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EFFECT OF HUMAN CHORIONIC GONADOTROPIN ON POST-OVULATORY MID-LUTEAL PROFILE AND SUBSEQUENT PREGNANCY RATE IN ANESTRUS BUFFALO USING TWO SYNCHRONIZATION REGIMES

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ABSTRACT

The present study was aimed to assess the impact of post-insemination human chorionic gonadotropin (hCG) administration on mid-luteal functions and conception rate in Murrah buffalo. The study was conducted on 2 buffalo treatment groups *viz*., estradiol + controlled internal drug releaser (CIDR) + gonadotropin releasing hormone (GnRH, n = 27) and ovsynch + CIDR (n = 22). Half of the animals in each group received intramuscular (i.m.) hCG (3000 IU, i.m.) on day 12 post-ovulation while groups served as control. The CG administration on day 12 post-ovulation improved corpus luteum size and progesterone level as well as first service conception rate after timed insemination in buffaloes.

Key words: Anestrus buffalo, conception rate, corpus luteum, endocrine profile, estrus synchronization, hCG

INTRODUCTION

Maintaining adequate level of progesterone hormone in bovines is crucial for the implantation of embryo following successful insemination. A number of specific strategies have been proposed to stimulate the growth of corpus luteum with greater progesterone producing capacity during critical periods, increase in anti-luteolytic stimulus by conceptus and decrease in uterine luteolytic capacity. Luteal inadequacy is an established cause of early embryonic mortality which is a major reproductive wastage affecting reproductive efficiency of buffalo (Lynch *et al.,* 2010; Pandey *et al.,* 2010). Treatment with human chorionic gonadotropin (hCG) and gonadotropin releasing hormone (GnRH) during early luteal phase improves luteal functions (Vijayarajan, 2009; Pandey *et al.,* 2010). More studies are required to assess the role of luteal profiles and their improvement after ovulation, especially in buffalo, to improve their conception rate. The present study was aimed to assess the effect of post-insemination hCG administration on luteal functions and conception rate following estradiol + controlled internal drug releaser (CIDR) protocol and ovsynch + CIDR protocol in buffaloes.

MATERIALS AND METHODS

The study was conducted on 49 Murrah buffaloes maintained in two dairy farms at Threke village of Ludhiana district (Punjab) from September 2011 to June 2012. The selected buffaloes had the history of anestrum since last 4-6 months. On the basis of treatment protocol, animals were divided into two groups i.e., group I (received estradiol + CIDR + GnRH treatment) and group II (ovsynch + CIDR received treatment). In group I, the buffaloes on day 0 were administered CIDR device (1.38 g P_4) and concurrently received 1.5 mg estradiol-17β through i.m. inj. On day 9, CIDR was removed and a prostaglandin ($PGF_{2\alpha)}$) analogue (500 µg, cloprostenol sodium, i.m) was administered. On day 11, buffaloes received GnRH analogue (20 µg, bucerelin acetate, i.m.) and were inseminated twice i.e., 12 and 24 hr post-GnRH injection. Group II animals received conventional ovsynch + CIDR (day 0, GnRH + CIDR; day 7, CIDR removal + $PGF_{2\alpha;}$ day 9, GnRH) and insemination procedures were same as treatment group I. Group I and II animals were further divided into sub-groups Ia (n = 15) and Ib (n = 12) and IIa (n = 12) and IIb (n = 10)). In estradiol-based protocol, group-Ia buffaloes were subjected to i.m. hCG inj. (3000 IU, chorulon) on day 12 post-ovulation (day 0: day of ovulation) whereas group-Ib served as control. In ovsynch-based protocol, group IIa (n = 12) buffaloes were subjected to i.m. hCG inj. (3000 IU, chorulon) on day 12 post-ovulation, whereas group-IIb served as control. In all the sub-groups, transrectal ovarian ultrasonography and blood sampling were carried out on days 0 (day of ovulation), 5, 12 and 16 post-ovulation. Ovaries were systematically examined for the diameter of visible corpora lutea (CL) and development of accessory CL. Plasma progesterone was assayed with a solid-phase radioimmunoassay using antisera raised in laboratory (Ghuman *et al.*, 2009). Sensitivity of assay was 0.1 ng mL^{-1}; intra- and inter-assay variation coefficients were 6.2 and 9.5%, respectively. Plasma estradiol-17β (E_2) was estimated using a commercially available direct immuno-enzymatic assay kit (Monobindinc. USA, Accubind estradiol ELISA microwells), using anti-estradiol IgG coated wells.

RESULTS AND DISCUSSION

The luteal profile was improved on day16 post-ovulation in group-Ia and group IIa as compared to the control animals (group Ib and group IIb) on day 16 post-ovulation. In group Ia, both the CL diameter and plasma progesterone showed increase on day 16 post-ovulation following the administration of hCG on day 12 post-ovulation (Table 1). In contrast the animals of group IIa showed non-significant increase in CL diameter ($p>0.05$) but significant rise in plasma progesterone following hCG injection on day 12 post-ovulation. Accessory corpora lutea (1.5 ± 0.3) were observed under ultrasonography following hCG administration in some animals (n = 12) which belonged to both the groups (8 animals of group Ia and 4 animals of group IIa). The plasma estradiol levels were almost similar before and after the hCG supplementation. It has been reported that treatment of anestrual high-producing dairy cows with CIDR increases the probability of onset of estrous cycles; and approximately 50% anestrous cows do not respond to the treatment (Chebel *et al.*, 2006). Further, the response in pregnancy per AI does not improve in anestrual cows (Galvao *et al.*, 2004). However, ovsynch + CIDR-based fixed time AI protocol is highly successful in inducing synchronized ovulation in repeat breeding cattle and yields an appreciable high conception rates (61%) [Ghuman *et al.*, 2010]. The literature regarding the use of such protocols is sparse in buffaloes and the strategies to improve luteal profiles are desired to enhance the conception rate in this species. The use of hormones during post-ovulatory luteal phase results in the luteinisation of large follicles in mid luteal phase to increase progesterone profiles. Pandey *et al.* (2010) reported that in buffalo hCG administration on day 12 resulted in the formation of accessory corpora luteal with subsequent rise in plasma progesterone. The present study revealed a rise in progesterone level after hCG administration which might be due to the increase in CL diameter and formation of accessory CL (n = 6). The pregnancy rate was also high which might be attributed to the high level of progesterone after hCG administration. It is well established that LH like activity of hCG provides luteotropic stimulation to CL through conversion of small luteal cells to large luteal cells by increase in the size of luteal cells (Khan *et al.,* 2007). Campanile *et al.* (2007) have suggested that hCG increased progesterone concentrations on day 15. Also, hCG did not stimulate increased P_4 secretion from the existing CL but rather induced ovulation and formation of an accessory CL, at least in a proportion of buffaloes (Pandey *et al.,* 2010). These reports support the observed better luteal profiles in hCG treated animals.

In present study no significant change was observed in plasma estradiol concentration on day 16 post-ovulation after hCG administration. This suggested that hCG treatment on day12 post-ovulation has no impact on estradiol concentration. Reports indicate decreased estradiol profiles and no impact on luteolytic response by administering hCG or GnRH (Pandey *et al.,* 2010). The reduction in estradiol level may be attributed to the elimination of large follicles by luteinisation which reportedly suppresses luteolytic response by reducing the development of oxytocin receptor in endometrium for the release of luteolytic pulse of PGF (Robinson *et al.,* 2001). In present study no significant difference in estradiol levels was observed revealing that luteinisation apparently does not contribute to decrease in estradiol levels. It appears that hCG is useful during post-ovulatory luteal phase for luteinisation of large follicles and increases progesterone profiles which may ultimately increase conception rate in buffalo.

Table 1: Effect of administration of hCG on day 12 post ovulation on luteal profiles and estradiol level in buffalo

Days post-ovulation	Group I (Estradiol + CIDR + GnRH)		Group II (Ovsynch + CIDR)	
	Group Ia (hCG)	Group Ib (control)	Group IIa (hCG)	Group IIb (control)
Effect on size of CL (mm)				
5	10.97 ± 0.46	10.56 ± 0.63	11.53 ± 0.41	11.68 ± 0.44
12	12.05 ± 0.40^a	10.13 ± 0.56	12.80 ± 0.22	12.18 ± 0.54
16	13.14 ± 0.30^b	10.32 ± 0.68	13.38 ± 0.21	13.05 ± 0.38
Effect on plasma P_4 concentration (ng mL^{-1})				
5	0.75 ± 0.18	0.86 ± 0.14	1.10 ± 0.46	2.32 ± 1.45
12	1.26 ± 0.21^c	1.20 ± 0.13	1.18 ± 0.12^c	1.46 ± 1.13
16	1.98 ± 0.28^d	1.31 ± 0.15	1.97 ± 0.27^d	0.91 ± 0.20
Effect on plasma E_2 concentration (ng mL^{-1})				
5	0.23 ± 0.43	0.20 ± 0.36	--	--
12	0.27 ± 0.55	0.45 ± 0.12	0.63 ± 0.19	0.56 ± 0.19
16	0.29 ± 0.13	0.37 ± 0.18	0.39 ± 0.60	0.61 ± 0.42
1st service CR	53.33%^{A}	41.67%^{B}	50%^{C}	33%^{D}

$^{a\ vs\ b}p<0.05$, within the column for size of CL, $^{c\ vs\ d}p<0.05$, within the column for P_4 level , $^{A\ vs\ B}p<0.05$ and $^{C\ vs\ D}p<0.05$, within the row for FSCR; Post-ovulatory Corpus luteum (CL), plasma progesterone (P_4); plasma estradiol (E_2)

The first service conception rate (FSCR) was higher in the animals administered hCG on day 12 post-ovulation (group Ia and IIa) as compared to the animals non-administered hCG (group Ib and IIb). The improvement in conception rate might be attributed to the induction of ovulation of large follicles, their luteinisation and subsequent formation of accessory corpora lutea (ACL). The impacts of hCG on FSCR and overall conception rates did not differ significantly between groups Ia and IIa. The comparable conception rates achieved following hCG administration indicated that improvement in conception rate was solely due to luteotropic action of hCG, no matter which of the two protocols were followed in buffalo. The information regarding the effect of hCG administration during post-ovulatory period on conception rate following estradiol- or ovsynch-based protocols is not available. The present study revealed a positive impact of hCG on day 12 post-ovulation on first service conception in anestrus buffaloes subjected to either of the protocols. In conclusion, hCG on day 12 post-ovulation improved CL size and progesterone level during post-ovulatory luteal phase following estradiol-based protocol. Supplementation of hCG raised progesterone levels without any effect on the size of corpus luteum in ovsynch-based animals. FSRC was improved in both the groups with hCG supplementation.

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