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Role of Various Estrus Induction and Synchronization Protocols in Influencing Plasma Progesterone, Metabolic Profile and Fertility in Acyclic Buffaloes

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Abstract

A study was carried out under field conditions on 50 acyclic/ anestrus buffaloes to evaluate the efficacy of four standard estrus synchronization protocols, viz., Doublesynch, Estradoublesynch, Ovsynch, and Ovsynch Plus (10 buffaloes in each protocol, and in untreated control group) in terms of estrus induction response, conception rates at induced estrus with FTAI and monitoring plasma progesterone, protein and cholesterol profile at different time intervals during treatment and day 12 post-AI. All the animals received pre-synchronization treatment, i.e., Inj. 100 mg ivermectin s/c, Inj. tono-vita 20 ml, and multi-minerals 1 bolus daily for 7 days. The conception rates obtained at induced estrus (FTAI) were 50.0, 40.0, 30.0 and 50.0 % with Doublesynch, Estradoublesynch, Ovsynch and Ovsynch Plus protocol, respectively. The rests were taken as non-conceived ones. The plasma progesterone concentrations monitored on day 0 (start of treatment), 7/9 ($PGF_2\alpha$ injection), 10/12 (FTAI) and on day 12 post-AI revealed significant ($p<0.01$) effect of sampling days in all four protocols with higher values on day of $PGF_2\alpha$ injection and on day 12 post-AI compared to other days. Moreover, the plasma progesterone concentrations were significantly ($p<0.05$) higher in conceiving than the non-conceiving buffaloes on day 12 post-AI in all 4 protocols. The mean plasma protein and cholesterol profile did not differ significantly between days in any of the protocols. The animals under Doublesynch protocol however had significantly higher protein values as compared to Ovsynch and Ovsynch Plus protocols. Moreover, the non-conceiving buffaloes under Ovsynch Plus protocol had significantly ($p<0.05$) lower mean plasma protein (5.73±0.15 vs. 6.49±0.13 g/dl) and cholesterol (57.42±1.19 vs. 76.68±1.85 mg/dl) concentrations compared to their counterparts. It was concluded that all four hormonal protocols improved plasma progesterone profile and conception rates in acyclic buffaloes without altering plasma protein and cholesterol profile. The maximal benefit was with Doublesynch and Ovsynch plus protocols, hence these can be practiced under field conditions to manage acyclic buffaloes.

Introduction

Anestrus is one of the most important reproductive disorders in buffaloes devastating the economy of dairy industry in India and Asia

(El-Wishy, 2007). The results of measures adopted to induce and synchronize estrus in such animals, though variable, are encouraging (Baruselli and Carvalho, 2005; Patel *et al.,* 2018) and could be the solution to overcome the difficulty of estrus detection in buffaloes. Among the causes, nutritional and endocrine status appears most significant (Sahoo *et al.,* 2017; Patel *et al.,* 2018). Progesterone hormone is responsible for stimulation of cyclicity, follicular development and also for continuation of pregnancy. The energy status and macro-micro nutrients synergistically potentiate these physiological events and response to therapy. Estimation of plasma progesterone levels denote either the presence or absence of CL and its functional competency which is directly related with fertility of the female. The females suffering from either irregular cycling - silent/ poor/weak heats or acyclicity will be having low or irregular levels of progesterone unless evoked by endogenous or exogenous hormones. The plasma progesterone assay is useful in determining ovarian dynamics, i.e. the detection of estrus, subestrus, anestrus, anovulation, early embryonic mortality and pregnancy, while plasma proteins and cholesterol are building blocks of muscle mass and precursors of hormones (Mungad *et al.,* 2016), and thereby throws some light on nutritional status and fertility criteria. Hence, this study was designed to evaluate therapeutic efficacy of four estrus induction protocols by monitoring estrus response, conception rate and progesteron, plasma protein and cholesterol in anestrus buffaloes under field condition.

Materials and Methods

This investigation was carried out during August 2016 to February 2017 on animals under field conditions of Anand as well as Mahisagar districts of Gujarat. Fifty postpartum (>90 days) acyclic/anestrus buffaloes that were confirmed on the basis of smooth small inactive ovaries and atonic uterus on per rectal palpations twice 10 days apart were included in the study. All infertile animals identified received Inj. Ivermectin 100 mg s/c and injection organic phosphorus (Inj. Tonophosphan, MSD Animal Health) and multivitamins AD_3E (Inj. Intavita-H, Intas Pharma) 10 ml each i/m and multi-minerals (Minotas, Intas Pharma) 1 bolus daily for 7 days. They were then randomly divided into 5 equal groups (n=10 each) and were subjected to the four standard estrus induction/ synchronization protocols, viz., Ovsynch, Ovsynch plus, Doublesynch and Estradoublesynch protocols, including blood sampling, keeping one group as untreated control as described earlier (Prajapati *et al.,* 2018).

Animals inseminated at induced/spontaneous estrus if not returned to estrus were followed for pregnancy per-rectum 60 days post-AI. The plasma progesterone concentration was determined by employing standard Radio-Immuno-Assay (RIA) technique. Labelled antigen (I^{125}), antibody coated tubes and standards were procured from Immunotech-SAS, France. The plasma total protein and total cholesterol concentrations were determined by Biuret method and CHOD/PAP method, respectively, using standard procedure and assay kits procured from Crest Bio-system, Goa with the help of chemistry analyzer (Mindray, BS 120, China).

Statistical Analysis:

The data on plasma profiles were analyzed statistically using ANOVA, DMRT and t-test on IBM SPSS 20.00 statistics to know the variations between sampling days, groups and conceived/ non-conceived status. The animals found pregnant at 60 days post-FTAI were taken as conceived and rests as non-conceived, and their blood profile was compared retrospectively to evaluate whether it has any role in estrus response and conception following different protocols.

Results and Discussion

Conception rates at synchronized estrus/ FTAI

Out of 10 anestrus buffaloes each subjected to Doublesynch, Estradoublesynch, Ovsynch and Ovsynch Plus protocols, 90-100 % buffaloes exhibited behavioural estrus within mean 48 to 76 h from $PGF_2\alpha$ injection. The estrus induction rate of 90 % achieved with Doublesynch and Estradoublesynch protocols was in harmony with the earlier results of Miramahmoudi and Prakash (2012), Miramahmoudi *et al.* (2014) and Patel *et al.* (2018); however Parida *et al.* (2015) found lower (70 and 83 %) response using same protocols in acyclic buffaloes. The estrus synchronization rate of 100 % achieved with

Ovsynch protocol was in harmony with the earlier results of Nakrani *et al.* (2014[a]), Mungad *et al.* (2016) and Savani *et al.* (2017) in acyclic buffaloes. The estrus induction rate of 90 % achieved with Ovsynch Plus protocol coincided well with the result of 93.7 to 100 % reported by Kumar *et al.* (2016[a]) in anestrus buffaloes.

The conception rates obtained at induced estrus/FTAI in acyclic buffaloes following Doublesynch, Estradoublesynch, Ovsynch and Ovsynch Plus protocols were 50, 40, 30 and 50 %, respectively. In control group only two buffaloes (20 %) exhibited estrus after 28 and 52 days of initiation of experiment and conceived. The present conception rate of 50 % obtained in anestrus buffaloes at induced estrus with Doublesynch protocol concurred well with earlier reports of Mirmahmoudi and Prakash (2012) and Kumar *et al.* (2016[b]) as 55 and 48 %, respectively. The conception rate of 40 % obtained at induced estrus with Estradoublesynch protocol was quite lower than the earlier results of 60-64 % obtained by Mirmahmoudi *et al.* (2014) in anestrus buffaloes. Patel *et al.* (2018) however found only 27.27% conception rate at induced estrus with both these protocols in anestrus Surti buffaloes. The 30 % conception rate obtained at induced estrus with Ovsynch protocol was quite similar with earlier results of 29.41 to 37.50 % reported by Ali and Fahamy (2007) and Buhecha *et al.* (2016[a]), but lower than 41.67 to 66.66 % reported by others (Nakrani *et al.,* 2014[a]; Mungad *et al.,* 2016; Savani *et al.,* 2017) in anestrus buffaloes. The conception rate of 50 % obtained with Ovsynch plus protocol at induced estrus closely concurred with the earlier report of 53.30 % by Kumar *et al.* (2016[b]) in anestrus buffaloes, but was higher than 28.00 % reported by Rathore *et al.* (2015). Present results reflect that additional injection of $PGF_2\alpha$ or PMSG 2 days before standard Ovsynch protocol, though did not influence the estrus response, has definite advantage of improving conception rates in anestrus buffaloes.

Plasma Progesterone Profile

The mean plasma progesterone (ng/ml) concentrations were low towards basal values (<0.5 ng/ml) in acyclic buffaloes on day 0, i.e., on the day of initiation of treatment in all four protocols. The levels rose significantly ($p<0.01$) to the peak values on day 7/9, i.e., just before $PGF_2\alpha$ injection in buffaloes of all four protocols. Thereafter the levels dropped suddenly and significantly within 48 h to the basal values coincident to induced estrus, when FTAIs were done. These levels again increased significantly ($p<0.01$) on day 12 post-AI in all the groups due to estruses being ovulatory with development and maintenance of CL and establishment of pregnancy in some of these animals. Thus, significantly ($p<0.05$) higher plasma progesterone concentrations were recorded on day 9 of treatment and day 12 post-AI as compared to day 0 and day of FTAI in all the protocols (Table 1). The trend and levels of plasma progesterone observed following doublesynch and estradoublesynch protocols closely concurred with Mirmahmoudi *et al*. (2014) and Patel *et al.* (2018).

The rise in mean plasma progesterone concentrations noted in acyclic buffaloes under all four protocols on day 7/9 over initial values might be due to luteinization of some of the growing/dominant follicles and/or ovulation of dominant follicle and formation of CL, under the influence of first GnRH injection, simulating diestrum phase. The sudden drop in plasma progesterone on the day of FTAI in all the groups could be attributed to luteolytic effect of $PGF_2\alpha$ injection given 2 days before, on the luteinized follicles and/or CLs, if any, with emergence of next wave of follicles. Moreover, second GnRH injection as such or the exogenous estradiol benzoate used on day 10 in Estradoublesynch protocol, probably helped by positive feedback effect on pituitary and hypothalamus in triggering the ovulatory LH surge and thereby better synchronized ovulation timing in treated animals (Mirmahmoudi *et al.,* 2014).

Further among different periods, the mean plasma progesterone concentrations differed significantly ($p<0.05$) between conceiving and non-conceiving buffaloes only on day 12 post-AI, being higher in conceiving ones in all the four protocols (Table 1). The present findings on plasma progesterone profile in acyclic buffaloes with respect to different protocols closely corroborated with the observations of Mirmahmoudi and Praklash (2012), Savalia *et al.* (2014), Parmar *et al.* (2015), Buhecha *et al.*

Table 1: Plasma progesterone concentrations (ng/ml) in anestrus buffaloes during different days of various estrus synchronization protocols and on day 12 post-AI

Estrus induction protocol	Status	No.	Plasma progesterone on days from treatment/AI			
			D-0	Day of $PGF_2\alpha$ Inj.	Day FTAI	D-12 post-AI
Doublesynch	Conceived	5	0.26±0.05	2.50±0.54	0.12±0.02	4.92±0.58^{y}
	Non-concd	5	0.45±0.11	1.12±0.26	0.40±0.27	1.94±0.75^{x}
	Overall	**10**	**0.36±0.06^{p}**	**1.81±0.36^{q}**	**0.26±0.14^{p}**	**3.43±0.66^{r}**
Estra-doublesynch	Conceived	4	0.51±0.13	2.52±0.59	0.39±0.05	4.39±0.47^{y}
	Non-concd	6	0.37±0.12	1.24±0.42	0.14±0.02	2.43±0.64^{x}
	Overall	**10**	**0.42±0.09^{p}**	**1.75±0.38^{q}**	**0.24±0.04^{p}**	**3.21±0.51^{r}**
Ovsynch	Conceived	3	0.10±0.06	3.13±0.37	0.26±0.08	5.83±0.76^{y}
	Non-concd	7	0.24±0.74	1.87±0.58	0.45±0.23	3.06±0.69^{x}
	Overall	**10**	**0.20±0.05^{p}**	**2.25±0.45^{q}**	**0.39±0.16^{p}**	**3.89±0.66^{r}**
Ovsynch plus	Conceived	5	0.69±0.25	2.24±0.45	0.33±0.08	5.28±0.44^{y}
	Non-concd	5	0.14±0.02	2.30±0.66	0.22±0.07	3.00±0.62^{x}
	Overall	**10**	**0.42±0.15^{p}**	**2.27±0.37^{q}**	**0.28±0.05^{p}**	**4.14±0.52^{r}**

D-0 = Starting the treatment. Means of protocol bearing uncommon superscripts within the row (p,q,r) and column (a,b) and conceived & non-conceived (x,y) subgroups differ significantly (p<0.05).

Table 2: Plasma total protein concentrations (g/dl) in anestrus buffaloes during different days of various estrus synchronization protocols and on day 12 post-AI

Estrus induction protocol	Status	No.	Plasma protein on days from treatment/AI			
			D-0	Day of $PGF_2\alpha$ Inj	Day FTAI	D-12 post-AI
Doublesynch	Conceived	5	6.75±0.08	6.69±0.31	6.92±0.28	6.53±0.15
	Non-concd	5	6.55±0.21	6.66±0.16	6.66±0.21	6.50±0.27
	Overall	**10**	**6.65±0.11^{c}**	**6.68±0.16**	**6.79±0.17^{b}**	**6.51±0.14**
Estra-doublesynch	Conceived	4	5.94±0.10	6.46±0.37	6.43±0.15	6.46±0.37
	Non-concd	6	5.98±0.17	6.17±0.27	6.37±0.26	6.48±0.20
	Overall	**10**	**5.97±0.10^{ab}**	**6.29±0.21**	**6.39±0.16^{ab}**	**6.47±0.18**
Ovsynch	Conceived	3	6.11±0.40	6.33±0.20	6.44±0.33	6.92±0.29
	Non-concd	7	6.41±0.13	6.51±0.13	6.53±0.19	6.50±0.09
	Overall	**10**	**6.32±0.14^{bc}**	**6.45±0.11**	**6.50±0.16^{ab}**	**6.63±0.11**
Ovsynch plus	Conceived	5	6.20±0.32^{y}	6.76±0.27^{y}	6.27±0.10	6.74±0.26
	Non-concd	5	5.27±0.19^{x}	5.66±0.18^{x}	5.91±0.38	6.08±0.35
	Overall	**10**	**5.73±0.23^{a}**	**6.21±0.24**	**6.09±0.19^{a}**	**6.41±0.23**

D-0 = Starting the treatment. Means of protocol bearing uncommon superscripts within the column (a,b,c) and conceived & non-conceived (x,y) subgroups differ significantly (p<0.05).

(2016[a]) and Patel *et al.* (2018) in anestrus buffaloes following CIDR, Doublesynch, Estradoublesynch, and Ovsynch protocols. They all found significantly (P<0.05) higher plasma progesterone (ng/ml) concentrations on day 7 for CIDR and/or Ovsynch protocols as compared

to the corresponding values obtained on day of initiation of treatment, on day of induced estrus, and even on day 20/21 post-AI, with higher ($p<0.05$) mean values only on day 12 or 20/21 post-AI in conceived than the non-conceived animals.

Plasma Total Protein Profile

The plasma protein profile neither varied significantly between days in any of the protocols nor between the protocols. It was little higher in conceived than non-conceived buffaloes in Doublesynch, Estradoubesynch and Ovsynch protocols. However, on day 0, the mean plasma protein concentrations observed in anestrus buffaloes under Doublesynch and Estradoublesynch protocols were significantly lower ($p<0.05$) as compared to observations of Sahoo *et al.* (2017), who reported the plasma protein values as 8.27±0.28 and 7.18±0.75 g/dl, respectively for these two protocols.

In Ovsynch Plus protocol, the conceiving buffaloes had consistently higher mean plasma total protein concentrations as compared to non-conceiving ones with significant ($p<0.05$) differences on day 0 and on day 10 only, probably due to variation in individual animal's nutritional status. However, no such differences were noted in other groups. These observations concurred with Prajapati *et al.* (2018) in crossbred cattle under same protocols. Ali *et al.* (2012), Savalia *et al.* (2014), Nakrani *et al*. (2014[b]), Mungad *et al*. (2016) and Patel *et al.* (2018) also reported non-significant variations in the mean plasma total protein concentrations in buffaloes between days (day 0, 7, 9/10 of treatment and day 21 post-AI) or between conceived and non-conceived subgroups under different protocols, viz., Ovsynch, Doublesynch, Triu-B/CIDR and Crestar.

Plasma Total Cholesterol Profile

The plasma total cholesterol profile also neither varied significantly between sampling days in any of the protocol/group nor between the protocols. The conceiving buffaloes had significantly ($p<0.05$) higher mean plasma total cholesterol concentrations as compared to non-conceiving ones in Ovsynch Plus group on all four days, but no such trend was seen in other protocols (Table 3).

The mean cholesterol concentrations observed on day 0 in anestrus buffaloes under Doublesynch and Estradoublesynch protocols

Table 3: Plasma total cholesterol concentrations (mg/dl) in anestrus buffaloes during different days of various estrus induction/ synchronization protocol and on day 12 post-AI

Estrus induction protocol	Status	No.	Plasma cholesterol on days from treatment/AI			
			D-0	Day of $PGF_2\alpha$ Inj	Day FTAI	D-12 post-AI
Doublesynch	Conceived	5	74.78±4.18	76.17±3.67	71.96±4.31	74.93±4.35
	Non-concd	5	68.68±5.55	77.33±3.00	72.47±4.57	68.72±7.70
	Overall	**10**	**71.73±3.43**	**76.75±2.24**	**72.21±2.96**	**71.83±4.29**
Estra-doublesynch	Conceived	4	71.22±3.36	65.57±3.00	66.07±5.12	75.52±4.19
	Non-concd	6	69.64±5.52	69.54±3.46	74.33±5.16	76.43±5.26
	Overall	**10**	**70.27±3.42**	**67.95±2.37**	**71.03±3.76**	**76.07±3.40**
Ovsynch	Conceived	3	79.30±6.67	72.44±6.11	79.46±7.26	82.66±6.16
	Non-concd	7	69.70±4.58	73.97±4.18	68.77±6.45	74.42±5.17
	Overall	**10**	**72.58±3.86**	**73.51±3.27**	**71.98±5.06**	**76.90±4.07**
Ovsynch plus	Conceived	5	$77.14±4.12^{y}$	$78.66±2.98^{y}$	$76.20±3.93^{y}$	$74.74±4.67^{y}$
	Non-concd	5	$55.20±3.14^{x}$	$58.31±1.76^{x}$	$59.77±2.37^{x}$	$56.40±2.33^{x}$
	Overall	**10**	**66.17±4.39**	**68.49±3.76**	**67.99±3.49**	**65.57±3.92**

D-0 = Starting the treatment. Means between periods or protocols did not differ significantly ($p>0.05$). Means with superscripts (x,y) differ between conceived & non-conceived status.

were relatively similar to the report of Sahoo *et al.* (2017) and Patel *et al.* (2018). The later authors further noted significant increase in values during luteal phase as compared to anestrus phase in animals under both the protocols. Savalia *et al.* (2014) recorded higher values of plasma total cholesterol in non-conceived and conceived anestrus buffaloes treated with CIDR protocol, but not with Ovsynch protocol. Like present findings. Parmar *et al.* (2015) and Nakrani *et al*. (2014[b]) also reported non-significant effect of periods and treatment protocols on plasma total cholesterol profile in anestrus buffaloes. As compared to present study, relatively higher or lower plasma total cholesterol levels obtained by some of these workers may be due to difference in the season, nutritional and health status of animals and the assay kits and procedures followed. Cholesterol the most important sterol is synthesized from acetate and it is an essential precursor for steroid hormone synthesis in the body. The higher cholesterol level in the cycling animals is indicative of more secretion of steroids during estrus due to increased ovarian activity. However, in the present study no such trend was seen in plasma cholesterol profile.

Conclusion

Based on the results, it was concluded that all four hormonal protocols, viz., Doublesynch, Estradoublesynch, Ovsynch, and Ovsynch Plus improved plasma progesterone profile and conception rates in acyclic buffaloes without altering plasma protein and cholesterol profile. The maximal benefit was with Doublesynch and Ovsynch plus protocols; hence these can be practiced under field conditions to manage acyclic buffaloes.

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Conflict of Interest:

All authors express no conflict of interest.

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