The Haryana Veterinarian (September, 2023) **62(SI-2),** 69-72 ISSN 0033-4359 (P) **Research Article**

STUDY ON EFFICACY OF MODIFIED CO-SYNCH PROTOCOL FOR ESTRUS INDUCTION IN SAHIWAL COWS

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ABSTRACT

Anestrous is a condition when the female does not exhibit regular estrous cycle, due to insufficient Gonadotrophin releasing hormone release from the hypothalamus to stimulate and maintain gonadotropin secretion. In present study different hormonal protocols were used to test the reproductive response in anestrus Sahiwal cows. Eighteen true anestrus Sahiwal cows of Rewa district aged between 3-5 years were selected and divided into the three groups each containing six animals. The selected animals were grouped as: Group-1 (Co-Synch Protocol), Group-2 (Modified Co-Synch plus protocol),Group-3 (Modified Co-Synch plus protocol with progesterone). Number of animals that shows estrus signs were 3, 4 and 6 in Group-1, Group-2 and Group-3, respectively. Conception rate was 66.66, 75.00 and 83.33% in Group-1, Group-2 and Group-3, respectively. Within the groups, serum estrogen level was significantly ($p<0.05$) higher on the day 9 for all groups. Serum progesterone level was significantly ($p<0.05$) higher on the day 18 onwards in Group-1 and Group-2, while in Group -3, Serum progesterone level was significantly ($p<0.05$) higher on the day 7.

Keywords: Anestrus, Estrus induction, Fertility response, Hormone

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Anestrous is a condition when the female does not exhibit regular estrous cycle due to insufficient GnRH release from the hypothalamus to stimulate and maintain gonadotropin secretion. The ovaries are relatively inactive and neither ovulatory follicles nor corpus luteum are present. In fact, post-partum anestrous is the lack of estrous signs (with estrous detection) within 60 days after calving, while normal cows show estrous signs in exactly same conditions after calving (Madhuri *et al.*, 2017). Numbers of estrous synchronization protocols are available in cattle based on the use of various hormones like progesterone, $PGF_{2\alpha}$ and their various combinations with other hormones like estrogen and gonadotrophin releasing hormones (Islam, 2011). In addition, some estrous synchronization protocols (progestin-based protocols) can induce a proportion of anestrous cows to begin estrous cycle. The study was aimed to study the efficacy of Modified Co-synch plus protocol and Modified Co-synch plus protocol with progesterone for estrous induction in Anestrus Sahiwal cattle.

MATERIALS AND METHODS

Selection of Animal: Eighteen true anestrus Sahiwal cows of Rewa district Madhya Pradesh aged between 3-5 years were selected. The animals which were selected were having the history of normal parturition and there were no signs of pathological condition of the reproductive organs.

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Ovarian screening by manual two rectal examinations 10 day interval was done to ensure absence of any palpable structure on either of the ovary (Corpus luteum or follicles). The selected cows were randomly divided into three Groups with six animals in each Group. All three Groups of animals were administered with the three different estrus induction protocols by using a combination of hormonal preparations as described below.

Group-1 (Co-Synch Protocol): The cows in this Group were administered GnRH (Buserelin acetate 10 µg, i.m) on day 0, $PGF_{2\alpha}$ (cloprostenol, 500 µg, i.m) on day 7th and (Buserelinacetate, 10 µg, i.m) on day 9th. Thereafter, detection of estrus and artificial insemination was done.

Group-2 (Modified Co-synch plus protocol): The cows in this Group were administered with eCG (equine serum gonadotropin) (400 IU i.m) on 3rd days before the first GnRH, GnRH (Buserelin acetate, 10 µg, i.m) on day 0, $PGF_{2\alpha}$ (cloprostenol, 500 µg, i.m) on day 7th, and hCG (human chorionic gonadotrophin) (2000 IU i.m) on day 9th. Thereafter detection of estrus and artificial insemination was done.

Group-3 (Modified Co-Synch plus Protocol with progesterone): The cows in this Group were administered with eCG (400 IU i.m) on 3 days before the first GnRH, GnRH on day 0, prostaglandin F_{2alpha} ($PGF_{2\alpha}$) (cloprostenol,

500 µg, i.m) on day 7th, and human chorionic gonadotropin (hCG) (2000 IU i.m) on day 9th. Additionally, a progesterone device (TRIUB) was inserted in vagina on day 0 and was kept in situ for 7 days and removed on day 7 of the protocol. Due to the unpredictable nature of ovulation timing, such treatment protocols require at least two inseminations to achieve adequate conception rates (Barile *et al.*, 2001).

Estrus Detection and AI: The estrus signs showed by the individual animal were recorded during morning and evening hours for confirmation of onset of estrus. The onset of estrus in cows was detected by cervico vaginal discharge and per-rectal examination by Gunasekaran (1998) method. Gunasekaran (1998) suggested that uterine tone and turgid or coiled cornuae on rectal palpation could be used for confirmation of estrus. Cows in estrous were inseminated with frozen semen after 12h of estrous and for co-synchanimal were inseminated at the time of second injection of GnRH.

Pregnancy Diagnosis: Pregnancy diagnosis in cows of all groups was done on day 60 post-insemination by per-rectal examination.

Conception Rate: Conception rate after A.I (artificial insemination) was calculated as number of cows conceived at A.I. out of all animals inseminated and over all conception rate was calculated as number of cows conceived at A.I. and subsequent AI in next cycle out of all animals inseminated.

Blood Collection and Storage: Blood samples were collected by jugular venipuncture on day -3, 0, 7, 9, 18 and 28. Serum sample for hormonal analysis is taken in plain sterile glass tube. Serum was separated from plain tube by centrifugation at 3000 rpm for 10 minutes and stored at -20 ºC until.

Serum Progesterone Estimation by ELISA: Serum progesterone concentration (ng/ml) was estimated on -3, 7th, 9th, 18th and 28th day after start of treatment for confirming the result obtained on gynaeco-clinical examination. The results of progesterone assay were also correlated with the fertility response to treatment. The quantitative determination of progesterone concentration in serum was performed by Pg (Progesterone) ELISA (Elabscience) kits.

Serum Estrogen Estimation by ELISA: Serum Estrogen was estimated on -3,7th, 9th, 18th and 28th day after start of treatment for confirming the result obtained on gynaeco-clinical examination. by QuicKey Pro Mouse E2 (Estradiol) ELISA Kit (Elabscience). The data was analyzed using standard statistical procedure as per (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Estrus detection: The animal exhibited the estrus of Group-1, Group-2 and Group-3 during experimental period has been presented in table no. 3. Estrus efficacy (%) was 50.00% in Group-1, 66.66% in Group-2 and 100.00% in Group-3, respectively. Exhibition of estrus was higher in Group-3 as compared to Group-1 and 2.

Pregnancy diagnosis: Pregnancy diagnosis in cows of all groups was performed on day 60 post-insemination by per-rectal examination. In group-1, two animals were found pregnant. In group-2, three animals were found pregnant and in group-3, five animals were found pregnant.

Estrogen: The change in the mean±S.E. values of serum estrogen of Group-1, Group-2 and Goup-3 during experimental period has been presented in table no. 2. The mean serum estrogen (pg/ml) concentration in Group-1 were recorded as 3.35±0.49, 4.41±0.72, 8.86±0.64, 18.01±0.96, 3.88±0.59, 4.78±0.82 on day -3 , 0, 7, 9, 18 and 28, respectively. The mean serum estrogen (pg/ml) concentration in Group-2 were recorded as 4.60±0.45, 5.27±0.35, 10.87±0.82, 19.17±2.56, 4.75±0.63, 5.12±0.97 on day -3 , 0, 7, 9, 18 and 28, respectively. The mean serum estrogen (pg/ml) concentration in Group-3 were recorded as 4.41±0.68, 6.02±0.51, 9.83±0.83, 20.86±1.28, 4.98±0.57, 5.01±0.58 on day -3 , 0, 7, 9, 18 and 28, respectively. In all groups significantly ($p<0.05$) higher concentration of serum estrogen was observed on day 9 than other days of sampling.While among the groups, numerically highest increase in serum estrogen was observed in Group-3, on day 9 post treatment. This higher estrogen value was due to more number of cyclic cows in group 3 than other group. Estrogen is produce by the follicles, which located on the ovary, as the follicle grows, more estrogen is produced. Itact in positive feedback mechanism and responsible for LH surge and ovulation in animals. The lack and irregular production of estrogen seen in group-3 are consistent with previous studies of norgestomet (Gonzalez-Padilla *et al.*, 1975).

Progesterone: The change in the mean±S.E. values of serum progesterone of Group-1, Group-2 and Group-3 during experimental period has been presented in table no. 02. The mean serum progesterone (ng/ml) concentration in Group-1, were recorded as 3.16±0.60, 3.27±0.48, 3.51±0.56 , 1.67±0.33, 7.17±0.75, 7.67±3.08 on day -3 , 0, 7, 9, 18 and 28, respectively. In Group-1, significantly ($p<0.05$) higher concentration of serum progesterone was observed on day 18 and 28 than other days of sampling. The mean serum progesterone (ng/ml) concentration in Group-2 were recorded as 3.33±0.71, 3.41±0.58, 3.53±0.26, 1.58±0.33, 7.33±0.49, 8.11±2.29, on day -3 , 0,

Table 1. Mean±S.E. value of Estrogen (pg/ml) by hormonal protocol in anestrus Sahiwal cows

Group	Days					
	-3	0	7	9	18	28
1.	3.35 ± 0.49^{A}	4.41 ± 0.72^{A}	8.86 ± 0.64^{B}	18.01 ± 0.96^{C}	3.88 ± 0.59^{A}	4.78 ± 0.82^{A}
2.	4.60 ± 0.45^{A}	5.27 ± 0.35^{A}	10.87 ± 0.82^{B}	19.17 ± 2.56^{C}	4.75 ± 0.63^{A}	5.12 ± 0.97^{A}
3.	4.41 ± 0.68^{A}	6.02 ± 0.51^{A}	9.83 ± 0.83^{B}	20.86 ± 1.28^{C}	4.98 ± 0.57^{A}	5.01 ± 0.58^{A}

Means bearing different superscripts (capital letters in row) differ significantly ($p< 0.05$).

Table 2. Mean±S.E. value of Progesterone (ng/ml) by hormonal protocol in anestrus Sahiwal cows

Group	Days					
	-3	0	7	9	18	28
1.	3.16 ± 0.48^{A}	3.27 ± 0.48^{A}	3.51 ± 0.56^{Aa}	1.67 ± 0.33^{A}	7.17 ± 0.75^{Ba}	7.67 ± 3.08^{Ba}
2.	3.33 ± 0.71^{A}	3.41 ± 0.58^{A}	3.53 ± 0.26^{Aa}	1.58 ± 0.33^{A}	7.33 ± 0.49^{Ba}	8.11 ± 2.29^{Ba}
3.	4.03 ± 0.61^{B}	4.11 ± 0.73^{B}	12.53 ± 1.12^{Cb}	1.54 ± 0.18^{A}	11.67 ± 0.75^{Cb}	12.33 ± 1.41^{Cb}

Means bearing different superscripts (small letters in column and capital letters in row) differ significantly ($p< 0.05$)

Table 3. Percent conception rate following AI at induced estrus and over all pregnant animals of Group 1, Group 2 and Group 3

Group	No. of animals	No. of animals comes in estrus	AI done in animals	Pregnant animals	Conception rate (%)
1	6	3	3	2	66.66
2	6	4	4	3	75.00
3	6	6	6	5	83.33

7, 9, 18 and 28, respectively. In Group-2 significantly ($p<0.05$) higher concentration of serum progesterone was observed on day 18 and 28 than other days of sampling. The mean serum progesterone (ng/ml) concentration in Group-3 were recorded as 4.03±0.61, 4.11±0.73, 12.53±1.12, 1.54±0.18, 11.67±0.75, 12.33±1.41 on day -3 , 0, 7, 9, 18 and 28, respectively. In group-3 significantly ($p<0.05$) higher concentration of serum progesterone was observed on day 7 and 28 than other days of sampling. Among the groups, the highest increase in serum progesterone was observed in Group-3, on day 28 post treatment.

Progesterone level is directly related with functioning CL, estrous cycle control and fertility of the cow. It is responsible for stimulation of cyclicity, follicular development and for continuation of pregnancy. In normal cyclic animals, progesterone levels were expected to be high, during diestrous stage while subsequently should alter during estrous stage (Singh and Singh, 2006). Similar findings were observed in the present study. The lowest mean P4 values in all groups on day 9 were probably because animals were cyclic. Constant rise in the concentration might be due to the functional activity of corpus luteum and the decreasing trend might be due to initiation of luteolysis (Hafez, 2008). The mean progesterone levels dropped suddenly and significantly after $PGF_{2\alpha}$ injection to the basal values in all three treatment Groups at the time of AI indicated lysis of progesterone releasing source i.e. CL. These levels again increased significantly ($p<0.05$) post-AI in all the Groups. This is understandable as the induced estrous wasovulatory with the development and maintenance of corpus luteum (CL) and also the establishment of pregnancy in some animals (Dhami *et al.*, 2015). Mean Serum progesterone level on day 18 in Group 3 was significantly ($P\leq0.05$) higher than Group 1 and 2 it may be because of more number of pregnant animal in Group 3 than other Groups after different treatment.

Conception Rate: The conception rates of Group-1, Group-2 and Group-3 during experimental period have been presented in table no. 3. Conception rate was 66.66 in Group-1, 75.00 in Group-2 and 83.33% Group-3. Similarly, 66.66, 60 and 22.7% conception rate observed by Barolia *et al.* (2016), Amle *et al.* (2015) and Melendez *et al.* (2006) using Ovosynch protocol. The variation in our finding regarding the conception rate might be due to selection of animals from different management condition i.e. from Gaushala's, villages, and organized dairy farms. In present study, through Cosynch protocol the conception rate was somewhat higher than reported by earlier by Barolia *et al.* (2016) (66.66%) and but similar findings were reported by Neglia *et al.* (2003) and Baruselli *et al.* (2001).

CONCLUSIONS

Co-synch protocol was found to be effective in induction of estrus and pregnancies in Sahiwalcows. Modified co-synch protocol was proved to be most successful with use of progesterone priming in anestrus cows. Highest fertility parameters were recorded in

Modified co-synch protocol with progesterone treated Sahiwal acyclic cows.

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