

## Fertility Management of Pubertal Anoestrus In Marathawadi Buffalo Heifers By Using Various Therapeutic Methods

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### ABSTRACT

The present research work was undertaken to study the efficacy of area specific mineral mixture and ovarian massage, inj. GnRH alone and inj. Progesterone primed GnRH therapy, Bio-assay heat detection for fertility improvement in pubertal anoestrus Marathawadi buffalo heifers in and around Udgir tehsil. Total 32 pubertal anoestrus Marathawadi buffalo heifers were selected from villages of Latur districts viz. Dawangoan, Shekapur, Kodali, Dongarshelki during January to June 2014. All the buffalo heifers were examined per rectally and heifers with smooth and round well-developed ovaries, few animals with CL on ovaries were randomly divided into four groups i.e. Group I, II, III and IV comprising of eight buffalo heifers in each. The buffalo heifers in Group I (n=8) were treated with deworming followed by area specific Mineral mixture and ovarian massage for 15 days with 5 days interval, Group II (n=8) were administered inj. GnRH @ 10 mcg on 0 day and if not showing heat symptoms, then repeat on 10<sup>th</sup> day, Group III (n=8) were administered inj. Progesterone @ 250 mcg on 0 day and inj. GnRH 10 mcg on 10<sup>th</sup> day, the Group IV (n=8) were kept as untreated control. The estrus duration of animal was Av. 9.87±0.75 hrs; Av. 9.86±0.31hrs, Av. 11 hr and Av. 9±0.22 hrs, respectively and conception rate observed was 75 per cent, 50 per cent and 25 per cent respectively. The accuracy rate of bioassay heat detection kit was found Av. 84.37 per cent.

**Key words:** Pubertal anoestrus, Ovarian massage, Feeding of mineral mixture, GnRH; Progesterone primed GnRH therapy and Bio-assay.

### INTRODUCTION

Buffalo plays an important role in livestock production of India as it provides milk, meat and draught power. Buffaloes therefore are of high economic importance for farmers in several developing countries. The reproductive performance is poor although the buffalo can adapt to harsh environment and live on poor quality forage. The reproductive efficiency is often compromised by such conditions, resulting in late sexual maturity, long postpartum anoestrus, poor expression of estrus, poor conception rates and long calving interval [1].

Pubertal anoestrus is a condition in which animal fails to exhibit heat signs after completing 41-45 months of age. Pubertal anoestrus is a condition mainly found in heifers and it is encountered under village management conditions [2].

The main factors which influence the age of attainment of puberty are the genotype, nutrition, management, thermal environment, year or season of birth, parasites and disease. With good nutritional diet, most heifers attain their puberty weight between 8-13 months of age. Maturation involves a complex interplay of endocrine factors which brings about

development of reproductive tract. Maturation depends on genetic and environmental factors. In heifers, first ovulation is triggered when the hypothalamic pituitary axis loses its sensitivity to the negative feedback effect of oestradiol-17  $\beta$ , allowing an LH surge to occur [3].

Max (2001) reported the uterine and ovarian massage is simple and relatively viable treatment method that should be considered especially when dealing with economically disadvantaged farmers [4]. Use of synthetic analogue of GnRH, Busereline causes a rapid secretion of LH and FSH from the pituitary with subsequent elevation of the concentration of these hormones in peripheral blood circulation [5]. Continuous administration of progesterone followed by sudden withdrawal elicits negative feedback on hypothalamus resulting in Ovulatory heat and the rapid drop in circulatory concentration of P4 promotes the release of GnRH as the negative feedback of P4 abolished, followed by FSH and LH release with subsequent resumption of ovarian cyclicity [6].

## MATERIALS AND METHODS

Total thirty-two pubertal anoestrus Marathawadi buffalo heifers with history of absence of estrus sings and no any conception after attaining 3 – 4 ½ years of age and with silent estrus with developed genitalia were selected. The animals were screened by rectal palpation for detection of any uterine pathology, anatomical abnormalities, adhesions, etc. after thorough examination; normal pubertal anoestrus buffalo heifers were selected.

### Treatment Schedule

Thirty-two pubertal aneustrus Marathawadi buffalo heifers were grouped in various groups viz. feeding of mineral supplement and ovarian massage, various hormonal treatments.

**Table.1: Experimental Design**

Sr. no.	Group	No. of animals	Treatment schedule
1.	I	08	Deworming, area specific mineral supplementation @ 50 gm./day and ovarian massage for 15 days with interval of five days.
2.	II	08	Inj.GnRH @ 10 mcg i/m on 0 day if not showing heat symptoms repeat on 10 <sup>th</sup> day inj. GnRH.
3.	III	08	Inj. Progesterone @ 250 mg i/m on 0 day followed by inj. GnRH @ 10 mcg on 10 <sup>th</sup> day
4.	IV	08	Untreated control

### Body Weight Calculation

Body weight of animals was calculated on the basis of formula given by Schaffer's in which body girth and length was recorded in cm.

Schaffer's Formula (for Cattle and Buffaloes).

$$\text{Live weight} = (L'') \times (G'')^2 / 300.$$

(L= length, G = Girth) (In inches) [7].

All the animals selected for research work was having body weight 250-400 kg.

### **Age Determination of Animal**

Age determination of animal was carried out by dentition formula of animal.

- 1) At 1 ½ yrs. of Age – Loss of central pair of temporary incisors.
- 2) At 1 yrs. 10 Month of Age – Appearance of central pair of permanent incisors.
- 3) At 2 ½ yrs. of Age – Appearance of second pair of permanent incisors.
- 4) At 3 yrs. – Appearance of third pair of permanent incisors.
- 5) At 3 ½ yrs. – Appearance of fourth pair of permanent incisors. [8]. All the Animals selected for research work had with Age within the range of 3-4 yrs.

### **Estrus Detection of Animals**

Estrus detection was carried out by bull parading and by using behavioral signs of estrus and physical characteristics of vaginal discharge.

### **Estrus Detection and Conformation by Fern Pattern**

Small drop of cervical mucus was spread on clean dry glass slide. It was air dried and then observed under low power (10X) and high power (40X) of microscope for fern pattern. It was classified as follows as described by [9].

- A) Atypical: Fern pattern with primary and secondary branches.
- B) Typical: Fern pattern with primary, secondary and tertiary branches.
- C) Nil: There were no primary, secondary and tertiary branches

### **Bioassay Heat Detection Kit**

Bioassay heat detection kit was used for the heat detection and early pregnancy diagnosis.

### **Principle**

Bioassay heat detection kit is based on the principle of ELISA, the micro titer plates contains precoated progesterone wells (known antigen) when we add milk sample or serum sample (antibodies), antigen A and B to wells it forms a antigen-antibody complex and gives a color reaction.

### **Content of Bioassay Heat Detection Kit**

- 1) Firmly packed and coated wells plate.
- 2) Dropper bottles containing reagents A and B.
- 3) Sharp razor blade.
- 4) Droppers.
- 5) Tissue papers pack.

### **Procedure of Performing the Test**

- 1) Remove kit from fridge and bring to room temperature.
- 2) Cut foil lid from required wells with sharp knife/ razor blade and peel off foil. Empty liquid and tap dry on tissue paper.
- 3) Add 2 drops of milk sample or serum sample to each well to be tested with the dropper supplied. Use separate dropper for each milk sample or serum sample.
- 4) Add 4-5 drops of reagent A to each well, the well should be just over half full. The plate should be placed at room temperature for one hour.
- 5) Empty wells and wash three times with drinking water. At each time empty the well and wash it again, then tap and wave dry.
- 6) Add 4-5 drops of reagent B in each well to fill it over half full. Result will be ready within 10-20 minutes of reagent B addition.

**Table. 2: Bio-Assay Heat Detection Results**

Colour	Interpretation	Remark
Dark/Strong pink	Low Progesterone	This means the cow/buffalo is most likely in heat and certainly not pregnant
Very Pale Pink/Colorless	High Progesterone	This means that the cow/ buffalo may be pregnant or in mid cycle.

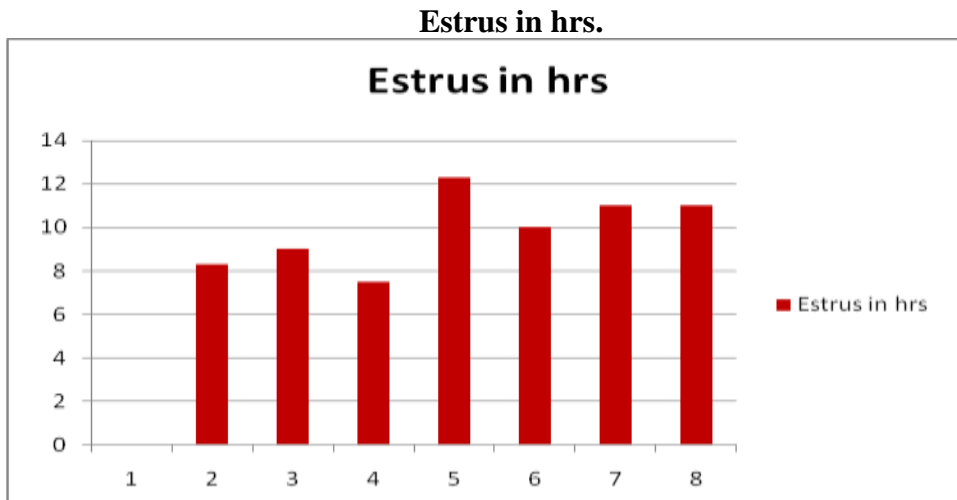
**Pregnancy Diagnosis**

Pregnancy was diagnosed after 60 days natural service on the basis of per rectal palpation of genitalia, gradual enlargement of uterine horn, asymmetry of uterus, double membrane feel, slippery feel, live feel and presence of corpus verum.

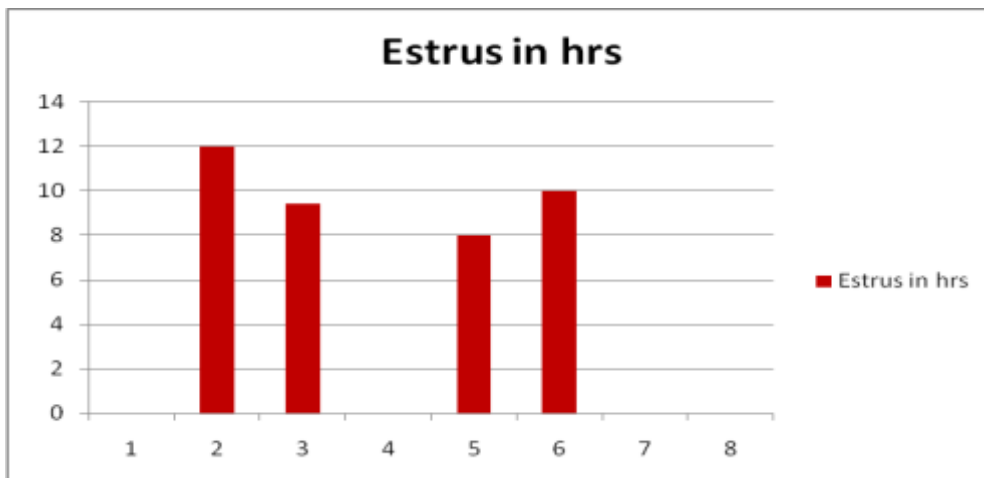
**Statistical Analysis**

The statistical analysis was carried out for interpretation of the research findings as per the method described by [10].

**RESULT AND DISCUSSION**



*Fig. 1: Estrus duration (Group- I)*



*Fig. 2. Estrus duration (Group- II)*

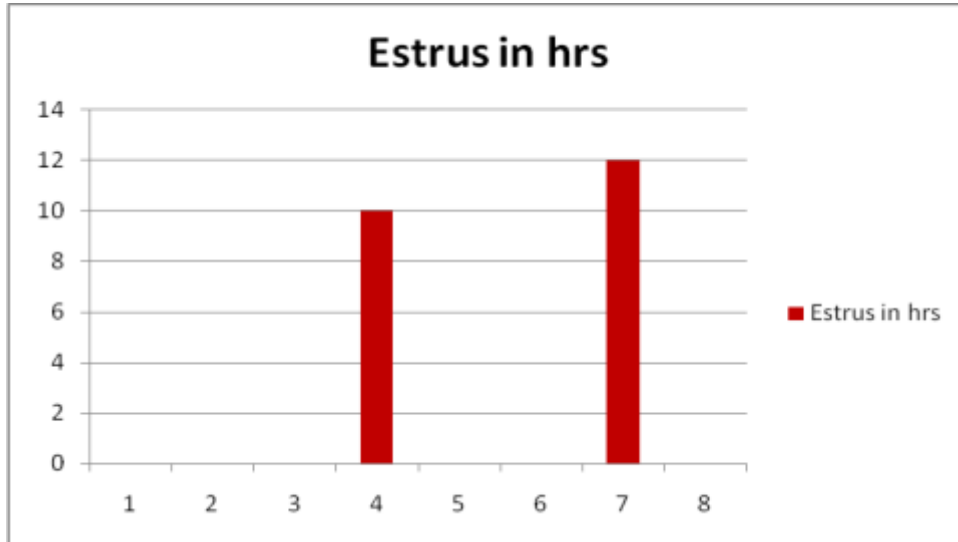


Fig. 3: Estrus duration (Group- III)

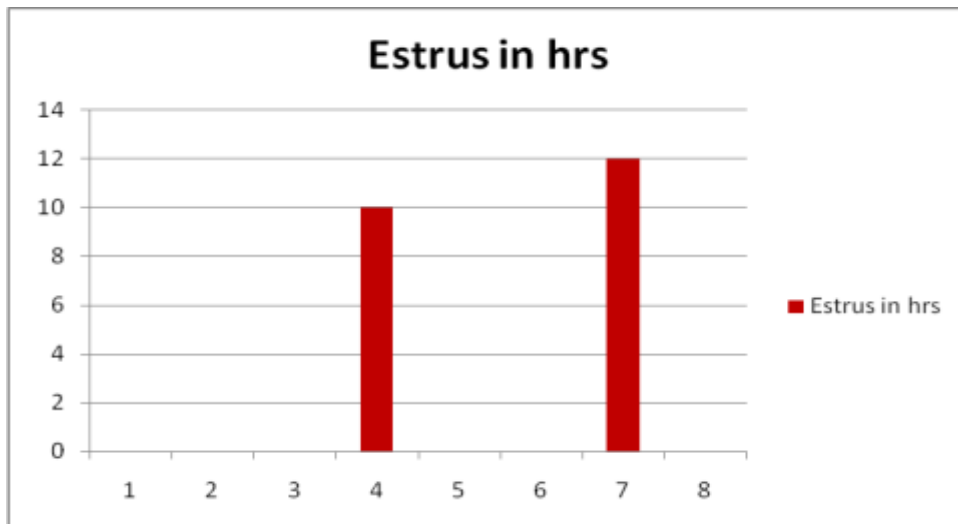


Fig. 4. Estrus duration (Group- IV)

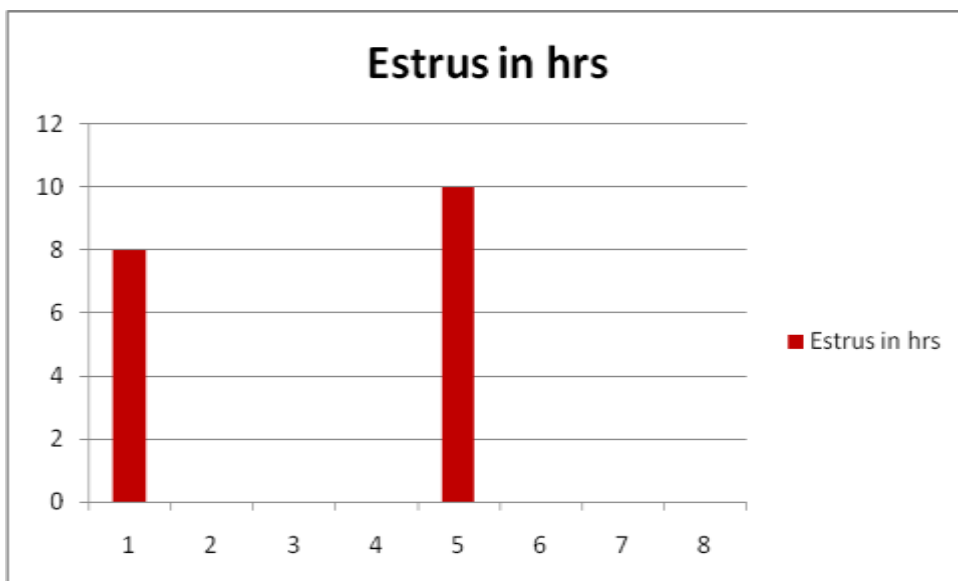


Fig. 5. Estrus duration (Group- IV)

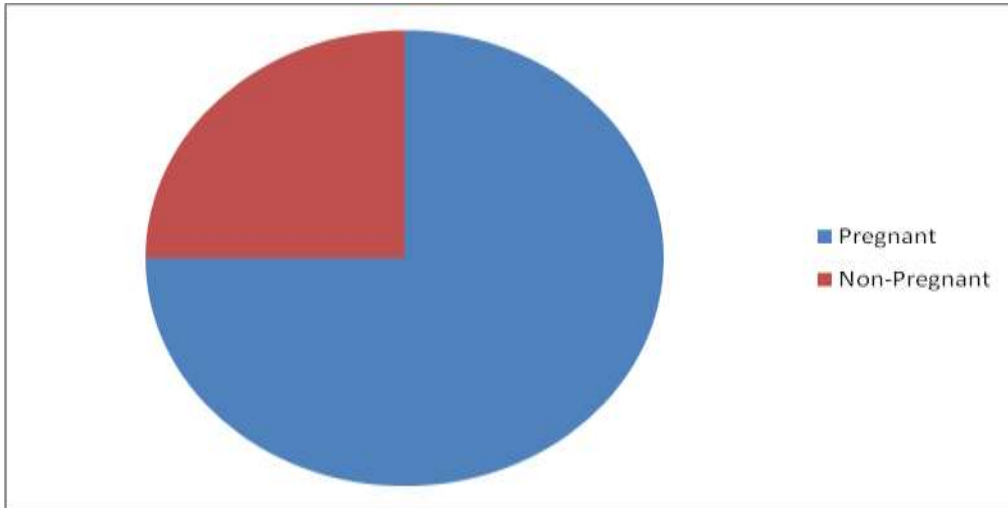


Fig. 6. Group I: Response of Feeding of Mineral mixture and ovarian massage

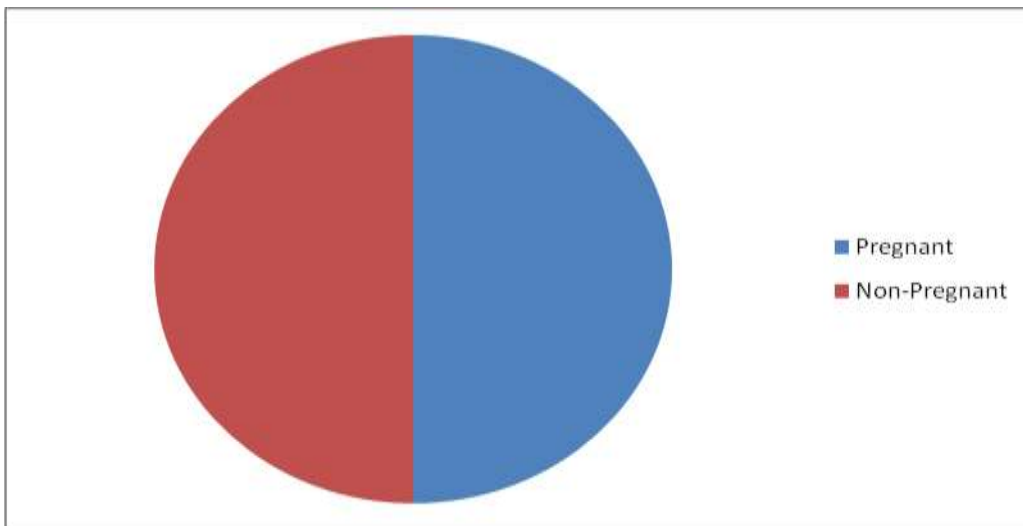


Fig. 7 Group II: Response of inj. GnRH 100 mcg on 0 and 10<sup>th</sup> day

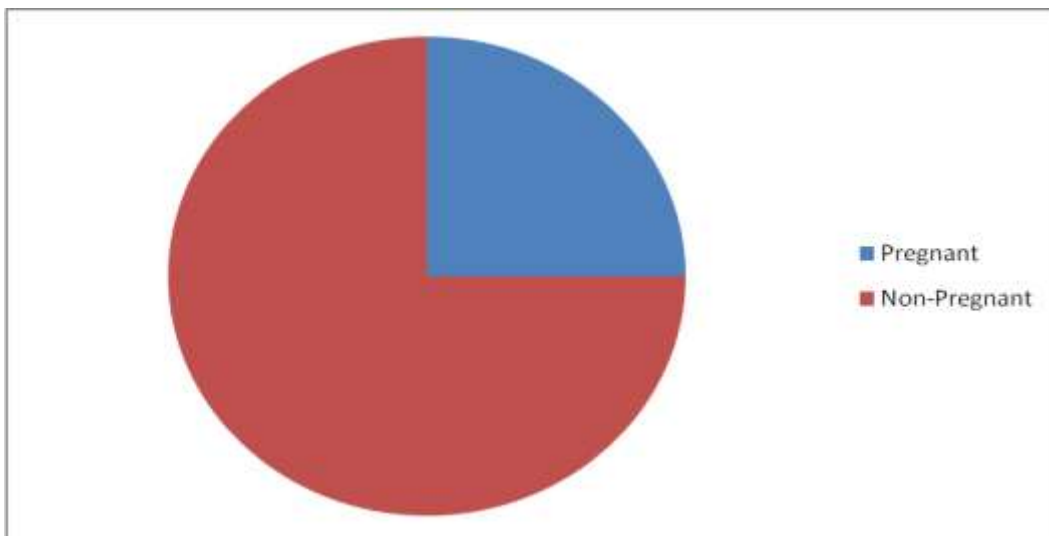


Fig. 8. III Response of inj. Progesterone on 0 day and Inj. GnRH on 10<sup>th</sup> day

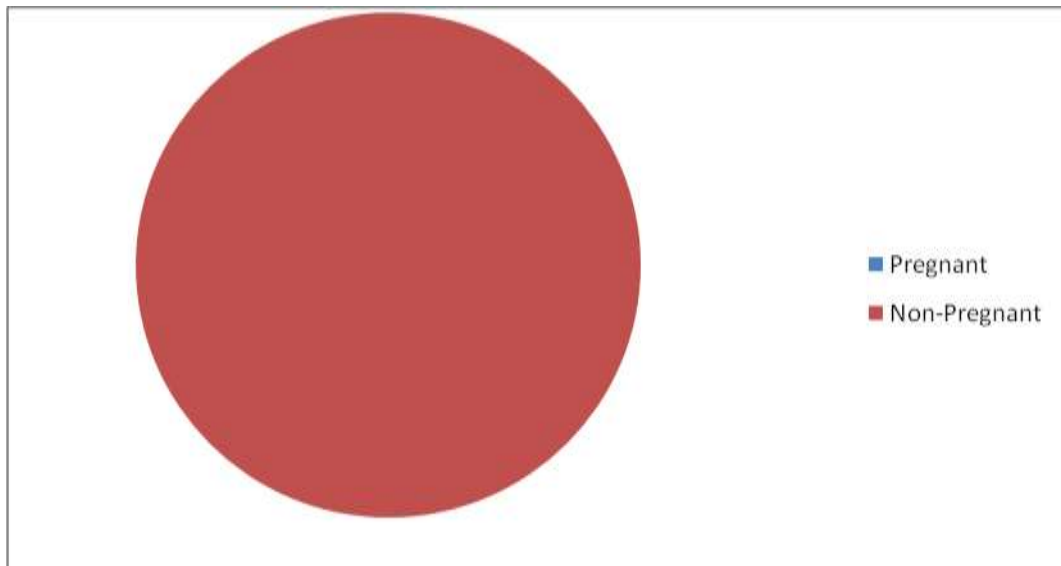


Fig. 9. IV Response of Control Group

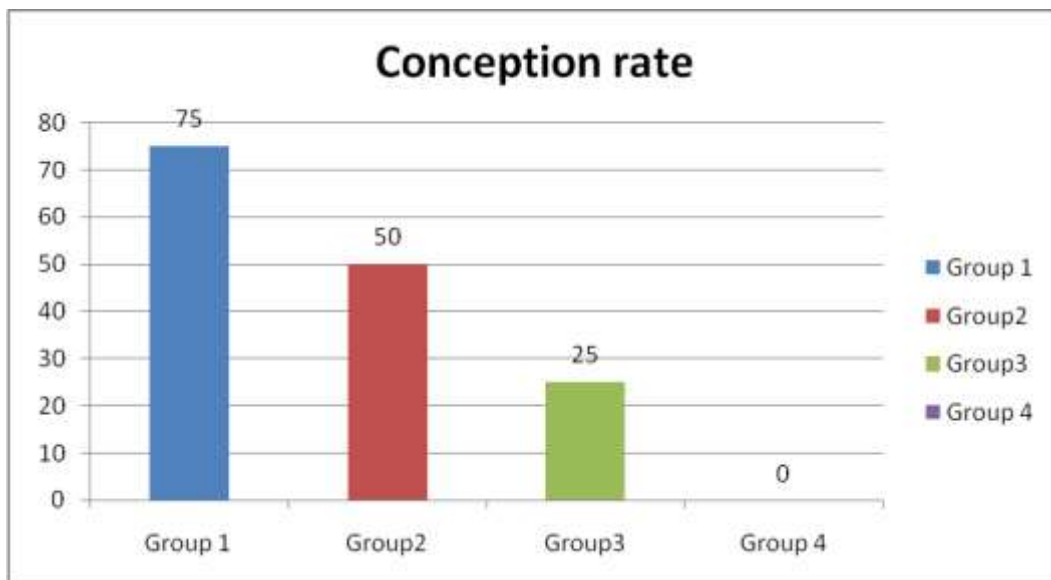


Fig. 10. Comparative conception rate in percent (Group I, II, III, IV).

All the pubertal anoestrus Marathawadi Buffalo heifers from the Group I was dewormed followed by feeding of area specific mineral supplementation with concentrate mixture and ovarian massage was carried out for 15 days with interval of 5 days. Out of eight treated buffalo heifers, seven buffalo heifers were exhibited estrus; the average duration of estrus was  $9.87 \pm 0.75$  hrs. Out of seven buffaloes six were found to be pregnant with 75.00 per cent conception rate. The present result is in accordance with the findings of [10, 11, 12, 13, 14].

Singh *et al.* (2006) reported lower overall conception rate in their GnRH treatment group and a higher overall conception rate in the mineral supplementation group [11].

The present findings are in close agreement with Singh *et al.* (2011) reported the effect of minerals supplementation on estrus induction and conception in various species [11]. Feeding of commercial mineral mixture and iodized salt in concentrate ration for 25 days resulted in ovulatory estrus in 132 crossbreed heifers. After supplementation 93.33 per cent heifers (124/132) were examined. Whereas, Naidu *et al.* (2009) studied in 80 graded Murrah buffalo heifers aged 4-5 years and found 60 per cent

conception rate in Group-II Multimineral supplementation with inorganic phosphorus and tab. Cofecu 2 tabs per day for 10 days orally, 30.40 per cent conception rate in Group I – heifers were subjected to utero-ovarian massage for 5 minutes at 5 days interval on two occasions and 20 per cent conception in Group – III Burserule acetate 10 mcg i/m once only. Group – IV Control animals.

Rahawy (2009) reported the application of the massage method of the uterus and ovaries by rectal palpation to treat true anoestrus in local Iraqi buffaloes and result of the present study indicated a significant effect of the massage method for the uterus and ovaries in heifers by producing estrus (80.82%).

Mansoor *et al.* (2011) studied in 96 Holstein Friesian cows presented in al-fayha station-Babylon, Iraq suffering from inactive ovaries in dairy cows. The study was designed to show the effect of different method for treatment of inactive ovaries in dairy cows. The animals were divided into four equal groups (n=24). The first Group were treated with 3000 IU eCG i/m. The second group was treated with 0.5 mg GnRH i/m. The third group was administered manual massage to ovaries and uterus once weekly per rectum for three times. The fourth group was artificially inseminated post treatment after signs of estrus appeared on the animals. Response to different treatments was 83.3 per cent, 50 per cent, 58.3 per cent, and 8.3 per cent respectively. It was concluded that from this study that treated with eCG and massage of ovaries was effective methods for treatment of inactive ovaries in dairy cows.

Syed (2013) reported highest estrus induction and conception rate 45 and 70 per cent respectively, in trace Mineral mixture feeding along with Folmin C Bolus @ 2 twice daily for 6 days than Illiren, and GnRH treatment groups in 27 Egyptian Murrah buffaloes.

Pubertal anoestrus Marathawadi Buffalo heifers from the Group II were treated with inj. GnRH @ 10 mcg on 0 day and non responded animals repeated with inj. GnRH @ 10 mcg on 10<sup>th</sup> day. one animal was responded within 24 hrs of treatment. Out of eight pubertal anoestrus Marathawadi buffalo heifers, four buffalo heifers exhibited estrus and the duration of estrus was Av.  $9.86 \pm 0.31$  hrs., and four animals becomes pregnant, the conception rate was 50 per cent. Observations of the present study are in accordance with the findings of [15, 16, 17].

Shama *et al.* (1991) In Receptal alone group 100 per cent cows were manifested estrus at an average post treatment interval of  $21.00 \pm 6.37$  days with 57.14 per cent C.R. The corresponding values for Receptal primed with estradiol and Receptal pretreated with Tonophosphone groups were 85.71 per cent,  $12.83 \pm 7.18$  days, 66.67 per cent and 100 per cent and  $23.57 \pm 6.29$  days, 71.43, per cent respectively. Based on the response obtained, Receptal alone for the treatment of anoestrus with smooth ovaries.

Gupta *et al.* (2012) observed 90 per cent estrus induction rate of inj. GnRH treatment than cloprostenol treatment (Vetmate) 50 per cent and conception rate 80 per cent and 78 per cent. The study was carried out in 20 post partum anoestrus cows.

Parmar (2012) treated postpartum aneustrous buffaloes (n=52) and divided into four groups. Group A: supplementation of cheated mineral mixture orally @ 30 gm. per day per animal for one month (n=14). Group B: Injection inorganic phosphorus and injection vitamin A, D3, E 10 ml each i/m (n=14) at weakly interval for 3 weeks. Group C: Injection GnRH 5 ml i/m once (n=2). Group D: Untreated control (n=12). The highest conception rate 80%, shortest service period ( $123.60 \pm 2.69$  days) and highest reproductive efficiency 66.66% was observed for group C buffaloes followed by group B, A and control groups. Injection of Receptal has beneficial effect on reproductive performance with improved conception rate and reduced service period as compared to other treatment.



All the pubertal anoestrus Marathawadi Buffalo heifers from the Group III were treated with inj. Progesterone @ 250 mg i/m on 0 day followed by inj. GnRH @ 10 mcg on 10<sup>th</sup> day. Out of eight pubertal anoestrus buffalo heifers, two buffalo heifers exhibited estrus and estrus duration was Av.11.00 hrs. Two buffalo heifers were found to be pregnant; Conception rate was 25 per cent. Observation of present study is in accordance with [18-22].

Mavi and Bahga (2007) recorded eight true anoestrus mature buffalo heifers aged 3-4 years and weighing 300-325 kg were randomly divided into two groups. Group I was injection with 100 mg progesterone (Duraprogen) daily for 10 days while, group II got two doses of 500 mg progesterone at 5 days interval. All animals were injected with increase in ovarian size and symptoms of estrus were exhibited in 75 per cent and 66 per cent animals in group I and II respectively. The number of pregnancies that occurs in group I and II were 0 and 75 per cent, respectively. Serum sodium, potassium, calcium and phosphorus levels were increase from 1 day to day 10, decrease on day 12 and again increase on day 21, which was the expected day of estrus. Calcium: Phosphorus (Ca: P) ratio also increase with progesterone administration.

Kumar *et al.* (2009) treated 34 post partum anoestrus cross breed cows. All the animals were dewormed with Fenbendazole. Animal were divided into five groups, consisting six animals in each group. Group I: 2 ml of normal saline solution intramuscularly. Group II, III, IV, V: received respectively 500 mg progesterone on day 5<sup>th</sup> and 10<sup>th</sup> as well as 20 µg GnRH on 15<sup>th</sup> day observed that estrus detected in only one animal (16.6%) of group II and estrus in five out of six animals (88.33%) in group III and estrus in five animals (83.3%) out of six animals of group IV and 100 per cent estrus induction in animals of group V.

Shugutta *et al.* (2009) recorded thirty-four post-partum anoestrus buffalo maintained under rural management system were selected and examined per rectum to conform their reproductive status. All the animals were dewormed with Fenbendazole. All the animals were administered (day 0) a single injection of prostaglandin (25 mg) and were observed closely for their estrus behavior. Two animals exhibited estrus and were excluded from the experiment. From rest 32 animals, 30 anoestrus buffaloes were equally divided into five groups (group I to V). Animals of Group I received normal saline solution (2 ml, i/m) and animals of group II, III, IV and V were injected with progesterone and GnRH. The animals were monitored for behavioral symptoms of estrus. Animals were allowed to be bred by natural service method. Group I received 25 mg PGF2 $\alpha$  i/m on day 0 and 2ml NSS i/m on day 5<sup>th</sup>, day 10<sup>th</sup>, day 15<sup>th</sup>. Group II received 25mg PGF2 $\alpha$  i/m on day 0, 250 mg progesterone i/m on day 5, 500 mg on day 10. Group III received 25 mg of PGF2 $\alpha$  i/m on day 0, 500mg of progesterone i/m on day 5<sup>th</sup>. Group IV received 25 mg PGF2 $\alpha$  i/m on day 0, 250 mg progesterone i/m on day 5, 500 mg progesterone on day 10 and GnRH 20 µg i/m on day 15<sup>th</sup>. Group V received 25 mg PGF2 $\alpha$  i/m on day 0, 500 mg progesterone i/m on day 10. There are 5 animals become pregnant from Group IV. It is concluded that anoestrus buffalo cows can be brought into fertile estrus through combined treatment of prostaglandin and progesterone on day 0 and 5<sup>th</sup> or 10<sup>th</sup> day respectively. The administration of GnRH in progesterone primed buffalo's 5<sup>th</sup> days later brought the anoestrus buffaloes to fertile estrus within 2 to 5 days. Twenty-four Parous and lactating cows, aged between 4 -10 yrs and having a body weight between 270-305 kg belonging to District Dairy Demonstration farm of the college of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhyay Pashuchikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura. The first group consists of 8 animals whereas; second and third group consists of 9 and 7 animals respectively. Group first were treated with Receptal (Buserelin – 0.004 mg/ml) @ 0.02 mg/animal i/m as a single dose, while the animal of second group received Duraprogen (17  $\alpha$  Hydroxyprogesterone caproate 250 mg i/m followed by injection of Prognone Depot @ 500 µg i/m after 48 hrs of hydroxyl progesterone injection. The animal of the third group were kept as untreated control. The animals were examined twice daily as morning evening schedule for signs of estrus. GnRH treatment was found effective in cows. Only 5 (62.5%) animals' responds to anoestrus treatment with mean time period of 88.80 $\pm$ 25.00 hr and in second group all the animals (100%) responded to the treatment within a mean time period of 57.44 $\pm$ 9.47 from last injection. While in control animals none of them came into estrus.

So, it was concluded that the condition of post partum anoestrus can effectively be treated with using P4 and E2 therapy with reasonably good conception rate and cost effectiveness compared to GnRH therapy [10]

All the pubertal anoestrus Marathawadi buffalo heifers in Group IV were of control group and no treatment was given. Out of eight animals two animals exhibited estrus, estrus duration was  $Av. 9 \pm 0.22$  hrs. None of the animal was become pregnant, the conception rate was 0.00 per cent.

## SUMMARY

Pubertal anoestrus Marathawadi buffalo heifers responded well to feeding of area specific Mineral supplementation, ovarian massage and hormonal therapy. The deworming followed by area specific Mineral supplementation and ovarian massage for 15 day with interval of 5 days could be used for treatment of pubertal anoestrus Marathawadi buffalo heifers with conception rate of 75.00 % with  $9.87 \pm 0.75$  hrs. estrus duration. The hormone therapy of inj. GnRH could be useful in treatment of pubertal anoestrus Marathawadi buffalo heifers with conception rate of 50% with  $9.86 \pm 0.31$  hrs. estrus duration. The efficacy of deworming followed by area specific Mineral supplementation and ovarian massage for 15 days with interval of 5 days was better than Inj. GnRH alone @ 10 mcg and Progesterone primed GnRH therapy. Deworming followed by area specific Mineral supplementation and ovarian massage was an economical method than the other hormonal treatments for improvement of fertility in pubertal anoestrus Marathawadi buffalo heifers.

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