EFFECT OF PRETREATMENT WITH INSULIN ON OVARIAN AND FERTILITY RESPONSE IN TRUE ANESTROUS BUFFALOES TO GONADOTROPIN-RELEASING HORMONE

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INTRODUCTION

Anestrous is the most prevalent, frustrating and challenging problem encountered in buffaloes. The present study was conducted to evaluate the effect of a pretreatment with insulin on ovarian and fertility response in true anestrous buffaloes with to gonadotrophin-releasing hormone (GnRH). Eighteen buffaloes with inactive ovaries were randomly allocated into three groups: Group-I treated with GnRH (G1, n=6) where each buffalo received an intramuscular injection of 20 μg of Buseralin acetate (Day 0); Group-II treated with insulin plus GnRH (G2, n=6) in which each buffalo received a subcutaneous injection of long acting bovine insulin at the dose rate of 0.25 IU/kg bodyweight once daily for 3 consecutive days, followed by an intramuscular injection of 20 μg of Buseralin acetate on Day 4 ; and the control group (G3, n=6) in which each buffalo given an intramuscular injection of 2.5 ml of sterile saline on Day 0. In response to the treatment, estrus was 33.33% and 66.66% of the animals within 9.5 ± 3.18 and 26.5 ± 6.61 days from the start of the treatment, respectively, in G-1 and G-2; however, estrus was induced in none of the control animals. The conception rate at induced estrus was 100% in both the treatment groups. The mean serum progesterone (P₄) concentration was less than 1 ng/ml on day before the start of treatment, day 7, and 14 following the start of treatment in all the experimental animals. After returning to its basal level at induced estrus (i.e. 0.19 ± 0.04 and 0.32 ± 0.03 ng/ml) the P₄ concentration increased significantly (i.e. 4.47 ± 0.58 and 4.27 ± 0.35 ng/ml) on day 10 post estrus, respectively, in G-1 and G-2 (p<0.05). No significant difference in serum estradiol-17β (E₂) concentration was recorded on the day before the start of treatment, day 7 and day 14 following the start of treatment either within or between the treatment groups. The serum E₂ concentration increased significantly at induced estrus, i.e. 47.69 ± 3.92 and 47.15 ± 2.93 pg/ml, respectively in G-1 and G-2, and again returned to its basal level, i.e. 13.08 ± 1.32 and 10.88 ± 0.99 pg/ml, significantly at day 10 post estrus, respectively in both the treatment groups (P<0.05). It is concluded that pretreatment with insulin for 3 days before GnRH injection increases estrous induction rate and enhances ovarian and fertility response in true anestrous buffaloes.

Keywords: insulin, anestrous, buffaloes, GnRH, fertility response

ABSTRACT

The present study was conducted to evaluate the effect of a pretreatment with insulin on ovarian and fertility response in true anestrous buffaloes with to gonadotrophin-releasing hormone (GnRH). Eighteen buffaloes with inactive ovaries were randomly allocated into three groups: Group-I treated with GnRH (G1, n=6) where each buffalo received an intramuscular injection of 20 μg of Buseralin acetate (Day 0); Group-II treated with insulin plus GnRH (G2, n=6) in which each buffalo received a subcutaneous injection of long acting bovine insulin at the dose rate of 0.25 IU/kg bodyweight once daily for 3 consecutive days, followed by an intramuscular injection of 20 μg of Buseralin acetate on Day 4 ; and the control group (G3, n=6) in which each buffalo given an intramuscular injection of 2.5 ml of sterile saline on Day 0. In response to the treatment, estrus was 33.33% and 66.66% of the animals within 9.5 ± 3.18 and 26.5 ± 6.61 days from the start of the treatment, respectively, in G-1 and G-2; however, estrus was induced in none of the control animals. The conception rate at induced estrus was 100% in both the treatment groups. The mean serum progesterone (P₄) concentration was less than 1 ng/ml on day before the start of treatment, day 7, and 14 following the start of treatment in all the experimental animals. After returning to its basal level at induced estrus (i.e. 0.19±0.04 and 0.32±0.03 ng/ml) the P₄ concentration increased significantly (i.e. 4.47±0.58 and 4.27±0.35 ng/ml) on day 10 post estrus, respectively, in G-1 and G-2 (p<0.05). No significant difference in serum estradiol-17β (E₂) concentration was recorded on the day before the start of treatment, day 7 and day 14 following the start of treatment either within or between the treatment groups. The serum E₂ concentration increased significantly at induced estrus, i.e. 47.69±3.92 and 47.15 ±2.93 pg/ml, respectively in G-1 and G-2, and again returned to its basal level, i.e. 13.08±1.32 and 10.88 ±0.99 pg/ml, significantly at day 10 post estrus, respectively in both the treatment groups (P<0.05). It is concluded that pretreatment with insulin for 3 days before GnRH injection increases estrous induction rate and enhances ovarian and fertility response in true anestrous buffaloes.

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INTRODUCTION

Anestrous is the most prevalent, frustrating and challenging problem encountered in buffa-
loes. Clinical surveys in India have revealed higher incidences of anestrous due to inactive ovaries in buffaloes in comparison to cattle, and this affects production potential in the form of huge economic losses (Tanwar et al., 2003). In the search for fruitful remedies for anestrous, many hormonal as well as non-hormonal preparations have been tried by several workers, and each has its own merits and demerits.

Application of insulin to modulate reproduction in livestock is a fairly recent development. Recombinant growth hormone and positive energy balance altered the ovarian activity with increase in circulatory IGF-1 and insulin as well intrafollicular IGF-1 concentration (Gong et al., 1994, 1997; Downing et al., 1995). The exogenous insulin was found to enhance steroidogenesis and folliculogenesis by modulating autocrine/paracrine action of follicular growth factor system and steroidal hormones (Simpson et al., 1994). Exogenous insulin was first reported as an effective measure for management of anoestrus in cattle (Shukla et al., 2005) with the hypothesis that insulin can be a factor regulating growth, maturation and ovulation of antral follicle. However, such studies are lacking in buffaloes. Insulin is cheap and easily available, so it seems that it would be useful in the management of anestrous in the buffalo. Therefore, the present experiment was designed with the following objectives: 1) To study ovarian functions and fertility response after administration of insulin in anestrous buffaloes. 2) To study the blood estradiol-17β and progesterone profile before and after insulin treatment.

**MATERIALS AND METHODS**

**Animals and management:**

The present experiment was conducted at the Composite Livestock Farm, Adhartal, JNKVV, Jabalpur (MP) India. Eighteen apparently healthy, non-suckling lactating, acyclic buffalo cows (>90 days post-partum), weighing approximately 450-550 kg and 4-8 years of age were used in the study. The experimental period extended from November to March, when the relative humidity was 70-80% and the ambient temperature was 25-30°C. The buffaloes were stall-fed and housed in concrete sheds with standard managerial norms of the Livestock Farm. The animals were let loose daily at least for 2 h in morning and evening in the paddock for water splashing following milking and had access ad lib to clean drinking water. They were fed a standard diet calculated to meet both their maintenance and milk production requirements.

**Animal grouping and treatments:**

The confirmation of anestrous was made on the basis of history, gynaecological examination of genitalia twice at an interval of a week, and serum progesterone assay. Animals having clinically smooth ovaries (without palpable corpus luteum and follicle) but normally developed genitalia in both the rectal examinations, and serum progesterone concentrations less than 1 ng/ml were selected and divided randomly into three groups, each comprising six (n=6) animals. Animals of Group-1 (G-1) received an intramuscular injection of 20 µg buserelin acetate (GnRH analog). Animals of Group-2 (G-2) received a subcutaneous injection of long-acting bovine insulin at a dose of 0.25 IU/kg bodyweight once daily for 3 consecutive days, followed by an intramuscular injection of 20 µg buserelin acetate (GnRH analog). Animals of Group-3 (G-III) were injected with 2.5 ml sterile saline and served as the control.

Reproductive management and fertility status:

All the animals were observed for signs
of estrus and acceptance of buffalo teaser bull twice daily at morning and evening. The estrus induction rate was determined based on the results of visual observations for estrus signs, teasing results and changes in serum progesterone and estradiol concentrations for each group. All the animals were examined per rectally at an interval of 7 days after treatment to monitor the ovarian and uterine changes. Buffaloes were bred by natural service at induced estrus using a fertile buffalo bull. Ovulatory response was studied by rectal examination and serum progesterone level at day 10 post estrus for the presence of corpus luteum on the surface of ovary. All the animals were examined per rectally at 60 days post service for confirmation of pregnancy. The time taken for onset of estrus following withdrawal of treatment, occurrence of ovulation and fertility at induced estrus were calculated and analyzed.

Blood sampling and hormone assay:

The blood samples were collected aseptically by jugular vein puncture from all the animals before the start of treatment; on day-7 and day-14 following start of treatment, on the day of estrus, and day 10 post estrus. The collected blood samples were centrifuged at 1500 g for 15 minutes and the harvested serum samples were stored at -20°C until hormones were estimated.

Serum progesterone and estradiol assay:

The quantitative determination of progesterone and estradiol-17β concentrations in serum was made by enzyme-linked-immunosorbent-assay (ELISA) using kits supplied by Biotron Diagnostics Inc, Hemet, California, USA. A standard curve was obtained by plotting the concentration of the standard versus the absorbance. The validity tests and standardization of the ELISA was performed by preparing the standard curve and working out the accuracy, sensitivity, intra- and inter-assay variation for all the assays. The sensitivities of progesterone and estradiol-17β kit were 0.3 ng/ml and 10 pg/ml, respectively. The intra- and inter-assay coefficients of variation were, respectively, 15.86 and 8.6% for the progesterone kit and 15.74 and 4.43% for the estradiol-17β kit. The accuracies of the progesterone and estradiol-17β assay kits were 94 and 91.21%, respectively.

Statistical analysis:

The data generated were analyzed statistically for the mean and standard error. Comparison of estradiol-17β and progesterone levels at different days was done using CRD (Snedecor and Cocharan, 1989).

RESULTS AND DISCUSSION

Ovarian and fertility response:

Results pertaining to the effect of insulin on ovarian functions and fertility response in anestrous buffaloes have been presented in Table 1. The induction of estrus following administration of GnRH and pretreatment with insulin in anestrous buffaloes was higher (66.66% G-2) as compared to GnRH treated animals (33.33% G-1); however, estrus was induced in none of control animal. The time taken for onset of estrus in G-I was shorter (9.5±3.18 days) as compared to G-2 (26.5±6.61 days). The ovulation and conception rate at induced estrus was 100% in animals of both the treated groups; however, no control animal ovulated.

The beneficial effect of pretreatment of insulin with GnRH on induction of estrus, resumption of ovarian cyclicity and fertility in anestrous buffaloes are comparable to the findings
of Shukla et al. (2005); this may be due to its effects on folliculogenesis and steroidogenesis. In vitro studies have also supported such findings where insulin and IGF-1 have been demonstrated as important regulators of folliculogenesis and steroidogenesis (Gong et al., 1994; Stewart et al., 1995) through growth and proliferation of granulosa, theca and luteal cells present in the ovary (Spicer et al., 1993; Stewart et al., 1995), either acting through specific insulin and IGF-1 or both types of receptors. Structural and functional relationship of IGF-1 and insulin (Blundell et al., 1978), suggested the role of insulin in synthesis of IGF-1, which is a potent ovarian growth factor and acts through autocrine and paracrine manner (Stewart et al., 1995), and thus enhances the production of estradiol-17β. This may be the reason for increased level of estradiol concentration and pronounced uterine tonicity following insulin administration observed in the present study.

GnRH and its analoge has been used for induction of estrus and fertility in anestrous bovines by various workers, who have reported 22 to 87% induction of estrus with an average interval of 4 to 29 days, 75 to 100% ovulation and 9 to 66.7% of conception rate (Pattabiraman et al., 1986, Dhoble and Gupta 1986; Sonwane et al., 1994, Thompson et al., 1999; Markanadaya and Patil, 2003; Sirmour et al., 2006). The results of the present study in respect to the time taken for onset of estrus, ovulation and conception rate at induced estrus was comparable to Pattabiraman et al. (1986), Rao and Venkatramian (1991), Nasar et al. (1983), Rathour (2004) and Ramoun et al. (2007). GnRH induces LH surge and ovulation when given to post partum dairy animals (Britt et al., 1974; Foster et al., 1980). This would explain the findings of a higher ovulation rate obtained in the present study.

Serum progesterone profile:
Results on serum progesterone concentration before start of treatment, day 7, and day 14 following the start of treatment; at estrus and on day 10 post estrus have been presented in Table 2. The serum progesterone concentration on the day before the start of treatment ranged between 0.43±0.09 to 0.44±0.11 ng/ml, i.e. less than 1 ng/ml in all the experimental animals, confirming the anestrous state of animals and the results of rectal palpation. Similar observations in anestrous cattle have been reported by Shukla et al. (2005). The mean serum progesterone concentration ranged from 0.37±0.11 to 0.57±0.11 at day 7 and 0.56±0.07 to 0.73±0.13 ng/ml at day 14 following the start of treatment, indicating no significant difference in serum progesterone concentration neither within nor between the treatment and control groups; this may have been due to absence of corpus luteum or lack of luteal cells in anestrous ovaries or progesterone produced by theca cells (Stewart et al., 1995). Serum progesterone concentration, after fluctuating at basal level (0.19±0.04 to 0.32±0.03) at estrus, increased thereafter and reached the highest level (4.27±0.35 to 4.47±0.58 ng/ml) at day 10 post estrus in all the treatment groups, indicating presence of functional CL and confirming the results of rectal palpation. Our results are in agreement with the findings of Bachlaus et al. (1979), Singh and Chaudhary (1992), Shukla et al. (2005).

Serum estradiol-17β profile:
Results of serum estradiol-17β (E2) concentration on the day before the start of treatment, day 7, 14 after the start of treatment, at estrus and on day 10 post estrus have been presented in Table 3. The mean serum E2 concentration on the day before the start of treatment ranged between 14.59±0.84 to 14.88±0.73 pg/ml in all experimental
animals; our results are in consistent with the findings of Batra and Pandey (1982) and confirm the results of rectal palpation and anestrous state of all the experimental animals.

The mean serum estradiol-17β concentration ranged from 11.56±1.18 to 14.53±0.88 at day 7, 11.57±1.00 to 14.55±0.83 pg/ml at day 14 following the start of treatment, indicating no significant difference in neither within nor between the treatment groups and the control on the same days. The serum estradiol 17β concentration significantly (P<0.05) increased at estrus, ranging from 47.15±2.93 to 47.69±3.92 pg/ml in all the treatment groups as compared to day before treatment, on the day 7, day 14 following the start of treatment and day 10 post estrus. Similar finding has been reported by Batra and Pandey (1982). This may be due to aromatization of progesterone into estradiol by theca cells (Adashi et al., 1985).

After estrus, the concentration of estradiol 17β significantly (p<0.05) declined day 10 post estrus, ranging from 10.88±0.99 to 13.08±1.32 pg/ml, in all the treated animals, similar to the findings of Batra and Pandey (1982).

**REFERENCES**


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**Table 1. Ovarian functions and fertility response.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Attributes</th>
<th>Group-1 GnRH alone</th>
<th>Groups-2 Insulin + GnRH</th>
<th>Groups-3 Control (N.S.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Animal Treated (n)</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>Duration of Treatment (Days)</td>
<td>1</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Animal Induced in estrus</td>
<td>2/6 (33.33%)</td>
<td>4/6 (66.66%)</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Ovulation rate in induced animals</td>
<td>100%</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>On set of estrus interval following start of treatment (days)</td>
<td>9.5±3.18 (5-14)</td>
<td>26.5±6.61 (10-47)</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Animals Bred</td>
<td>2/2</td>
<td>4/4</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Animals conceived at induced estrus</td>
<td>2/2</td>
<td>4/4</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Over all conception rate (CR)</td>
<td>100%</td>
<td>100%</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2. Serum progesterone (ng/ml) profile before and after insulin treatment.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Days before Treatment</th>
<th>Following start of treatment</th>
<th>At estrus</th>
<th>Day 10th post estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1 (GnRH alone)</td>
<td>0.44±0.11</td>
<td>0.51±0.07</td>
<td>0.73±0.13</td>
<td>0.19±0.04</td>
</tr>
<tr>
<td>Group-2 (Insulin +GnRH)</td>
<td>0.43±0.01</td>
<td>0.37±0.11</td>
<td>0.72±0.19</td>
<td>0.32±0.03</td>
</tr>
<tr>
<td>Group-3 (Control)</td>
<td>0.47±0.13</td>
<td>0.57±0.11</td>
<td>0.56±0.07</td>
<td>-</td>
</tr>
</tbody>
</table>

S.E = 0.09

C.D = 0.22

Mean values bearing superscript (a, b) in columns and (A, B) in a rows differ significantly P<0.05.

Table 3. Serum estradiol-17β (pg/ml) profile before and after insulin treatment.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Days before Treatment</th>
<th>Following start of treatment</th>
<th>At estrus</th>
<th>Day 10th post estrus</th>
<th>SE/CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1 (GnRH alone)</td>
<td>14.59±0.84</td>
<td>13.58±0.90</td>
<td>14.55±0.83</td>
<td>47.69±3.92</td>
<td>13.08±1.32</td>
</tr>
<tr>
<td>Group-2 (Insulin+GnRH )</td>
<td>14.88±0.73</td>
<td>14.53±0.88</td>
<td>11.57±1.00</td>
<td>47.15±2.93</td>
<td>10.88±0.99</td>
</tr>
<tr>
<td>Group-3 (Control)</td>
<td>13.90±0.92</td>
<td>11.56±1.18</td>
<td>12.13±0.93</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean values bearing superscript (a, b) in rows differ significantly P<0.05.


