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ORIGINAL ARTICLE

Study on Effect of Hormonal and Mineral Supplementation on Mineral Status of Infertile Cattle of Coastal Districts of Odisha

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ABSTRACT
Area specific mineral mixture and Two different hormonal protocols were tried in cross breed animals (n=30) in coastal districts of Odisha for their response. The anoestrus & repeat breeding animal group were treated either with mineral mixture or along with double synch / estra double synch protocol yielded highest conception rate of 66.66% followed by 50% in repeat breeding animal group supplemented with mineral mixture alone and 33.33% in anoestrus animal group supplemented with mineral mixture alone. The conception rate in repeat breeding animals (58.33%) was better than the anoestrus animals (50%). The pre treatment values for serum calcium (mg/dl), phosphorous (mg/dl), zinc (ppm), copper (ppm) and manganese (ppm) are 6.16 ± 0.18, 4.38 ± 0.15, 0.73 ± 0.01, 0.75 ± 0.01 and 0.33 ± 0.01 respectively and corresponding post treatment serum mineral values are 7.51 ± 0.21, 5.24 ± 0.17, 1.37 ± 0.07, 1.26 ± 0.05 and 0.57 ± 0.02 respectively. The pre treatment values for estrual mucus copper (ppm), iron (ppm), zinc (ppm), and manganese (ppm) are 0.80 ± 0.09, 0.48 ± 0.04, 1.03 ± 0.06 and 0.15 ± 0.01 respectively and corresponding post treatment estrual mucus mineral values are 1.12 ± 0.04, 0.65 ± 0.03, 1.27 ± 0.02 and 0.19 ± 0.01 respectively. The serum and mucus mineral profile differed significantly ($p < 0.01$) between these class of animals. There was no significant difference ($p < 0.01$) in pre and post treatment values in serum calcium and phosphorous except in repeat breeding animals supplemented with mineral mixture along with the use of hormonal protocol. Serum calcium, zinc and manganese concentration and estrual mucus iron concentration was low in most of the animals. It was concluded that supplementing area specific mineral mixture and employing estra double synch or double synch protocols might be of value to improve fertility in crossbred animals.
Keywords: mucus and serum mineral profile, cattle, and estrual mucus iron concentration

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INTRODUCTION

In Odisha, cattle is the predominant dairy species contributing 85.21% of the total milk production in the state, while at the national level it is buffalo which is the major species contributing 51% of the total milk production [1,2,3]. Hence, all efforts should be made to address the problems that impede the performance of the cattle species in Odisha. The State of Odisha though is endowed with a rich resource of dairy animal wealth, performance of these animals has been found very low. As per 19th quinquennial livestock census 2012, cattle population of Odisha stood at 11.6 million and buffalo 0.726 million as against the National figure of 190.9 million and 108.7 million, respectively. The total milk yield in Odisha was 1.93 MMT as against the National figure of 155.49 MMT during the year 2013-14 [3].

It is an established fact that minerals do have a positive role in normal reproduction. Moreover toxic levels of certain minerals do have deleterious effect on reproduction. Sometimes the animals which were synchronised or inducted with different hormonal protocols do not respond well to the treatment which might be due to the inadequate or toxic levels of certain minerals. Moreover, the bioavailability of minerals after supplementation of minerals to the animals also varies greatly from animal to animal and

from estrus to diestrus phase of the cycle. Hence the present study is conducted with the following objectives.

1) To study the macro and micro mineral status in serum and uterine luminal fluid of infertile cattle.
2) To study the effect of hormonal and mineral supplementation on macro and micro mineral status of infertile cattle.
3) To assess the conception rate following mineral and Hormonal supplementation.

MATERIAL AND METHODS

The purposed study was conducted for six months from November 2017 to April 2018 in the Department of Animal Reproduction Gynaecology and Obstetrics, College of veterinary science and animal husbandry, OUAT, BBSR in collaboration with All India Coordinated Research Project (AICRP) on Nutritional and Physiological Approaches for Enhancing Reproductive Performance in Animals (NPAERPA).

Crossbred cattle with the history of anoestrus and repeat breeding were considered for the present investigation. Cows owned by farmers in rural condition in undivided Cuttack and Puri districts of Odisha, primarily constituted the materials for the present experiment. The animals belonging to rural farmers were maintained by traditional animal husbandry practices. The animals were subjected to standard feeding and managemental practices with provision of quality mineral supplement provided by the project.

Experimental design

Groups		Treatment Protocol
Group I (n=6)	Normal cyclic cows	-
Group II (n=6)	Anoestrus cows treated with mineral mixture	Mineral mixture fed for 2 months
Group III (n=6)	Anoestrus cows treated with both mineral mixture and hormonal protocol	Mineral mixture fed for 2 months along with double synch or estra double synch protocol
Group IV (n=6)	Repeat breeding cows treated with mineral mixture	Mineral mixture fed for 2 months
Group V (n=6)	Repeat breeding cows treated with both mineral mixture and hormonal protocol	Mineral mixture fed for 2 months along with double synch or estra double synch protocol

Figures in the parenthesis indicate the number of animals

The animals after being properly restrained in a trevis, blood was collected from pre and post-treatment (on day of oestrus) for estimation of Serum macro and micro mineral profile. 7ml blood was collected from each of the animal from jugular vein adopting proper collection procedure with clean and sterilized syringe and needle. The collected blood sample was put in a sterilized clot activator.

After collection of blood in sterilized clot activator it was kept in a slanting position for 30 to 60 min without any disturbances. When serum oozed out, it was collected by the help of a micro pippet and kept in a vial without contamination and centrifuged at 2500 rpm for 10 minutes. Four ml of serum was collected and transferred to a dry sterile cryo-vial and finally kept at -20^{0}C in deep freeze for future experiment.

External genitalia of the animal was cleaned using light antiseptic solutions and imped with cottons. Mucus samples were collected by aspiration using a sterile catheter connected with a disposable 50 mL syringe. Catheter was inserted through vagina and cervix followed by back racking and stimulation to os-cervix.

Serum calcium

Calcium was estimated with OCPC method with the help of a estimation kit*

Serum phosphorous

Serum phosphorus level was estimated using Molybdate U.V method with the help of estimation kit.

Estimation of serum (Copper, Manganese and Zinc) and estrual mucus (Copper, Iron, Zinc and Manganese) minerals

It was done by Inductively coupled plasma - optical emission spectrometry ICP-OES (model-Avio-200 Perkin Elmer)

Prior to digestion, all samples were vortexed thoroughly to provide a homogeneous matrix for digestion. Samples were immediately pipetted to prevent settling prior to removing the sample. A sample volume of 250 µL of serum and Estrual mucus was dispensed into an acid-washed glass microwave digestion vessel. Reagent blanks were prepared by addition of deionized water in place of the samples.

For both samples, a volume of 300 µL concentrated nitric acid (HNO_3) (Ultrex purity, Fisher), 200 µL concentrated hydrochloric acid (HCl) (Ultrex, Fisher), and 100 µL of non-stabilized 30% hydrogen peroxide (H_2O_2) solution (Ultrex, Fisher) was added to each vial. Deionized water was added to provide a

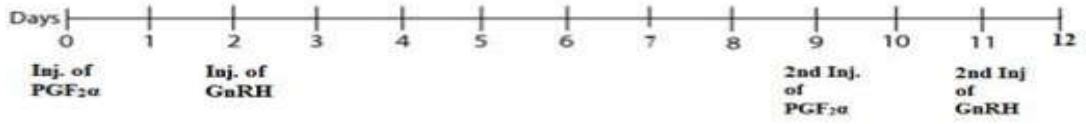
final volume of 2.0 mL. An acid-washed stir-bar was added to each vial for the purpose of stirring during digestion. Samples were then sealed, placed in the microwave and digested following the program described in Table .At the end of digestion, all samples were removed from the microwave and allowed to cool to room temperature. In the clean hood, samples were quantitatively transferred to acid-washed 15 mL polypropylene sample tubes, spiked with a multielement internal standard to provide a final concentration of 10 ng/mL Indium, Scandium, and Praesodymium, and diluted to the final volume with deionized (DI) H_2O. Samples were stored in a monitored refrigerator at a nominal temperature of -20 °C until analysis.

All sample volumes were made upto 13 ml in centrifuge tubes, Red cap ie., diluted 52 times using ultrapure water (Resistivity (25 °C) >18.18 MΩ·cm)

Finally the value obtained is multiplied by the dilution factor and expressed in standard units.

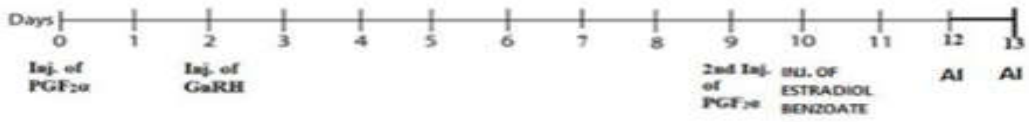
Double synch protocol

The animals in this group were injected with Cloprostenol* 500 mcg i/m on the day of examination (0 day). Inj. of Buserelin acetate** 10 mcg was advocated by i/m route after 48 hr i.e. on Day 2. Injection of cloprostenol* 500 mcg i/m was repeated after a week (Day 9). Similarly Buserelin acetate** 10 mcg was repeated after two days i.e. Day 11.



DOUBLE SYNCH PROTOCOL

The animals in this group were injected with Cloprostenol* 500 mcg i/m on the day of examination (0 day). Inj. of Buserelin acetate** 10 mcg was advocated by i/m route after 48 hr i.e. on Day 2. Injection of cloprostenol* 500 mcg i/m was repeated after a week (Day 9). Inj. of Estradial Benzoate*** 2 mg was administered on the following day (Day 10).



ESTRA DOUBLE SYNCH PROTOCOL

The animals were inseminated in the induced estrus with good quality frozen semen supplied by ARD Dept. In case of animals inducted with double synch protocol, fixed time insemination was conducted at 16 hrs and 28 hrs of last injection of Buserelin acetate. Similarly animals inducted with estra double synch protocol were inseminated twice at 48 and 72 hrs of injection of estradiol benzoate. The control animals were inseminated at estrus following AM-PM rule.

Pregnancy diagnosis was conducted 45-60 days post insemination by rectal palpation.

Conception rate was calculated as the percentage of pregnant animals out of the total inseminated animals.

All the data generated in the above experiments were statistically analyzed using SPSS (1996) computer package. Charts were done with the help of Data analysis tool of Office 2010 of Microsoft in the computer.

RESULTS

For test of significance, pre treatment serum calcium values (mg/dl) in different experimental groups were 6.34 ± 0.83, 6.05 ± 0.75, 6.23 ± 0.84, 5.86 ± 0.49 and 6.34 ± 0.82 for Group I, Group II, Group III, Group IV and Group V respectively. Corresponding values for post treatment serum calcium (mg/dl) in different experimental groups were estimated to be 6.89 ± 0.83, 7.90 ± 0.75, 7.35 ± 0.84, 8.00 ± 0.49 and 7.39 ± 0.82 in same order. Analysis of variance indicated no significant difference between any groups. However, serum calcium values between pre and post treatment in Group IV showed significantly ($P<0.01$) higher values in post treatment sampling as revealed from test of significance.

For test of significance, pre treatment serum phosphorous values (mg/dl) in different experimental groups were 3.97 ± 0.21, 4.18 ± 0.45, 4.78 ± 0.51, 4.02 ± 0.28 and 4.98 ± 0.47 for Group I, Group II, Group III, Group IV and Group V respectively. Corresponding values for post treatment serum phosphorous (mg/dl) in different experimental groups were 4.32 ± 0.21, 5.24 ± 0.45, 5.71 ± 0.51, 5.25 ± 0.28 and 5.71 ±

0.47 for Group I, Group II, Group III, Group IV and Group V respectively. Analysis of variance of serum phosphorous did not reveal any significant difference among groups. However, serum phosphorous values between pre and post treatment in Group IV showed significantly (P<0.01) higher values in post treatment sampling as revealed from test of significance.

For test of significance, pre treatment serum zinc values (ppm) in different experimental groups were 0.73 ± 0.04, 0.69 ± 0.08, 0.74 ± 0.12, 0.74 ± 0.13 and 0.73 ± 0.14 for Group I, Group II, Group III, Group IV and Group V respectively. Corresponding values for post treatment serum zinc (ppm) in different experimental groups were 0.73 ± 0.04, 1.50 ± 0.08, 1.61 ± 0.12, 1.40 ± 0.13 and 1.61 ± 0.14 for Group I, Group II, Group III, Group IV and Group V respectively. Analysis of variance indicated higher zinc value in all groups than control significantly (P<0.01), irrespective of treatment. Test of significance indicated significantly higher (P<0.01) post treatment zinc level in all treatment groups irrespective of treatment.

For test of significance, pre treatment serum copper values (ppm) in different experimental groups were 0.77 ± 0.001, 0.77 ± 0.08, 0.74 ± 0.05, 0.76 ± 0.10 and 0.73 ± 0.08 for Group I, Group II, Group III, Group IV and Group V respectively. Corresponding values for post treatment serum copper (ppm) in different experimental groups were 0.77 ± 0.001, 1.43 ± 0.08, 1.42 ± 0.05, 1.27 ± 0.10 and 1.39 ± 0.08 for Group I, Group II, Group III, Group IV and Group V respectively. Analysis of variance indicated a significantly (P<0.01) higher post treatment serum copper value than control in all treatment groups irrespective of treatment adopted. Similarly test of significance indicated significantly higher (P<0.01) post treatment value in all groups irrespective of treatment.

For test of significance, pre treatment serum manganese values (ppm) in different experimental groups were 0.34 ± 0.04, 0.32 ± 0.04, 0.33 ± 0.03, 0.33 ± 0.05 and 0.35 ± 0.06 for Group I, Group II, Group III, Group IV and Group V respectively. Corresponding values for post treatment serum manganese (ppm) in different experimental groups were 0.35 ± 0.04, 0.64 ± 0.04, 0.64 ± 0.03, 0.58 ± 0.05 and 0.65 ± 0.06 for Group I, Group II, Group III, Group IV and Group V respectively. Analysis of variance and test of significance indicated significantly (P<0.01) higher post treatment value from the control and its pre treatment sampling in all groups irrespective of treatment .

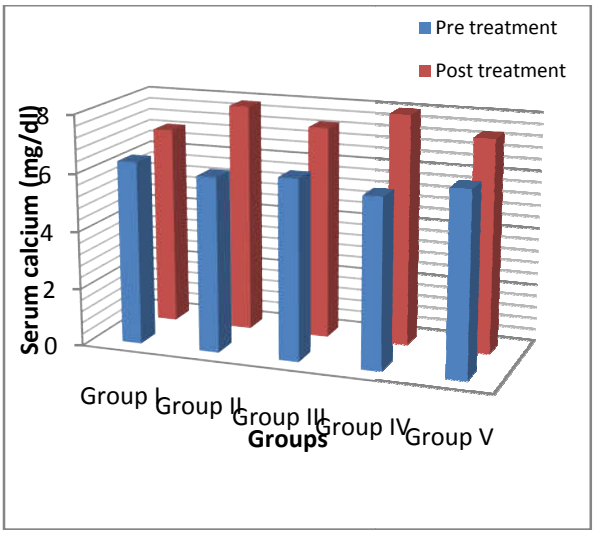
Analysis of variance revealed a significantly higher (P<0.01) pre treatment estrual copper level in control animals. In the post treatment stage the control value was significantly higher (P<0.01) than the group IV animals. Test of significance indicated a significantly higher (P<0.01) post treatment value of copper in both the treatment groups.

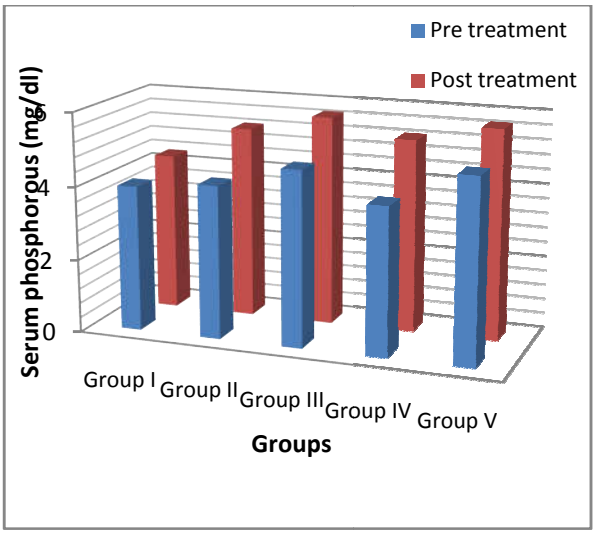
For test of significance, pre treatment estrual mucus iron values (ppm) in different experimental groups were 0.73 ± 0.02, 0.63 ± 0.03 and 0.34 ± 0.03 for Group I, Group IV and Group V respectively. Corresponding values for post treatment estrual mucus iron (ppm) in different experimental groups were 0.74 ± 0.02, 0.58 ± 0.03 and 0.62 ± 0.03 for Group I, Group IV and Group V respectively. Analysis of variance revealed a significantly (P<0.01) lower value of pre treatment iron in both the treatment groups than the control. The test of significance did not show post treatment increase in iron value in any of the experimental groups including control.

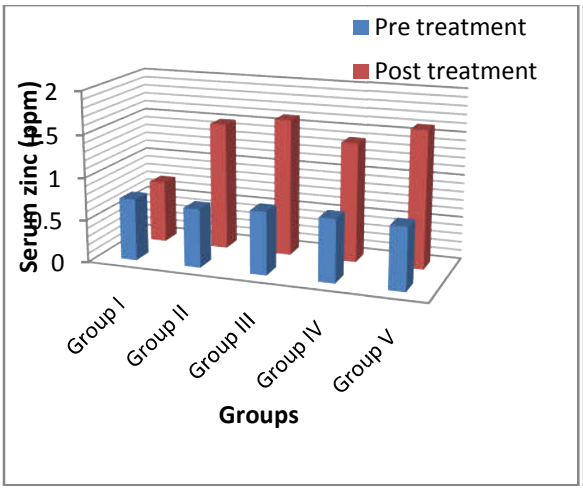
For test of significance, pre treatment estrual mucus zinc values (ppm) in different experimental groups were 1.38 ± 0.06, 0.89 ± 0.03 and 0.84 ± 0.03 for Group I, Group IV and Group V respectively. Corresponding values for post treatment estrual mucus zinc (ppm) in different experimental groups were 1.36 ± 0.06, 1.21 ± 0.03 and 1.24 ± 0.03 for Group I, Group IV and Group V respectively. Analysis of variance revealed a significantly (P<0.01) lower value of pre treatment zinc in both the treatment groups from the control. The test of significance showed a significant (P<0.01) rise in post treatment zinc level in both the treatment groups.

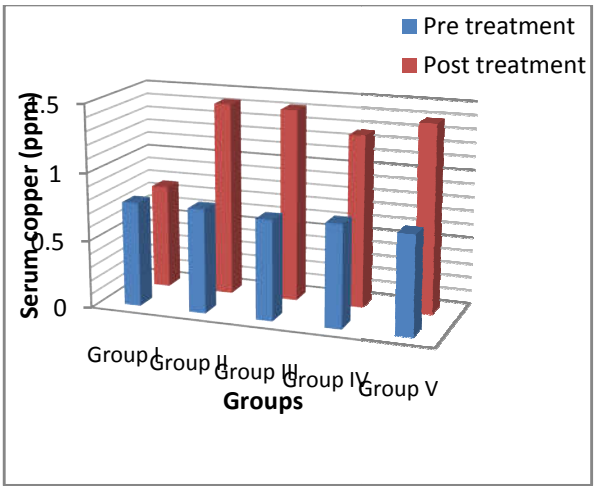
For test of significance, pre treatment estrual mucus manganese values (ppm) in different experimental groups were 0.20 ± 0.01, 0.13 ± 0.01 and 0.12 ± 0.01 for Group I, Group IV and Group V respectively. Corresponding values for post treatment estrual mucus manganese (ppm) in different experimental groups were 0.20 ± 0.01, 0.18 ± 0.01 and 0.20 ± 0.01 for Group I, Group IV and Group V respectively. Analysis of variance indicated no significant difference among experimental groups in pre or post treatment level of manganese in estrual mucus. However test of significance revealed a significant (P<0.01) increase in post treatment value in both the treatment groups.

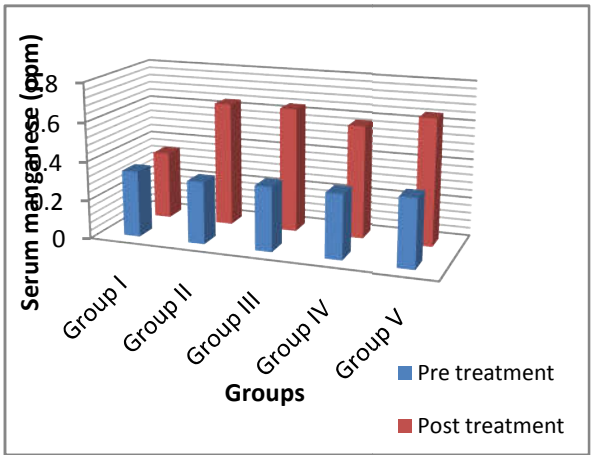
The conception rate achieved in different experimental groups is presented in Table 21. In group II and the control (group I) group, conception rate of 33.33 per cent has been recorded. Similarly, group III and V recorded the highest of 66.66 per cent each. Group IV animals recorded a moderate value of 50.00 per cent conception rate. However, chi-square analysis revealed no significant difference in conception rate among different experimental groups.

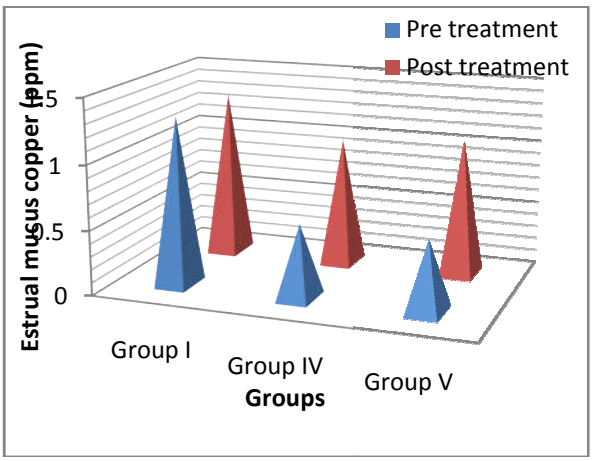












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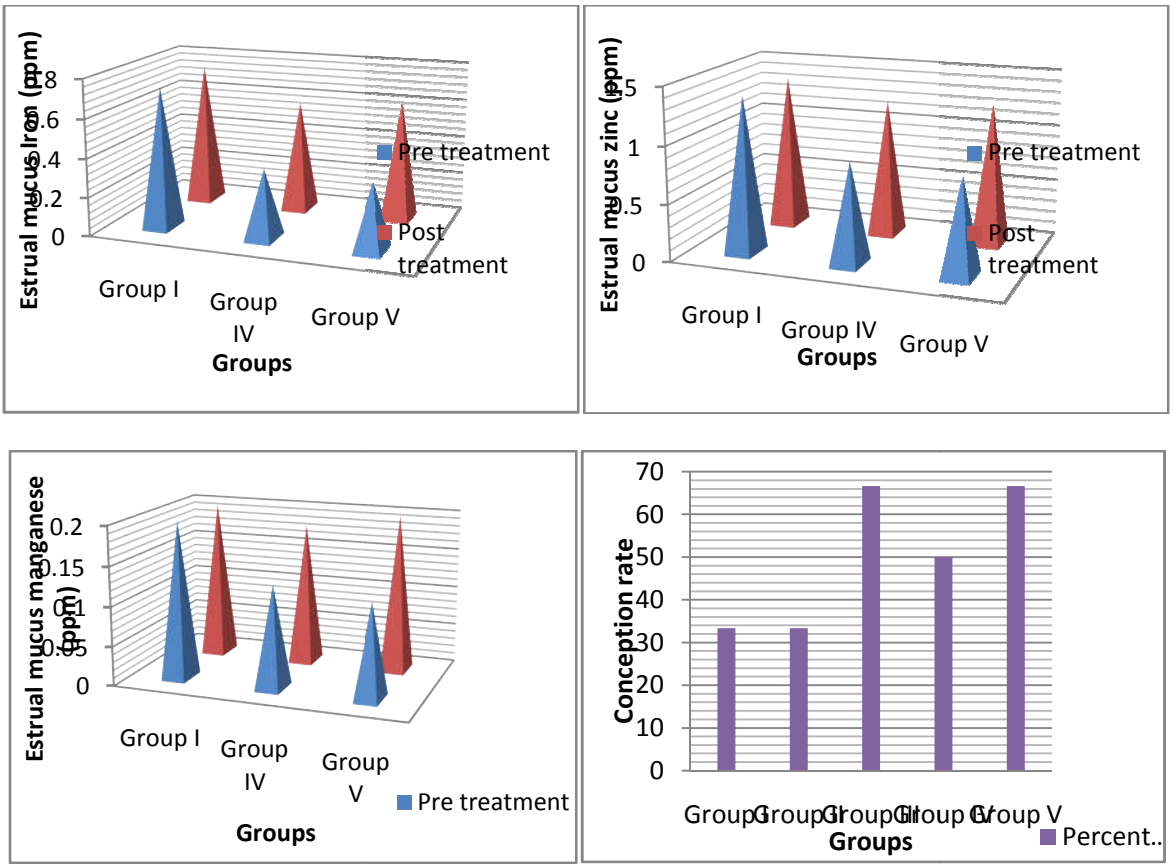

Fig 1: Effect Of Hormonal And Mineral Supplementation On Mineral Status Of Infertile Cattle

DISCUSSION

During the present study in Kakatapur and Balipatna block, the infertile cattle covering both anoestrus and repeat breeding groups, have been treated with either mineral supplementation alone or in combination with one of the recently developed hormonal protocols namely double synch and estra-double synch. The farmers of rural Odisha are not using mineral supplementation as a routine practice. Moreover, soil and fodder of the irrigated belt is also deficient in different macro and micro minerals. Further, the rural farmers do not rear their cattle under scientific feeding and management practices. As such, the probability of mineral deficiency is very high. Obviously supplementation of good quality mineral mixture @ 50g/day for a period of 60 days to the animals ought to be reflected in the response / conception rate among experimental animals. The hormonal protocol used during the study was intended to improve the response / conception rate further.

The post treatment serum calcium level subsequent to mineral and /or hormonal supplementation during the present study is comparable to the earlier reports [9]. However, higher post treatment values (9.39 ± 0.53) has also been reported.

Many researchers are of view that neither calcium nor phosphorous level alone are responsible for causing infertility conditions. Rather calcium : phosphorous ratio which is more important should be taken into account.

The test of significance indicated a highly significant difference ($P < 0.01$) in all the supplemented groups indicative of substantial increase of zinc in the post treatment regimen. The analysis of variance for zinc also showed a similar trend with all the experimental groups which differed significantly from the control group of animals in the post treatment phase. However, the treatment groups did not differ among themselves.

In the present experiment also significantly higher level of copper has been envisaged during the post treatment regimen which might have resulted in higher response of conception rate. It is an established fact that Copper and Iron contribute significantly to the level of haemoglobin and total erythrocyte count which might have resulted in better conception rate.

Test of significance between pre and post treatment values of manganese indicated highly significant difference ($P < 0.01$) among all the experimental groups irrespective of treatment adopted. Similarly,

analysis of variance indicated a significant (P < 0.01) post treatment increase than the control animals in all the groups irrespective of the treatment adopted. The post treatment increase in the manganese level is in consistence with the findings of Gouda *et al*. [5] in the supplemented groups.

Cervical or uterine discharge collected during pre treatment stage in normal cyclic at repeat breeding animals is presented in table 2 along with the normal values. All the trace minerals like copper, iron, zinc and manganese are well within the physiological range [6].

The pre treatment values of copper in estrual mucus in normal cyclic control animals is significantly higher than the Group IV and Group V animals. In post treatment regimen the copper level in estrual mucus has improved which is still significantly (P<0.01) lower in Group IV animals than in control ones. The variation in copper concentration might be due to formation of copper-iron and copper-zinc bio-complexes which are not bio-available [4].

The analysis of variance indicated no variation in post treatment level of iron among treatment groups and the control. A significantly (P<0.01) higher pre treatment value in the control group is indicative of iron saturation, as the areas come under iron rich coastal zone of the state.

Comparison of zinc in serum and mucus indicated that the significant rise in levels of zinc in the post treatment phase is uniform both in serum and mucus.

Perusal of literature indicated a comparable value of manganese in the mucus by other workers [7]. However, the figure in infertile cattle during the present experiment is lower than earlier report [7]. The comparison of manganese level in serum and mucus revealed a similar trend both in pre and post treatment samplings.

Though the hormone treated groups of animals achieved numerically higher conception rate, the chi-square analysis of conception rate did not reveal any significant difference among different experimental groups including control. A bigger trial involving higher number of animals might highlight the success of treatment in future.

CONCLUSION

In the present study, area specific mineral mixture and two different hormonal protocols were tried in 24 crossbred cattle in traditional husbandry to improve their fertility. Parameters like serum mineral profile, estrual mucus mineral profile and conception rate were assessed. The study revealed the following salient findings.

- All treatment groups provided higher conception rate. Conception rate varied from 33.33% to 66.66% with highest in group of repeat breeding and anoestrus animals supplemented with both mineral mixture and hormonal protocol followed by RB animals supplemented with mineral mixture alone (50%), anoestrus animals (33.33%).
- Serum **calcium, zinc** and **manganese** concentration and estrual mucus **iron** concentration of cattle in the area of research (Undivided Puri and Cuttuck Districts) are less than the normal standard values.
- The serum calcium and phosphorous level did not differ much among groups. However there is a significant change in post treatment serum zinc, copper and manganese mineral status in all groups compared to normal cyclic animals.
- Effect of copper, iron and zinc concentration were less in estrual mucus compared to normal cyclic animals.
- Post treatment estrual mucus copper concentration in repeat breeders found to be still than normal physiological level.
- Supplementing mineral mixture alone to anoestrus animals didn't improve in conception and pregnancy rate compared to normal cyclic animals.
- Since the crossbred cattle need more attention in feeding and management than desi breeds of animals to countermand the stress due to climate and production, still their fertility can be maintained in Odisha condition by employing both area specific mineral mixture and hormonal supplementation.

It can be concluded that supplementing ASMM, along with estra double synch or double synch hormonal protocols might be of value to improve fertility in crossbred cattle of Odisha.

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