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BIOCHEMICAL PARAMETERS, PROGESTERONE CONCENTRATION and CONCEPTION RATES in POSTPARTUM ANESTRUS BUFFALOES TREATED WITH MODIFIED OVSYNCH and MODIFIED COSYNCH PROTOCOLS

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ABSTRACT

The present study was planned to evaluate the biochemical parameters, progesterone concentration and conception rates in postpartum anestrus buffaloes treated with modified Ovsynch and Cosynch protocols.A total of 120 postpartum buffaloes reared under field conditions in Jabalpur (M.P.) were surveyed during the study. The buffaloes with the history of not showing signs of oestrus for 60 days or more postpartum were considered to be suffering from postpartum anestrus. These buffaloes were per rectally examined twice at 10 days apart to confirm ovarian activity.Twenty four postpartum anestrus buffaloes were randomly selected from the buffaloes found positive for anestrus. These animals were again randomly divided into 4 groups (n=6 animal/group). Group I, II and III animals were treated with CIDR plus Ovsynch, CIDR plus Cosynch and eCG plus Cosynch protocols, respectively, while the control group (Group IV) animals were not given any treatment. All treatment group animals were bred by fixed time artificial insemination while control animals were bred on observed oestrus by artificial insemination. Serum progesterone (ng/ml) levels were estimated by ELISA and biochemical analysis (like Calcium, Phosphorus, Magnesium, Total protein and Total cholesterol) were done by semi-automatic analyzer on 0 day and day 21 post artificial insemination. Pregnancy diagnosis was done by per rectal examination 60 days post insemination. Mean serum total cholesterol concentration on day 21 post insemination varied significantly between group I and III. Mean serum progesterone concentration decreased significantly ($p<0.05$) in group II on day 21 post insemination as compared to day 0. The conception rates in group I, II, III and IV were 50.00, 33.33, 66.67 and 33.33 per cent, respectively. It was concluded that eCG+ Cosynch protocol was better as compared to CIDR+Cosynch and CIDR + Ovsynch in postpartum anestrus buffaloes.

Keywords: Biochemical parameters, Progesterone, CIDR, Cosynch, Ovsynch, Postpartum anestrus

INTRODUCTION

Reproductive efficiency is the primary factor affecting the productivity and buffalo has a reputation of being a shy breederdue totheir late puberty, long postpartum ovarian dormancy,absence of oestrus symptoms and long calvingintervals. Anestrus is one of the most commonreproductive problems in buffaloes affecting livestock productivity and economics to a

great extent (Mujawar et al.,2019).It is normal for an animal to not exhibit ovarian cyclicity immediately postpartum, but, prolongation of this period reduces the reproductive efficiency of the animal. The delay in onset of cyclicity and initiation of ovulation during postpartum period in buffaloes constitute a major problem and results into long postpartumanestrus and delayed breeding with consequent serious economic losses in the milk production and efficient reproduction.

The hemato-biochemical parameters are key indicators of the physiological condition of the animals; which also reflect production and reproduction. Progesterone in cyclic animals acts as a regulator of dioestrus period, because as soon as the corpus luteum fails to secrete progesterone, development of follicles begins leading to proestrus phase. There are reports of high incidence of postpartum anestrus which are associated with the deficiencies of cholesterol, glucose and total protein etc.

Effective treatment of postpartum anestrus in buffaloes is the efficient means of improving their milk productivity. Estrus induction protocols using various hormones such as gonadotropin releasing hormone (GnRH), progesterone (CIDR, Crestar), estradiol, equine chorionic gonadotropin (eCG) and prostaglandins with fixed-time artificial insemination (FTAI) have been tried for the treatment of anestrus with variable success rates (Murgavel et al.2009 and Kumar et al.2014). Keeping this in view, the present study was planned to evaluate thebiochemical parameters, progesterone concentration and conception ratesin postpartum anestrus buffaloes treated with modified Ovsynch and modified Cosynch protocols.

MATERIALS AND METHODS

The present research work was carried out in the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Jabalpur (M.P.) and different villages of Jabalpur (M.P.), India.

SELECTION OF ANIMAL

A total of 120 postpartum buffaloes (BCS >2.5) reared under field conditions in different villages of Jabalpur (M.P.) were surveyed during the study. The buffaloes with the history of not showing signs of oestrus for 60 days or more postpartum were considered to be suffering from postpartum anestrus. These buffaloes were per rectally examined twice at 10 days apart to confirm ovarian activity. Twenty four postpartum anestrus buffaloes were randomly selected from the buffaloes found positive for anestrus. These animals were randomly divided into four groups i.e. three treatment group and one untreated control group (n=06 animals/group) and were dewormed. The animals of treatment groups were subjected to various synchronization protocols after 07 days of deworming as follows:

Table 1: Treatment protocols for different groups

Groups	Treatment regimen	Schedule

(n=06 animals /group)		
I (Modified Ovsynch protocol / CIDR+Ovsynch)	Inj. GnRH (Buserelin acetate 20µg, I/M) + CIDR Implant	Day 0
	Inj. $PGF_{2\alpha}$ (Cloprostenol 500µg, I/M) + Removal of CIDR Implant	Day 07
	Inj. GnRH (Buserelin acetate 10µg, I/M)	Day 09
	FTAI	Day 10 (24 hrs. after 2nd GnRH injection)
II (Modified Cosynch/ CIDR+Cosynch)	Inj. GnRH (20µg, I/M) + CIDR Implant	Day 0
	Inj. $PGF_{2\alpha}$ (Cloprostenol 500 µg, I/M) + Removal of CIDR implant	Day 07
	Inj. GnRH (Buserelin acetate 10µg, I/M) + FTAI	Day 09
III (Modified Cosynch/ eCG+Cosynch)	Inj. eCG (400 IU, I/M)	Day -3 (3 days prior to day 0)
	Inj. GnRH (Buserelin acetate 20µg, I/M)	Day 0
	Inj. $PGF_{2\alpha}$ (Cloprostenol 500 µg, I/M)	Day 07
	Inj. GnRH (Buserelin acetate 10µg, I/M) + FTAI	Day 09
iv (Untreated Control)	No treatment	AI on estrus

APPLICATION & REMOVAL of CIDR

The buffaloes were restrained in trevis and CIDR was inserted into the vagina. Extra nylon tail attached to the end of the CIDR was cut to prevent pulling out by neighboring animals. The CIDR was removed on day 7 of treatment by pulling out the nylon tail that is exposed from the reproductive tract.

ESTRUS DETECTION & BREEDING

Detection of oestrus was carried out twice daily (morning and evening) by visual observations. Buffaloes at induced oestrus were bred by artificial insemination (AI) on the fixed day of treatment protocols.

COLLECTION of BLOOD

Approx. 5ml of blood was collected aseptically from the external jugular vein from all the experimental buffaloes on of initiation of treatment (day 0)and day 21 post AI. Serum was separated at 1500 rpm for 30 minutes and stored in labelled vials in deep freezer at -20 ^{0}C till estimation of biochemical parameters and progesterone estimation.

SERUM PROGESTERONE ASSAY

Serum progesterone concentration (ng/ml) was estimated on the day of initiation of treatment (0 day) and day 21 post AI for confirming the results obtained on gynaeco-clinical examination. The results of progesterone assay were correlated with the fertility response to treatment in terms of conception rates.The quantitative determination of progesterone concentration in plasma was done by ELISA using commercial kit manufactured by Alkor Bio Inc., Russia.

ESTIMATION of BIOCHEMICAL PARAMETERS in SERUM SAMPLES

The levels of minerals, viz. calcium, phosphorus, magnesium in serum were determined on day 0 (day of initiation of treatment) and day 21 post AI by auto analyzer. Serum calcium, phosphorus, cholesterol and total protein were analysed in semi auto analyser by using commercial kit manufactured by Erba Manheim, Transasia biochemical (India) Pvt. Ltd. Serum magnesium was analysed on semi auto analyser by using commercial kit manufactured by LAB-CARE diagnostics (India) Pvt. Ltd.

CONCEPTION RATE

Pregnancy diagnosis was done by per rectal examination on 60 days post artificial insemination to determine the conception rate.

STATISTICAL ANALYSIS

The data was analysed on R platform (R Core Team, 2018) using “dplyr” library. Data from different experiments were presented as Mean ±SE. The pair-wise comparison of means was carried out using Fisher’s multiple comparison test as per standard statistical method described by Snedecor and Cochran (1994). The results of conception rates were expressed in percentage.

RESULTS

MEAN SERUM PROGESTERONE CONCENTRATION (ng/ml)

Serum progesterone concentration is specifically related with CL function and fertility of the buffaloes. The progesterone concentration is responsible for stimulation of cyclicity, follicular development and also for continuation of pregnancy. In normal cyclic buffaloes, serum progesterone level is expected to be high during diestrus stage and subsequently should reduce during oestrus stage. Anestrus buffaloes carry irregular levels of progesterone which create

uncertainty in exhibition of next oestrus and the stage continues for months together unless disrupted by endogenous or exogenous hormones.

Mean serum Progesterone (P_4) concentrations (ng/ml) were studied in postpartum anestrus buffaloes on day 0 of the treatment and day 21 post AI in different treatment groups and the results are presented in table 2.

Table 2: MEAN SERUM PROGESTERONE CONCENTRATION (NG/ML) IN CONTROL AND DIFFERENT TREATMENT GROUPS

Group (n=06/group)	Day 0	Day 21 post AI
I	4.19±1.33	3.91±0.92
II	4.72^{a}±1.23	2.97^{b}±0.70
III	4.91±0.93	4.21±0.44
IV	2.31±0.80	2.2±0.37

Mean values bearing different superscripts (a,b) in a row differ significantly ($p<0.05$)

The mean serum progesterone (ng/ml) concentration were >1ng/ml on day 0 in group I, II, III and IV (4.19±1.33, 4.72±1.23, 4.91±0.93 and 2.31±0.80, respectively), suggestive of the fact that most of the buffaloes were having embedded functional persistent luteal structure on their ovaries resulting in postpartum anoestrus. These levels subsequently decreased non-significantly on day 21 post insemination (3.91±0.92, 4.21±0.44 and 2.2±0.37, respectively), except in group II. In group II animals the mean serum progesterone concentration decreased significantly ($p\geq0.05$) on day 21 post insemination as compared to day 0 of treatment.

The progesterone concentration might have risen after oestrus and AI is attributed to CIDR reinsertion complementing the gradual endogenous production of P_4 from the CL formed after 2nd GnRH injection. Serum progesterone levels (>1ng/ml) from oestrus to days 21 post-AI indicated successful fertilization, embryonic development and great improvements in pregnancy rate. The trend of mean serum progesterone is also inflicted in the form of conception rate in the present study in different treatment groups where significant decrease in mean serum progesterone shows lower conception rates of treatment group II as compared to treatment group I and III. Furthermore, progesterone plays various important roles during synchronization of ovulation with CIDR, as source of exogenous P_4, simultaneously administered with Ovsynch protocol for FTAI. There is improvement of oocyte quality, follicular growth, and prevention of premature ovulation improving conception. Timed AI program applied focuses on the follicular development and the attainment of the right size of preovulatory follicle at the time of AI (Baruselli et al., 2012). Follicle ovulating at the desired diameter (13-14mm) can result in the formation of larger corpus luteum and serves as source of endogenous P_4 following AI.

However, some protocols may not work as expected; yielding smaller ovulatory follicle with small CL formed resulting in insufficient endogenous P_4 production. Essentially, the present study underscores the roles P_4 generally played in buffalo reproduction; from oocyte development, follicular growth, early embryonic development, and maintenance of pregnancy finally leading to successful development to term of the fetus. In sum, breeding program and FTAI protocols which ensures high P_4 levels post-AI support early embryonic development and uterine implantation thus is highly recommended towards greater productivity and profitability from buffalo rearing and dairy-based enterprise activities.

The result of the present study suggested that estimation of the plasma progesterone levels by ELISA is helpful tool to detect the current reproductive/cyclical status of the animals and to diagnose cause of anoestrus, early pregnancy with reasonable accuracy in buffaloes after AI.

SERUM BIOCHEMICAL PARAMETERS

Minerals affect various physiological activities as they function as cofactors, activators of enzymes, or stabilizers of secondary molecular structure. Minerals are absorbed only mainly through the small intestine. A deficiency of mineral elements like Ca, P, Mg, etc. is associated with subnormal fertility, decreased ovarian activity, irregular oestrus and anestrus condition. The imbalance of minerals leads to anestrus and inactive ovaries associated with increased estrogen production by ovarian follicles. Animals having a deficiency of minerals are known to exhibit delayed sexual maturity and prolonged postpartum anestrus period.

The mean serum profile of minerals, viz., calcium, phosphorus and magnesium recorded on day 0 and day 21 post insemination CIDR+Ovsynch (group I), CIDR+Cosynch (group II), eCG plus Cosynch (group III) and Control groups (group IV) in postpartum anestrus buffaloes are presented in table 3 to 7.

MEAN SERUM CALCIUM CONCENTRATION

Table 3: MEAN SERUM CALCIUM CONCENTRATION (MG/DL) IN CONTROL AND DIFFERENT TREATMENT GROUPS

Group (n=06/group)	Day 0	Day 21 post AI
I	8.68^{AB}±0.97	8.71±0.97
II	5.90^{B}±0.39	6.77±0.41
III	9.35^{A}±0.77	9.05±0.86

IV	8.25^{AB}±1.05	9.97±1.14

Mean values bearing different superscripts (A,B) in a column differ significantly ($p<0.05$)

The results presented in table 3 depicts that the mean serum calcium (mg/dl) concentration on day 0 in group I, II, III and IV were 8.68±0.97, 5.90±0.39, 9.35±0.77, 8.25±1.05, respectively, suggestive of the fact that most of the buffaloes were having normal range of serum calcium. These levels subsequently increased non-significantly on day 21 post insemination (8.71±0.97, 6.77±0.41, 9.05±0.86, 9.97±1.14, respectively), except in group III in which concentration decreased non-significantly from day 0 to day 21 post insemination. The mean serum calcium concentration on day 0 and day 21 post AI were within normal physiological range except group II buffaloes.

The mean serum calcium concentration in normal cyclic and postpartum anoestrus buffaloes has been recorded to be 8.12±0.25 mg/dl and 7.87±0.32 mg/dl (Hafez, 2019). The mean calcium concentration in group II was significantly lower i.e. 5.9±0.39 mg/dl on day 0 and 6.77±0.41 on day 21 post insemination. The lower values of mean serum calcium concentration in group II buffaloes may be due to feeding and managemental practices followed at farms, parity and period of lactation.

Mean serum calcium concentration on day 0 showed significant variation ($p<0.05$) between treatment group II and III while this variation was non-significant ($p\geq0.05$) between group I and II, I and III, I and IV, II and IV, III and IV. The levels on day 0 in different treatment group were within normal physiological range except group II. While, significant variation ($p<0.05$) in mean calcium concentration between group II and III may be attributed to feeding and managemental practices followed at different farms, stage of lactation and smaller sample size.

Similar results were found by Saikiran et al. (2020) in which it was observed that the calcium levels were 8.58±0.76 and 10.16±0.27 mg/dl in PPA and normal cyclic animals with no significant ($p\geq0.05$) difference between groups. Kumar et al. (2015b) reported calcium concentration of 12.9±4.34 and 12.45±0.63 mg/dl in oestrus and anoestrus water buffaloes with no significant difference between groups. In contrary, Kumar et al. (2016a) reported significantly lower levels of calcium in postpartum anoestrus buffaloes compared to cyclical buffaloes (7.418±0.621 vs 11.756±0.860 mg/dl).

MEAN SERUM PHOSPHORUS CONCENTRATION

Deficiency of phosphorus influences the level of the pituitary and ovarian hormones and thereby causes the aberrations in the normal reproductive rhythm. The results of mean serum phosphorus (mg/dl) concentration on day 0 of the treatment and day 21 post AI observed in different treatment groups are summarized in table 4.

Table 4: SERUM PHOSPHORUS CONCENTRATION (MG/DL) IN CONTROL AND DIFFERENT TREATMENT GROUPS

Group (n=06/group)	Day 0	Day 21 post AI
I	6.46±1.82	6.32±0.56
II	6.66±1.60	7.75±1.60
III	7.80±0.54	6.86±0.42
IV	7.15±0.43	6.24±0.52

Mean serum phosphorus concentration did not vary significantly between day 0 and day 21 post AI in different groups. The values of mean serum phosphorus concentration were within normal physiological range on day 0 and day 21 post insemination. Non-significant variation was observed for mean serum phosphorus concentration between different treatment groups on 0 and day 21 post insemination.

In contrary to the present findings, the mean serum phosphorus concentration in normal cyclic and postpartum anoestrus buffaloes has been recorded to be 5.29±0.28 mg/dl and 3.24±0.03 mg/dl (Hafez, 2019). The phosphorus concentration in present study was found to be higher as compared to the Hafez, 2019 and Saikiran et al., 2020. Similar non-significant variations were observed with lower concentration of serum phosphorus in the study of Kumar et al. (2015b) in which no significant difference in the concentration of phosphorus between postpartum anoestrus and oestrus buffaloes. Kumar et al. (2015b) recorded serum phosphorus values of 4.00±0.20 and 4.12±0.36 mg/dl in oestrus and anoestrus water buffaloes.

MEAN SERUM MAGNESIUM CONCENTRATION

Magnesium usually does not have direct impact on the reproductive status of animals, since in body it remains in almost antagonistic relation with calcium and any disturbance in Ca-P-Mg homeostasis can impart some influence on reproduction. Moreover, reduced reproductive efficiency encountered is due to loss of appetite as a result of magnesium deficiency (Kumar, 2003).

The results of mean serum magnesium (mg/dl) concentration on day 0 of the treatment and day 21 post AI observed in different treatment groups are summarized in table 5.

Table 5: MEAN SERUM MAGNESIUM CONCENTRATION (MG/DL) IN CONTROL AND DIFFERENT TREATMENT GROUPS

Group	Day 0	Day 21 post AI

(n=06/group)		
I	4.17±0.24	4.01±0.31
II	4.11±0.77	3.91±0.56
III	3.68±0.27	4.53±0.29
IV	3.73±0.38	3.70±0.32

Non-significant ($p \geq 0.05$) variation was observed in mean serum magnesium (mg/dl) concentration on day 0 of the treatment and day 21 post AI within the groups. Similarly, non-significant ($p \geq 0.05$) variation in mean serum magnesium (mg/dl) concentration on day 0 of the treatment and day 21 post AI was observed between the groups.

Similar trend was reported by Savalia et al. (2013) in Ovsynch and CIDR groups. The present non-significant differences observed in mean serum magnesium concentration on day 0 and day 21 post AI corroborated with the reports of Sharma et al. (1999) in Murrah buffaloes, Paul et al. (2000) Nilli-Ravi buffaloes and Butani et al. (2011) in Surti buffaloes. Latif et al. (1993) and Newer et al. (1999) reported the non- significant difference in the mean plasma magnesium levels between cyclic and anoestrus animals in Mehsana and Swamp buffaloes, respectively. The imbalance of other minerals such as calcium, phosphorus and potassium may decrease magnesium absorption from the gut of ruminants.

MEAN SERUM TOTAL PROTEIN CONCENTRATION

Ideal protein level is vital for improvement of endocrine and sex organs. The diminished protein has antagonistic impact on reproduction is through pituitary and sex organs (Patel et al. 2018). The results of mean serum total protein (g/dl) concentration on day 0 of the treatment and day 21 post AI observed in different treatment groups are summarized in table 6.

Table 6: MEAN SERUM TOTAL PROTEIN CONCENTRATION (G/DL) IN CONTROL AND DIFFERENT TREATMENT GROUPS

Group (n=06/group)	Day 0	Day 21 post AI
I	5.55±0.51	6.36±0.49
II	5.45±0.71	5.75±1.09
III	5.66±0.91	5.53±0.71

IV	7.16±0.74	6.94±0.43

Non-significant (p≥0.05) variation in mean serum total protein (g/dl) concentration on day 0 of the treatment and day 21 post AI was observed within groups I, II, III and IV. Non-significant (p≥0.05) variation in mean serum total protein (g/dl) concentration on day 0 of the treatment and day 21 post AI was observed between groups I, II, III and IV.

In the current study, the mean serum total protein concentration of anoestrus buffaloes was in agreement with the findings of Parmar et al. (2015) and Kalasariya et al. (2017) in buffaloes. Depending upon the feed intake of the animal the serum protein levels change with different phases of reproduction. Protein deficiency retarded the reproductive organs improvement and was regarded as a factor responsible of the postponement in beginning or failure of postpartum estrus (Patel et al., 2018).

MEAN SERUM CHOLESTEROL CONCENTRATION

Cholesterol is incorporated from acetic acid derivation with a progression of intermediate substances. It is a critical predecessor of the ovary, testis and adrenal cortex steroid hormone synthesis (Sesh and Meur, 2013). The results of mean serum cholesterol (mg/dl) concentration on day 0 of the treatment and day 21 post AI observed in different treatment groups are summarized in table 7.

Table 7: MEAN SERUM CHOLESTEROL CONCENTRATION (MG/DL) IN CONTROL AND DIFFERENT TREATMENT GROUPS

Group (n=06/group)	Day 0	Day 21post AI
I	131.47±8.38	153.92^{A}±9.03
II	101.10±5.55	115.31^{AB}±11.67
III	108.64±16.57	99.15^{B}±8.81
IV	107.71±7.25	131.06^{AB}±19.23

Mean values bearing different superscripts (A, B) in a column differ significantly ($p<0.05$)

The mean serum cholesterol (mg/dl) concentration values observed on day 0 were within normal physiological range of serum cholesterol. Mean serum cholesterol increased non-significantly on day 21 post insemination as compared to day 0 in all the treatment groups except in group III in which concentration decreased non-significantly between day 0 and day 21 post insemination. The variation in mean serum cholesterol concentration was within normal physiological range.

Similarly non-significant ($p \geq 0.05$) variation was observed on day 21 between group I and II, I and IV, II and III, II and IV and III and IV while this variation was significant ($p < 0.05$) between group I and III. The variation in mean serum cholesterol concentration was within normal physiological range.

The mean serum cholesterol concentration in normal cyclic and postpartum anoestrus buffaloes has been recorded to be 138.78±9.73 mg/dl and 76.47±4.82 mg/dl (Hafez, 2019). High cholesterol level in normal cyclic animals compared to anoestrus ones might be a sign of improved steroid secretion due to increased ovarian activity (Sesh and Meur, 2013). Cholesterol acts as a precursor for the formation of steroid hormones in theca and luteal cells in the ovarian. The mechanism by which estrogen affect the interrelationships of pituitary-thyroid-adrenal functions is by affecting the carbohydrate metabolism that in turn increase the production of cholesterol in endocrine gland tissue from acetate, and that explains the increase in serum cholesterol during estrus (Yadav et al., 2006).

CONCEPTION RATE

The conception rates obtained in postpartum anestrus buffaloes after artificial insemination are depicted in table 8.

Table 8. CONCEPTION RATES IN CONTROL AND DIFFERENT TREATMENT GROUPS IN POSTPARTUM ANESTRUS BUFFALOES

Groups (N=06/Group)	Conception rate (per cent)
I (CIDR+Ovsynch)	50 (3)
II (CIDR+Cosynch)	33.33 (2)
III (eCG+Cosynch)	66.67 (4)
IV (Control)	33.33 (2)

Figures in parenthesis indicate number of animals

In the present study, the conception rate is quite similar to Kalwar et al. (2015) and Baruselli et al. (2007) who reported 52.94 per cent and 57.5 per cent conception rate in anestrus buffaloes when treated with CIDR plus Ovsynch protocol. However, comparatively lower conception rate has been reported by Ravikumar et al. (2008) and Azawi et al. (2012) i.e. 18.18 per cent and 26.40 per cent in postpartum anestrus buffaloes, respectively. A very low conception rate of 4.70 per cent has also been reported by De-Rensis et al. (2005) after synchronized ovulation with Ovsynch in non-cyclic buffaloes but conception rates were significantly increased to 30 per cent when CIDR was also combined with Ovsynch protocol. Comparatively higher conception rates were reported by Naikoo et al. (2010) in Mehsana buffaloes (83.33 per cent), Kumar et al. (2015a) in postpartum anestrus buffaloes (66.66 per cent) and Vikash et al. (2014) in anestrus buffaloes (74.43 per cent) in Haryana.

A variety of progestational compounds have been administered (Malik 2005) to mimic the luteal function by blocking the release of gonadotrophins from pituitary, so that subsequent withdrawal of these compounds may result in release of gonadotrophins to initiate follicular activity in ovaries with establishment of estrus cycles.

In CIDR+Cosynch protocol (group II), the conception rate was 33.33 per cent. In similar experiments, Patil et al. (2020) recorded conception rates of 53.35, 19.34 and 10.58 per cent for buffaloes in first, second and third oestrous cycles respectively, achieving overall conception rate of 62.89 per cent which was quite higher than that reported in group II in present study.

Since buffaloes have a problem with delayed ovulation relative to cattle, there is currently very little information available about the effective timing of concurrent A.I./GnRH in CIDR Co-synch protocol in buffaloes. The best time of TAI in Nili Ravi heifers using CIDR Co-synch (Haider et al. 2021) was 84 hours (after CIDR removal), yielding 65 per cent pregnancy rates and these results were in close agreement with the results of other studies in buffalo (Kumar et al. 2016b).

High progesterone near at the time of fixed time artificial insemination also leads to decreased fertility (Wiltbank et al. 2012) as high progesterone produces incompetent oocyte whereby poor fertilization or poor embryo quality production and finally reduced pregnancy which could had happened with present study. Hence, in such researches hormonal assay of progesterone, follicle stimulating hormone, estradiol and luteinizing hormone are also required to correlate and interpret the results in a more precise way. Besides, conception is governed by many factors like semen quality, maternal recognition of pregnancy and early embryonic deaths etc. which were beyond the objective of this study.

In present study, conception rate in eCG+Cosynch protocol (group III) was 66.67 per cent. Similar results were obtained by Dhaka et al. (2019) in which they evaluated efficacy of Co-synch Plus eCG and Co-synch+CIDR+eCG Protocol yielding the conception rate of 69.20 and 86.60 per cent in Murrah buffaloes.

Lower conception rates were obtained with addition of eCG in different CIDR based synchronization protocol. Murugavel et al. (2009) in their study in postpartum Nilli-Ravi buffaloes using eCG+CIDR and CIDR yielded pregnancy rate of 40.60 and 27.30 per cent, respectively. While, Naseer et al. (2013) in postpartum anestrus buffaloes reported conception rate of 50.00 and 60.00 per cent using eCG alone and eCG+CIDr protocol.

Kumar et al. (2016b) evaluated the efficiency of three different protocols for estrus induction and conception rate in postpartum anestrus buffaloes and reported that cosynch protocol with addition of eCG results comparatively better pregnancy rate. They reported estrus induction response of 93.7 per cent, conception rate of 53.3 and 75 per cent at subsequent estrus.

The combined effect of progesterone priming of the brain for estradiol receptors and high endogenous estradiol with eCG administration is mainly responsible for behavioural signs. Similarly, conception rate was better in Group III following Cosynch-plus treatment as eCG

injection 3 days prior to first GnRH injection ensured sufficient follicle diameter (Malik 2012) which was essential for better conception rate.

CONCLUSION

It can be concluded that CIDR+Ovsynch protocol was found to be better as compared to CIDR+Cosynch in buffaloes suffering from postpartum anestrus. The addition of eCG to a GnRH and prostaglandin based protocol substantially improved the conception rates in postpartum anestrus buffaloes.

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REFERENCES

- Azawi, O.I., Ali, M.D., Oday, S.A., Salih, A., Al-Hadad, A.S. and Mouayad, S.J. (2012). Comparative efficacy of different CIDR protocols for the treatment of postpartum anestrous in Iraqi buffaloes. Veterinary World, 5(4): 201-205.
- Baruselli, P.S., Carvalho, N.A.T., Gimenes, L.U. and Crepaldi, G.A. (2007). Fixed-time artificial insemination in buffalo. Italian Journal of Animal Science, 6:107-118.
- Baruselli, P.S., Sa Filho, M.F., Ferreira, R.M., Sales, J.N.S., Gimenes, L.U., Vieira, L.M., Mendanha, M.F. and Bo, G.A.(2012). Manipulation of follicle development to ensure optimal oocyte quality and conception rates in cattle. Reproduction in Domestic Animals, 47:134-141.
- Butani, M.G., Dhami, A.J. and Kumar, R. (2011. Comparative blood profile of progesterone, metabolites and minerals in anestrus, suboestrus, repeat breeding and normal cyclic buffaloes. Indian Journal of Field Veterinarians, 7(2): 20-24.
- De Rensis, F., Ronci, G., Guarneri, P., Nguyen, B.X., Presicce, G.A., Huszenicza, G. and Scaramuzzi, R.J. (2005). Conception rate after fixed time insemination following Ovsynch protocol with and without progesterone supplementation in cyclic and non-cyclic Mediterranean Italian buffaloes (Bubalus bubalis). Theriogenology,63(7): 1824-1831.
- Dhaka, A.P., Phogat, J.B., Singh, S., Pandey, A.K., Sharma, K. and Kumari, S. (2019). Efficacy of modified co-synch plus protocol with or without progesterone device for estrus induction and conception rate in Murrah buffaloes under field conditions during summer season. Buffalo Bulletin, 38(2): 353-361.
- Hafez, M.H. (2019). Serum hormonal, metabolic and minerals profile in normal cyclic and postpartum anestrus egyptian buffaloes. Alexandria Journal for Veterinary Sciences, 60(2): 102-108.

- Haider, S., Chishti, G.A., Mehmood, M.U., Jamal, M.A., Mehmood, K., Shahzad, M. and Tahir, M.Z. (2021). The effect of GnRH administration/insemination time on follicular growth rate, ovulation intervals, and conception rate of Nili Ravi buffalo heifers in 7–day-CIDR Co-synch. Tropical Animal Health and Production, 53(6): 1-7.
- Kalasariya, R.M., Dhami, A.J., Hadiya, K.K., Borkhatariya, D.N. and Patel, J.A. (2017). Effect of peripartum nutritional management on plasma profile of steroid hormones, metabolites, and postpartum fertility in buffaloes. Veterinary World, 10(3): 302-310.
- Kalwar, Q., Ahmed Memon, A., Bhutto, M.B., Kunbhar, H.K., Mirani, A.H., Anwar, M. and AhmedWagan, S. (2015). Estrus response and fertility rate in Kundhi buffaloes following estrus synchronization in breeding season. Journal of Advanced Veterinary and Animal Research, 2(3): 362-365.
- Kumar, A., Sharma, U., Pandey, A.K., Kumar, S. and Kumar, S. (2015a). Induction of ovarian cyclicity and conception rate in postpartum anestrus buffaloes treated with different hormonal protocols. Buffalo Bulletin, 34(4): 441-445.
- Kumar, L., Phogat, J.B., Pandey, A.K., Phulia, S.K., Kumar, S. and Dalal, J. (2016b). Estrus induction and fertility response following different treatment protocols in Murrah buffaloes under field conditions. Veterinary World, 9(12): 1466-1470.
- Kumar, P.R., Singh, S.K., Kharche, S.D., Govindaraju, C.S., Behera, B.K., Shukla, S.N., Kumar, H. and Agarwal, S.K. (2014). Anestrus in cattle and buffalo: Indian perspective. Advances in Animal Veteriary Science,2(3): 124-138.
- Kumar, R., Ghosh, M., Rawat, S.S., Kumar, P. and Kumar, S. (2016a). Plasma mineral profile of normal cyclic and postpartum anestrous murrah buffaloes in organized farms. Animal Science, 10(2): 12-14.
- Kumar, S. (2003). Management of infertility due to mineral deficiency in dairy animals. In: ICAR summer school on Advance Diagnostic Techniques and Therapeutic Approaches to Metabolic and Deficiency Diseases, Izatnagar, 15 july-14 august, pp 128.
- Kumar, S., Balhara, A.K., Kumar, R., Kumar, N., Buragohain, L., Baro, D., Sharma, R.K., Phulia, S.K. and Singh, I. (2015b). Hemato-biochemical and hormonal profiles in post-partum water buffaloes (Bubalus bubalis). Veterinary World, 8(4):512.
- Latif, A., Siddiquee, G.M. and Vadodaria, U.P. (1993). Biochemical studies in postpartum anestrus in Mehsana buffaloes. Indian Journal of Animal Reproduction, 14(1): 64.
- Malik, R.K. (2005). Studies on ovarian follicular dynamics during early postpartum period, anestrus condition and hormonal therapies for induction of estrus in Murrah buffaloes (Bubalus bubalis). Ph.D. thesis(Gynaecology and Obstetrics), Chaudhary Charan Singh Haryana Agricultural University, Hisar (India).

- Malik, S.K. (2012). "Ovsynch-plus" and a ''modified Ovsynch-plus" treatment of anestrous buffalo heifers during summer and winter seasons: study of ovarian activity and post-treatment fertility. Ph.D. thesis (Animal Reproduction, Gynaecology and Obstetrics), Lala Lajpat Rai University, Hisar.
- Mujawar, A.S., Razzaque, W.A.A., Ramteke, S.S., Patil, A.D., Ali, S.S., Bhikane, A.U., Khan, M.A. and Mogal, I.R. (2019). Estrus induction and fertility response in postpartum anestrus marathwadi buffaloes using hormonal protocol along with vitamin E and selenium. International Journal of Livestock Research, 9(3): 289-296.
- Murugavel, K., Antoine, D., Raju, M.S. and Lopez-Gatius, F. (2009). The effect of addition of equine chorionic gonadotropin to a progesterone based estrous synchronization protocol in buffaloes (Bubalis bubalis) under tropical conditions. Theriogenology, 71: 1120-1126.
- Naikoo, M., Patel, D.M., Sarvaiya, N.P. and Killader, A. (2010). Estrous synchronization in postpartum anestrous Mehsana buffaloes using different hormone protocols. Indian Journal of Field Veterinarians, 6(2): 1-4
- Naseer, Z., Ahmad, E., Ullah, N., Yaqoob, M. and Akbar, Z. (2013). Treatment of anestrous Nili–Ravi buffaloes using eCG and CIDR protocols. Asian Pacific Journal of Reproduction, 2(3): 215-217.
- Newer, S., Baruah, K.K., Baruah, A., Bhuyan, D. and Kalita, D.J. (1999). Studies on certain macromineral status in anestrus and cyclic postpartum swamp buffaloes. Indian Veterinary Journal, 76(7): 671-672.
- Parmar, S.C., Khasatiya, C.T., Chaudhary, J.K., Patel, R.V. and Dhamsaniya, H.B. (2015). Serum metabolic and minerals profile in norgestomet primed postpartum anestrous surti buffaloes. Veterinary World, 8(5): 625-630.
- Patel, A.J., Patel, J.A., Dhami, A.J., Prajapati, J.P. and Parmar, S.C. (2018). Estrus induction, fertility and biochemical profile in true anestrus surti buffalo following different estrus synchronization protocols. The Indian Journal of Animal Reproduction, 39(2): 36-39.
- Patil, S.K., Tandle, M.K., Bijurkar, R.G., Patil, N.A., Kumar, D., Kulkarni, S. and Naveen Kumar, S. (2020). Efficacy of Co-Synch Plus CIDR Oestrus Synchronization Protocol on Fertility in Anestrus Cows and Buffaloes. International Journal of Current Microbiology and Applied Sciences, 9(11): 3488-3495.
- Paul, S.S., Chawla, D.S. and Lall, D. (2000). Serum mineral profile and its relationship with reproductive disorders in Nili-Ravi buffaloes. The Indian Journal of Animal Nutrition, 17(4): 324-327.
- R Core Team. (2018). R: A language ad environment for statistical computing. Vienna: R Foundation for Statistical Computing.

- Ravikumar, K., Asokan, S.A. and Veerapandian, C. (2008). Effect of ovsynch plus CIDR protocol on fertility in postpartum subestrus and anestrus buffaloes. Indian Veterinary Journal, 85(2): 140-142.
- Saikiran, B.V.S., Vasantha, S.K.I., Nikhil, K.T.J., Lavanya, S., Chandra Prasad, B., Rama Goury, M. and Srinivasa Prasad, C.H. (2020). Assessment of hemato biochemical, mineral and oxidative changes in postpartum anestrous (true anoestrous) Murrah buffaloes. International Journal of Chemical Studies, 8(6): 29-33.
- Savalia, K.K., Dhami, A.J., Patel, K.R. and Hadiya, K.K. (2013). Influence of controlled breeding techniques on fertility and plasma macro-minerals profile in conceived and non-conceived anestrus and repeat breeding buffaloes. Indian Journal of Field Veterinarian,9(2): 28-35.
- Sesh, P.S.L. and Meur, S.K. (2013). Role of cholesterol and its fractions in buffalo ovarian follicular atresia. International Journal of Advanced Veterinary Science and Technology, 2: 35-46.
- Sharma, K.B., Nayyar, S., Malik, V.S., Singh, R. and Sodhi,S.P.S. (1999). Level of hormones and minerals in cyclic, anestrus and sub-oestrus buffalo heifers. International Journal of Animal Science,69(4): 214-216.
- Snedecor, G.W. and Cochran, W.G. (1994). Statistical Methods, 8th Edn., The lowa state university press, Ames, Lowa, USA, pp. 491.
- Vikash, V.M., Malik, R.K. and Singh, P. (2014). Impact of CIDR in combination with different hormones for treatment of anestrus in buffaloes under field conditions of Haryana. Haryana Vet, 53: 28-33.
- Wiltbank, M.C., Souza, A.H., Giordano, J.O., Nascimento, A.B., Vasconcelos, J.M., Pereira, M.H.C., Fricke, P.M., Surjus, R.S., Zinsly, F.C.S., Carvalho, P.D., Bender, R.W. and Sartori, R. (2012). Positive and negative effects of progesterone during timed AI protocols in lactating dairy cattle. Animal Reproduction,9(3): 231-241.
- Yadav, K.V.S., Ansari, M.R. and Kumaresan, A. (2006). Profile of macro, microelement, total protein and cholestrol in serum of cyclic and acyclic murrah buffaloes. The Indian Journal of Veterinary Research, 15(2): 10-13.