OPEN ACCESS Journal of Animal Health and Production

Research Article

Effect of Insulin on Post-AI Circulating Estrodiol-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 estrodiol-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

Editor | Asghar Ali Kamboh, Sindh Agriculture University, Tandojam, Pakistan.
Received | May 22, 2018; **Accepted** | October 11, 2018; **Published** | November 06, 2018
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Citation | Sharma M, Gupta HP, Prasad S (2018). Effect of Insulin on post-AI circulating estrodiol-17β and LH in crossbred cattle. J. Anim. Health. Prod. 6(4): 103-107.
DOI | http://dx.doi.org/10.17582/journal.jahp/2018/6.4.103.107
ISSN (Online) | 2308-2801; **ISSN (Print)** | 2309-3331

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 (Luteinising 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. Sarath 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 ovines. 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 of estrous cycle in group I, II and III. Fourth group served as control and PBS was injected in equal amount to insulin suspension. Blood samples were collected on day 0, 5, 10, 16, and 21 of estrous cycle. Animals were detected in estrus and artificially inseminated with frozen semen.

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				
	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			
	Group I	Group II	Group III	Control Group
	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[a]	12.90±4.99[ac]	15.44±3.46[b]	26.84±4.09[b]
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).

Table 3: Blood concentration (Mean±SE) of Luteinizing Hormone (m IU/ml) during estrous cycle in insulin treated crossbred cows

Treatment Groups	Days of estrous cycle				
	Day 0	Day 5	Day 10	Day 16	Day 21
Group I (n=12)	3.41± 0.71	3.25± 0.62	2.04± 0.30	3.62± 0.96	3.18± 0.63
Group II (n=12)	3.33± 0.54	3.11± 0.89	3.74± 1.37	3.67± 0.91	3.53± 0.74
Group III (n=12)	4.08± 0.77	4.23± 0.97	4.07± 0.77	3.95± 0.94	4.58± 1.88
Group IV (control) (n=12)	3.21± 0.99	3.52± 0.70	5.20± 1.37	3.94± 0.66	6.07± 1.83

Table 4: Blood concentration (Mean±SE) of Luteinizing Hormone (m IU/ml) during estrous cycle in insulin treated pregnant crossbred cows

Days of estrous cycle	Treatment Groups			
	Group I	Group II	Group III	Control Group
	Pregnant (n=7)	Pregnant (n=3)	Pregnant (n=5)	Pregnant (n=2)
Day 0	2.65±0.52	3.58±1.25	4.16±0.50^{ac}	0.15±0.15
Day 5	2.97±0.71	2.33±0.51	4.46±0.68^{a}	1.20±1.20
Day 10	2.30±0.43	3.47±1.62	4.56±1.56^{a}	8.75±5.45
Day 16	4.46±1.52	3.14±0.87	1.76±0.81^{cb}	5.30±2.39
Day 21	4.19±0.89	2.89±0.15	0.12±0.12^{b}	1.45±1.45

Means bearing different superscripts within the column (a,b,c) differs significantly (P<0.05)

High level of LH in group I, II and III compared to control on day 0 and 21 was due to high rise of LH just before ovulation under the influence of E2 hence affecting corpus luteum formation as well as maturation and had positive effect on rise of progesterone too as in pregnant animals of control group the P4 level was also low since Luteinizing hormone (LH) is the major luteotropin in domestic ruminants (Niswender et al., 1985) and cattle (Oshea, 1987).

In pregnant animals the serum concentration of LH in group III on day 0, 5 and 10 was significantly (P<0.01) higher from day 21. Serum LH concentration on day 5, 10 was also significantly (P<0.05) higher from day 16. LH level decreases with advancement of pregnancy due to the negative feedback effect of P4 on LH secretion (Convey et al., 1977).

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 GnRH and LH secretion (Hacket and Hafs 1969). Randel and Erb (1971) reported that plasma LH levels were decreased in those animals having increased plasma progesterone.

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 6^{th}–10^{th} and 11^{th} –16^{th} 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.

ACKNOWLEDGMENTS

Authors are thankful to the Dean, College of Veterinary and Animal Sciences and Joint Director, IDF, DES GBPUA & T, Pantnagar, for facilities and financial assistance extended during the course of study.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.

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