

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.710.112>

Studies on Biochemical and Mineral Profile of Anestrus Murrah Buffaloes Treated with Different Hormonal Protocols

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ABSTRACT

Keywords

Biochemical, Mineral profile, Anestrus Murrah buffaloes, Hormonal protocols

Article Info

Accepted:

10 September 2018

Available Online:

10 October 2018

Eighteen anestrus Murrah buffaloes were randomly selected and equally allotted to three groups. Animals were subjected to treatment with CIDR in group I and Ovsynch protocol in group II. Rest of the six animals was kept in control group. The mean value of cholesterol was found significantly higher ($P < 0.05$) in conceived animals of CIDR group in comparison with animals conceived in Ovsynch group. The mean cholesterol value was found non-significantly higher in Ovsynch group in comparison with control non-conceived animals. Likewise, mineral estimation from serum like calcium, phosphorous, magnesium and copper, no significant difference was observed within the groups and in between the groups on different days of sampling.

Introduction

Alterations in various biochemical constituents are responsible for reproductive failures (Prabha *et al.*, 2000). Cholesterol is the most important sterol and is the essential precursor for steroid hormone synthesis controlling reproduction. Minerals also play an important role in regulation of reproduction and production in animals. Lower concentration of circulatory minerals mainly Ca, P and Mg results in impaired reproductive function leading to cessation of cyclic activity (Martson *et al.*, 1972). Micronutrients also play a vital role in the control of reproduction.

The minerals like calcium and phosphorous are very important in animal production and reproduction. Calcium and phosphorous play intermediate role in the action of reproductive hormones and enzymes at a sub cellular level in an integrated fashion in the initiation of estrus in animals (Ali *et al.*, 1991). Rogers (1992) opined that the imbalance or deficiency of trace elements (Zn, Cu, etc.) leads to inactive ovaries and repeat breeding in dairy animals.

This study was planned to study the biochemical and mineral changes due to two different hormonal treatment protocols in anestrus Murrah buffaloes.

Materials and Methods

The study was conducted on Eighteen Indian buffaloes (age: 3-6 years) reared at Instructional dairy farm, Ranchi Veterinary College, Kanke and local khatals near Kanke, Ranchi. Animals with smooth and quiescent ovaries were identified. The selected animals were allotted to 3 groups consisting of 6 animals in each group and subjected to the therapeutic management as per the following protocol.

Group-I: CIDR (controlled internal drug release) Protocol (n=6), an intra-vaginal device (0.96g hydroxyprogesterone in elastic rubber molded over a nylon spine) was inserted into vagina with the help of the applicator and was kept in situ for 7 days. The CIDR device was removed on 7th day and 2 ml of PGF2 α (Cloprostenol I.P 263 μ g) was injected I.M. on the 7th day after removal of CIDR device. Fixed Time Artificial Insemination (FTAI) was done with frozen thawed semen 48 hrs after the withdrawal of the device.

Group-II: Ovsynch Protocol (n=6), The animals of this group was treated under Ovsynch protocol where 5 ml GnRH analogue (Buserelin IP – 0.004 mg) was injected I.M to each animal on day 0 followed by 2 ml PGF2 α analogue (Cloprostenol IP 263 μ g) on day 7th, again on day 9th 5 ml of GnRH analogue was injected and all the animals were inseminated with frozen semen after 24 hrs (FTAI) on 10th day.

Group-III: Anestrus Control (n=6) six normal but acyclic buffaloes each were injected with normal saline; 5 ml, i/m on days 0 and 9. They were followed for onset of natural estrus and insemination. Specimen numbers of hormonally treated true anestrus buffaloes were studied for their nutritional status. For this, jugular blood samples approximately 5ml were collected in slanting test-tubes four times

from true anestrus buffaloes among groups. i.e., on day 0 – just before start of treatment, on day 7 just before PGF2 α administration, on day 9/10 (day of A.I) and day 60th post treatment. The serum samples were centrifuged at 2500 rpm for 15 minutes, and serum separated out was transferred to 4.5 ml storage vials and stored in deep freeze at -20°C with a drop of Merthiolate (0.1%), as preservative until analyzed.

Results and Discussion

During the present study the overall cholesterol level up to 60 days were obtained to be 65.82, 63.19, and 61.15 mg/dl in CIDR, Ovsynch and Control group anestrus buffaloes respectively. Buhechha *et al.*, (2016b) recorded relatively higher plasma cholesterol level in anestrus buffaloes under same protocol while comparatively much lower values were recorded by Parmar (2013) and Nakrani *et al.*, (2014b). This may be due to differences in the nutritional statuses of animals and the assay kits and procedures followed. The higher level of cholesterol in animals may be attributed to the effect of GnRH, which is said to have influence on lipoprotein metabolism in a positive manner (Kumari *et al.*, 2015). Slightly lower concentration of cholesterol observed both in CIDR treated buffaloes and Ovsynch protocol group animals on day 0, 7, 9/10 suggested that cholesterol might have been utilized in the steroidal hormone synthesis.

In the present study, at all days/period mean cholesterol concentration was non-significantly higher in conceived than non-conceived animals for both CIDR and Ovsynch groups. These results between conceived buffaloes under both protocols are in line with the earlier observation of Savalia *et al.*, (2014), Nakrani *et al.*, (2014b) and Shivajibhai (2016). Plasma calcium values neither varied significantly between sampling days, within protocol nor between the

protocols. The non-significant variation in plasma calcium level was also recorded between conceived and non-conceived buffaloes in all three treatment groups. The present observations of non-significant variation between sampling days within the protocol and or between the protocol are comparable with the recent studies documented by some of the workers using Ovsynch and CIDR in buffaloes (Savalia *et al.*, 2013 and Shivajibhai, 2016). In contrast, Buhecha *et al.*, 2016 reported significantly higher plasma Calcium concentration under CIDR protocol as compared to the values of other treatment groups. However, the calcium level obtained in the present study are within normal range as reported by various authors in cyclic and non-cyclic anestrus buffaloes (Ali *et al.*, 2012b and Shivajibhai, 2016). The concentration of calcium in the body fluids depends on the mineral contents of the feed and forage, the level of dietary sources intake and the availability of minerals (Suttle, 2010). This indicates a direct plant-animal relationship. The altered Ca: P ratio may affect ovarian function through its blocking action on pituitary gland, which results in prolongation of first oestrus and ovulation ultimately reducing the reproduction efficiency (Satish kumar, 2003). The levels of plasma phosphorous neither varied significantly between sampling days in any of treatment protocol nor between conceived and non-conceived buffaloes in any group. The present findings are in coroboration with the report of Shivaji bhai (2016) in cases of buffaloes after CIDR/Ovsynch protocol. However comparatively lower values have been reported by some workers using CIDR and Ovsynch protocol in anestrus buffaloes including normal cyclic groups (Savalia *et al.*, 2013, Nakrani *et al.*, 2014 and Buhecha *et al.*, 2016b). Bhaskaran and Ichaa (1981) documented that the marginal deficiency of phosphorous is enough to cause disturbance in

pituitary-ovarian-axis. Butani *et al.*, (2011) and Savalia *et al.*, (2013) did not find variation in the mean plasma phosphorous levels on day of GnRH and PG treatment at induced oestrus and on day 21 post AI in anoestrus or sub-oestrus buffaloes which also supports the present findings.

The plasma magnesium concentration neither varied significantly between sampling days nor between the treatment groups. The value also didn't vary significantly between conceived and non-conceived animals within the group under any protocol. Similar trend was reported by Savalia *et al.*, (2013) and Shivaji bhai (2016) in Ovsynch and CIDR group. The present non-significant differences observed in plasma magnesium profile on different dates of sampling 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 anestrus 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.

The levels of Plasma Copper content recorded on day 0, 7, 9/10 and day 60 in CIDR group, Ovsynch group and Control group were estimated. No significant difference was observed within days in these groups with respect to copper content. The mean plasma Copper content in conceived and non-conceived groups in all the three experimental groups were also found to be statistically similar and non-significant on day 0, 7, 9/10 and 60. The influence of estrus induction protocols like CIDR and Ovsynch on Copper levels in buffaloes could not be seen in literature reviewed (Table 1).

Table.1 Plasma biochemical and mineral profile in different days of sampling of anestrus buffaloes treated with CIDR and Ovsynch protocol (Mean ± S.E)

Biochemical and Mineral Profiles	Group/ Estrus induction protocol	Different days of the treatment					Average Value
		N	Day 0	Day 7	Day 9/10	Day 60	
Cholesterol	CIDR	6	63.18 ± 1.26	65.41 ± 1.44	66.25 ± 1.37	68.43 ± 2.02	65.82 ± 0.82^a
	Ovsynch	6	60.00 ± 1.11	62.62 ± 1.82	63.67 ± 1.88	66.47 ± 1.74	63.19 ± 1.00^b
	Control	6	60.23 ± 1.81	60.18 ± 1.99	61.22 ± 1.36	62.95 ± 1.59	61.15 ± 0.85^b
Calcium	CIDR	6	8.35 ± 0.11	8.43 ± 0.22	8.45 ± 0.13	8.54 ± 0.16	8.44 ± 0.08
	Ovsynch	6	8.15 ± 0.05	8.48 ± 0.26	8.33 ± 0.09	8.49 ± 0.11	8.37 ± 0.05
	Control	6	8.15 ± 0.12	8.22 ± 0.11	8.42 ± 0.15	8.55 ± 0.14	8.33 ± 0.07
Phosphorous	CIDR	6	5.11 ± 0.03	5.13 ± 0.05	5.12 ± 0.04	5.12 ± 0.10	5.12 ± 0.03
	Ovsynch	6	5.24 ± 0.05	5.32 ± 0.05	5.07 ± 0.04	4.71 ± 0.17	5.08 ± 0.07
	Control	6	4.76 ± 0.30	4.68 ± 0.28	4.86 ± 0.26	4.81 ± 0.26	4.78 ± 0.13
Magnesium	CIDR	6	2.06 ± 0.05	2.24 ± 0.14	2.28 ± 0.28	2.28 ± 0.32	2.21 ± 0.04
	Ovsynch	6	2.13 ± 0.16	2.19 ± 0.16	2.27 ± 0.24	2.32 ± 0.23	2.22 ± 0.03
	Control	6	2.33 ± 0.21	2.30 ± 0.18	2.20 ± 0.19	2.16 ± 0.13	2.25 ± 0.09
Copper	CIDR	6	0.39 ± 0.05	0.41 ± 0.06	0.43 ± 0.12	0.46 ± 0.05	0.42 ± 0.02
	Ovsynch	6	0.38 ± 0.01	0.43 ± 0.01	0.39 ± 0.01	0.43 ± 0.01	0.41 ± 0.01
	Control	6	0.42 ± 0.02	0.42 ± 0.01	0.43 ± 0.01	0.48 ± 0.01	0.44 ± 0.01

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However, the level of copper estimated during the present study was near the normal serum copper concentration (0.5-1.0 ppm) studied under different physiological conditions under farm conditions (Geogievskii, 1982). Copper is an essential trace element for normal reproduction performance of the buffaloes and deficiency of copper is generally accompanied by anemia, debility and lack of appetite and also results in failure of estrus and delayed onset of puberty (Underwood, 1981). It indicates that the reproductive problems may be associated with the reduced copper level in animals.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary Science and Animal Husbandry, Kanke, Ranchi for providing necessary facilities.

On the basis of above result, it can be concluded that none of the hormonal protocol had any significant effect on blood biochemical and mineral profiles on any day of sampling.

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How to cite this article:

Kumar Rohit, Sinha, Manoranjan Prasad and Prasad Raju. 2018. Studies on Biochemical and Mineral Profile of Anestrus Murrah Buffaloes Treated with Different Hormonal Protocols. *Int.J.Curr.Microbiol.App.Sci*. 7(10): 1010-1015. doi: <https://doi.org/10.20546/ijemas.2018.710.112>