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Modulation of ovarian response in anoestrus cattle treated with insulin alone and in combination with gonadotropin releasing hormone

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ABSTRACT

The influence of insulin alone and in combination with gonadotropin releasing hormone (GnRH) on ovarian response as well as glucose and progesterone-profile was studied on 28 anoestrus cows divided randomly into 4 groups i.e. insulin treatment (G-I), GnRH treatment (G-II), insulin plus GnRH (G-III) and control (G-IV), each comprising 7 animals. In insulin treatment group cows were injected subcutaneously long acting purified bovine insulin at 0.25 IU/kg body weight/day for 5 consecutive days. In GnRH treatment group cows were injected intramuscularly single 10 µg dose of buserelin acetate. In insulin + GnRH treatment group cows were injected insulin for 5 days similar to G-I and a single injection of GnRH 48 hrs after the last insulin injection. In control group cows were not administered any drug. The induction of estrus was higher in insulin treated cows (85.7%) ($P<0.05$) as compared to GnRH (57.14%), insulin + GnRH (42.85%) and control (14.3%). A remarkable degree of conception was recorded using insulin in anoestrus cows. Overall conception rate was 40, 100 and 33.3% in G-I, -II and -III, respectively, none got conceived in control group. The plasma glucose concentration was at the normal level before start of treatment (43.500±1.552 to 51.243±2.932 mg/dl) and did not significantly differ among the groups during different days of treatment. Serum progesterone concentration was at the basal level (<1 ng/ml) before start of treatment; day 5, 10 and 15 after treatment in all the groups, progesterone concentration was lowest at estrus and thereafter, it increased at higher level in insulin treatment group (7.115±0.723 ng/ml) as compared to GnRH (5.660±0.315 ng/ml), insulin + GnRH (4.160±0.834 ng/ml) and control (3.59±0.00 ng/ml) ($P>0.01$). These results indicated beneficial effects of insulin on ovarian response and restoration of fertility in anoestrus cattle.

Key words: Anoestrus, cattle, insulin, GnRH, plasma glucose, serum progesterone

Anoestrus is one of the most important functional ovarian disorders in livestock affecting adversely the economics of milk production by delaying age of first calving and calving interval. Incidence of anoestrus in cattle has been reported from 5 to 45% (Luktuke and Sharma, 1978; Naidu and Rao, 1981; Narledkar *et al.*, 1994). Causes of anoestrus have been attributed to factors viz. nutritional deficiency, seasonal changes, environmental and lactation stress, ageing and pathological disorders ultimately leading to endocrine disturbances. Insufficient Luteinizing hormone (LH) secretion associated with inadequate energy intake has been reported to be one of the major causes of postpartum anoestrus in cattle (Lucy *et al.*, 1991). It has been found that prolong postpartum anoestrus is due to failure of dominant follicle to ovulate rather than delay in their development (McDougall *et al.*, 1995; Roche *et al.*, 1998).

In search of fruitful remedy for anoestrus in cattle, several therapies have been proposed with their own merits and demerits. Exogenous insulin enhances diameter of dominant follicle as well as intrafollicular insulin like growth factor-1 (IGF-1) and estradiol concentration in cattle (Simpson *et al.*, 1994). Local ovarian action of insulin and IGF-1 in folliculogenesis and steroidogenesis (Spicer and Echternkamp, 1995) as well as gonadotropic action to release LH from pituitary has been reported in mammals (Tanaka *et al.*, 2000; Bucholtz *et al.*, 2000). Use of insulin to modulate reproductive functions especially to overcome problems of post partum anoestrus in dairy cattle is meager. Therefore, the present experiment was designed to study effect of insulin alone and in combination with GnRH on restoration of cyclicity and fertility in anoestrus cattle.

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MATERIALS AND METHODS

The present experiment was conducted on twenty eight healthy, parous, non suckling, crossbred dairy cattle with the history of normal calving but not resumed estrus to 60 days postpartum. The confirmation of anoestrus was done on the basis of history, gynaecological examination of genitalia twice at weekly interval and serum progesterone profile (i.e. <1 ng/ml). Animals with the history of anoestrus having normal developed genital tract without palpable corpus luteum and follicle were selected for the experiment.

Treatment groups : The experimental animals were divided randomly into 4 -groups each comprising 7 animals

Group I : Animals of this group were administered bovine insulin (Longact-B™; M.J. Biopharma Pvt. Ltd., Mumbai, India); subcutaneously at the dose of 0.25IU/ Kg body weight once daily for consecutive 5 days.

Group II: Animals were administered only buserelin acetate (Receptal™; Intervet) at the dose of 2.5 ml as a single intramuscular injection.

Group III: Animals were administered bovine insulin for 5 days; similar to group I followed by a single intramuscular injection of buserelin acetate (Receptal™) at the dose of 2.5 ml after 48 hrs to the last injection of insulin.

Group IV: Animals of this group were not administered any drug. Only normal saline was injected as a placebo and served as control.

Detection of estrus and ovulatory response : The animals were subjected to detection of estrus twice daily morning and evening using a teaser bull and visual observation of estrus. All the animals were examined per rectally at 5 days interval to monitor the ovarian and uterine changes for ascertaining estrus following the treatment. The animals detected in estrus were inseminated twice at 10 to 12 hrs interval. Ovulatory response was studied by rectal examination 10 days post estrus for the presence of corpus luteum on the surface of ovary and confirmed with concomitant rise in the serum progesterone level. Pregnancy diagnosis was done 60 days post insemination. Fertility response at induced and subsequent estrus were calculated and analyzed, accordingly.

Blood glucose and serum progesterone assay : Blood samples were collected by aseptic puncture of jugular vein on day before start of treatment; day 5th, 10th and 15th following start of treatment; at induced estrus and day 10th post estrus. Serum was separated for progesterone assay. In another tube blood was collected using EDTA and sodium fluoride and plasma was separated by centrifugation with in two hrs for glucose estimation. Both serum and plasma samples were stored at -20°C in sterilized micro centrifuge tubes until further assay.

The plasma glucose estimation was done using standard diagnostic kit (Enzopak; Reckon Diagnostic Pvt. Ltd; Baroda, India).

Serum progesterone profile was analyzed using 125I- radio immuno assay (RIA) technique with the help of standard diagnostic kit (Immuno-techTM, France).

Statistical analysis : Data for estrus and fertility response were analyzed using z-test. Data for serum progesterone and glucose were analyzed by ANOVA for difference between groups (Snedecor and Cochran, 1994). The statistical model included effects between treatments and within the treatment (error term). A probability of $P<0.05$ was set as the significance level. All values are expressed as mean±S.E.

RESULTS AND DISCUSSION

Animals in estrus following administration of insulin (G-I) in anoestrus cattle was higher (85.7%) as compared to control (14.3%, G-IV) ($p<0.05$), GnRH (57.14%, G-II) and insulin +GnRH (42.85%, G-III) (Table 1). The time taken for onset of estrus in insulin treated group (G-I) was shorter (180±42.82 hrs) than G-II (186±45.29); and was slightly higher but comparable with insulin +GnRH treated group (G-III, 176±80 hrs). The ovulation rate at induced estrus using insulin (83.3%, G-I) was comparable to G-II (100%), G-III (100%), and control (100%). The conception rate at induced estrus using insulin was better than other treatment groups; however it was lower than GnRH treatment group. Animals not conceiving at induced estrus, returned to subsequent 1st estrus (80, 33.3, 100 and 100%, respectively) with an average interval of 21.5±3.79, 18±0.00, 20.6±7.62 and 21±0.00 days, respectively, in G-I, G-II, G-III and control, indicating restoration of cyclicity without any aberrations in the estrus cycle. The overall conception rate was higher in animals treated with insulin (40%) than insulin+ GnRH (33.3%) and lower than GnRH (100%) treatment group. However, none got conceived in control groups.

Table 1. Ovarian response and restoration of fertility using insulin alone and in combination with GnRH in anoestrus cattle

Attributes	G-I (Insulin)	G-II (GnRH)	G-III (Insulin+GnRH)	Control
Animals treated (n)	7	7	7	7
Animals induced in estrus	6/7 (85.7%)[a]	4/7 (57.14%)	3/7 (42.85%)	1/7 (14.3%)[b]
Onset of estrus interval following start of treatment (hrs).	180±42.82 (96-336)	186±45.29 (96-264)	176±80.00 (96-336)	168±0.00 (168)
Animals ovulated at induced estrus	5/6 (83.3%)	4/4 (100%)	3/3 (100%)	1/1 (100%)
Animals inseminated (n)	2/6	1/4	1/3	1/1
Animals conceived at induced estrus	1/2 (50%)	1/1 (100%)	0/1 (0.0%)	0/1 (0.0%)
Animals exhibited 1st subsequent estrus	4/5 (80%)	1/3 (33.3%)	3/3 (100%)	1/1 (100%)
Interval between induced and Ist subsequent estrus (days)	21.5±3.79	18.0±0.00	20.7±7.62	21.0±0.00
Animals inseminated (n)	3/4	1/1	2/3	1/1
Animals conceived at Ist subsequent estrus	1/3 (33.3%)	1/1 (100%)	1/2 (50%)	0/1 (0.0%)
Overall conception	2/5 (40%)	2/2 (100%)	1/3 (33.3%)	0/0 (0.0%)

Values bearing different superscript (a,b) in a row differ significantly (P<0.05)

The beneficial effect of insulin on induction of estrus, restoration of cyclicity and fertility in anoestrus cows may be due to its effect on folliculogenesis and steroidogenesis. In vitro studies have demonstrated insulin and IGF-1 as important regulators of folliculogenesis and steroidogenesis (Gong *et al.*, 1993; Lucy *et al.*, 1993; Stewart *et al.*, 1995; Spicer and Echternkamp, 1995). Insulin enhances growth and proliferation of ovarian cells (Gong *et al.*, 1993; Gutierrez *et al.*, 1995), thus enhances folliculogenesis (Simpson *et al.*, 1994) either acting through specific insulin and or IGF-1 or both types of receptors (Poretsky *et al.*, 1985; Samota *et al.*, 1993). A considerable homology between IGF-1 and insulin suggested the role of insulin in synthesis of IGF-1 in the follicles (Telford *et al.*, 1990; Simpson *et al*, 1994). IGF-1 is a potent ovarian growth factor acts through autocrine and or paracrine manner and enhances production of estradiol (Spicer and Echternkamp, 1995; Stewart *et al.*, 1995; Hamilton *et al.*, 1999). Although, we have not estimated blood estradiol-17β concentration but enhanced uterine tonicity following insulin administration has been observed during rectal examination of genital tract indicating enhanced secretion of estradiol-17β from granulosa cells affecting turgidity of uterine musculature. The estradiol-17β is necessary for pulsatile LH secretion, a prerequisite for maturation and ovulation of follicle and exhibition of estrus. Insulin along with IGF-1 enhances growth and proliferation of theca cells leading to production of progesterone as well as androstenedion (Stewart *et al.*, 1995), thus enhanced production of estradiol from androstenedion by the process of aromatisation (Dorrington *et al.*, 1987). Insulin enhanced pulsatile LH secretion acting through CNS has been reported (Tanaka *et al.*, 2000; Bucholtz *et al.*, 2000).

GnRH and its analogue have been used for induction of estrus and fertility in anoestrus cattle by the various workers. GnRH induces LH surge and ovulation when given to post partum dairy cows (Kesler *et al.*, 1977). This would explain the findings of higher ovulation rate obtained in the present study. The onset of estrus in insulin+GnRH group (G-III, 42.85%) was lower but comparable to GnRH alone group (G-II, 57.14%) and lower than insulin alone group (G-I, 85.7%) (P>0.05). The onset of estrus interval in insulin+GnRH group was

shorter than GnRH treated group (176±80 Vs 186±45.29 hrs) and was comparable to insulin alone group. In insulin+GnRH treatment group (G-III) out of 3 animals, 2 were exhibited estrus within 96 hrs due to the influence of insulin where as only one animal exhibited estrus at 552 hrs indicating beneficial effects of insulin on folliculogenesis but lower synergistic action between insulin and GnRH. The exhibition of 1st subsequent estrus was higher in insulin +GnRH than GnRH and insulin alone groups (100% vs. 33.3 and 80%, respectively) but none could conceive in insulin + GnRH group at induced estrus. The overall conception rate using insulin+GnRH was lower (P>0.05) than insulin and GnRH alone (33.3% vs. 40, 100%, respectively).

Plasma glucose level before start of treatment was within the normal range (43.50±1.552 to 51.244±2.932 mg/dl) in all experimental animals indicating free from hypoglycemic state (Table 2). Although, some authors also reported lower blood glucose level in anoestrus cattle (Butler and Smith, 1989; Gong *et al.*, 2002) which has not been observed in the present study. This may be due to well fed and good health of experimental animals. Lucy *et al.* (1991) reported that low blood glucose level during the first 2 to 3-weeks returned to normal by day 40 postpartum. There was no statistical significant difference (P>0.05) in plasma glucose concentration during different days within and between the treatment groups which may be due to the fact that the blood sampling was done 24 hrs after insulin administration and by that time the hemopoietic system maintained the normal blood glucose level. However, in our pilot trial for deciding the dose of insulin i.e. 0.25 IU/kg body weight produced 50% reduction in blood glucose level within 5-10 min of its administration and the glucose level rebound to normal within 8-10 hrs. Significant reduction in blood glucose concentration for a period of about 12 hrs after long acting insulin administration has been reported in cows (Simpson *et al.*, 1994). Similarly, the glucose was rebound to normal in insulin-injected animals by 24 hours of its administration (Beam and Holcombe, 1992). The results from glucose profile of present study indicated the role of insulin in modulation of reproduction either by local intra-ovarian and or central gonadotropic effect through direct insulin and or IGF-1 receptors (Gong *et al.*, 1993; Tanaka *et al.*, 2000).

Serum progesterone concentration before start of treatment ranged between 0.279±0.107 to 0.662±0.179 ng/ml, i.e. <1ng/ml in all the experimental animals confirming the anoestrus state in the animals (Table 2). Following start of treatment progesterone concentration did not differ significantly (P>0.01) neither within nor between the treatment groups and control, indicating lack of structure and function of corpus luteum in anoestrus ovaries. The serum progesterone level was lowest (0.020±0.00 to 0.256±0.086 ng/ml) at estrus in all experimental groups and did not differ significantly among the groups (P>0.05). The mean progesterone concentration at day 10th post estrus was higher (p>0.01) in insulin group (7.115±0.723 ng/ml) as compared to other treatment groups and control, indicating beneficial effect of insulin on steroidogenesis. Insulin may have a direct effect in stimulating progesterone secretion during late

Table 2. Plasma glucose and Serum progesterone profile

Group	Before start of treatment	Following start of treatment			At estrus	Day 10th post estrus
		Day 5th	Day 10th	Day 15th		
G-I						
Glucose(mg/dl)	51.243±2.932	50.629±2.499	50.800±3.331	53.280±4.653	52.383±2.929	52.550±4.090
Progesterone(ng/ml)	0.452±0.121	0.506±0.218	0.474±0.116	0.270±0.000	0.256±0.086	7.115±0.723
G-II						
Glucose(mg/dl)	50.871±2.694	50.743±2.896	51.250±4.188	51.750±2.396	51.050±5.718	50.275±4.855
Progesterone(ng/ml)	0.662±0.179	0.662±0.179	0.662±0.179	0.733±0.565	0.161±0.052	5.680±0.315
G-III						
Glucose(mg/dl)	49.943±2.783	49.314±2.727	49.150±3.658	49.183±2.811	48.867±3.553	51.433±4.712
Progesterone(ng/ml)	0.279±0.107	0.239±0.103	0.349±0.121	0.411±0.179	0.117±0.075	4.160±0.834
Control						
Glucose(mg/dl)	43.500±1.552	43.600±1.05	43.567±1.538	45.317±1.519	45.200±0.000	50.200±0.000
Progesterone(ng/ml)	4.160±0.834	0.427±0.168	0.426±0.144	0.479±0.227	0.020±0.000	3.590±0.000

luteal phase as insulin receptor has been demonstrated in luteal cells (Sauerwein *et al.*, 1992). Lucy *et al.* (1993) and Selvaraju *et al.* (2002) have also reported the increased peripheral concentration of progesterone due to the effect of insulin on follicles and corpus luteum. The higher concentration of progesterone during luteal phase would justify the reasonable degree of fertility obtained in insulin treated animals.

A remarkable response for induction of estrus with the reasonable degree of fertility has been found in insulin treated anoestrus cows which are somewhat comparable to GnRH treatment group. The present study could not produce very authentic data regarding conception at induced estrus because the less number of animals were inseminated due to dirty vaginal discharge. Thus it may desire further experimentation on this aspect to generate more data before recommendation of insulin for management of postpartum anoestrus in cattle.

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