

ENHANCEMENT OF CONCEPTION RATE THROUGH LUTEOTROPIC HORMONES WITH SPECIAL REFERENCE TO METABOLIC, BIOCHEMICAL PROFILE AND CERVICAL MUCUS PROPERTIES IN NON-INFECTIOUS REPEAT BREEDING MURRAH BUFFALOES

**Umesh Balkrishna Kumbhar*, Vaibhav Prakashrao Deshpande,
Sarita Ulhas Gulavane and Sandeep Mahadeo Gaikwad**

ABSTRACT

The present research work was carried out to evaluate efficacy luteotropic hormones with special refence to metabolic, biochemical profile and cervical mucus properties in non-infectious repeat breeding Murrah buffaloes. The research was conducted at Department of Animal Reproduction, Gynaecology and Obstetrics, Bombay Veterinary College, Mumbai and Murrah buffalo dairy farms of Aarey Colony, Goregaon, Mumbai. Out of 60 repeat breeding Murrah buffaloes of 6 to 8 years old, thirty two pluriparous Murrah buffaloes of non-infectious origin were selected on the basis of white side test for the present study. The incidence of infectious and non-infectious repeat breeding buffaloes was 44.66% and 53.33%, respectively in 60 repeat breeding buffaloes of Aarey colony. The cervical mucus properties, serum metabolic and biochemical profile was performed in 32 Murrah buffaloes. The overall mean serum phosphorus, glucose, BUN concentration was 4.29 ± 0.13 mg/dL, 45.51 ± 0.91 mg/dL, 13.37 ± 0.46 mg/dL respectively. In present study, out of 32 non-infectious repeat breeder buffaloes, 21 (65.63%), 10 (31.25%) and 1 (3.12%) buffaloes showed typical, atypical and nil fern pattern of cervical mucus, respectively. The mean pH of cervical mucus in conceived

buffaloes was 7.54 ± 0.09 while in non-conceived buffaloes it was 7.44 ± 0.06 . Out of 32 non-infectious repeat breeder buffaloes, 16 (50.00%), 6 (18.75%) and 10 (31.25%) buffaloes showed thin and thick consistency of cervical mucus discharge, respectively. All the 32 (100%) non-infectious repeat breeder buffaloes of all the four groups showed clear cervical mucus. The Murrah buffaloes were randomly divided into four groups with eight animals in each group. The Group I was treated with Inj. GnRH 20 μ g while Group II and Group III were treated with Inj. hCG 1500 IU and 3000 IU, respectively on day 7 after Artificial Insemination. Group IV was kept as control. The pregnancy was confirmed after 2 months of Artificial Insemination by per rectal examination. In present research work, the conception rates in Murrah buffaloes of Group I, II, and III were 50.00% (4/8), 50.00% (4/8) and 62.50% (5/8), respectively. In Group IV none of the eight Murrah buffaloes was conceived. The overall conception rate in the present study was 40.67% (13/32). From present research it is concluded that the higher conception rate can be expected when non-infectious repeat breeding buffaloes shows typical fern pattern with thin consistency of mucus, alkaline pH and colour was transpernt. The serum biochemical estimation of blood urea nitrogen, glucose and phosphorus

was in normal concentration. Also there was no significant difference in the values of these components among conceived and non-conceived buffaloes. All the three blood constituents were found to have no effect on conception. The hormones GnRH and hCG both can be used to treat the repeat breeding Murrah buffaloes of non-infectious origin when administered on day 7 after artificial insemination. The efficacy of human chorionic gonadotropin 3000 IU is more than that of GnRH 20 µg and hCG 1500 IU to enhance the conception rate. The treatment protocols of present studies were found beneficial to increase the serum progesterone concentration when given on day 7 after AI therefore administration of hCG or GnRH on day 7 after AI could be used as a management tool to enhance the progesterone concentration and thereby to improve conception rate in non-infectious repeat breeding Murrah buffaloes.

Keywords: *Bubalus bubalis*, buffaloes, non infectious repeat breeding, BUN, glucose, phosphorus, cervical mucus, white side test, GnRH, hCG, conception

INTRODUCTION

Buffalo is considered as black diamond due to its great position among the milch animals. They are triple purpose animals, being suitable for milk, meat and draught. In India, buffalo has eminent position among the milk producing animals. India has 108.7 million Murrah buffaloes contributing more than 50% of total milk production in the country (Livestock Census, India, 2012). One of the most important and commonly encountered sub fertile conditions in buffalo which plays a vital role in dairy economics is repeat breeding.

Repeat breeding is among reproductive disorders which hinder favourable productivity in Murrah buffaloes (Sah and Nako, 2006). A repeat breeder buffalo is an animal which does not conceive with 3 or more than 3 consecutive natural services or artificial inseminations. It exhibits normal signs of estrous every 18 to 24 days but require more than 3 services to become pregnant (Hafez, 2000). Luteal dysfunction leading to inadequate progesterone production post-breeding could be a cause of embryonic death. It results in fertilization failure and early embryonic mortality (Diskin and Morris, 2008) that ultimately cause repeat breeding. Studies have shown that administration of GnRH, GnRH agonist and hCG after AI can stimulate CL function, increase progesterone, reduce estradiol production with a consecutive positive effect on embryonic survival (Bartolome, 2005). In present study, efficacy of two luteotropic hormones GnRH and hCG at two different doses with special reference to metabolic, biochemical profile and cervical mucus properties in non-infectious repeat breeding Murrah buffaloes have been studied.

MATERIALS AND METHODS

The present research work was conducted in 6 to 8 years old, pluriparous, 32 Murrah buffaloes with history of repeat breeding syndrome. To identify non infectious repeat breeding Murrah buffaloes, total 100 Murrah buffaloes were screened with the white side test and 32 Murrah buffaloes showing negative result to white side test were selected for the present study from Aarey colony, Goregaon, Mumbai. The per-rectal examination was performed twice 8 days apart to confirm the cyclicity.

The cervical mucus was collected by a

sterile AI sheath connected with 20 ml syringe was inserted per-vaginally and the cervical mucus which represented the endometrial secretion was aspirated from the os or mid cervix and then transferred to a sterilized test tube to perform white side test to diagnose repeat breeder buffaloes having uterine infection. For white side test one ml of the cervical mucus was mixed with one ml of 5% sodium hydroxide (5% NaOH) solution in a test tube and heated up to the boiling point and subsequently cooled in running tap water. The appearance of yellow colour was taken as positive indication of infection. The buffaloes showing negative result to the white side test i. e. showing no color on performing the test were selected for the study. Physical characters of the cervical mucus namely color, consistency, pH and fern pattern were examined immediately after the collection of sample on the day of estrous. Normal color of cervical mucus is clean and transparent. Any variation from normal color was noted with visual examination. Consistency denotes the degree of thickness of cervical mucus. consistency was recorded as thin, medium and thick. A narrow range pH paper was used to determine the pH of cervical mucus. The strip was dipped into the test tube containing cervical mucus. Then it was compared with the standard color pH strip and was noted. The fern pattern of cervical mucus was evaluated on clean, dry glass slide and observed under low power (10X) of microscope for fern pattern. It was noted as typical, atypical and nil. Blood samples of all 32 buffaloes were collected aseptically from jugular vein on day of estrous for the estimation of BUN, glucose and phosphorus concentration. The serum biochemical constituents of all buffaloes; namely blood urea nitrogen (BUN), glucose and phosphorus were estimated by using readymade kits and by employing standard

laboratory methods.

The animals were randomly divided into four groups with eight Murrah buffaloes in each group. All the animals were inseminated after estrous detection by AMPM rule. The Group I (n=8) Murrah buffaloes were injected with Inj. Gyn arich (Buserelin acetate) 20 µg intramuscularly on day 7 after Artificial Insemination. Group II (n=8) Murrah buffaloes were injected Inj. Chorulon (Human Chorionic Gonadotropin) 1500 IU intramuscularly on day 7 after Artificial Insemination. Group III (n=8) Murrah buffaloes were injected Inj. Chorulon (Human Chorionic Gonadotropin) 3000 IU on day 7 after Artificial Insemination while Group IV (n=8) Murrah buffaloes were kept untreated after AI. The pregnancy diagnosis was performed by per-rectal examination after 60 day of Artificial Insemination.

The conception rate of the buffaloes among all the groups under study was calculated by following formula:

$$\text{Conception rate (CR) \%} = \frac{\text{Number of animals conceived in a group}}{\text{Total number of animals inseminated in group}}$$

The recorded data was organized systemically and analyzed statistically by using suitable design to draw appropriate conclusion according to Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Efficacy of different hormonal protocols on conception rate

In present study, hormonal treatment was employed to treat the noninfectious repeat breeding Murrah buffaloes. The conception rates were studied in all the four groups. Group I was treated with GnRH hormone 20 µg, Group II was

treated hCG hormone 1500 IU and Group III was treated with 3000 IU while Group IV was kept untreated which served as control.

In present research work, the conception rates in Murrah buffaloes of Group I, II, and III were 50.00% (4/8), 50.00% (4/8) and 62.50% (5/8), respectively (Table 1). In Group IV none of the eight Murrah buffaloes was conceived. The overall conception rate in the present study was 40.67% (13/32) in non-infectious repeat breeding Murrah buffaloes.

The present finding of Group I is in agreement with Pandey (2016) who reported 51.30% conception rate in Murrah Murrah buffaloes when 20 µg GnRH was given on the day of AI. Close findings were reported by Kharche and Shrivastava (2006) who reported 45.00% conception rate when GnRH was given 20 µg after AI in repeat breeder cows and also with Rao (2000) who reported 55% conception rate when GnRH was given 20 µg on the day of insemination in repeat breeder cows. Hailu *et al.* (2015) also reported conception rate of 55% when GnRH was given on day 12 after AI in repeat breeding cows. Lower findings were reported by Zain (1996) 40.90% conception rate in repeat breeding Murrah buffaloes when GnRH was given on day 6 to 8.

The findings of Group II are similar to that of Sianangama (1992) who reported 55.00% conception rate in cows when treated with hCG intramuscularly 1500 IU on day 14 while 62.00% conception rate was also reported by the same author in cows treated with hCG intramuscularly 1500 IU on day 7 which is higher finding than present observations. The present research work findings are slightly higher than Kumar *et al.* (1994) who reported 46.15% conception rate by using hCG 1500 IU in repeat breeding crossbred cows

The present findings of Group III are in agreement with Pandey (2016) who reported 66.70% conception rate by using hCG 3000 IU on the day of AI in Murrah Murrah buffaloes. The conception rate achieved with 3000 IU hCG is also in agreement with Sandhu and Singh (1992) who reported 67.20% conception rate using hCG 3000 IU intravenously on the day of insemination in repeat breeding crossbred cows.

The present results shows that both GnRH and hCG hormone can be effectively used on day 7 after AI to treat repeat breeding Murrah buffaloes of non-infectious origin. It may be due to luteotropic action of GnRH and hCG hormone on CL and thereby preventing early luteolysis. However the difference in the conception rate reported by different researchers could be due to the difference in the species, reproductive status, dose rate or day of treatment.

White side test

White side test was performed to select the repeat breeding buffaloes of non-infectious origin. In Aarey colony from three farms, out of 600 buffaloes, 60 buffaloes were found with the history of repeat breeding syndrome. The incidence of non-infectious origin of repeat breeding syndrome in buffaloes of Aarey colony was calculated from selected buffaloes with history of repeat breeding.

Present study shows that, out of 60 screened buffaloes having history of repeat breeding, 28 buffaloes were suffering from repeat breeding syndrome of infectious origin while 32 buffaloes were suffering from repeat breeding syndrome of non-infectious origin. The incidence of infectious and non-infectious repeat breeding buffaloes was 44.66% and 53.33%, respectively in 60 repeat breeding buffaloes of Aarey colony. The 32 repeat breeding buffaloes of non-infectious

origin obtained with white side test were further selected for the present research work.

Study of properties of cervical mucus

The properties of cervical mucus are related to the fertility in bovines. In the present study, cervico-vaginal mucus of 32 selected repeat breeding buffaloes was evaluated on the day of estrous when AI was done for its physical properties namely color, consistency, pH and fern pattern. The results are discussed as follows;

Colour

The color of cervical mucus of all 32 non-infectious repeat breeding buffaloes was evaluated visually. It was evaluated with three categories; clear, turbid and dirty. The findings were also co-related with conception. The results are given Table 2.

In present study, all the 32 (100%) non-infectious repeat breeder buffaloes of all the four groups showed clear cervical mucus (Table 2). It is also clear that 13 (40.62%) conceived as well as 19 (59.38%) non-conceived buffaloes both showed clear color of cervical mucus. The present findings are in agreement with Kumar *et al.* (2011) who reported 100% clear color of cervical mucus in repeat breeding buffaloes. Sharma *et al.* (1978); Rangnekar *et al.* (2002) also reported clear color of estrual discharge in 100% repeat breeding cows. The findings of present study are higher than Verma *et al.* (2014) who reported 85.10% clear color of cervical mucus in normal buffaloes and are also higher than Enkhia and Kohli (1982); Sharma and Tripathi (1987); Malik *et al.* (2014) who reported 50%, 75% and 35.66% clear color of cervical mucus in repeat breeder cows, respectively. The present findings differed from Samad *et al.* (2002) who reported 50% clear, 38.33% translucent

and 6.67% whitish discharge in repeat breeding buffaloes. This difference in the results may be due to infectious origin of repeat breeding syndrome in some buffaloes and cows. In present study overall conception rate in non-infectious repeat breeding buffaloes with clear cervical mucus was 40.62%. The present results are less than Agarwal and Purbey (1983) who reported maximum conception rate (55%) when buffaloes had clear cervical mucus discharge. Saphale *et al.* (1993) also reported higher fertility (80%) in repeat breeding cows with clear cervical mucus discharge. This difference could be due to the repeat breeding syndrome in present experimental buffaloes. However, in present study both conceived and non-conceived repeat breeder buffaloes showed clear color of cervical mucus, hence it can be said that color of cervical mucus does not play any role in predicting fertility of the non-infectious repeat breeder buffaloes but clear color of cervical mucus is suggestive of non-infectious origin in repeat breeding buffaloes.

Consistency

The consistency of the cervical mucus in present study was evaluated by taking small quantity of cervical mucus on glass slide and then turning the slide at 45° to see the movement of mucus. It was categorized into thin, medium and thick depending upon the movement of cervical mucus on slide. The consistency was also correlated with the conception. The results are given in Table 3.

In present study, out of 32 non-infectious repeat breeder buffaloes, 16 (50.00%), 6 (18.75%) and 10 (31.25%) buffaloes showed thin and thick consistency of cervical mucus discharge, respectively (Table 3). In Group I, II, III and IV consistency of cervical mucus discharge was thin in 4 (50.00%), 3 (37.50%), 4 (50.00%) and 4 (50.00%)

buffaloes, medium in 1 (12.50%), 2 (25.00%), 2 (25.00%) and 1 (12.50%) buffaloes while thick in 3 (37.50%), 3 (37.50%), 2 (25.00%) and 3 (37.50%) buffaloes, respectively (Table 3). The present findings are in agreement with Samad *et al.* (2002) who reported thin consistency in 50% in repeat breeder buffaloes. Kumar *et al.* (2011); Sharma *et al.* (1978) reported 55.55% thin consistency of cervical mucus in repeat breeder buffaloes and 55.81% thin consistency in repeat breeder cattle, respectively, which were close findings to the present results. The present findings of thin consistency are higher than the findings noted by Saphale *et al.* (1993); Jadhav (1996); Rangnekar *et al.* (2002); Malik *et al.* (2014); Verma *et al.* (2014) who reported thin consistency in 40.00%, 34.28%, 35.00%, 15.66% and 15.96%, respectively in repeat breeding cows. The findings are lower than the findings given by Enkhia and Kohli (1982) who reported 80% thin consistency in repeat breeder cows.

However In present study, consistency of cervical mucus discharge was thin, medium and thick in 6 (50.00%), 3 (23.08%) and 4 (30.77%) buffaloes, respectively from 13 conceived buffaloes (Table 3). Out of 19 non-conceived buffaloes, cervical mucus discharge was thin, medium and thick in 10 (52.63%), 3 (15.79%) and 6 (31.58%) buffaloes, respectively (Table 3). The percentage of conception was higher among buffaloes having thin consistency of cervical mucus than those having medium and thick consistency. This finding was in agreement with Kumar *et al.* (2011) but was in contrast to the Dhaliwal and Sharma (1988) who reported higher conception rate with thick cervical mucus. This higher conception with thin cervical mucus could be due to easy sperm penetration.

pH (Hydrogen ion concentration)

The pH of the cervical mucus of all the

buffaloes was calculated by using narrow range pH paper. The values of the pH were correlated with conception.

Present findings are in agreement with Samad *et al.* (2002) who reported mean pH value of cervical mucus 7.49 in repeat breeding buffaloes. In repeat breeding cows slightly higher findings were reported by, Reddy (1974) 7.89, Wani *et al.* (1981) 7.93, Rangnekar *et al.* (2002) 7.71, while slightly lower findings were reported by Sinha (1974) 7.27, Bishnoi *et al.* (1983) 7.38 and Pandey *et al.* (1983) 7.25.

In the present study, pH of cervical mucus of all the 32 non-infectious repeat breeding buffaloes was alkaline and this finding is in agreement with Rangnekar *et al.* (2011) who reported pH of the repeat breeding animals more alkaline in reaction. The mean pH of cervical mucus in conceived buffaloes was 7.54 ± 0.09 while in non-conceived buffaloes it was 7.44 ± 0.06 . This data was analyzed statistically and no significant difference was found in the pH of cervical mucus among conceived and non-conceived buffaloes.

Fern pattern

Fern pattern/arborization pattern of cervical mucus of all the buffaloes under study was classified into three categories; typical, atypical and nil fern pattern. The findings are noted in Table 5.

In present study, out of 32 non-infectious repeat breeder buffaloes, 21 (65.63%), 10 (31.25%) and 1 (3.12%) buffaloes showed typical, atypical and nil fern pattern of cervical mucus, respectively (Table 5). In Group I, II, III and IV fern pattern was typical in 4 (50.00%), 6 (75.00%), 5 (62.50%) and 6 (75.00%) buffaloes, respectively while atypical in 3 (37.50%), 2 (25.00%), 3 (37.50%) and 2 (25.00%) non-infectious repeat breeder

buffaloes, respectively (Table 5). Only one buffalo (12.50%) from Group I showed nil fern pattern which remained non-pregnant.

The present results are quite similar to Rangnekar *et al.* (2002) who reported 60% typical, 35% atypical and 5% nil fern pattern of cervical mucus in repeat breeding cows. The present findings of typical fern pattern are higher than Samad *et al.* (2002) who reported 48.33% typical fern pattern in repeat breeding buffaloes and Kumaresan *et al.* (2001) who reported it 40.00% in normal buffaloes. However, present findings of atypical fern pattern are lower than Samad *et al.* (2002) who reported 45.00% atypical fern pattern in repeat breeding buffaloes. The present findings are close to Sharma *et al.* (1978); Malik *et al.* (2014) who reported typical fern pattern 60.46% and 60.00% in repeat breeding cows, respectively. The difference in these observations may be due to species difference, breed difference, age difference, difference in number of calving or presence of infection.

This fern pattern was also correlated with the conception. In the present study out of 13 conceived buffaloes, 10 (76.92%) showed typical fern pattern and 3 (23.08%) showed atypical fern pattern (Table 5). From Table 5, it is clear that maximum conception was observed with typical fern pattern than atypical and nil fern pattern and this finding of higher conception with typical fern pattern is in agreement with Agarwal and Purbey (1983); Kumaresan *et al.* (2001); Verma *et al.* (2014) who reported maximum conception rates 67%, 57.50% and 54.90%, respectively, with typical fern pattern than atypical fern pattern. Non-conceived buffaloes showed both typical (57.89%) as well as atypical (36.84%) fern pattern (Table 5). Hence it can be concluded that higher conception rate can be expected when non-infectious repeat breeding

buffaloes shows typical fern pattern.

Serum biochemical estimation of BUN, glucose and phosphorus

The standard laboratory methods were applied for the estimation of serum concentration of Blood urea nitrogen, glucose and phosphorus. The values obtained were co-related with findings of other researchers and its co-relation with conception was also studied. The mean values of BUN, glucose and phosphorus are shown in Table 6.

Blood urea nitrogen

In present research work, mean serum concentration of blood urea nitrogen in Group I, II, III and IV were 13.63 ± 1.13 , 12.71 ± 1.03 , 13.54 ± 1.12 and 13.76 ± 0.97 mg/dL, respectively. The overall serum BUN concentration in 32 non-infectious repeat breeder buffaloes was 13.37 ± 0.46 mg/dL (Table 6). The blood urea nitrogen values of the non-infectious repeat breeding buffaloes were within normal range. The present results are similar to the findings of Couch *et al.* (2017) who reported average mean value of BUN in African buffalo as 13.66 ± 0.36 mg/dL and were also in agreement with Amle *et al.* (2014) who reported mean BUN value of 13.44 ± 1.15 mg/dL in repeat breeding crossbred cows. The present findings are lower than Parmar *et al.* (1986) who reported blood BUN range of 17.41 ± 2.61 to 28.88 ± 1.87 mg/dL in repeat breeder cows. In present study, the mean serum concentration of BUN in 13 conceived buffaloes was 13.24 ± 0.64 mg/dL and in non-conceived buffaloes it was 13.46 ± 0.65 mg/dL. This data was analyzed statistically. There was no significant difference in the mean values of serum BUN concentration of conceived and non-conceived repeat breeding buffaloes of non-

infectious origin. Hence the blood urea nitrogen concentration was found to have no significant correlation with conception.

Glucose

In the present study, the average mean values of serum glucose concentration in Group I, II, III and IV were 45.44 ± 2.54 , 43.89 ± 1.16 , 46.45 ± 1.97 , 46.08 ± 1.58 mg/dL, respectively (Table 6). The overall mean serum glucose concentration of 32 non-infectious repeat breeding buffaloes was 45.51 ± 0.91 mg/dL (Table 6). The serum Glucose levels of all the non-infectious repeat breeding buffaloes were within normal range. The present findings are lower than Arshad *et al.* (2005) who reported mean average value of blood glucose as 123.55 ± 3.5 mg/dL in normal buffaloes and 61.2 ± 1.02 mg/dL in repeat breeding buffaloes, respectively. The present findings are in agreement with Ramkrishna (1996) who reported average mean value of blood glucose 45.6 ± 1.52 mg/dL and Mondal and Paul (2012) who reported mean serum glucose concentration of 49.2 mg/dL in repeat breeder cows. The mean serum glucose concentration in conceived buffaloes was 43.33 ± 1.08 mg/dL while in non-conceived buffaloes it was 46.70 ± 1.26 mg/dL. This data was analyzed statistically. There was no significant difference in the mean serum glucose concentration among conceived and non-conceived non-infectious repeat breeding buffaloes hence, in present study, no significant correlation was found between serum glucose concentration and conception.

Inorganic phosphorus

In the present study, the average mean value of serum phosphorus concentration in Group I, II, III and IV were 3.63 ± 0.26 , 4.17 ± 0.16 , 4.54 ± 0.19 and 4.54 ± 0.25 mg/dL, respectively (Table 6). The

overall mean serum phosphorus concentration was 4.29 ± 0.13 mg/dL (Table 6). The serum phosphorus concentration of most of the non-infectious repeat breeding buffaloes was within normal range while few showed slightly lower concentration than normal. The present findings are similar to Chaurasia *et al.* (2010) who reported 4.50 ± 0.16 mg/dL mean phosphorus concentration in repeat breeder buffaloes and also with Ramkrishna (1996); Amle *et al.* (2011) who reported 4.51 ± 0.18 mg/dL and 4.44 ± 0.17 mg/dL mean phosphorus value in repeat breeder cows, respectively. The present results are higher than Akhtar (2014) who reported average mean value of phosphorus 3.8 ± 0.31 mg/dL in normal buffaloes and 3.71 ± 0.19 mg/dL in repeat breeding buffaloes, respectively. In repeat breeding cows, Rupde (1993); Barui *et al.* (2015) reported lower average mean value of phosphorus 3.375 ± 0.22 mg/dL and 2.08 ± 0.10 mg/dL respectively. The present findings are within the range 3.82 to 5.29 mg/dL reported by Jaychandran *et al.* (2007) in repeat breeding buffaloes.

The present results are lower than Shrivastava and Kharche (1986); Shahzad *et al.* (2016) who reported 6.60 mg/dL and 5.55 ± 0.32 mg/dL mean serum phosphorus concentration in repeat breeding buffaloes, respectively. Also the present findings are lower than Ceylan *et al.* (2008); Kumar *et al.* (2009); Das *et al.* (2009) who reported mean serum phosphorus concentration 5.19 ± 0.22 , 8.90 ± 0.21 and 5.45 ± 0.15 mg/dL, respectively in repeat breeder cows. The mean serum concentration of serum inorganic phosphorus in conceived buffaloes was 4.46 ± 0.19 mg/dL and in non-conceived buffaloes 4.17 ± 0.16 mg/dL. This data was analyzed statistically. There was no significant difference in the mean serum level of inorganic phosphorus among the conceived and non-conceived non-infectious repeat

Table 1. Conception rates in different hormonal protocols.

Sr. No.	Group	No. of animals treated	No. of animals conceived	Conception rate (%)
1.	Group I	8	4	50.00
2.	Group II	8	4	50.00
3.	Group III	8	5	62.50
4.	Group IV	8	0	0.00
Total/Overall		32	13	40.67%

Table 2. Details of Color of cervical mucus of repeat breeding buffaloes.

Group	No. of animals	Clear
Group I	8	8 (100%)
Group II	8	8 (100%)
Group III	8	8 (100%)
Group IV	8	8 (100%)
Overall	32	32 (100%)
Color of cervical mucus with relation to conception		
Conceived	13 (40.62%)	13 (100%)
Non-conceived	19 (59.38%)	19 (100%)

Table 3. Details of consistency of cervical mucus of repeat breeding Murrah buffaloes.

Group	No. of animals	Thin	Medium	Thick
Group I	8	4 (50.00%)	1 (12.50%)	3 (37.50%)
Group II	8	3 (37.50%)	2 (25.00%)	3 (37.50%)
Group III	8	4 (50.00%)	2 (25.00%)	2 (25.00%)
Group IV	8	4 (50.00%)	1 (12.50%)	3 (37.50%)
Overall	32	16 (50.00%)	6 (18.75%)	10 (31.25%)
Consistency of cervical mucus in relation to conception				
Conceived	13	6 (46.15%)	3 (23.08%)	4 (30.77%)
Non-conceived	19	10 (52.63%)	3 (15.79%)	6 (31.58%)

Table 4. Details of pH value of cervical mucus.

Group	No. of animals	Mean + SE pH value
Group I	8	7.56±0.10
Group II	8	7.58±0.11
Group III	8	7.43±0.10
Group IV	8	7.35±0.08
Overall	32	7.48±0.05
Mean pH value in relation to conception		
Conceived	13 (40.62%)	7.54±0.09
Non-conceived	19 (59.38%)	7.44±0.06

Table 5. Details of fern pattern of cervical mucus.

Group	No. of animals	Typical	Atypical	Nil
Group I	8	4 (50.00%)	3 (37.50%)	1 (12.50%)
Group II	8	6 (75.00%)	2 (25.00%)	0
Group III	8	5 (62.50%)	3 (37.50%)	0
Group IV	8	6 (75.00%)	2 (25.00%)	0
Overall	32	21 (65.63%)	10 (31.25%)	1 (3.12%)
Fern pattern in relation to conception				
Conceived	13	10 (76.92%)	3 (23.08%)	0
Non-conceived	19	11 (57.89%)	7 (36.84%)	1 (5.26%)

Table 6. Average mean serum BUN, glucose and phosphorus concentration study.

Group	No. of animals	BUN (mg/dL)	Glucose (mg/dL)	Phosphorus (mg/dL)
Group I	8	13.63±1.13	45.44±2.54	3.63±0.26
Group II	8	12.71±1.03	43.89±1.16	4.30±0.27
Group III	8	13.54±1.12	46.45±1.97	4.54±0.19
Group IV	8	13.76±0.97	46.08±1.58	4.54±0.25
Overall	32	13.37±0.46	45.51±0.91	4.29±0.13
BUN, glucose, phosphorus in relation to conception				
Conceived	13	13.24±0.64	43.33±1.08	4.46±0.19
Non-conceived	19	13.46±0.65	46.70±1.26	4.17±0.16

breeding buffaloes. this finding is in agreement with Balakrishnan and Balagopal (1994) who reported no difference in the levels of blood serum phosphorus in regular and repeat breeder buffaloes. In the present study serum inorganic phosphorus concentration found to have no correlation with conception.

From present research it is concluded that the higher conception rate can be expected when non-infectious repeat breeding buffaloes shows typical fern pattern with thin consistency of mucus, alkaline pH and colour was transparent. The serum biochemical estimation of blood urea nitrogen, glucose and phosphorus was done by standard laboratory techniques. All the 32 non-infectious repeat breeding buffaloes under study showed normal concentration of BUN, glucose and phosphorus. Also there was no significant difference in the values of these components among conceived and non-conceived buffaloes. All the three blood constituents were found to have no effect on conception. From the literature cited in present research work it is seen that the more study is expected related to BUN, glucose and phosphorus levels in repeat breeding buffaloes. The hormones GnRH and hCG both can be used to treat the repeat breeding Murrah buffaloes of non-infectious origin when administered on day 7 after artificial insemination. The efficacy of human chorionic gonadotropin 3000 IU is more than that of GnRH 20 µg and hCG 1500 IU to enhance the conception rate. The treatment protocols of present studies were found beneficial to increase the serum progesterone concentration when given on day 7 after AI therefore administration of hCG or GnRH on day 7 after AI could be used as a management tool to enhance the progesterone concentration and thereby to improve conception rate in non-infectious repeat breeding Murrah buffaloes.

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