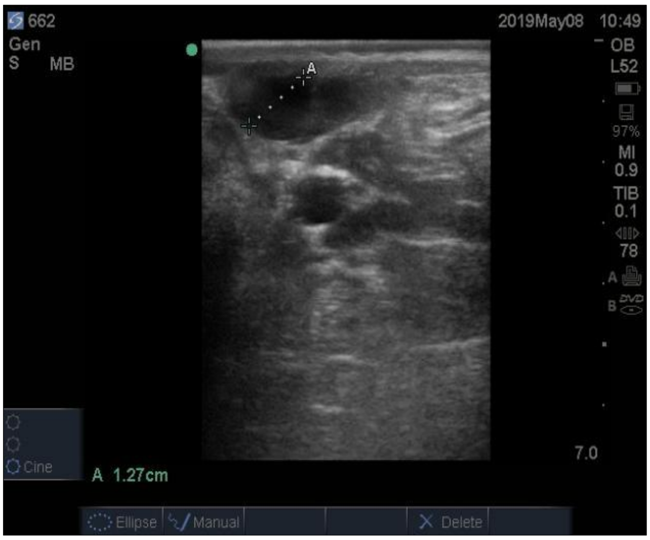

Image 2: USG of ovary shows presence of follicle of diameter 1.27 cm diameter (Day 6)

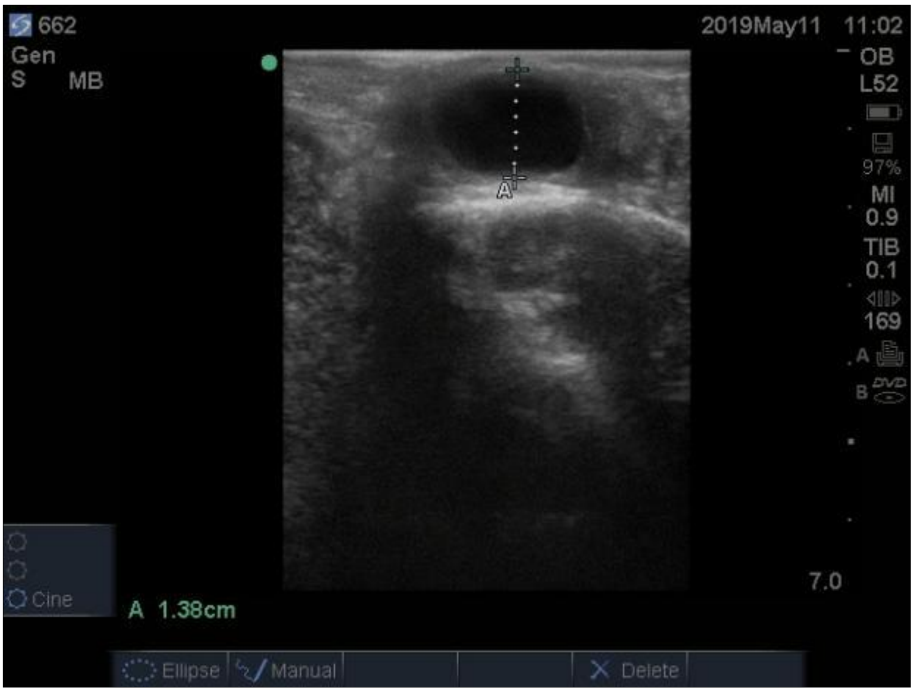

Image 3: USG of ovary shows presence of follicle of diameter 1.38 cm diameter (Day 9)

Image 4: USG of ovary with color doppler shows presence of matured corpus luteum with increased blood supply

Intensity of estrus

The intensity of estrus was assessed based on the score card described by Rao and Rao (1981) [19] in crossbred cows with slight modifications, since the cows were not let loose because of the hilly terrain condition nearby Khanapara area, North-east region of India. The details of the score card are given below.

Table 1: Score Card

S. No.	Parameters	Points		
1.	**Behavioural changes**			5
	a. Restlessness and alertness	1		
	b. Homosexuality	1		
	c. Tail raising	1		
	d. Bellowing	1		
	e. Off feed	1		
2.	**Physiological changes**			5
	a. Vulval edema and congestion		2	
	▪ High	2		
	▪ Moderate	1		
	▪ No edema and congestion	0		
	b. Urination		1	
	c. Genital discharge		2	
	▪ Large volume (copious), ropy	2		
	▪ Moderate volume, stringy	1		
	▪ Less volume/no mucus	0		
3.	**Gynaecological observations**			5
	a. Fern pattern		2	
	▪ Typical fern pattern	2		
	▪ Atypical fern pattern	1		
	b. Cervical relaxation		1	
	c. Uterine tonicity		2	
	▪ High	2		
	▪ Moderate	1		
	▪ No tone	0		
	Total	15		
	Based on the above score card, the intensity of estrus was classified as follows.			
	Intense	:	10-15 points	
	Intermediate	:	5-10 points	
	Weak	:	0-5 points	

Results and Discussion

Intensity of oestrus

In the present study, the intensity of oestrus in anestrous crossbred cows treated with various protocols are presented in Table 1 and Fig 1. Most of the animals responding to treatment showed intermediate oestrus behavior in all the treatment protocols.

In the present investigation the intensity of oestrus was recorded as intense in 14.20, 25.00, 12.50 and 25.00 intermediate in 71.43, 62.50, 75.00 and 62.50 and weak in 14.20, 12.50, 12.50 and 12.50 percent crossbred cows treated with Ovsynch, Ovsynch fortified with mineral and bypass fat, Kisspeptin and Kisspeptin fortified with mineral and bypass protocols respectively. Ravikumar *et al*. (2005) [20] reported intensity of oestrus as intense (16.67%), intermediate (66.67%) and weak (16.67%) in postpartum subestrus buffaloes treated with Ovsynch protocol which was comparable to that observed in the present investigation. Bhoraniya *et al*. (2012) [2] and Alyas *et al*. (2013) [1] also reported similar observation in anoestrous Kankrej cows treated with Ovsynch protocol while higher intensity of oestrus (33.33-58.33%) than that of the present investigation was reported by Chaudhari *et al*. (2012) [6] and Nevkar *et al*. (2012) [14] in acyclic crossbred cows treated with Ovsynch protocol. Velladurai *et al*. (2014) [25] recorded the intensity of oestrus as intense, intermediate and weak in 50.00, 37.50 and 12.50 percent cows treated with Ovsynch protocol which was found higher than that recorded in the present investigation. In contrary to the present observation, Ramana *et al*. (2013) [18] recorded the intensity of oestrus as weak in 9.10%, normal in 90.90% and intense in 0.00% Ongole cows treated with $PGF_{2\alpha}$ which was found lower in comparison to the present observation. The intensity of oestrus was influenced by various factors like management practices, season of study, genetic makeup, postpartum condition, nutrition, environment and level of reproductive hormones of the experimental animals (Tomar 1970, Verma and Kharche, 1982, Khattab *et al*. 1988, Chauhan *et al*.1981) [24, 26, 12, 7].

Table 2: Frequency of occurrence of intense, intermmediate and weak intensity of oestrus following treatment with different oestrus induction protocol\

Treatment Protocol	No. of cows treated	Ovulatory response	Intensity of oestrus		
			Intense	Intermmediate	Weak
Ovsynch	10	7	14.20 (1)	71.43 (5)	14.20 (1)
Ovsynch Protocol + mm + bpf	10	8	25.00 (2)	62.50 (5)	12.50 (1)
Kisspeptin protocol	10	8	12.50 (1)	75.00 (6)	12.50 (1)
Kisspeptin protocol + mm + bpf	10	8	25.00 (2)	62.50 (5)	12.50 (1)
Control	10	0	0.00 (0)	0.00 (0)	0.00 (0)

Figures in parentheses indicate number of animals observed

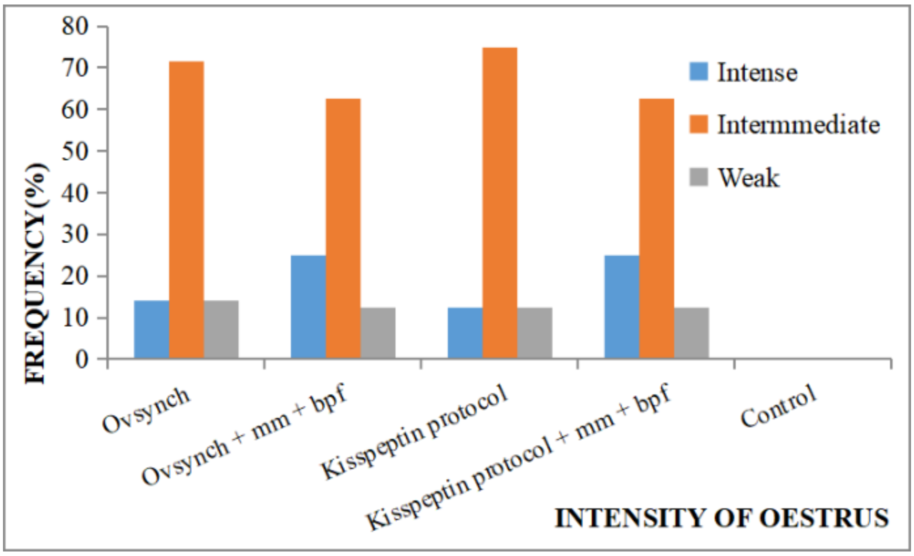

Fig 5: Variation in intensity of oestrus in anestrous crossbred cows following treatment with different oestrus induction protocols

Ovulation rate

It was observed in the present investigation that 40 percent of animal treated with Ovsynch protocol and Ovsynch protocol + MM + BPF ovulated on day 10 of treatment while 30 and 40 percent animals ovulated after treatment with Ovsynch protocol and Ovsynch + MM + BPF on day 11 of treatment, respectively while no animal was recorded to ovulate after 11 day following treatment with Ovsynch and Ovsynch + MM+BPF protocol. In the present study ovulation was recorded in 20.00, 40.00 and 20.00 percent of animal on 16, 17 and 18 day post treatment, respectively with Kisspeptin protocol. The corresponding figures for Kisspeptin + MM+BPF protocol were 50.00, 20.00 and 10.00 percent. No animal was recorded to ovulate after 18 day following treatment with Kisspeptin protocol and Kisspeptin + MM + BPF protocol. Thus from the present investigation it can be concluded that Kisspeptin with or without fortification lead to delayed ovulation and prolonged oestrus with a dose rate of 1.3mcg/kg in the treatment of postpartum anestrous.

Mondal *et al*. (2018) [13] observed Kisspeptin concentration during different days of oestrus cycle in crossbred cows and reported that the Kisspeptin level increase on a day before ovulation and declined to basal level on day 4 and again increase on day 6 which might be responsible for ovulation on day 16 to 18 as observed in the present investigation while Chaikhun *et al*. (2019) [5] in a study on cyclic buffalo observed that there was increase in the level of LH concentration after GnRH administration but no such increase in the level of LH was observed after injection of Kisspeptin-10 at the dose rate of 1.3 µg/kg body weight.

Pottapenjera *et al*. (2018) [17] reported that the kisspeptin induced LH peak was short lived and it reached within 15-30min which returned to basal values by 1-2 hours. The Kisspeptin induced increase in LH level was less compared to buserelin induced increase in LH level which sustained over time. Kisspeptin did not enhance FSH release while buserelin resulted in a gradual increase over time. Buserelin induced an increase in the number of follicles while Kisspeptin induced an increase in the growth rate of the follicle. He further stated that both the drugs increased plasma level of LH however buserelin injection can cause greater increase in the LH level. Higher ovulation rate observed in the Ovsynch and Ovsynch fortified with mineral and bypass fat might be due to incorporation of buserelin in both the protocols.

Conclusion

In the present study the fortification prior to start the hormonal protocol effectively increases the intensity of oestrus and ovulation rate. Fortified Ovsynch protocol shows better intensity and ovulation rate than Kisspeptin protocol. It was found in our investigation that animal treated with kisspeptin showed ovulation failure and prolonged oestrus has been recorded. Although, fortified Ovsynch gives better result than Kisspeptin. Kisspeptin can be a potent substitute once dose rate of kisspeptin is determined for the induction of oestrus which gives the future researcher a prospective to determine the dose rate of kisspeptin for the treatment of postpartum anestrous cows.

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