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Effect of Post-Mating GnRH Treatment on Serum Progesterone Profile and Conception Rate in Buffaloes

Research Article

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ABSTRACT

The present study was designed to investigate the impact of exogenous administration of GnRH (Receptal VET®, Intervet Pharmaceuticals Ltd., India) on serum progesterone profile and conception rates during mid luteal phase of estrous cycle in buffaloes. Estrus was induced using Cyclix 2 mL. i/m. (Intervet India Pvt. Ltd., India) and animals were bred naturally during observed estrus. The buffaloes (n=40) were grouped as control (n=10) on day 0 and treatment groups (I, II and III, n=10 in each group). GnRH (2.5 mL) was administrated on day 0, 11 and 13 of estrous cycle. Progesterone concentration was significantly higher ($P<0.05$; 1.782±0.046 ng/mL) in group I on day 5 and in group II on day 13 (4.514±0.038 ng/mL) and day 18 (6.173±0.015). However, in group III progesterone concentration was significantly higher on day 18 (6.554±0.0993) compared to control on day 5 (1.390±0.587 ng/mL), day 13 (3.770±0.103 ng/mL) and day 18 (5.114±0.009 ng/mL), respectively. Although progesterone concentration increased in all the three treatment groups compared to control, it was significantly higher ($P<0.05$) in pregnant animals of group I on days 5, 11, 12, 13 and 18 than non-pregnant animals of same group. Nevertheless, these differences were significant on days 13 and 18 in group II and on day 18 in group III in pregnant animals compared to non-pregnant animals, respectively. Similarly the conception rate was also significantly higher ($P<0.05$; 80% *vs.* 60%) in animals at group III compared to those at control. Comparison of data on progesterone profile between pregnant and non-pregnant (within group) animals of various treatment and control groups showed significantly higher levels of serum progesterone in pregnant animals on days 11, 12, 13 and 18 as compared to non-pregnant animals of the same group. Thus, the above study revealed positive impact of GnRH administration on progesterone profile during mid luteal phase of estrous cycle, which could be used to improve fertility in buffaloes.

KEY WORDS buffalo, corpus luteum, estrous cycle, GnRH, hormonal response, progesterone.

INTRODUCTION

The buffalo population in India is about 98.6 million out of 180.7 million in world, which represent 54.56 percent of the total world's buffalo population. Reproductive efficiency is the primary factor affecting productivity which is hampered in the female buffalo by inherent late maturity, poor estrous expression, distinct seasonal reproductive pattern and prolonged calving intervals (Singh *et al.* 2000). Early embryonic mortality is one of the predominant causes for repeat breeding in dairy animals (Santos *et al.* 2004; Diskin and Morris, 2008). In fact, losses due to early or late (<25 or > 25 to 45 days after fertilization, respectively) embryonic mortality could range between 20 to 44 and 8 to 17 per cent, respectively (Humblot, 2001). In cattle majority of embryonic loss occurs during pre-implantation stage,

i.e. during the first 20 days of pregnancy, when 75-80% of fertilized eggs are lost. One of the causes of embryonic loss is thought to be the inadequate luteal function (Willard *et al*. 2003). Abnormal corpus luteum (CL) function in early and mid luteal phase of estrous cycle results in low production of progesterone (Bullman and Laming, 1978) in peripheral circulation, which may cause early embryonic mortality.

Various hormonal treatments have been attempted to reduce embryonic loss or improve pregnancy rate in cattle. However, recent interest has been focused on the use of gonadotrophin releasing hormone (GnRH). But its effects on pregnancy rates and plasma progesterone profile are inconsistent. It is reported that injection of GnRH agonist, buserelin, between days 11 and 13 after insemination in lactating dairy cattle resulted in extended interestrous intervals and elevated levels of progesterone in serum (Rettmer *et al*. 1992b; Stevenson *et al*. 1993).

On the contrary, Young and Swanson (1988) could not confirm such effects. Further injection of a GnRH during mid luteal phase after insemination induces sufficient release of LH (indicate the meaning of the acronym LH) and FSH (indicate the meaning of the acronym FSH) to increase the life span of corpus luteum by counteracting luteolysis through disruption of normal follicular growth and secretion of estrogen, thereby permitting maternal recognition of pregnancy to occur (Willard *et al*. 2003).

Mac-Millan *et al*. (1986) suggested that GnRH have luteotropic and luteoprotective effects, thereby enabling maternal recognition of pregnancy. It is thought that GnRH treatment may increase the chances of embryo survival by improving luteal function and / or interfering with the luteolytic mechanism (Beck *et al*. 1994; Birnie *et al*. 1997; Cam *et al*. 2002).

Therefore, it seemed reasonable to evaluate this strategy in buffaloes as available information regarding progesterone secretion following GnRH administration on different days of estrous cycle mostly pertains to cows and is scanty in buffaloes. Hence, the present work was conducted to elucidate the efficacy of GnRH agonist (buserelin) administration at the time of natural breeding or between 11 to 13 days after natural breeding on pregnancy rates in buffaloes.

MATERIALS AND METHODS

A total of 40 cyclic buffaloes (*Bubalus bubalis*), 4 to 7 years of age, weighing 350-450 kg were selected from Instructional Dairy Farm (I.D.F.), Nagla, G.B. Pant University of Agriculture and Technology, Pantnagar-263145, District-Udham Singh Nagar (Uttarakhand). Buffaloes had a history of normal calving and had no clinically detectable. All the experimental animals were kept at I.D.F. under uniform feeding and management conditions throughout the period of experimentation.

The animals having mature CL were given 2 mL (263 µg/mL) intramuscular injection of Cloprostenol sodium (Cyclix®, Intervet India Pvt. Ltd., India) to synchronize the estrus cycle. The estrus was detected at every morning and evening by close observation for external signs, and it was confirmed by per-rectal palpation of genital organs. All the animals of treatment and control groups detected in heat were served by fertile breeding buffalo bull. The animals were randomly allotted to one of the following four treatment groups: treatment group-I (n=10), treatment group-II (n=10), treatment group-III (n=10) and control group-IV (n=10).

Buffaloes were given PBS 2.5 mL and GnRH agonist (Receptal) 2.5 mL on day 0, 11 and 13, respectively. Blood samples were taken by jugular venipuncture on day 0, 5, 10, 11, 12, 13 and 18 of estrous cycle. About 4-5 mL blood without anticoagulant was collected in sterilized glass tubes and kept at room temperature as a slant for 6-8 hours for separation of blood serum.

Blood serum was separated, centrifuged at 3.000 rpm for 15 minute and it was transferred into sterilized serum vials. All samples were stored at -20 ˚C till analysis. Serum progesterone was estimated by liquid phase Radioimmunoassay (RIA) procedure using progesterone antisera raised in the Department of Veterinary Gynecology and Obstetrics, GADVASU, Ludhiana, Punjab, India.

Pregnancy was checked by per-rectal examination of the genital organs after 60 days of service. The data obtained in the present study were analyzed statistically and subjected to the test of significance using the methods described.

RESULTS AND DISCUSSION

The effect of buserelin injection on pregnancy rates at the time of estrus or 11 and 13 days after natural breeding are presented in Table 1. Pregnancy rates to first natural insemination for buffaloes in groups I, II, III and group IV were 6.10 (60%), 7.10 (70%), 7.10 (70%) and 8.10 (80%), respectively. Thus, conception rate was higher in treatment group-III followed by treatment group-II and I compared to control.

There were significant ($P<0.05$) differences in conception rate between treatment groups III and groups I and II compared to control. Thus, GnRH treatment on day 13 was better as compared to other days. Experimental results showed that there were significant differences ($P<0.05$) in serum progesterone levels in treatment group-I on day 5 (1.782±0.046 ng/mL), treatment group-II on day 13 (4.514±0.0838 ng/mL) and 18 (6.173±.01050 ng/mL) and treatment group-III on day 18 (6.554±0.0993 ng/mL) in comparison to con-

trol group on the same days i.e. day 5 (1.390±0.587 ng/mL), 13 (3.770±0.103 ng/mL) and 18 (5.114±0.099 ng/mL) (Table 2).

The results also showed that there were significant differences in progesterone concentrations in pregnant animals of group-I on day 5 (1.865±0.0242 ng/mL), group-II on day 13 (4.617±.073 ng/mL) and 18 (6.362±.057 ng/mL) and group-III on day 18 (6.666±.0827 ng/mL) as against the pregnant animals of control on day 5 (1.505±.0539 ng/mL), 13 (3.994±.0640 ng/mL) and 18 (5.306±.0961 ng/mL) (Table 3).

Among all the treated groups there was much higher progesterone concentration on day 18 in treatment group-III compared to the rest of the groups on the same days. However, this difference was non significant.

The study investigated the positive relationship between rise in progesterone after GnRH treatment and pregnancy rate. Treatment of buffaloes with buserelin, a GnRH agonist at the time of insemination, improved pregnancy rates compared with control group. Karimi *et al.* (2007) showed 10% improvement in conception rate (70% *vs.* 80%) when GnRH (5 mL) was administered to dairy heifers at day of estrous.

Mandal *et al.* (2009) showed an improvement in the conception rate (87.5% *vs.* 75%) of buffaloes treated with GnRH on day of estrous as compared to control. In another study, conducted by Iftikhar *et al.* (2009) an improvement in conception rate (37.5% *vs.* 68.75%) was observed when 5 mL GnRH was injected at the time of insemination which is also in accordance with our study (60% *vs.* 70%). Rangnekar *et al.* (2002) also reported 70% conception rate in 10 repeat breeder Holstein-Friesian cows.

This improvement could be due to amplified preovulatory LH surge (Lucy and Stevenson, 1986; Yoshioka *et al.* 2001). Stevenson *et al.* (1993) observed that injections of GnRH stimulated the transformation of follicular cells to luteal cells, which was required at least 2 to 3 days for optimum progesterone production.

Failure or delay of ovulation might be prevented and conception rate might increase by GnRH administered at AI (indicate the meaning of the acronym AI). The higher conception rate in buffaloes treated with GnRH on day of estrus in our study could possibly be related to better synchrony of preovulatory LH surge (Tanabe *et al.* 1994) and ovulation (Senthikumar and Rajasekar, 1998).

Furthermore, GnRH at estrus may potentiate conversion of small luteal cells to large luteal cells resulting into development of large sized functional CL required for embryo survival through enhanced progesterone secretion. But in a few other studies, GnRH administration failed to exhibit a positive impact on pregnancy rate (Anderson and Malmo, 1985; Young and Swanson, 1988; Perry and Perry, 2009).

A reason for non-impact on fertility could be due to GnRH-induced ovulation of physiologically immature follicles that had a negative impact on pregnancy rates and lead to late embryonic/fetal survival (Perry *et al.* 2005; Busch *et al.* 2008; Lynch *et al.* 2010).

Treatment of buffaloes with buserelin acetate on days 11-13 after natural breeding improved its pregnancy rates. Batavani and Eliasi (2004) found a 33% improvement in conception rate in buffaloes treated with GnRH analogue i.e. gonadorelin during days 11-13 after insemination, which is in accordance with our study.

Mandal *et al.* (2004) showed an improvement in the conception rate in buffaloes (75% *vs.* 45%) treated with GnRH on day 12 of estrous cycle compared to control at first insemination, which is in accordance with our results. Such improvement in the conception rate was also observed by Zain and Nakao (1996) in buffaloes and Saratsis *et al.* (1998) in cattle, but contradictory to reports of Jubb *et al.* (1990) and Thatcher *et al.* (1993).

Mac-Millan *et al.* (1985) suggested that GnRH analogue has both luteotropic and luteoprotective effect which helps in maternal recognition of pregnancy. Mann *et al.* (1995) reported that GnRH administered at mid-luteal phase suppressed the small pulses of PGF2α occurring from day 12 onwards and thus reduces the strength of luteolytic drive. This resulted in prolonged life span of CL by secretion of sufficient progesterone required for pregnancy maintenance. Results from our research reveal that in buffaloes, there may be probably incidence of higher embryonic mortality between days 13 to day 17. The reason for this mortality may be luteal insufficiency, which was cared by supplementing the exogenous GnRH on day 13. The significant improvement in conception rate in treatment group-III compared to the rest of the groups could be due to reduced oestradiol-17β secretion during maternal recognition of pregnancy i.e. days 14-17 (Thatcher *et al.* 1993). In non pregnant ruminants, during the normal estrous cycle the synthesis and release of prostaglandin F2α from the endometrium is stimulated by luteal oxytocin to cause regression of the corpus luteum (Mann *et al.* 1995). Oxytocin receptor concentrations increase during the luteal phase of the cycle, stimulated by oestradiol-17β produced from waves of ovarian follicular growth. If pregnancy occurs, both secretion of luteal oxytocin and the development of endometrial oxytocin receptors are suppressed. Inhibition of oxytocin secretion and suppression of receptor development may serve to save corpus luteum for pregnancy. It seems that the mechanism involved in the effect of GnRH agonist on pregnancy rates is its ability to luteinize or induce ovulation of ovarian follicle at mid cycle following the acute onset of LH release after GnRH injection. This may reduce estradiol secretion from the ovarian follicles.

Table 1 Conception rate (%) in control and treatment groups

Treatment groups	Day of estrous cycle	Number of animals	Pregnant animals	Overall CR (%)
I	Day-0	10	7	70
II	Day-11	10	7	70
III	Day-13	10	8*	80
IV	Control	10	6	60

* Means within a column with different superscript are significantly different (P<0.05).

Table 2 Mean ± (SE) serum progesterone concentration in GnRH treated and control buffaloes

Days of estrous cycle	Progesterone concentration (ng/mL)			
	GnRH treated			
	Group-I	Group-II	Group-III	Control
0	0.499±0.015	0.561±0.013	0.637±0.014	0.682±0.018
5	1.782±0.0460*	1.665±0.089	2.581±0.073	1.390±0.587
10	4.076±0.0990	3.976±0.104	4.100±0.046	3.868±0.075
11	4.219±0.0860	4.013±0.092	4.184±0.037	3.902±0.092
12	4.450±0.116	4.184±0.0496	4.167±0.1020	4.017±0.148
13	4.547±0.127	4.514±0.0838*	3.983±0.1390	3.770±0.103
18	5.978±0.087	6.173±0.1050*	6.554±0.099*	5.114±0.099

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Mean ± (SE) serum progesterone concentration in pregnant and non pregnant buffaloes of GnRH treated and control groups buffaloes

Days of estrous cycle	Progesterone concentration (ng/mL)							
	GnRH treated							
	Group-I		Group-II		Group-III		Control	
	Pregnant	Non pregnant	Pregnant	Non pregnant	Pregnant	Non pregnant	Pregnant	Non pregnant
0	0.5120±.0184	0.4690±.0224	0.584±.009	0.5083±.0064	0.652±.0130	0.580±.003	0.718±0.093	0.629±.025
5	1.865±.0242*	1.586±.0375	1.807±0.068	1.336±.0406	2.680±.0380	2.185±.061	1.505±.0539	1.2190±.047
10	4.212±.0872	3.756±.1532	4.162±.0.059	3.543±.0730	4.153±.0368	3.885±.039	4.019±.0422	3.640±.097
11	4.372±.0509*	3.862±.0618	4.186±.041	3.611±.0182	4.230±.0265	4.001±.015	4.068±.0444	3.651±.156
12	4.650±.0755*	3.982±.0793	4.268±.037	3.989±.0081	4.306±.0472	3.614±.150	4.284±.139	3.618±.167
13	4.793±.0335*	3.972±.0259	4.617±.073*	4.272±.1600	4.096±.0395	3.53±0.19	3.994±.0640	3.434±.0879
18	6.115±.0772*	5.658±.0343	6.362±.057*	5.733±.0584	6.666±.0827*	6.105±.019	5.306±.0961	4.826±.0730

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Lack of estradiol late in the estrous cycle probably changes in uterine receptor concentrations of oxytocin as well as other events that are prerequisite for luteolysis (Thatcher *et al.* 1993; Beck *et al.* 1994; Drew and Peters, 1994; Harvey *et al.* 1994; Arikan and Pamukcu, 2001). Delaying luteolysis with a GnRH agonist treatment increases the chances for embryos to establish signals for maternal recognition of pregnancy before a new wave of estrogen secreting follicles develop and initiates luteolysis (Rettmer *et al.* 1992a).

Delaying the onset of luteolysis may also improve pregnancy rate by giving embryos more time to produce sufficient quantities of interferon-tau (IFN-τ; Komar *et al.* 2001; Inskeep, 2004) and by effectively blocking the luteolytic signal (Khan *et al.* 2007).

The suggested approach is to administer GnRH analogue in the second half of luteal phase viz., day 11-14 after estrus to remove the source of estradiol (Willard *et al.* 2003). Administration of GnRH during any stage of the estrous cycle leads to an LH surge (Campanile *et al.* 2008; Yildiz *et al.* 2009).

Between days 11-14 after estrus, GnRH administration promotes the ovulation of second-wave dominant follicle or will induce luteinization and / or atresia of pre-dominant follicles (Thatcher and Chenault, 1976; Thatcher *et al.* 1993; Guilbaust *et al.* 1990).

Administration of buserelin acetate during mid-luteal phase increases cycle length, plasma LH and progesterone in cattle (Mac-Millan *et al.* 1985). The net effect is a transitory increase in plasma progesterone and estradiol concentrations, followed by a prolonged decrease in plasma estradiol concentrations (Thatcher *et al.* 1989; Rettmer *et al.* 1992b; Ryan *et al.* 1994; Mann and Lamming, 1995; Beck *et al.* 1996a, b) and delay in luteolysis (Mac-Millan and Thatcher, 1991). In the present study, we found that serum progesterone concentration remained higher in treatment group-I in comparison to control irrespective of pregnancy status of animals. However, on day 5 the difference was significant (P<0.05). In treatment group-II, progesterone concentration was significant on day 13 and 18 (P<0.01). The serum progesterone concentration remained higher on day 5 to day 18 in treatment group-III compared to control

irrespective of pregnancy status of animals. Nevertheless, the difference was only significant on day 18 (P<0.05). Pregnant animals of treatment group-II had significantly higher (P<0.01) serum progesterone levels on days 13 and 18 post-breeding compared to pregnant animals of control group. Pregnant animals of treatment group-III had significantly higher serum progesterone levels on days 13 (P<0.05) and 18 (P<0.01) post-breeding compared to pregnant animals of control group. Progesterone concentration was higher in non-pregnant animals of treatment group-I compared to non-pregnant animals of control. However, these differences were not significant. In non-pregnant animals of treatment group-II, concentration of progesterone was significantly higher (P<0.05) on days 13 and 18 compared to non-pregnant animals of control. In non-pregnant animals of treatment group-III, concentration of progesterone was significantly higher (P<0.05) on day 18 compared to non-pregnant animals of control. Karimi *et al.* (2007) found a significant increase in progesterone concentration on day 5 when 5 mL of GnRH was administered on day 0 to dairy heifers (1.42±0.14 *vs.* 5.43±2.3) which is comparable with our findings.

They also reported significantly higher concentration of progesterone on day 19 when GnRH was given on day 12 (5.50±0.8 *vs.* 9.43±2.15). Selvaraj and Kumar (2001) also reported higher concentration of progesterone from day 8 to day 22 when GnRH was injected to repeat breeder cows on day of estrus. The mean concentrations of progesterone on day 8 and 16 were 4.12±0.47 ng/mL and 4.57±0.47 ng/mL, respectively while as corresponding values in non-pregnant animals were obtained 1.73±0.23 ng/mL and 3.62±0.88 ng/mL, respectively (Selvaraj and Kumar, 2001). Mandal *et al.* (2009) also observed significant increase in progesterone when GnRH was given on day of estrus in buffaloes (1.75±0.22 ng/mL) which is in consistent with our study (1.782±0.046 ng/mL). The increased progesterone concentration in treatment-I could be explained by firstly that the GnRH induces an additional LH surge to enhance active luetinization of granulose and theca cells to ensure adequate production of progesterone in developing CL. Secondly, GnRH may have acted on the developing CL to promote the conversion of small luteal cells to large luteal cells, which are responsible for about 85% of basal progesterone secretion at luteal phase (Niswender *et al.* 1985).

The administration of GnRH at estrus induces release of both LH and FSH in buffaloes (Aboul-Ela *et al.* 1985) which causes maturation of ovarian follicles and ovulation. This might also act by enhancing or altering theca lutein cells in the pre- and post-ovulatory follicles or on developing CL to promote conversion of small lutein cells into large lutein cells, resulting into development of large sized functional CL, enhancing progesterone secretion required for embryo survival. GnRH administration at the time of insemination is associated with increased plasma progesterone during post-insemination luteal phase (Lee *et al.* 1983; Kaim *et al.* 2003).

Increased progesterone is explained by GnRH-induced additional LH surge that enhances active luteinization of granulose and theca cells to ensure adequate production of progesterone from the developing CL. Moreover, GnRH injection at or near the time of estrus increases plasma progesterone during the first 7 days of estrous cycle (Ullah *et al.* 1996; Sharma *et al.* 2006; Vijayarajan *et al.* 2009) and increases the proportion of large luteal cells in the CL on day 10 of estrous cycle.

Mandal *et al.* (2009) administered GnRH on day-12 and they found significant difference in progesterone concentration (P<0.05) on day 21 compared to control and group-I in which GnRH was administered on day 0. In our study, there were significant differences in progesterone concentrations (P<0.05) on days 13 and 18 when GnRH was administered on day 11 and it was also found a significant increase (P<0.05) in progesterone concentration on day 18 when GnRH was administered on day 13 compared to control on same days.

Mandal *et al.* (2009) administered GnRH on day 12 and found significant difference in progesterone concentration (P<0.05) on day 21 compared to control and group-I in which GnRH was administered on day 0. The results are also comparable with the observations of Ryan *et al.* (1994) where progesterone concentration was higher following GnRH administration at mid-luteal phase, but different with the finding of Mann *et al.* (1995) where progesterone concentration was similar on day 8 or 10 in both treated and control animals.

Administration of GnRH during any stage of the estrous cycle leads to an LH surge (Campanile *et al.* 2008; Yildiz *et al.* 2009). Between days 11-14 after estrus, GnRH administration promotes the ovulation of second-wave dominant follicle or induces luteinization and / or atresia of predominant follicles (Thatcher and Chenault, 1976; Thatcher *et al.* 1993; Guilbaust *et al.* 1990). The net effect is a transitory increase in plasma progesterone and estradiol concentrations, followed by a prolonged decrease in plasma estradiol concentrations (Thatcher *et al.* 1989; Rettmer *et al.* 1992b; Ryan *et al.* 1994; Mann and Lamming 1995a; Beck *et al.* 1996a, b) and a delay in luteolysis and an increase in the life span of CL which gets more time to secrete progesterone (Mac-Millan and Thatcher, 1991).

CONCLUSION

The results of the present study show that GnRH agonist administration improves conception rate in buffaloes when

administered on day 13 post-breeding compared to day 0 or day 11 probably due to its beneficial effect on embryo survival by enhancing luteal function. However, its role in reducing the luteolytic drive during maternal recognition of pregnancy cannot be overlooked from the present study. Hence further study should be carried out to examine this effect.

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