Effect of Insulin on Post-AI Circulating Estradiol-17β and LH in Crossbred Cattle

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Abstract | The present study was conducted to evaluate the effect of insulin on blood level of estradiol-17β (E2) and LH. The experiment was conducted using forty-eight cows and divided into Treatment (I, II and III) and control groups each consisting of 12 animals. Insulin was injected @0.25 IU/Kg body weight subcutaneously on day 0-3, 4-7 and 8-11 of estrous cycle in group I, II and III, respectively and PBS was injected in control animals. Blood samples were collected on day 0, 5, 10, 16 and 21 of estrous cycle. Serum was separated and stored at -20°C till analysis. In pregnant animals, E2 level in blood on day 5 was significantly (P<0.01) lower in group I compared to control and group III. For the same day it was significantly (P<0.05) lower in group II compared to group III and control. Serum concentration of LH, in pregnant animals on day 0, 5 and 10 was significantly (P<0.01) higher from day 21 in group III. Serum LH concentration on day 5 and 10 was also significantly (P<0.05) higher than day 16. These results indicated that insulin treatment declined the estrogen at day 5 and enhanced LH secretion up to day 10 reflecting its beneficial effect in corpus luteum survival and further conception.

Keywords | Insulin, Crossbred, Estradiol 17-β, Cow, Post-AI, LH etc

INTRODUCTION

Application of metabolic hormones i.e. somatotrophin, insulin and insulin like growth factors (IGF-1) in regulation of ovarian functions in livestock is fairly a recent development (Purkayastha et al., 2015). Insulin and IGF-I either alone or in combination with gonadotrophins have been found to have a profound effect on steroidogenesis of cultured bovine granulosa cells (Singh et al., 2010, Gong et al., 1993b, Spicer et al., 1993), Garzo and Darrington (1984), Poretsky and Piper (1994) and Willis et al. (1996) suggested that acting at the ovarian level, insulin appears to potentiate the steroidogenic response to gonadotropins, both in vitro (Baithalu et al., 2013) and in vivo. In granulosa cells, this effect may be mediated by an increase in LH (Luteinizing Hormone) receptor number, since insulin in association with FSH (Follicle stimulating Hormone) increases ovarian LH-binding capacity (Adashi et al., 1985; Davoren et al., 1986). In vitro studies have shown that insulin directly stimulates both mitosis and steroid production of cultured bovine granulosa (Gutierrez et al., 1999), theca (Stewart et al., 1995) and luteal cells (Mamluk et al., 1999). Willis et al. (1996) reported that insulin stimulates ovarian steroidogenesis by both granulosa and thecal cells, increasing production of androgens, estrogens and progesterone in vitro.

Selvaraju et al. (2002) recorded significantly higher plasma progesterone concentrations on day 10 of estrous cycle in insulin treated repeat breeding cattle than control.
et al. (2008) reported a higher progesterone concentration in insulin-treated goats on days 12, 16, 20 and 24. Stewart et al. (1995) suggested that insulin enhances growth and proliferation of theca cells leading to production of progesterone. Increase in progesterone might be due to direct effect of insulin on corpus luteum or by increasing the ovulation rate. Donaldson and Hart (1981) reported that administration of estradiol-17-β increased blood insulin concentration in farm animals. Gong et al. (1994) and Monniaux and Pisselet (1992) reported that IGF-I enhances FSH stimulated estrogen and progesterone production in granulosa cells of bovines and ovinus. Ramoun et al. (2007) found that pretreatment with insulin for 3 days before gonadotrophin-releasing hormone agonist injection increased the size of the largest follicle and the oestrus induction rate in buffaloes suffering from summer acyclicity.

Insulin stimulates the follicle production and estrogen synthesis (Gupta et al., 2010). Information regarding the effect of insulin administration during follicular and luteal phase of the cycle on conception rate in cows is limited. Therefore, the present study was designed to observe the effect of insulin on circulating LH and estradiol 17-β in crossbred cows.

**MATERIALS AND METHODS**

The present study was conducted on 48 crossbred cyclic cows aged 4-12 years, maintained at Instructional Dairy Farm, GBPUA&T, Pantnagar (Uttarakhand). Ethical approval was taken to conduct the study. Cows were divided into four groups (n=12), as per the day of insulin (0.25 IU/kg subcutaneously) treatment. It was day 0-3, 4-7 and 8-11 into four groups (n=12), as per the methods described by Snedecor and Cochran (1994) and results were presented as Mean±SE.

Estradiol 17-β was assayed by RIA kits (Immunotech, France) as per guidelines of manufacturer of RIA kits. Luteinizing hormone (LH) level in blood was determined by ELISA. Samples stored at -20 °C were thawed and used for qualitative and quantitative determination of various hormones in blood.

The data obtained in the present study was analyzed statistically as per the methods described by Snedecor and Cochran (1994) and results were presented as Mean±SE.

**RESULTS AND DISCUSSION**

Serum estradiol 17-β concentration (pg/ml) in animals of different groups was estimated (Table 1). Analysis of variance indicated that E2 concentration on day 0, 5, 10, 16 and 21 in all groups and among group I, II, III and IV varied non-significantly on different days.

Serum estradiol 17-β concentration was also studied in insulin treated pregnant animals (Table 2). Blood level of E2 on day 5 varied significantly (P<0.01) among all the groups. E2 level in blood on day 5 was significantly (P<0.01 and P<0.05) lower in group I compared to Control and group III, respectively. For the same day it was significantly (P<0.05) lower in group II compared to group IV. E2 level in pregnant animals on day 5 was significantly lower in group I and II compared to IV and in group I compared to group III might be due to suppressing effect of insulin on immature follicles during luteal phase of estrous cycle. E2 concentration on day 0, 5, 10, 16 and 21 was studied and analysed between pregnant and non-pregnant animals of each group.

### Table 1: Blood concentration (Mean±SE) of estradiol 17-β (pg/ml) during estrous cycle in insulin treated crossbred cows (n=12).

| Treatment Groups | Days of estrous cycle | Days of estrous cycle |
|------------------|----------------------|----------------------|
|                  | Day 0 | Day 5 | Day 10 | Day 16 | Day 21 |
| Group I          | 10.35±3.15 | 7.82±1.99 | 8.46±1.79 | 8.35±1.23 | 7.11±1.73 |
| Group II         | 12.53±2.59 | 11.60±3.28 | 11.65±2.58 | 13.22±3.29 | 11.49±2.45 |
| Group III        | 17.43±3.05 | 13.94±1.81 | 14.93±2.49 | 16.13±2.0 | 15.11±3.26 |
| Group IV         | 9.63±1.72 | 12.89±2.45 | 10.46±2.22 | 12.72±1.57 | 12.17±2.38 |

### Table 2: Blood concentration (Mean±SE) of estradiol 17-β (pg/ml) during estrous cycle in insulin treated pregnant crossbred cows

| Days of estrous cycle | Treatment Groups | Treatment Groups |
|----------------------|------------------|------------------|
|                      | Pregnant (n=7)   | Pregnant (n=3)   |
|                      | Pregnant (n=5)   | Pregnant (n=2)   |
| Day 0                | 10.17±5.54       | 9.19±4.34        | 22.67±6.45       | 18.94±1.55  |
| Day 5                | 4.66±2.05        | 12.90±4.99       | 15.44±3.46       | 26.84±4.09  |
| Day 10               | 7.16±1.42        | 4.57±2.19        | 11.95±2.82       | 13.42±9.43  |
| Day 16               | 8.27±1.53        | 11.51±6.97       | 17.92±2.40       | 17.48±0.27  |
| Day 21               | 6.36±2.75        | 11.83±4.60       | 19.47±5.06       | 17.03±3.55  |

Means bearing different superscripts within the rows (a,b,c) differs significantly (P<0.05)
E2 might be in correlation with LH level as suggested by earlier researchers that the effect of insulin and LH on bovine granulosa cells is likely physiologically relevant (Spicer, 1998). Average concentrations of insulin and LH in beef and dairy cattle are usually less than 10 ng/ml (Richards et al., 1989; 1991) except at the time of the ovulatory surge of LH during which LH concentrations can achieve > 30 ng/ml (Richards et al., 1991). Studies in vivo have shown that insulin injections increase estradiol concentrations in follicular fluid of cattle (Simpson et al., 1994), and that estradiol concentrations in follicular fluid decrease after the LH surge in cattle (Voss and Fortune, 1993). Thus, insulin and LH may be physiologically relevant regulators of ovarian follicular estradiol production in cattle (Spicer, 1998).

Serum LH concentration (mIU/ml) in insulin treated crossbred cows during estrous cycle was estimated (Table 3) while it was also studied and analysed in insulin treated pregnant and non-pregnant animals (Table 4).

Serum concentration of LH on day 0, 5, 10, 16 and was analysed between pregnant and nonpregnant animals for all the groups. Its concentration on day 16 and 21 varied significantly (P<0.05) for group III. Concentration of LH on day 16 and day 21 in group III was significantly (P<0.05) lower in pregnant animals compared to nonpregnant animals. It was due to the negative feedback effect of P4 on LH secretion (Convey et al., 1977).

Level of LH in blood on day 10 was also significantly (P<0.05) higher in pregnant animals of group I compared to pregnant animals of control. LH supports the P4 secretion since luteinizing hormone treatment increased progesterone secretion in 6th–10th and 11th–16th days of the estrous cycle as suggested by Rekawiecki (2007).

CONCLUSION

Insulin treatment declined the estrogen at day 5 and enhanced LH secretion up to day 10 reflecting its beneficial effect in corpus luteum survival and further conception.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.
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