EFFECT OF GnRH DURING VARIOUS STAGES OF ESTRUS CYCLE ON FERTILITY AND PLASMA PROGESTERONE IN BUFFALOES

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ABSTRACT

This study was aimed to see the effect of GnRH administration on progesterone profile and conception in buffaloes. Forty parous buffaloes were divided into five groups each of eight animals. Animals in group I, II and III were administered GnRH (2.5 ml. Receptal) on day-0, day-5 and day-12 of estrus cycle, respectively, whereas group IV animals received same dose of GnRH on all three days, ie day-0, 5 and 12 of cycle. Group V received normal saline on the same days as above and served as control. Progesterone concentration was significantly higher ($P<0.05$) in group I on days 12, 13 and 21, whereas in group II, III and IV from day 6 to 21 compared to control. Although P4 was higher in all the four treatment groups than control, but it was significantly higher ($P<0.05$) among the animals of group III and IV compared to group I and II also on day 21. Similarly conception rate was also higher in the animals of all the treatment groups but significantly higher ($P<0.05$) in group III and IV than group I, II and control. Service per conception was also significantly lower ($P< 0.05$) in group III and IV than control group. Inspite of three times GnRH administration in group IV animals, conception rate was similar to group III animals. Thus the study reveals that single GnRH administration at day 12 might be sufficient for improvement of conception in buffaloes

Key words: Buffaloes, GnRH, Progesterone, Fertility.

INTRODUCTION

Corpus luteum (CL), a temporary endocrine gland on ovary secrets an important hormone progesterone (P_4), which is essential for maintenance of early pregnancy in almost all species. Abnormal CL function in early and mid luteal phase of estrus cycle results in low P_4 concentration (Bulman and Laming, 1978) in peripheral circulation, which may cause early embryonic mortality. GnRH has been administered at various stages of estrus cycle as a measure for correcting this reproductive failure.

GnRH administration at the time of insemination induces release of endogenous LH and FSH in buffaloes (Aboul-Ela et al., 1983) and thus ensures follicle development and ovulation. Administration of GnRH during met-estrus (day 5 or 6) in cows causes ovulation of first wave dominant follicle (FWDF) with the formation of induced CL or luteinization of mature follicle and thereby enhances plasma P4 concentration. GnRH administration during midcycle (day 11 or 12) decreases number of large follicles and causes premature luteinization resulting in increased P_4 secretion. However, Thatcher et al. (1989) proposed formation of accessory corpora lutea as a second mechanism for action of GnRH when administered on day 11 or 12 of estrus cycle

Available information regarding P_4 secretion following GnRH administration on different days of estrus cycle, mostly pertains to cows and is scanty in buffaloes, hence present experiment was designed.

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MATERIALS AND METHODS

Selection of animals

The study was conducted on 40 apparently healthy postpartum buffaloes maintained at livestock farm of the Institute, Izatnagar. The buffaloes weighed 450±40 kg and were of 2nd to 4th parity and age group of 4.5 to 6.5 years. The animals were kept under loose housing system in sheds with one fourth covered roof and maintained on balanced ration.

Estrus detection

Estrus was detected by parading vasectomized bull in the sheds thrice daily for one hour each time. Buffaloes observed standing for mounting by a teaser bull, were considered to be in good heat. Genital organs were also palpated per-rectally to rule out any gross abnormality. Animals with normal genital tract and clear mucus discharge were selected for the experiment.

Experimental design

Selected buffaloes were divided randomly into five groups comprising of eight animals in each. Buffaloes in group I, II and III were given 2.5 ml receptal (10 µg buserelin), i.m. on day 0 (day of estrus), day 5 and day 12 of estrus cycle, respectively, whereas group IV animals received above treatment on all the three days i.e. day 0, 5 and 12. In Vth group, buffaloes were given 2.5 ml normal saline solution at three ocassions similar to treatment group IV, and served as control.

Blood collection and progesterone estimation

For estimation of progesterone, jugular blood was collected on days 0, 5, 6, 12, 13 and 21 of the cycle in a heparinized glass tubes. This was centrifuged at 3000 rpm for 15 minutes and plasma was stored at –20° C till use. Progesterone concentrations were estimated following standard protocol provided with RIA kit (ICN pharmaceuticals INC., New York). Percentage binding in the tube was counted by gamma counter (Packard Instrument Company). P4 concentration (ng/ml) was read from standard curve plotted on logit-log paper.

Artificial insemination

All the buffaloes observed in estrus were inseminated once at 12-15 hrs after onset of estrus, using frozen semen straws from a single proven bull. Inseminated buffaloes were monitored carefully and inseminated again if returned to estrus.

Pregnancy diagnosis

Buffaloes were examined per-rectum at 45 to 60 days post A I, to confirm pregnancy. The conception rate (CR) was calculated by percentage of female conceived out of total inseminated at first and subsequent A I.

Statistical analysis

Effect of GnRH on conception rate and service per conception between treatment and control animals, was statistically analyzed using chi-square test whereas on progesterone concentration using student's 't' test according to Snedecor and Cochran (1989)

RESULTS AND DISCUSSION

Progesterone concentration increased significantly ($P<0.05$) on days 12,13 and 21 but non-significantly on days 5 and 6 in group I than control group when GnRH was administered at day 0 (Table 1). The result is comparable with Mee et al. (1993) but different from the findings of Taponen et al. (1999). The administration of GnRH at estrus induces release of both LH and FSH in buffaloes (Aboul-Ela et. al. 1983) 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, thereby increased P4 in this group (Mee et.al., 1993).

In group II, P_4 concentration increased significantly ($P<0.05$) from day 6 to day 21 as compared to control (Table 1) when GnRH was administered on day 5. The result is in agreement with the Schmitt et. al. (1996) but differs from the findings of Martin et.al. (1990) whereas increase in progesterone level could not be

detected after GnRH administration on day 7 or 8. Administration of GnRH during metestrus induces ovulation of first wave dominant follicle and formation of accessory corpora lutea or luteinization of mature follicle (Schmitt, et. al.,1993). Rusbridge et al. (1992) reported formation of accessory CL in 75% Holstein heifers after administration of GnRH on day 6 of estrus cycle. Thus the increase in plasma P_4 concentration in this group might be either due to luteiotrophic support of original CL function or due to increased luteinization of small follicles.

In group III, significant difference ($P<0.05$) was observed on days 12, 13 and 21 compared to control group. Concentration was also significantly higher ($P<0.05$) on day 21 compared to same days of groups I and II. The result is similar to the observations of Ryan et. al. (1994) where P_4 was higher following GnRH administration at mid luteal phase, but different with the finding of Mann et. al. (1995) where P_4 concentration was similar on day 8 or 10 in both treated and control animals. Administration of GnRH on day 12 of estrus cycle results in release of LH comparable to preovulatory surge (Bostedt and Okyere, 1988) which induces luteinization / atresia of follicles or ovulation and formation of accessory corpora lutea (Harvey et. al. 1994). Increase in P_4 concentration in group III buffaloes of present study, might be due to P_4 secreted by accessory corpora lutea but we could not confirm it by ultrasonographic study, which has to be ascertained.

GnRH was administered on day-0, day-5 and day-12 in group IV buffaloes. P_4 concentration was significantly higher ($P<0.05$) from day 5 to day 21 compared to control group of same day. There is no comparable information about the effect of GnRH treatment on days 0, 5 and 12 on P_4 concentration in buffaloes, but in cows progesterone level was elevated on day 7 after administration of GnRH at estrus (Mee et. al., 1993), during day 6 to 13 after GnRH administration on day 5 (Schmitt et al. 1996) and during day 12, 13 and 21 after administration on day 12 (Rettmer and Stevenson 1991). The reason might be as already described for group I, II and III.

Administration of GnRH increased conception rate in all the treatment groups compared to control. It was non-significantly higher in group I and II whereas significantly ($P<0.05$) higher in group III and IV (Table 2). Services per conception was also significantly lower ($P<0.05$) in group III and IV (1.25) than control group (2.67). This was probably due to synchrony between timely release of preovulatory LH surge (Tanabe et al. 1994) and ovulation (Taponen et al. 1999). Ryan et al. (1994) reported a secondary LH surge when GnRH was administered at estrus / AI. Improvement in CR in group II was also probably due to development of large healthy dominant follicles for ovulation and development of accessory corpus luteum. These results are similar to the observations of Zain and Nakao (1996) reported in buffaloes. GnRH administered at mid luteal phase suppresses the small pulses of $PGF_{2\alpha}$, occurring from day 12 onwards (Mann, et al., 1995). This also gets support from significantly higher ($P<0.05$) P_4 concentration in animals of group III and IV than group V on different days.

In present experiment, the higher P_4 concentration among buffaloes of group I to group IV compared to control from day 12 to day 21 was responsible for increased pregnancy. The concentration also increased in group II, III and IV from day 6 to day 21 as compared to group I (Table 1). This confirms the reason of higher pregnancy in these groups than group I. Although P_4 was significantly higher in group IV than group III, on day 6 but it was similar on days 12, 13 and 21. This might be a reason of similar pregnancy status in both the groups.

Thus the study reflects that single GnRH injection at day 12 compared to day of estrus or during metestrus might be more benifitial for achieving higher pregnancy in buffaloes..

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Table 1: Mean ± (SE) plasma progesterone concentration in GnRH treated and control buffaloes

Days of treatment	Progesterone concentration (ng / ml)				
	GnRH treated				Control
	Group - I	Group - II	Group - III	Group - IV	Group - V
0	0.46±0.06^{b}	0.55±0.08^{ab}	0.54±0.03^{ab}	0.52±0.06^{ab}	0.89±0.06^{a}
5	1.75±0.22^{bc}	2.29±0.11^{ab}	2.16±0.08^{ab}	2.63±0.09^{a}	1.55± 0.14^{c}
6	2.07±0.18^{cd}	2.58±0.11^{ab}	2.37±0.10^{bc}	2.77±0.12^{a}	1.97±0.06^{d}
12	4.27±0.24^{a}	4.81±0.10^{a}	4.24±0.15^{a}	4.54±0.24^{a}	3.53±0.14^{b}
13	4.50±0.31^{a}	5.02±0.11^{a}	4.62±0.15^{a}	4.73±0.22^{a}	3.72±0.14^{b}
21	6.51±0.96^{a}	6.28±1.09^{a}	7.33±1.10^{c}	7.44±1.13^{c}	5.34±0.82^{b}

Values in same row having different superscripts differ significantly (P<0.05)

Table 2: Conception rate in different groups of buffaloes after GnRH treatment

Treatment Groups	Day of Cycle	No. treated	No.pregnant	Overall C R(%)	Services per conception
I	Day – 0	I(n = 8)	7	87.5^{ab}	2.00^{ab}
II	Day - 5	II(n = 8)	7	87.5^{ab}	2.14^{ab}
III	Day - 12	III(n = 8)	8	100.0^{a}	1.25^{a}
IV	Day 0, 5, 12	IV(n = 8)	8	100.0^{a}	1.25^{a}
V	control	V(n = 8)	6	75.0^{b}	2.67^{b}

Values in same column having different superscripts differ significantly (P<0.05)

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