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Research Article

EVALUATION OF INSULIN AND DEXTROSE PRETREATMENT TO OVSYNCH PROTOCOL ON INDUCTION OF CYCLICITY AND CONCEPTION IN BUFFALOES DURING SUMMER

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ABSTRACT

A study was undertaken in 24 true anestrous Murrah buffaloes to determine the efficacy of insulin and dextrose pretreatment to GnRH on induction of cyclicity and conception during summer. The animals were of 7 to 10 years of age and at third to fifth parity having quiescent ovaries (i.e., absence of palpable corpus luteum and ovarian follicle) as confirmed by per rectal examination twice at 10 days intervals. The acyclic buffaloes were randomly divided in four treatment groups. The buffaloes in Group I (n=6) were treated with GnRH + $PGF_{2á}$ + GnRH (Ovsynch protocol); Group II (n=6) with GnRH + $PGF_{2á}$ + GnRH + insulin; Group III (n=6) with GnRH + $PGF_{2á}$ + GnRH + dextrose and Group IV (n=6) buffaloes served as control. Artificial insemination was performed in buffaloes of all groups that showed signs of estrous. Highest percentage of buffaloes (100%) exhibited estrous in group III as compared to other treatment groups (66.67% in group I, 50.00% in group II and 16.67% in group IV). Overall conception rate (%) was 33.33, 0.0, 66.67 and 16.67 in groups I, II, III and IV, respectively. The hormone levels determined on day 0, 7 and 9 by radio-immunoassay method revealed comparatively higher levels of progesterone and insulin on day 7 in groups II and III. However, rise in estradiol-17 β concentration was observed on day 9 after treatment with prostaglandin on day 7 in groups I, II and III, while there was no change in the levels of progesterone, estradiol-17 β and insulin in control group. This study revealed better manifestation of ovarian cyclicity and higher pregnancy rates in animals pretreated with dextrose. This might be due to the reason that positive energy balance by dextrose enhanced the ovarian activity due to optimum availability of energy level that helped the animal to combat environmental stress during summer.

Key words: Buffalo, dextrose, insulin, estrous, ovsynchprotocol, pregnancy rate

Despite of higher production potential, buffaloes are often considered as poor reproducers because of its low conception rates, inherent late maturity, silent/weak estrous, distinct seasonal reproductive pattern, long intercalving period and repeat breeding (Singh, 1988; Madan *et al.,* 1994). Although it is well known fact that buffaloes are polyestrous but their reproductive efficiency shows wide variation throughout the year and suffer from inherent reproductive problems thus limiting its lifetime production. A high incidence of anestrous due to inactive ovaries has been reported in buffaloes (Singh *et al*. 2000). Role of insulin (Shukla *et al*., 2005) and GnRH (Thatcher *et al*., 1993) was reported to control ovarian function and reproductive efficiency in cattle.

Postpartum reproductive function in dairy buffaloes seems directly dependent on the availability of nutrient energy rather than its utilization. Literature on effect of insulin, dextrose and GnRH in improving the reproductive efficiency in anestrous buffaloes is completely lacking, hence the present study was designed to determine the efficacy of insulin and dextrose pretreatment to GnRH on induction of cyclicity and conception in buffaloes during summer season.

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MATERIALS AND METHODS

The present study was undertaken on 24 true anestrous Murrah buffaloes of 7 to 10 years of age and in their third to fifth parity. These animals had quiescent ovaries (i.e. absence of palpable corpus luteum and ovarian follicle) on per rectal examination twice at 10 days interval. These 24 acyclic buffaloes were randomly divided into fourtreatment groups.

Group I (Ovsynch protocol): The animals (n=6) were treated with gonadotropin releasing hormone (GnRH) analogue (Buserelin acetate 10µg: Receptal- Intervet; I/M) on day 0, followed by prostaglandin ($PGF_{2\alpha}$) analogue (Cloprostenol sodium 500 µg: Cyclix - Intervet; I/M) on day 7, and an intramuscular injection of GnRH analogue on day 9.

Group II: The animals (n=6) were treated with human insulin (Biphasic isophanec insulin 125 IU: Human mixtard –Torrent Pharma; S/C) on days 6, 7 and 8 in addition to the treatment given as in group I.

Group III: The animals (n=6) were treated with dextrose solution (Dextrose 25% 540 ml: Woktrose 25 - Wockhardt; I/V) on days 6, 7 and 8 along with the treatment given as in group I.

Group IV: The animals (n=6) were not administered with any treatment and were kept as control.

Blood samples were collected on day 0 (i.e. prior to any treatment), day 7 (i.e. prior to PG injection) and day 9 (i.e. prior to second GnRH injection) from animals of all treatment groups for the estimation of hormones (progesterone, estrogen and insulin). Blood samples were allowed to clot and serum was separated and stored at -20°C until analysis. Serum progesterone and insulin concentrations (ng/ml) were estimated by radioimmunoassay (RIA method using bovine RIA kit supplied by BRIT, BARC, Mumbai) whereas estrogen (estradio l-17 β-pg/ml) concentration was determined by RIA kit (Immuno Tech, France).

Animals of all four groups were closely monitored for signs of estrus while parading with teaser bull and per rectal examination on days 9, 10 and 11. Animals found in estrous were inseminated twice at 12 h interval. and they were palpated per rectally for confirmation of pregnancy on day 60 post insemination. Data was analyzed statistically using one way ANOVA (CRD) among different treatment groups, whereas hormones (Insulin, progesterone and estrogen) between cyclic and acyclic animals were compared using 't' test as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Estrus Induction and Fertility Rate: Induction of estrous was higher (6/6, 100%) in group III followed by 66.67% (4/6) in group I, 50% (3/3) in group II and 16.67% (1/6) in group IV. Overall percentage of animals conceived in groups I, II, III and IV were 33.33 (2/6), 0.00 (0/6), 66.67 (4/6) and 16.67 (1/6), respectively. Conception rate was higher in group III (dextrose pretreatment in Ovsynch protocol) in comparison to other groups (I, II and IV).

In this study, the estrous induction in insulin pretreatment (group II) did not improve the estrous induction rather it was lower than Ovsynch protocol (group I) alone (50.00% vs 66.67%). This finding is in accordance with the findings of Gupta *et al*. (2011). Better response in term of estrous induction and higher conception rate after treatment with dextrose in anestrous buffaloes in this study may be due to optimized energy balance for fostering ovarian responsiveness to the gonadotropin stimulation during summer.

Hormonal Assay: Mean progesterone level on day 0 in true anestrous buffaloes were 0.50, 0.50, 0.90 and 0.50 ng/ml in groups I, II, III and IV, respectively (Table 1). Lower progesterone level on day 0 in animals of all the treatment groups indicated the absence of estrous thereby confirming true acyclic status of the ovaries. Progesterone level rose on day 7 after GnRH treatment in groups I, II and III (Table 1) which may be attributed to the effect of GnRH resulting in growth, maturation and luteinization of medium or large sized follicles by inducing the release of follicle stimulating hormone and luteinizing hormone from the anterior pituitary (Nanda *et al*. 1991; Palta and Madan, 1995). $PGF_{2\alpha}$ treatment resulted in decline of progesterone level in groups I, II and III on day 9 i.e. 48 h later which might be due to luteolysis of corpus luteum by $PGF_{2\alpha}$. No change was noticed in the level of progesterone in buffaloes of control group (Table 1).

Mean estrogen level on days 0 and 7 was almost similar among various groups (table 1). However, rise in the level of estrogen on day 9 after prostaglandin treatment in buffaloes of groups I, II and III might be due to folliculogenesis as a result of increase in follicle stimulating hormone and luteolysis induced by $PGF_{2\alpha}$ (Manik *et al.*, 2002). Mean estrogen level in control group (group IV) did not show any change throughout the protocol.

Mean insulin level (ng/ml) on days 0 and 7 i.e. prior to any treatment in group I was 12.83 and 12.75 while it rose to 13.33 ng/ml on day 9 (Table 1). Mean insulin level (ng/ml table 1) indicated that there was a transient rise in levels the of insulin on day 7 in buffaloes of group II and which were administered insulin (group II) or dextrose (group III) on day 6, 7 and 8. Rise in insulin might have stimulated folliculogenesis as insulin like growth factors (IGF-I) had been reported to stimulate granulosa cell mitogenesis, thus resulting in increased progesterone production (Harrinson and Randel, 1986; Spicer and Echternkamp., 1995; Beam and Butler, 1999; Butler *et al*., 2004). However, insulin administration

Table 1
Serum progesterone, estradiol-17 β and insulin concentration in anestrous buffaloes

Hormone	Day	Group I	Group II	Group III	Group IV
Progesterone (ng/ml)	0	0.50 ± 0.00^{ae}	0.50 ± 0.00^{af}	0.90 ± 0.22^{bg}	0.50 ± 0.00^{ah}
	7	1.73 ± 0.65^{bf}	2.46 ± 1.52^{bg}	2.10 ± 0.43^{bh}	0.50 ± 0.00^{ch}
	9	0.67 ± 0.20^{ce}	0.50 ± 0.00^{cf}	1.05 ± 0.67^{dg}	0.50 ± 0.00^{ch}
Estradiol-17 β (pg/ml)	0	6.00 ± 0.00^{ae}	6.00 ± 0.00^{af}	6.10 ± 0.13^{ag}	6.03 ± 0.04^{ah}
	7	6.66 ± 0.81^{be}	8.78 ± 2.23^{bf}	6.10 ± 0.13^{bg}	6.00 ± 0.00^{bh}
	9	16.70 ± 13.05^{cf}	14.83 ± 9.00^{cg}	12.16 ± 7.55^{ch}	6.00 ± 0.00^{dh}
Insulin (ng/ml)	0	12.83 ± 0.25^{ae}	19.25 ± 3.46^{af}	18.75 ± 7.65^{ag}	12.66 ± 0.20^{ah}
	7	12.75 ± 0.30^{be}	65.58 ± 40.14^{cg}	55.25 ± 37.43^{ch}	12.50 ± 0.00^{bh}
	9	13.33 ± 1.02^{ce}	24.08 ± 6.86^{dg}	13.16 ± 0.81^{cg}	12.50 ± 0.00^{ch}

Group I=GnRH+$PGF_{2\alpha}$+GnRH (Ovsynch protocol); Group II=GnRH+$PGF_{2\alpha}$+GnRH+insulin; Group III=GnRH+$PGF_{2\alpha}$+GnRH+dextrose; Group IV=control.
Between groups, mean having different superscripts^{abcd} in row-wise differ significantly at 5% level of probability
Withina group, mean having different superscripts^{efgh} in column-wise differ significantly between days at 5% level of probability

did not support folliculogenesis as evidenced by poor estrous response and pregnancy rate in group II; it might have resulted in transient hypoglycaemia and ultimately not supporting folliculogenesis and estrous onset. From the present study, it may be concluded that dextrose pretreatment to Ovsynch protocol (GnRH-$PGF_{2\alpha}$-GnRH) in true anestrous buffaloes during summer appears to be comparatively more effective in inducing estrous and establishing pregnancy.

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