HAEMATO-BIOCHEMICAL AND HORMONAL PROFILE ALTERATIONS IN POSTPARTUM TRUE ANESTRUS MEHSANA BUFFALOES TREATED WITH INDIGENOUS HERBAL HEAT INDUCER

ABSTRACT

The present study was designed to explore the effects of a new Herbal heat inducer in Mehsana buffaloes with postpartum true anestrusand consecutive changes in Blood haematological, Biochemical and Hormonal profile. All the buffaloes were orally supplemented with Herbal heat inducer mixture on (Jantana powder-10gm)1st, (Metrali powder-20gm)2nd and (Hitali-powder-500mg)5th day for augmentation of oestrus. Out of thetotal treated animals, 10 animals expressed the signs of estrus within 30 days. Post-treatment analysis of the overall blood packed cell volume percent in conceiving buffaloes hada significantly($p \leq 0.05$)elevated.Overall blood plasma totalcholesterol concentration (mg/dl) was found to be a significantly ($p \leq 0.001$) elevated in all treated and both conceiving and non-conceiving buffaloes, respectively.Overall blood plasma progesterone concentration (ng/ml) was found to be asignificantly ($p \leq 0.0001$) and ($p \leq 0.001$) elevated in all treated and both conceived and non-conceived buffaloes,whichindicates the positive favorable the favourableeffect of all the drugs on postpartum true anestrus condition.However, Overall blood plasma tri-iodothyronine concentration (ng/ml) was found to be a significantly($p \leq 0.001$) and ($p \leq 0.01$) elevated in all treated and all conceived buffaloes.ThefFindings of the present study revealed that strategic use of Indigenous herbal heat inducerin feed mixture may resolve the postpartum true anestrusproblems in buffaloes.

Keywords: Mehsana Buffaloes, Postpartum true anestrus, Herbal heat inducer, Haematological profile, Blood Plasma profile

INTRODUCTION

The Buffaloes in tropical and subtropical countries are used as a source of milk, meat and draught purposes. Postpartum Trueanestrous(PPTAE) is one of the main reproductive constraints in lactating buffaloes, and is much higher in conventionally managed herds than in organized farms.The Rreproductive efficiency of the animal is the primary determinant for a sound and economic animal production system. A Large number of interacting factors viz. endocrine disturbances, poor nutrition, seasonal and systemic diseases might be attributed to the cause of primary infertility. Also,theanestrus is a multi-faceted problem, but inadequate nutrition, particularly dietary insufficiency of minerals, like calcium, phosphorus, copper, zinc and manganese, greatly contribute to anestrus (Kumar*et al.*, 2014), as these minerals play intermediate roles in the action of hormones and enzymes at cellular level, as reproductive cycle in animals are regulated by endocrine-neuroendocrine interactions between hypothalamic, gonadotropic, gonadal and other hormones (Terzano *et al.*, 2012).There are also certain biological and metabolic constituents that directly reflectCertain biological and metabolic constituents directly reflect the nutritional status and influence the reproductive performance of PPTAE either by acting as precursor of hormone synthesis or by stimulatingthe response of target tissues. Disturbances in haematological, hormonal and biochemical milieu due to

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deficiency of trace minerals also cause anestrus where their estimation in serum samples might be a potential aid in characterizing this problem in post parturient buffaloes.Several Indigenous Herbal ~~inducer~~ inducers~~are being~~have been used since dates back to augment the reproductive performances as traditional medicine. There is a paucity of information regarding the blood haematological, biochemical and hormonal profile in estrus and anestrus~~phase~~ phases.Based upon the above facts, the present study was designed to evaluate the influence of Herbal heat inducer mixture on reproductive performance of postpartum true anestrusMehsanabuffaloes.

MATERIALS AND METHODS

` The present investigation was carried out under field conditions of Banaskantha district north western part of the state of Gujarat and collaboration with the Banaskantha district co-operative Milk ~~producers unions limited~~Producers Unions Limited (Banas Dairy) to study the changes in haemato-biochemical and hormonal profile in postpartum true anestrus Mehsana buffaloes treated with Indigenous Herbal heat inducer.

The Mehsana buffaloes ~~with~~ ingood physical condition were gynaecologically examined. The buffaloes having smooth and inactive ovaries without palpable follicles or corpora lutea on either of ovaries and without pathological condition in the reproductive tract were randomly selected for the study. Repeated rectal examinations were made at 10-12 days interval until a definite conclusion could be drawn for cause (s) of anestrus. A total number of 10 buffaloes affected with postpartum true anestrous were selected for the study. All the buffaloes were orally supplemented with different Herbal heat inducer mixture Jantana powder-10gmon 1st day, Metrali powder-20gm on2ndday and Hitali-powder-500mgon 5th day of Fertikit, Mycon Pharma. All the buffaloes were observed for occurrence of estrous and were bred with fertile bull semen. Buffaloes which did not showed the signs of estrus following the treatment were examined per rectally for pregnancy diagnosis on 60th day post-insemination to confirm the pregnancy and the buffaloes which did not conceive at first insemination were re-inseminated at subsequent estrus cycles.

The blood samples from different groups were collected on day 0 before treatment and on 7th day after treatment. A 10 ml of blood was collected by jugular vein puncture into a heparinized polystyrene tube, and the sample was maintained at 4°C and transported to laboratory within 1-2 hrs of collection. Blood haemoglobin (Hb) and packed cell volume (PCV) was estimated as per standard protocol (Benjamin, 1985). Plasma was separated by centrifugation at 3000 rpm for 15 minute and was stored in deep freeze (-20 °C) until analyzed. Plasma progesterone, tri-iodothyronine and thyroxine were estimated by using commercially available ELISA kits (Sigma Diagnostics Pvt. Limited, USA) and plasma total protein and total cholesterol were estimated by using standard procedures using commercial available diagnostic kits (LiquiCHEKTM, AGAPPE Diagnostics Pvt. Limited, Kerala, India) in Biochemistry Analyzer RX-50V (Micro Lab, India). The data collected were suitably tabulated and analyzed by standard statistical Graphpadprism®Trial version 4 software. The data obtained pertaining to different groups were analyzed statistically using Two-way ANOVA. While the test of significance between and within the treatment groups were calculated by multiple comparison with boneferroni test (Bonferroni, 1960) with 95% confidence interval.

RESULTS AND DISCUSSION

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Blood Haematogical Profile

Overall haemoglobin concentration (gm/dl) was found to be 13.64±0.30 (11.7-16.8) in treatment group buffaloes.Statistical analysis revealed a non-significant difference within various periods in the treatment group buffaloes with an increase at day 7(Table 1).Statistical analysis also revealed a non-significant difference between and within the conceived and non-conceived buffaloes (Table 2)**.**Haemoglobin levels (gm/dl) at various periods observed in the present study is in agreement with the Das *et al.,* (2016) in buffaloes.In the present study, blood haemoglobin profile revealed a non-significant difference within the group, agreed well with the Ray *et al.,* (2016) who have reported that adequate haemoglobin level is required to transport enough oxygen and nutrients to the vital organs including the ovarian tissues to be prepared for sending the signal to hypothalamus, which is in agreement with an increase in haemoglobin concentration after treatment in all groups.

Table1. Blood Haemoglobin concentration (gm/dl) in postpartum true anestrousbuffaloes group (Mean±SEm).

Period	P-1 (0 day)	P-2 (7th day)	Overall
Group	12.98±0.36(11.7-14.9)	14.29±0.39(12.8-16.8)	13.64±0.30(11.7-16.8)

- Figures in parentheses indicate range.

Table2. Blood Haemoglobin concentration (gm/dl) inall conceived and non-conceived true anestrous buffaloes (Mean±SEm).

Period	P-1 (0 day)	P-2 (7th day)	Overall
Conceived	12.39± 0.53(8.1- 16.2)	13.46± 0.48(9.2- 16.8)	12.93±0.37(8.1-16.8)
Non-conceived	13.08 ± 0.73(9.2- 16.6)	14.26± 0.51(12.2- 16.8)	13.68±0.46(9.2-16.8)

- Figures in parentheses indicate range.

~~Overall~~Overall, per cent packed cell volume was found to be 34.27±0.83 (28.9-42.9) in treatment group buffaloes. Statistical analysis revealeda non-significant differencewithin various periods in the treatment group (Table.3). The overall packed cell volume per cent observed at different periods in all the conceived and non-conceived buffaloes show non-significant difference are presented in table.4. Statistical analysis revealed a significant (P≤0.01) difference within the conceived buffaloes with higher PCV per cent at day 7th(36.93±0.94)as compared to that ofon day 0 (33.51±0.88)(Table.4), similarly Das *et al.,* (2016) has reported in Mehsana buffaloes.An increase in PCV value was observed in all the groups parallel with an increase in haemoglobin concentration after treatment which is in agreement with Ray *et al.,* (2016) who have also reported in respective groups of postpartum anoestrus buffaloes.

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Table3.Blood Packed cell volume (PCV) per cent in postpartum true anestrousbuffaloes group (Mean±SEm).

Period	P-1 (0 day)	P-2 (7th day)	Overall
Group	32.32±0.76(28.9-36.8)	36.21±1.22(23.9-42.9)	34.27±0.83(28.9-42.9)

- Figures in parentheses indicate range.

Table4. Blood Packed cell volume (PCV) per cent inall conceived and non-conceived true anestrous buffaloes (Mean±SEm).

Period	P-1 (0 day)	P-2 (7th day)	Overall
Conceived	33.51±0.88^{p}(28.4- 41.1)	36.93±0.94^{q}(28.7- 42.9)	35.23±0.69(28.4-42.4)
Non-conceived	33.88±1.41(28.6- 40.9)	36.81±1.09(32.9- 42.6)	35.35±0.94(28.6-42.6)

- Figures in parentheses indicate range.
- Values bearing different superscripts within column differed significantly (P≤0.01).

Blood Plasma Biochemical Profile

Overall blood plasma total protein concentration (gm/dl) was found to be 8.81±0.34(5.99-11.22) in treatment group buffaloes. Statistical analysis revealed a non-significant difference within the treatment group(Table 5). The plasma total protein concentration (gm/dl) observed at various periods was non-significantly higher in conceiving as compared to non-conceiving buffaloes (Table 6).~~Which~~ This is ~~an~~ in agreement with the study of Singh *et al.*, (2012) who have reported that the plasma totalprotein levels at different periods which ~~gets~~ getthe support of is increasing post-treatment in bovine using herbal estrus inducer therapy because ~~its~~ itmight due toherbal medicines that contain some amounts of proteinsand concluded that the compound of herbal drugsinfluenced the biochemical profile of blood under normalrange which ultimately affects the estrus response inbovine.However, the level was non-significantly higher ~~at~~on 7th day in all the conceiving buffaloes than in non-conceivedbuffaloes under experiment.

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Table5. Blood plasma total protein concentration (gm/dl**)**in postpartum true anestrousbuffaloes group (Mean±SEm).

Period	P-1 (0 day)	P-2 (7th day)	Overall
Group	8.25±0.40(5.99-10.2)	9.36±0.49(6.2-11.22)	8.81±0.34(5.99-11.22)

- Figures in parentheses indicate range

Table6. Blood plasma total protein concentration (gm/dl) **in all conceived and non-conceived true anestrous buffaloes (Mean±SEm).**

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Period	P-1 (0 day)	P-2 (7th day)	Overall
Conceived	8.49±0.29(5.99-10.93)	9.21±0.35(5.99-11.22)	8.85±0.23(5.99-11.22)
Non-conceived	7.69±0.38(6.44-9.98)	8.73±0.39(7.5-10.88)	8.44±0.27(6.78-10.88)

- Figures in parentheses indicate range.

Overall blood plasma total cholesterol concentration (mg/dl) was found to be 118.08±4.15 (77.6-145.2) intreatment group buffaloes.Statistical analysis revealed an increase in total cholesterol at day 7 post treatment with a significantly (P≤0.001) higher value only in treatment group buffaloes (Table 7). However, significantly (P≤0.001) higher cholesterol level was also

observed at 7th day in all the conceiving and non-conceiving buffaloes under experiment (Table 8). Plasma total cholesterol concentration (mg/dl) level observed in the present study is in agreement with that of the111.55±10.12 (mg/dl) level at expressed heat and 88.91±10.77 (mg/dl) level in dairy bovinewhich not expressing heat after treatment for postpartum anoestrus using herbal heat inducer (Ray *et al.,* 2016) and 160.76±0.52 (mg/dl) level at estrus and 130.98±0.31 (mg/dl) level in regular cyclic Murrah buffaloes (Balamurugan *et al.,* 2015). Significantly higher cholesterol level in treatment group animals might be an indication of positive action of the drug in reducing the service period since higher plasma cholesterol level facilitates early expression of oestrus in dairy bovine, as lipids are the precursors of gonadal steroid hormones (Westwood *et al.,* 2002).

Table7. Plasma total cholesterol concentration (mg/dl) in postpartum true anoestrousbuffaloes group (Mean±SEm).

Period	**P-1 (0 day)**	**P-2 (7th day)**	**Overall**
Group	107.44±5.82^{p}(77.6-130.1)	128.72±5.17^{q}(99.7-145.2)	118.08±4.15(77.6-145.2)

- Figures in parentheses indicate range.
- Values bearing different superscripts within column differed significantly (P≤0.001).

Table8. Plasma total cholesterol concentration (mg/dl) **in all conceived and non-conceived true anestrous buffaloes (Mean±SEm).**

Period	**P-1 (0 day)**	**P-2 (7th day)**	**Overall**
Conceived	101.45±4.27^{p}(72.8-132.7)	121.26± 4.47^{q}(74.5-148.9)	111.35±3.42(72.8-3.42)
Non-conceived	106.28±6.45^{p}(77.6-133.5)	127.99±4.72^{q}(97.7-142.2)	117.13±4.68(77.6-142.2)

- Figures in parentheses indicate range.
- Values bearing different superscripts within column differed significantly (P≤0.001).

Blood Plasma Hormonal Profile

The overall blood plasma progesterone concentration (ng/ml) was found to be 1.37±0.11(0.80-1.99) in treatment group buffaloes. Statistical analysis revealed a significant (P≤0.0001) difference within in the treatment group buffaloes(Table 9).Statistical analysis also revealed a significant (P≤0.001) difference within the group of all conceived and non-conceived buffaloes (Table 10).However, the difference was non-significant between the groups in conceived as well as non-conceived buffaloes. A significant (P≤0.001) increase in P_4 at day 7 post treatment, in conceiving as well as non-conceiving buffaloes indicate the favorable effect of all the drugs on anoestrus condition.The treatment used in the present study have shown a significant increase in P4 levels in the manner of normal cyclic animals and also increased conception rate in anoestrus buffaloes under field condition in particular with the use of Herbal treatment.

Table9. Plasma progesteroneconcentration (ng/ml) **in postpartum true anoestrousbuffaloes group (Mean±SEm).**

Period	P-1 (0 day)	P-2 (7^{th} day)	Overall
Group	0.97 ± 0.04^{p}(0.80-1.18)	1.74 ± 0.13^{q}(0.93-1.99)	1.37±0.11(0.80-1.99)

- Figures in the parenthesis indicate range
- Values bearing different superscripts within column differ significantly (P≤0.0001).

Table10. Plasma progesterone concentration (ng/ml)in all conceived and non-conceived true anestrous buffaloes (Mean±SEm).

Period	P-1 (0 day)	P-2 (7^{th} day)	Overall
Conceived	0.99 ± 0.06^{p}(0.18- 1.50)	1.74 ± 0.14^{q}(0.93-2.98)	1.36±0.09(0.18-2.98)
Non-conceived	0.91 ± 0.04^{p}(0.82-1.18)	1.72 ± 0.10^{q}(0.99-1.98)	1.32±0.11(0.82-1.98)

- Figures in parentheses indicate range.
- Values bearing different superscripts within column differ significantly (P≤0.001).

Overall blood plasma tri-iodothyronine (T3) concentration (ng/ml) was found to be 5.92±0.26 (4.21-9.19) in treatment group buffaloes. Statistical analysis revealed that the T_3 levels were found to be significantly (P≤0.001) increase on day 7^{th}(6.58±0.38 ng/ml) after treatment as compared to (5.27±0.24 ng/ml) on day 0 (Table 11).

Statistical analysis revealed a non-significant difference for T_3 levels between the conceived and non-conceived buffaloes, but a significantly (P≤0.01) higher overall T_3 level was observed at day 7 in all the conceiving buffaloes (Table.12).The mean plasma tri-iodothyronine concentration (ng/ml) observed in the buffaloes under experiment is in agreement with that of the postpartum Egyptian buffaloes (Ashmawy *et al*., 2015). In the present study, the mean plasma tri-iodothyronine concentration (ng/ml) profile revealed a significant (P≤0.001) rise in the herbal group after treatment which is in agreement with the study of Dutt *et al*., (2011) in postpartum anoestrus buffaloes using herbal extract therapy.

Table11.Plasma tri-iodothyronine concentration(ng/ml)in postpartum true anoestrousbuffaloes group (Mean±SEm).

Period	P-1 (0 day)	P-2 (7^{th} day)	Overall
Group	5.27 ± 0.24^{p}(3.86-6.55)	6.58 ± 0.38^{q}(5.29-9.19)	5.92±0.26(4.21-9.19)

- Figures in parentheses indicate range.
- Values bearing different superscripts within column differed significantly (P≤0.001).

Table12. Plasma tri-iodothyronine concentration (ng/ml) in all conceived and non-conceived true anestrous buffaloes (Mean±SEm).

Period	P-1 (0 day)	P-2 (7^{th} day)	Overall
Conceived	5.45 ± 0.19^{p}(4.10- 7.11)	6.51 ± 0.19^{q}(5.36- 8.12)	6.08±0.15(3.86-8.12)
Non-	5.81±0.30(4.62- 7.52)	6.28±0.41(4.79- 9.19)	6.01±0.14(4.10-9.19)

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- Figures in parentheses indicate range.
- Values bearing different superscripts within column differed significantly (P≤0.01).

Overall blood plasma thyroxine (T_4) concentration (ng/ml) was found to be 53.83±4.59 (28.58-101.37) in treatment group buffaloes. Statistical analysis revealed a non-significant difference within treatment group buffaloes(Table.13). It was found to increase non-significantly at day 7 in treatment group.

Statistical analysis revealed a non-significant difference withinthe conceived and non-conceived buffaloes (Table.14).The mean plasma thyroxine concentration (ng/ml) observed in the buffaloes under experiment is in agreement with the study of Kumar *et al*., (2010) and Raja Kumar *et al*., (2010) in cows at normal cyclic condition and postpartum anoestrus. Whereas, lower plasma thyroxine concentration (ng/ml) in comparison to present findings have been reported in normal cyclic and postpartum true anestrous acyclic Nili-Ravi buffaloes by Muhammad *et al.*, (2016).In the present study, the profile of plasma thyroxine concentration (ng/ml) revealed the non-significant difference between and within the all conceived and non-conceived buffaloes but it was found to increase non-significantly at day 7 post-treatment in all conceived and non-conceived buffaloes indicating the response to herbal heat inducers.

Table13.Plasma thyroxine concentration (ng/ml)in postpartum true anoestrousbuffaloes group(Mean±SEm).

Period	**P-1 (0 day)**	**P-2 (7^{th} day)**	**Overall**
Group	50.10±5.60(28.58-80.97)	57.54±7.37(34.84-101.37)	53.83±4.59(28.58-101.37)

- Figures in parentheses indicate range.

Table14. Plasma thyroxine concentration (ng/ml) in all conceived and non-conceived true anestrous buffaloes (Mean±SEm).

Period	**P-1 (0 day)**	**P-2 (7^{th} day)**	**Overall**
Conceived	53.04±3.49(28.58-81.70)	53.87±3.42(28.78-95.22)	53.46±2.42(28.58-95.22)
Non-conceived	50.67±3.55(32.80-64.45)	68.19±8.98(28.65-101.37)	59.44±5.14(28.65-101.37)

- Figures in parentheses indicate range.

Fertility Response

The per cent estrus induction was 80.00 with days at 15.5 (8-30 days) and intensity of estrus in terms of intense: moderate: mild were 25.00: 37.50: 37.50 in treatment group buffaloes, The conception rates were 70.00 per cent in treatment group buffaloeswith lesser no of services per conception (1.57). The per cent buffaloes conceived with first two services were 85.72 in treatment group. Average days at fertile estrus post treatment was observed to be 29.57 (7/10) in conceived buffaloes of the treatment group. The major symptoms noticed were vaginal mucus discharge, edema, erythematic and wetness of vulva followed by excitement, occasionally bellowing and rarely off feed symptoms. Per cent conception rate following herbal mixture therapy in the present study agreed well with findings of Deshpande *et al*.,(2000);Hadiya *et al.*, (2015) andSahatpure*et al*.,(2016) in anestrus buffaloes.

The herbal treatment group buffaloes have shown a better overall breeding performance in terms of fertile days post treatment and a number of services required per conception. This effect of herbal drugs may be attributed to the collective beneficial effect of various ingredients as reported with their known effect in particular Vidang (Anthelmintic: useful against tapeworms, *Dama and Kirdak., 2002*), Palashbeej (recovers sexual dysfunction, Huxley *et al.*, 1992), Sonamukhi (heat inducer, anthelmintic and strengthen immunity), Gokharu (to treat utero-genital tract infections and sexual dysfunction, *Samani et al.*,*2016*), *Gorakhmundi (*anthelmintic and immunomodulatory and anti-inflammatory), Shatawari (antiinflammatory, nourishing the ovum and cures infertility,increases libido, *Alok et al.*,*2013*), Ashwagandha (useful to cure female and male sexual disorders), Kalonji (anti-inflammatory, analgesic, cleanser of uterus, anthelmintic, *Liu et al. (2016)*,Balshepa (increases the fertility rate, *Santos et al., 2002*), Gajar beej (useful in oligomenorrhea, dysmenorrhea and irregular estrus cycle, Afzal, *et al.*, 2013), Shivlingi (excellent anti-inflammatory, anti-infertility and sterility treatment infemale, provides nourishment to the female organs and support normal functioning, Verma *et al.*, 1993) which seem to be implicated in better health status and reproductive performance of the buffaloes of Herbal treatment group.

CONCLUSION

The haemoglobin concentration (gm/dl) and PCV per cent increased non-significantly at day 7 in treatment group. Whereas, per cent packed cell volume increase significantly at day 7 in conceived group of buffaloes. Total protein concentration levels ~~was~~were non-significantly higher in buffaloes of conceived as compared to non-conceived group. Overall blood plasma total cholesterol concentration (mg/dl) found to be significantly (P≤0.001) ~~increase~~ increasedat day 7 post treatment in treatment group buffaloes, However, a significantly (P≤0.001) higher cholesterol level was also observed ~~at~~ on 7^{th} day in all the conceiving and non-conceiving buffaloes under experiment. A significant rise in plasma progesterone concentration (ng/ml) ~~at 7th day~~on the day post-treatment indicates a positive effect of all the herbal heat inducers on cyclicity. A significant increase in tri-iodothyronine and total cholesterol was noticed using herbal heat inducer at day 7 post treatment. The conceived buffaloes had significantly higher tri-iodothyronine levels at day 7 post treatment.Average days at fertile estrus occurred earlier in the herbal treatment group with a lesser number of services per conception.Conclusively, orally supplemented Herbal heat inducer can be used to induce the ~~estrous~~ oestrousin postpartum true anestrus buffaloes.

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