

ENHANCEMENT OF CONCEPTION RATE THROUGH LUTEOTROPIC HORMONES AND ITS EFFECT ON PROGESTERONE CONCENTRATION IN NON-INFECTIOUS REPEAT BREEDING MURRAH BUFFALOES

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ABSTRACT

The present research work was carried out to evaluate efficacy luteotropic hormones and its effect on progesterone concentration in non-infectious repeat breeding Murrah buffaloes. The research was conducted at Department of Animal Reproduction, Gynaecology and Obstetrics and Radio Immunity Assay (RIA) Laboratory of Bombay Veterinary College, Mumbai and buffalo dairy farms of Aarey Colony, Goregaon, Mumbai. Out of 100 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 Murrah buffaloes were randomly divided into four groups with eight animals in each group. The Group I was treated with Inj. GnRH 20 µg 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). The mean serum progesterone concentration in Group I, II, III and IV on day 7 was 2.53±0.26, 2.57±0.39, 3.77±0.58, 2.54±0.28 ng/ml, respectively while on day 14 it was 5.74±0.19, 5.99±1.06, 7.34±0.90, 3.96±0.27 ng/ml, respectively. There was no significant difference in mean progesterone concentration between Group I, II, III and IV on day 7 but there was significant difference in mean serum progesterone concentration between Group I, II, III and IV at 5% level on day 14. All the groups treated with respective protocols showed significant increase in the mean serum progesterone concentration on day 14 from day 7. This significant increase clearly indicates that the hormones GnRH 20 µg, hCG 1500 IU and hCG 3000 IU can be effectively used to enhance the progesterone level in non-infectious repeat breeder Murrah buffaloes when given on day 7 after artificial insemination.

Keywords: *Bubalus bubalis*, buffaloes, RIA, progesterone, conception, GnRH, hCG

INTRODUCTION

Buffalo is considered as black diamond due

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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 along with its effect on progesterone concentration 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

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 animals were randomly divided into four groups with eight Murrah buffaloes in each group. All the animals were inseminated after estrous detection by AM PM rule. The Group I (n=8) Murrah buffaloes were injected with Inj. Gynarich (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.

For estimation of serum progesterone concentration, the blood samples of all the Murrah buffaloes were collected aseptically from the jugular vein on day 7 and 14 after AI. The serum was separated and stored at -20°C till the analysis. The blood samples on day 7 were collected before the treatment. The serum progesterone concentration was estimated by using Radio-immune assay technique. A readymade progesterone RIA kit "Beckman Coulter REF-IM1188" was used for this. 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

Progesterone profiles

The progesterone hormone is responsible for maintenance of pregnancy in bovines. In cyclic animals, the serum progesterone concentration is increased from metestrus to diestrus stage. For maintenance of pregnancy and survival of conceptus maintenance of high progesterone level in blood is necessary. The progesterone concentration in the present study was estimated by using RIA technique. The progesterone profile observed during present study is given in Table 1 and Table 2.

In present study, the mean serum progesterone concentration was increased on day 14 from day 7 in all the groups. The data was analyzed statistically to observe difference in the mean serum concentration on day 7 and day 14 in all the groups. There was significant increase in mean serum progesterone concentration from day 7 to day 14 at 5% level in Group I and II and in Group III and IV at 1% and 5% level.

There was no significant difference in the mean serum progesterone concentration between Group I, II, III and IV on day 7 which indicate that all the Murrah buffaloes had more or less similar status of progesterone level but significant difference was observed in mean serum progesterone concentration between Group III

and Group IV on day 14 at 5% level. However, there was non-significant increase in the mean serum progesterone concentration in Group I and II compared to Group IV. The mean serum progesterone concentration in Group I and Group II was statistically similar.

The mean serum progesterone concentration on day 14 in conceived Murrah buffaloes of Group I, II and III was 7.40 ± 1.03 , 7.97 ± 1.05 and 8.91 ± 0.75 ng/ml, respectively, while in non-conceived Murrah buffaloes of Group I, II, III and IV it was 3.35 ± 0.28 , 4.00 ± 0.57 , 4.73 ± 0.54 and 3.95 ± 0.26 ng/ml, respectively (Table 2).

In all the three treated groups, the mean serum progesterone concentration in conceived Murrah buffaloes was higher than non-conceived Murrah buffaloes. The difference in the serum progesterone concentration on day 14 in conceived and non-conceived Murrah buffaloes was analyzed statistically by t-test. There was significant difference in the mean serum progesterone concentration among the conceived and non-conceived Murrah buffaloes at 1% and 5% level in Group I and III while at 5% level in Group II. No buffalo from Group IV was conceived and hence there was no comparison was done in Group IV.

The data was further analyzed statistically by t-test. There was no significant difference among the conceived Murrah buffaloes of Group I, II and III on day 14. Also there was no significant difference in the serum progesterone concentration among non-conceived Murrah buffaloes of Group I, II, III and IV.

From the Table 2 it is clear that, all the treatment protocols have enhanced the serum progesterone concentration in the respective groups. Also there is increase in the progesterone concentration of Group VI which was kept untreated. The effect of GnRH and hCG hormone

at two different doses on serum progesterone concentration is discussed in depth as below.

Efficacy of GnRH on serum progesterone concentration

In present study the mean value of serum progesterone concentration in Group I treated with GnRH on day 7 after AI in non-infectious repeat breeding Murrah buffaloes was 2.53 ± 0.26 ng/ml on day 7 and 5.37 ± 0.91 ng/ml on day 14 (Table 1). The data was analyzed statistically and the progesterone level was found to be increased significantly at 5% level from day 7 (2.53 ± 0.26 ng/ml) to day 14 (5.37 ± 0.91 ng/ml). When compared to Group IV (Control), this increased mean serum progesterone concentration on day 14 in this Group I (5.37 ± 0.91) was higher than Group IV (3.96 ± 0.27) but this difference was statistically non-significant.

The present findings of progesterone concentration are in agreement with Howard *et al.* (2006) who reported significant increase ($P < 0.05$) in serum concentration of progesterone on day 13 (5.22 ± 0.46 ng/ml) after treating dairy cattle with GnRH on day 5 (1.64 ± 0.46 ng/ml) after AI. Pandey *et al.* (2016) also reported increase in the mean serum progesterone concentration on day 12 (1.17 ± 0.16 ng/ml) than day 5 (0.76 ± 0.10 ng/ml) when treated with GnRH on the day of AI.

The present results were similar to Karimi *et al.* (2007) who reported significant increase in serum progesterone concentration on day 19 post AI (9.24 ± 2.2 ng/ml) when treated with $20 \mu\text{g}$ GnRH on day 5 post AI in dairy heifers. The mean serum progesterone concentration in conceived Murrah buffaloes of Group I on day 14 was 7.40 ± 1.03 ng/ml while in non-conceived Murrah buffaloes it was 3.35 ± 0.28 ng/ml respectively (Table 2). There was significant difference in progesterone concentration at 1% and 5% level in conceived and non-conceived

Murrah buffaloes of Group I on day 14.

The significant increase in mean serum progesterone concentration on day 14 in Group I Murrah buffaloes can be due to the luteotropic effect of GnRH over corpus luteum when administered on day 7 after AI. However the mean serum progesterone concentration continued to be higher in conceived Murrah buffaloes on day 14 than non-conceived Murrah buffaloes. This could be because of low response of nonconceived Murrah buffaloes to the GnRH treatment.

Efficacy of hCG 1500 IU on serum progesterone concentration

In present study the mean values of serum progesterone concentration in Group II treated with hCG 1500 IU i.m. on day 7 after AI in non-infectious repeat breeding Murrah buffaloes was 2.57 ± 0.39 ng/ml on day 7 and 5.99 ± 1.06 ng/ml on day 14 (Table 1). There was increase in the mean serum progesterone concentration on day 14 from day 7.

The data was analyzed statistically and there was significant increase ($P < 0.05$) in the mean serum progesterone concentration from day 7 to day 14. This increased mean serum progesterone concentration on day 14 in this Group II (5.99 ± 1.06 ng/ml) was higher than Group IV (3.96 ± 0.27 ng/ml) but this difference was statistically non-significant.

The present findings are similar to Hanlon *et al.* (2005) who reported mean plasma progesterone concentration of 6.2 ± 2.7 ng/ml on day 12 when hCG was given 1500 IU i.m. in dairy cows. Santos *et al.* (2001) also reported increase in the progesterone concentration by 5.0 ng/ml when hCG was given on day 5 of after AI in cows. Companile *et al.* (2007) also reported significantly higher (4.51 ± 0.24 ng/ml) progesterone concentration in milk on day 15 after treating

Murrah buffaloes with hCG 1500 IU on day 5. Nascimento *et al.* (2013) reported similar increase in serum progesterone concentration (5.3 ng/ml) on day 12 with using hCG hormone.

The increase in the serum progesterone concentration from day 7 to day 14 of Group II (5.99 ± 1.06 ab ng/ml) was statistically non-significant to the increase in serum progesterone concentration of Group I (5.37 ± 0.91 ab ng/ml) while it was non-significantly lower than Group III (7.34 ± 0.90 ng/ml). This finding of similar increase in the progesterone concentration is in agreement with Companile *et al.* (2007) who reported similar concentration of progesterone in milk on day 15 in Murrah buffaloes treated with GnRH (12 µg) and hCG (1500 IU) on day 5.

When the present findings were compared with Group IV (Control), the serum progesterone concentration on day 14 in Group II (5.99 ± 1.06 ng/ml) was higher than Group IV (3.96 ± 0.27 ng/ml) but this difference was statistically non-significant.

The mean serum progesterone concentration in conceived Murrah buffaloes of Group II on day 14 was 7.97 ± 1.05 ng/ml while in non-conceived Murrah buffaloes it was 4.0 ± 0.57 ng/ml respectively. There was significant difference in progesterone concentration at 5% level on day 14 in conceived and non-conceived Murrah buffaloes of Group II. This difference can be contributed to the lower response of non-conceived Murrah buffaloes to the treatment.

From the present results, it is clear that the hCG hormone can be used 1500 IU to increase the serum progesterone concentration in non-infectious repeat breeding Murrah buffaloes. The luteotropic nature of hCG might be helpful in strengthening the CL which secretes progesterone hormone. This finding is in agreement with most of the researchers however difference in the

progesterone values among the present findings and findings of other researchers could be due to the difference in the species, reproductive status, dose of hCG or day of treatment.

Efficacy of hCG 3000 IU on serum progesterone concentration

In the present study the mean value of serum progesterone concentration in Group III treated with hCG 3000 IU i.m. on day 7 after AI in non-infectious repeat breeding Murrah buffaloes was 3.77 ± 0.58 ng/ml on day 7 and 7.34 ± 0.90 ng/ml on day 14 (Table 1).

The data was analyzed statistically and there was significant increase at 1% and 5% level in the mean serum progesterone concentration from day 7 to day 14. This increased mean serum progesterone concentration on day 14 in Group III (7.34 ± 0.90 ng/ml) was higher than Group I (5.37 ± 0.91 ng/ml) and Group II (5.99 ± 1.06 ng/ml) but this difference was statistically non significant.

When the present findings were compared with Group IV (Control), the serum progesterone concentration on day 14 in Group III (7.34 ± 0.90 ng/ml) was significantly higher than Group IV (3.96 ± 0.27 ng/ml) at 5% level.

The present findings are in agreement with Esfandabadi *et al.* (2007) who reported significant increase in the serum progesterone concentration on day 12 (16.07 ± 3.64 ng/ml) from day 5 (2.94 ± 0.65 ng/ml) when hCG was given 3000 IU on day 5 after AI. The present results are similar to Beltran *et al.* (2008) who reported increase in the serum progesterone concentration on day 12 (8.5 ± 0.5 ng/ml) from day 7 (5.7 ± 0.5 ng/ml) after giving hCG 2500 IU on day 5 after AI. The present finding of increase in the serum progesterone concentration on day 14 from day 7 in agreement of Rizos *et al.* (2012) who reported elevation in

serum progesterone concentration from day 7 to day 14 when hCG was given 3000 IU i.m. on day 5 of estrous cycle.

The mean serum progesterone concentration in conceived Murrah buffaloes of Group III on day 14 was 8.91 ± 0.75 ng/ml while in non-conceived Murrah buffaloes it was 4.73 ± 0.54 ng/ml, respectively (Table 2). There was significant difference in progesterone concentration at 1% and 5% level in conceived and nonconceived Murrah buffaloes of Group III on day 14. This difference could be due to the lower response of non-conceived Murrah buffaloes to the treatment.

From the present results, it is clear that the hCG hormone when used 3000 IU increases the serum progesterone concentration in non-infectious repeat breeding Murrah buffaloes and this can be contributed to the luteotropic property of hormone hCG. This finding is in agreement with most of the researchers however difference in the progesterone values among the present findings and findings of other researchers could be due to the difference in the species, reproductive status, dose of hCG or day of treatment.

Progesterone profile of untreated control group animals

In the present study the mean value of serum progesterone concentration in untreated control Group IV was 2.54 ± 0.28 ng/ml on day 7 and 3.96 ± 0.27 ng/ml on day 14 (Table 2).

In this group, all the Murrah buffaloes remained non-pregnant. There was significant increase in serum progesterone concentration on day 14 compared to day 7 in all non-conceived Murrah buffaloes at 1% and 5% level. This increase in the serum progesterone could be due to the normal cyclic CL secreting progesterone during the diestrous stage of estrous cycle.

Comparative efficacy of GnRH and different doses of hCG on serum progesterone profile

In present research work the mean serum progesterone concentration in Group I, II, III and IV on day 7 was 2.53 ± 0.26 , 2.57 ± 0.39 , 3.77 ± 0.58 , 2.54 ± 0.28 ng/ml, respectively while on day 14 it was 5.74 ± 0.19 , 5.99 ± 1.06 , 7.34 ± 0.90 , 3.96 ± 0.27 ng/ml, respectively (Table 1). There was no significant difference in mean progesterone concentration between Group I, II, III and IV on day 7 but there was significant difference in mean serum progesterone concentration between Group I, II, III and IV at 5% level on day 14.

From Table 1, it is clear that highest serum progesterone concentration was reported in Group III which was treated with hCG 3000 IU i.m. This increase in the progesterone concentration was significantly higher than Group IV at 5% level of significance but it was non-significantly higher than Group II and Group I treated with hCG 1500 IU and GnRH 20 μ g i.m., respectively. However, the increased serum progesterone concentration on day 14 was nonsignificant between Group I and Group II.

All the groups treated with respective protocols showed significant increase in the mean serum progesterone concentration on day 14 from day 7. This significant increase clearly indicates that the hormones GnRH 20 μ g, hCG 1500 IU and hCG 3000 IU can be effectively used to enhance the progesterone level in non-infectious repeat breeder Murrah buffaloes when given on day 7 after artificial insemination. These protocols might be helping in strengthening the CL formed after ovulation which is the source of progesterone in early luteal phase for maintenance of pregnancy. However the increase in the progesterone concentration in Group IV (control) could be the result of normal cyclicality of the non-infectious

repeat breeder Murrah buffaloes of that group.

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 3). 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 mg 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 mg after AI in repeat breeder cows and also with Rao (2000) who reported 55% conception rate when GnRH was given 20 mg 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 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.

From present research it is concluded that 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

Table 1. Overall progesterone profile of all repeat breeding Murrah buffaloes.

Sr. No.	Group	No. of animals	Progesterone (7 th day) (ng/ml)	Progesterone (14 th day) (ng/ml)
1.	Group I	8	2.53±0.26	5.37±0.91 ^{ab}
2.	Group II	8	2.57±0.39	5.99±1.06 ^{ab}
3.	Group III	8	3.77±0.58	7.34±0.90 ^a
4.	Group IV	8	2.54±0.28	3.96±0.27 ^b

Means with different superscripts in column differ significantly at 5% level (a, b),

Coefficient of variation = 3.51

Table 2. Comparison of progesterone concentration on day 14 in conceived and non-conceived Murrah buffaloes.

Group	Conceived (ng/ml)	Non-conceived (ng/ml)
Group I	7.40±1.03	3.35±0.28
Group II	7.97±1.05	4.00±0.57
Group III	8.91±0.75	4.73±0.54
Group IV	-	3.95±0.26

Table 3. 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%

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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