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# Ruling out Efficacy of Hormonal Protocols alone or Fortified with Mineral and Bypass Fat for Treatment of Postpartum Anoestrus in Cattle

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## ABSTRACT

**Background:** High milk production and excellent fertility is a dream of livestock owners.The major reproductive disorders which lead to decrease the productivity and reproductive ability of farm animals is postpartum anestrous.Recently, kisspeptin was reported as game changer in the animal reproduction. Kisspeptin increases the number of follicles at wave emergence, oestrus response rate and duration of oestrus. Thus, indicating its potentiality for induction of oestrus in anestrous animals.

**Methods:** The present study was conducted to compare the efficacy of Ovsynch and kisspeptin protocol and need of fortification for treatment of postpartum anestrous.Fifty postpartum anestrous cows were selected. The animals were divided into four treatment groups (10 cows in each) and treated with Ovsynch (Group I), Ovsynch fortified with mineral mixture (MM) and bypass fat (BPF) (Group II), kisspeptin (Group III) and kisspeptin fortified with MM +BPF (Group IV). The remaining 10 cows served as control (Group V). The oestrus response was recorded on the basis of behavioral signs, physical signs and clinico-gynaecological changes. The blood was collected following treatment for hormonal estimation. The pregnancy was confirmed on day 45 post AI using ultrasound.

**Result:** The oestrusresponse was pronounced in Ovsynch as compared to kisspeptin groups. Further, fortification has enhanced the response. The serum oestrogen concentration increased following treatment and was significantly high on day of onset of oestruswith highest concentration in group II. The results indicate Ovsynchdistinguishly succeeded in addressing postpartum anestrus with fortification enhances fertility response.However, kisspeptin also emerged as a potent candidate for the resumption of cyclicity.


**Key words:** Kisspeptin, Oestrogen, Ovsynch, Postpartum anestrus, Progesterone.

## INTRODUCTION

The enormous impact of postpartum anestrus in reproductive performance of an animal is well known. Factors such as reduced energy intake, poor body reserve and reproductive diseases can prolong the period of resumption of cyclicity (Peter *et al*., 2009; Dixit *et al*., 2020). The negative energy balance (NEB) results in poor release of reproductive hormones contributing in delayed postpartum oestrus and ovulation (Butler *et al*., 2003). Such cows are in greater risk of being culled by dairy producers in order to upkeep the economic benefit (Esslemont *et al*., 2001).

Attempts have been made to address prolonged postpartum anestrous in the dairy cattle using various protocol. Ovsynchprogram have been extensively evaluated for treatment of postpartum anestrus (Bhoraniya *et al*., 2012; Dhami *et al*., 2015). In recent years, new concepts for early onset of puberty, induction of oestrususing kisspeptin has been reported (Caraty *et al*., 2007; Redmond *et al*., 2011). Kisspeptin is a neuropeptide which is encoded by KiSS1 gene and its cognate. The KiSS1 gene is expressed in specific areas of the hypothalamus (Preoptic area and arcuate nucleus) that are critical for secretion of gonadal steroid hormone (Pielecka-Fortuna *et al*., 2008). The kisspeptin stimulates pulsatile release of GnRH which in turns causes release of LH and FSH. Earlier study had demonstrated increased number of follicles at wave emergence, diameter of dominant follicle, oestrus response rate and duration of oestrus following kisspeptin administration. The other authors had advocated the use of

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kisspeptin in anestrous animals (Pottapenjera *et al*., 2018).

The reproductive physiology and secretion of reproductive hormones is directly associated with energy status of animals. It has been reported that the cows with low body fat reserves are unlikely to respond to hormonal therapy (Peter *et al*., 2009). Earlier literature has reported beneficial effect of bypass fat (BPF) and mineral mixture (MM) on postpartum ovarian activity (Colazo *et al*., 2009). Hence, could be useful in augmentation of hormonal protocol against postpartum anoestrus. Therefore, the present study was designed with aim to evaluate the efficacy of Ovsynch and kisspeptin protocol and need of fortification for the treatment of postpartum anestrous in crossbred cows. Further, to best of our knowledge, information in the regard of kisspeptin as a therapeutic measure in postpartum anestrus cows is scanty.

## MATERIALS AND METHODS

The study was approved by the Institutional Animal Ethics Committee of College of Veterinary Science, Assam Agricultural University, Khanapara, Assam, India.

### Experimental animals

The present study was conducted during the period of one year at Instructional Livestock Farm of College of Veterinary Science, Assam Agricultural University, Khanapara, Assam. Fifty postpartum anestrous crossbred cows were utilized for the study. The animals with the history of absence of cyclicity for more than 90 days after parturition were examined per rectum for ovarian activity. The cows having smooth ovaries with no palpable corpus luteum of any stage with quiescent genital organ were diagnosed as postpartum anestrous. The ultrasonography was performed in experimental animals to confirm the status of the animal as postpartum anestrous. The experimental cows were randomly divided into four treatment groups (group I, II, III and IV) comprising 10 cows in each. While, ten animals receiving no treatment served as control. All the cows were between 3 to 4 parity and subjected to similar feed and managemental conditions throughout the study.

### Treatment

### Ovsynch protocol (group I)

The animals were subjected to Ovsynch protocol as described by Bhoraniya *et al*. (2012). The cows were injected with 0.020 mg of GnRH analogue (Buserelin Acetate, Gynarich®, Intas Pharmaceutical Ltd., Ahmedabad, India) on day 0 followed by 500 µg of cloprostenol sodium (Pragma™, Intas Pharmaceutical Ltd., Ahmedabad, India) on day 7 and 0.020 mg of GnRH on day 9 through intramuscular route. Fixed time AI was performed 16-24 hrs after 2nd GnRH injection.

### Fortified ovsynch protocol (group II)

The cows were fed 50 g/cow/day MM (Agrimin® forte, Virbac Animal Health India Pvt Ltd., Maharashtra, India) and 150 g/cow/day BPF (Fatomax®, Intas Pharmaceutical Ltd., Ahmedabad, India) orally for a period of 21 days. The supplementation was followed by Ovsynch protocol.

### Kisspeptin protocol (group III)

The animals were administered with kisspeptin-prostagladin-kisspeptin protocol. The cows were injected with kisspeptin (Metastin 45-54 calbiochem cat: 445888 U.S.A.) @ 1.3 µg/kg body weight (Khan *et al*., 2019) intravenously on Day 0 and followed by intramuscular injection of Cloprostenol sodium (Cloprostenol, Pragma™, Intas Pharmaceutical Ltd., Ahmedabad, 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.

### Fortified kisspeptin protocol (group IV)

Animals were supplemented with MM + BPF followed by kisspeptin. The therapeutic protocol was followed as described earlier.

### Control (group V)

The animals not receiving feed supplementation or treatment for induction of oestrus served as control.

### Assessment of oestrus response

All the experimental cows were closely observed during the period of treatment for the sign of oestrus. Oestrus was detected on the basis of behavioural, physical and clinico-gynaecological changes. The clinico-gynaecological examination was carried out 24 hrs after the $PGF_{2\alpha}$ injection and then at every 12 hrs to confirm oestrus in the experimental groups. The ultrasonography was performed to confirm oestrus and to check the status of preovulatory follicle and corpus luteum. Duration of oestrus was calculated from the time of first to last appearance of estrus signs and recorded in hours (Lopez *et al*., 2004).

### Endocrine profile

Blood samples were collected on day 0, 7, 9 and 10 of treatment from all the experimental animals. The serum was separated and stored at -20°C for serum oestrogen and progesterone assay. Serum oestrogen and progesterone level were analyzed using commercial oestradiol ELISA kit (Calbiotech ct., El Cajon, CA 92020, USA) and Progesterone ELISA Kit (Calbiotech ct., El Cajon, CA 92022, USA) as per manufacturer's instructions and the reading of optical densities were taken immediately after completion of assay procedure in microplate reader (Biorad, USA).

### Assessment of pregnancy rate

The pregnancy was determined 45 days post AI using real-time B mode ultrasound machine (M-SONOSITE, FUJIFILM inc Bothell, WA 98021-3904 USA).

### Statistical analysis

The data of behavioral, physical and clinico-gynaecological changes were compiled using Microsoft excel (version 10) and presented in percentage. The duration of oestrus and serum hormonal concentrations were presented as Mean±SEM using Microsoft excel (version 10). To estimate the difference between treatment groups, the duration of oestrus and endocrine profile were analyzed using two-way ANOVA by GraphPad Prism statistical software. The Chi-square test was used to analyze the conception rate. The level of significant was considered as P<0.05.

## RESULTS AND DISCUSSION

### Oestrus response

The oestrus response observed in the study was 70-80 per cent (Table 1). The oestrus response was observed in treated groups while, none of the cows in control group exhibited oestrusbehaviour. The prominent sign of oestrus was observed in groups fortified with MM+BPF. Similar to the present results, Naikoo *et al*. (2016) and Sahoo *et al*. (2016) reported oestrus response as 66% (Ovsynch) and 71% (MM + BPF supplementation), respectively. The 83.33% oestrus response following administration of kisspeptinin buffalo was observedby Pottapenjera *et al*. (2018). However, no earlier work on effect of kisspeptin on oestrus response in cattle has been reported to our knowledge. The higher oestrus response observed in group II and IV might be due to nutritional supplementation prior to hormonal treatment. Minerals have been reported to have a beneficial role in endocrine system and play an important role in resumption of follicular growth (Kor *et al*., 2013). The fortification might have increased the number of ovarian follicles and enhanced the follicular growth (Ambrose *et al*., 2006; Colazo *et al*., 2009). The oestrus response in group III is attributed to administration of kisspeptin. The kisspeptin is known as potent secretagogue of GnRH which might be responsible for onset of cyclicity in anoestrus cows in the present study.

The duration of oestrus in the study ranged from 18.57 to 32.75 hrs. The duration of oestrus observed in group I and II was similar to earlier reports of Dudi *et al*. (2017) and Ratnaparkhi *et al*. (2020) using Ovsynch and fortified Ovsynch protocol, respectively. The prolong duration of oestrus in group III and IV might be attributed to lower dose (1.3 µg/kg) of kisspeptin used in the present investigation. In contrast, lesser duration of oestrus (22.50 hrs) was observed following administration of Kisspeptin (20 µg/kg) by Pottapenjera *et al*. (2018) in buffaloes. The authors further elucidated that the kisspeptin induced LH release is short-term and lower than GnRH induced LH release. This might be the reason of prolong oestrus duration in Kisspeptin group as compared to Ovsynch group in our study.

### Signs of oestrus

The various behavioural signs of oestrus observed in present investigation have been shown in Table 2. The number of cows showing bellowing, mounting, frequent urination, loss of appetite and restlessness behaviourwere higher in group II. While, sniffing of vulva and raised tail were observed higher in group I and IV, respectively. The observed physical signs of oestrus in cows have been presented in Table 3. The population of cows with congested and swollen vulva was found higher in group II and IV. The copious quantity of mucus discharge was found greater in cows treated under group II. The clinico-gynaecological changes noted in animals responding to treatment with different oestrus

**Table 1:** Oestrus response and duration of oestrus in anestrous crossbred cows following treatment with different oestrus induction protocols.

| Groups | No. of cows treated | No. of cows responded | Oestrus response (%) | Duration of oestrus (hrs) |
|---|---|---|---|---|
| Group I | 10 | 7 | 70.00 | 18.57±1.02[a] |
| Group II | 10 | 8 | 80.00 | 20.75±1.06[a] |
| Group III | 10 | 8 | 80.00 | 32.75±0.92[b] |
| Group IV | 10 | 8 | 80.00 | 28.50±1.58[b] |
| Group V | 10 | 0 | 0.00 | - |

Data are presented as Mean±SEM. Mean bearing superscript a:b:c:d=$p$<0.05 in a column differ significantly.

**Table 2:** Frequency of occurrence of various behavioural signs of oestrus in anestrous crossbred cows responding to different oestrus induction protocols.

| Behavioural signs | Frequency of occurrence | | | |
|---|---|---|---|---|
| | Group I | Group II | Group III | Group IV |
| No. of cows responded | 7 | 8 | 8 | 8 |
| Bellowing | 1/7 (14.28) | 4/8 (50.00) | 2/8 (25.00) | 3/8 (37.50) |
| Mounting | 2/7 (28.57) | 3/8 (37.50) | 1/8 (12.50) | 1/8 (12.50) |
| Sniffing of vulva | 2/7 (28.57) | 2/8 (25.00) | 1/8 (12.50) | 2/8 (25.00) |
| Frequent urination | 3/7 (42.85) | 5/8 (62.50) | 4/8 (50.00) | 5/8 (62.50) |
| Restlessness | 3/7 (42.85) | 5/8 (62.50) | 3/8 (37.50) | 4/8 (50.00) |
| Loss of appetite | 1/7 (14.28) | 2/8 (25.00) | 2/8 (25.00) | 1/8 (12.50) |
| Tail raising | 1/7 (14.28) | 1/8 (12.50) | 1/8 (12.50) | 2/8 (25.00) |

Figures in parentheses indicate percentage of animal exhibited signs of oestrus.

induction protocols have been reported in Table 4. On examination, the open cervix, uterine tonicity and presence of large follicles with soft consistency were found in all treated animals. However, the proportion of cows with good uterine tone and soft follicular consistency was greater in group II as compared to other groups.

The behavioural signs of oestrus were observed to be higher in groups supplemented with MM and BPF. The supplementation might have showed a beneficial effect on resumption of ovarian cyclicity in anestrus animals. It is well known that minerals serve as co-factors in the steroidogenesis and play an important role in resumption ovarian activity (Smith and Akinbamijo, 2000). Similarly, feeding fat positively influence ovarian functions by enhancing the synthesis of steroidal hormones (Rahbar *et al*., 2014). The better behavioural signs of oestrus following fortification might be due to large size of ovulatory follicle and higher oestradiol production. In the present study, fortification resulted in higher percentage of cattle showing positive clinico-gynaecological changes such as relaxed cervix, turgid uterus and presence of large follicles. The swollen vagina with congested mucosa was also observed better in groups supplemented with MM and BPF. Similarly, copious discharge of cervico-vaginal mucus was found in the aforementioned groups. The fortification might have evoked better gonadal stimulation that resulted in higher elevation of oestrogen level which could bring about more conspicuous changes in the genitalia. The vaginal mucus discharge recorded (50-75%) were comparable to 61.7% observed by Negussie *et al*. (2002). In contrast, Nevkar *et al*. (2012) observed 45 per cent cows with mucus discharge, congested vulvar mucosa and swelling of vulva following treatment with Ovsynch protocol.

The changes in the genitalia were observed to be more pronounced in group I and II as compared to III and IV. The Ovsynch treatment in Group I and II might have evoked better gonadal stimulation leading to higher oestrogen level which could bring about more conspicuous changes in the genitalia (Roberts, 1971; Salisbury and Vandemark, 1961). As the oestrogen secreted from Graafian follicle is the reason of gradual relaxation of cervix and increased uterine tonicity (Jainudeen and Hafez, 1987). However, inferior results in Group III and IV treated animals might be due to low dose of kisspeptin (Chaikhun-Marcou *et al*., 2019). Further, the clinico-gynaecological changes were more pronounced in cows supplemented with MM and BPF. The supplements

**Table 3:** Frequency of occurrence of various physical signs of oestrus in anestrous crossbred cows responding to different oestrus induction protocols.

| | Physical signs | Frequency of occurrence | | | |
|---|---|---|---|---|---|
| | | Group I | Group II | Group III | Group IV |
| Vulva | Congested | 5/7 (71.42) | 6/8 (75.00) | 5/8 (62.50) | 6/8 (75.00) |
| | Pale | 2/7 (28.57) | 2/8 (25.00) | 3/8 (37.50) | 2/8 (25.00) |
| | Swollen | 7/7 (100.00) | 8/8 (100.00) | 7/8 (87.50) | 8/8 (100.00) |
| | Wrinkled | 0/7 (0.00) | 0/8 (0.00) | 1/8 (12.50) | 0/8 (0.00) |
| Quantity of vaginal mucus | Copious | 5/7 (71.42) | 6/8 (75.00) | 4/8 (50.00) | 5/8 (62.50) |
| | Scanty | 2/7 (28.57) | 2/8 (25.00) | 4/8 (50.00) | 3/8 (37.50) |
| | Absent | 0/7 (0.00) | 0/8 (0.00) | 0/8 (0.00) | 0/8 (0.00) |
| Consistency of vaginal mucus | Thin | 5/7 (71.42) | 6/8 (75.00) | 3/8 (37.50) | 4/8 (50.00) |
| | Watery | 2/7 (28.57) | 2/8 (25.00) | 5/8 (62.50) | 4/8 (50.00) |

Figures in parentheses indicate percentage of animal exhibited signs of oestrus.

**Table 4:** Clinico-gynaecological changes determined in anestrous crossbred cows responding to different oestrus induction protocol.

| Clinico-gynaecological changes | | Frequency of occurrence | | | |
|---|---|---|---|---|---|
| | | Group I | Group II | Group III | Group IV |
| Patency of cervix | Open | 7/7 (100.00) | 8/8 (100.00) | 8/8 (100.00) | 8/8 (100.00) |
| | Closed | 0/7 (0.00) | 0/8 (0.00) | 0/8 (0.00) | 0/8 (0.00) |
| Tone of uterus | Good | 3/7 (42.85) | 5/8 (62.50) | 2/8 (25.00) | 4/8 (50.00) |
| | Moderate | 4/7 (57.14) | 3/8 (37.50) | 6/8 (75.00) | 4/8 (50.00) |
| | No tone | 0/7 (0.00) | 0/8 (0.00) | 0/8 (0.00) | 0/8 (0.00) |
| Ovarian changes | Large follicle (>9 mm) | 7/7 (100.00) | 8/8 (100.00) | 8/8 (100.00) | 8/8 (100.00) |
| | Medium sized (5-8 mm) | 2/7 (28.57) | 3/8 (37.50) | 5/8 (62.50) | 5/8 (62.50) |
| | Small (<5 mm) | 4/7 (57.14) | 5/8 (62.50) | 8/8 (100.00) | 8/8 (100.00) |
| Consistency of follicular wall | Tense | 3/7 (42.85) | 2/8 (25.00) | 5/8 (62.50) | 6/8 (75.00) |
| | Soft | 4/7 (57.14) | 6/8 (75.00) | 3/8 (37.50) | 2/8 (25.00) |

Figures in parentheses indicate percentage of animals showing clinico-gynaecological changes.

might have positive influence on reproduction by altering both hormonal and gondal status (Mattos *et al*., 2000; Smith and Akinbamijo, 2000).

**Oestrogen concentration**

The serum oestrogen concentration at various days of treatment in different treatment groups has been presented in Table 5. The level of serum oestrogen varied significantly between treatment protocols on 7th, 9th and 10th with control group but remained similar (P>0.05) at day 0. The serum oestrogen concentration increased significantly (P<0.05) from day 0 to day 10 of treatment in all the treatment protocols. Except in group II and IV where serum oestrogen concentration was statistically non-significant on day 7 and 9. The level of oestrogen concentration was found higher in group II at day 7, 9 and 10. The oestrogen concentration was found statistically greater in group I and II as compared to group II and IV. However, the difference was non-significant (P>0.05) between corresponding groups.

The gradual increase in mean serum oestrogen concentration was observed from day 0 to day 10 in all treated groups. This might be due to 1st GnRH or kisspeptin injection which might have caused emergence of new follicular wave pursuing into growth and development of dominant follicle. Highest level of serum oestrogen concentration recorded on day 10 in all treatment protocol might be due to presence of pre-ovulatory follicle. The concentration of oestrogen was highest in Ovsynch groups which are in conformity to oestrus response of the animals. Poor response in kisspeptin groups is again accounted to inappropriate dosage. The better steroid secretion and follicular development in fortified groups is indicated by higher oestrogen concentration in fortified groups as compared to non-fortified ones.

**Progesterone concentration**

The serum progesterone concentration at 0, 7, 9 and 10 day of treatment in various groups has been presented in Table 6. The serum progesterone level was found to be higher (P<0.05) on day 7 in group I, II, IV while the difference was non-significant (P>0.05) in group III and V. The progesterone concentration was statistically similar between various groups at day 0 and 9. The serum progesterone concentration on day 7 and 10 was higher (P<0.05) in group II and IV, respectively. The level of progesterone was observed to be gradually increased from day 0 to day 7 of treatment and the progesterone level was found to be significantly higher (P<0.01) in group I, II and IV. However, mean progesterone level was found not to differ significantly in different days of treatment in group III.

Increase in serum progesterone level observed in the present investigation upto day 7 might be due to formation of CL or luteinization of follicles present in the ovary following GnRH and Kisspeptin administration. The significant reduction in serum progesterone levels on day 9 and 10 might be due to parenteral administration of $PGF_{2\alpha}$ on day 7 of treatment protocol leading to CL lysis which caused physiological declination of progesterone. However, no significant change in the level of progesterone was observed in different days of treatment in group III indicating absence of CL formation or lutenization of follicle due to low secretion of LH. In agreement to the present study, Chaikhun *et al*. (2019) reported that administration of kisspeptin at the dose rate of 1.3 µg/kg caused lower secretion of LH as compared to buserelin injection. Besides, kisspeptin at the dose rate of 20 µg/kg causes maximum elevation of LH (Pottapenjera *et al*., 2018). Luteal activity as indicated by serum progesterone concentration on day 7 of treatment might be due lower dose of kisspeptin (1.3 µg/kg) which failed to cause ovulation or luteinization of follicles.

**Pregnancy rate**

In the present investigation, the overall pregnancy rate was recorded highest in group II followed by group III and IV. However, lowest pregnancy rate was observed in group I (Table 7). The difference in number of cows those conceived

**Table 5:** Level of serum oestrogen (pg/ml) in crossbred cows responding to various oestrus induction protocols at different days of treatment.

| Groups | DAY 0 | DAY 7 | DAY 9 | DAY 10 |
|---|---|---|---|---|
| Group I | 39.87±0.81$^{D}$ | 44.26±0.92$^{Ca}$ | 46.97±0.64$^{Ba}$ | 52.36±1.17$^{Aa}$ |
| Group II | 39.08±0.88$^{C}$ | 45.50±0.56 $^{Ba}$ | 47.14±0.73$^{Ba}$ | 53.46±0.20$^{Aa}$ |
| Group III | 38.16±0.25$^{D}$ | 40.67±0.54$^{Cb}$ | 42.37±0.53$^{Bb}$ | 44.63±0.55$^{Ab}$ |
| Group IV | 39.28±0.43$^{C}$ | 41.79±0.68 $^{Bb}$ | 43.39±0.71$^{Bb}$ | 45.29±0.33$^{Ab}$ |
| Group V | 38.17±0.33 | 38.24±1.05$^{c}$ | 38.30±0.42$^{c}$ | 38.22±0.40$^{c}$ |

Data are presented as Mean±SEM. Mean bearing superscript A:B:C:D=*p*<0.05 in a row and a:b:c:d= *p*<0.05 in a column differ significantly.

**Table 6:** Level of progesterone (ng/ml) in crossbred cows responding to various oestrus induction protocols at different days of treatment.

| Groups | DAY 0 | DAY 7 | DAY9 | DAY 10 |
|---|---|---|---|---|
| Group I | 0.62±0.04$^{B}$ | 2.92±0.62$^{Aa}$ | 0.47±0.09$^{B}$ | 0.40±0.13$^{Ba}$ |
| Group II | 0.67±0.06$^{B}$ | 3.68±0.41$^{Aa}$ | 0.58±0.13$^{B}$ | 0.41±0.11$^{Ba}$ |
| Group III | 0.66±0.07 | 0.77±0.03$^{b}$ | 0.63±0.13 | 0.61±0.06$^{ab}$ |
| Group IV | 0.69±0.04$^{B}$ | 1.47±0.33$^{Ab}$ | 0.70±0.05$^{B}$ | 0.72±0.04$^{Bb}$ |
| Group V | 0.57±0.04 | 0.52±0.05$^{b}$ | 0.51±0.02 | 0.50±0.02$^{a}$ |

Data are presented as Mean±SEM. Mean bearing superscript A:B:C:D= p<0.05 in a row and a:b:c:d=*p*<0.05 in a column differ significantly

**Table 7:** Effect of different hormonal protocols on pregnancy rate in postpartum anestrous cows.

| Groups | No. of cows treated | No. of cows conceived | Induced estrus | Second cycle | Third cycle | Overall pregnancy rate (%) |
|---|---|---|---|---|---|---|
| Group I | 10 | 4 | 28.57% (2/7) | 40.00% (2/5) | 0.00% (0/3) | 40 |
| Group II | 10 | 6 | 37.50% (3/8) | 40.00%(2/5) | 33.33% (1/3) | 60 |
| Group III | 10 | 5 | 12.50% (1/8) | 42.85% (3/7) | 25.00% (1/4) | 50 |
| Group IV | 10 | 5 | 12.50% (1/8) | 57.14% (4/7) | 0.00% (0/3) | 50 |

Figures in parentheses indicate number of animals conceived.

did not differ significantly at induced estrus, second and third cycle. The pregnancy rate in cows inseminated at induced oestrus was higher in Ovsynch protocol. This might effectively persuade to generate LH surge by pre-ovulatory follicle. It was clearly mentioned that GnRH induced LH response is higher as compared to kisspeptin induced protocol (Pottapenjera *et al*., 2018; Chaikhun-Marcou *et al*., 2019). The lower dose of kisspeptin used in the study might have failed to generate LH surge (Decourt *et al*., 2014; McGrath *et al*., 2016). The higher pregnancy rate was observed in kisspeptin treated cows inseminated at second oestrus. The reason of better pregnancy rate at subsequent oestrus is still unclear. However, we could suspect that under given dosage the kisspeptin has successfully onset the cyclicity and has sustained hormonal milieu with fruitful conception in subsequent oestrus.

## CONCLUSION

The study utilized Ovsynch, fortified Ovsynch, kisspeptin and fortified kisspeptin protocol for the treatment of postpartum anestrous cows. The Ovsynch resulted in higher overall pregnancy rate as compared to kisspeptin protocol. The behavioural, physical and clinico-gynaecological changes were much prominent in fortified groups; fertility results of this protocol varied. The kisspeptin with or without fortification led to prolonged oestrus and delayed ovulation in crossbred cows with dose rate of 1.3 µg/kg body weight. Further, the dosage failed to generate LH surge. However, in subsequent oestrus, higher number of animals conceived in kisspeptin group. The mechanism for this differential pregnancy rate is unclear and need to be explored. Despite of lower dose of kisspeptin showed higher efficacy to bring animal into cyclicity. Thus, kisspeptin protocol can appear as valuable tool in the advancement of the reproductive technology. However, the higher dose rate ofkisspeptin is need to betargeted in future study.

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