THE EFFECTS OF INSULIN AND INSULIN-LIKE GROWTH FACTOR I ON BOVINE CORPORA LUTEA.

By

Alan Dale Ealy

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ABSTRACT

THE EFFECT OF INSULIN AND INSULIN-LIKE GROWTH FACTOR I ON BOVINE CORPORA LUTEA

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To determine the effect of low insulin on serum progesterone in heifers maintaining energy balance, sixteen Holstein heifers were assigned to isocaloric diets of 100% hay or 90% corn silage:10% soybean meal (CS). Jugular blood was sampled to quantify insulin and progesterone in serum. Area of insulin profiles was less in heifers fed hay than in heifers fed CS. Diet did not affect concentrations of progesterone. Thus, low postprandial concentrations of insulin do not affect serum concentrations of progesterone.

To determine effects of IGF-I on basal and LH-induced secretion of progesterone and number of luteal cells, corpora lutea were collected from ten Holstein heifers at d5 or d10 postestrus. Corpora lutea were incubated for 24h with LH (0, .1 or 1 ng) and IGF-I (0 or 500 ng). At d5 and d10 postestrus, media concentrations of progesterone and DNA content of cultures increased with 1 ng LH compared with basal and .1 ng LH. However, 500 ng IGF-I did not affect progesterone secretion or DNA content from luteal cells at d5 or d10 postestrus. Hence, secretion of progesterone and luteal cell numbers were not affected by IGF-I.

The encouragement and support of my parents during my Masters program was immeasurable. To demonstrate my appreciation, I dedicate this thesis to Harold and Suzanne Ealy.

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LIST OF ABBREVIATIONS

cAMP	cyclic Adenosine Monophosphate
DNA	Deoxyribonucleic Acid
FSH	Follicle-Stimulating Hormone
IGF-I	Insulin-like Growth Factor I
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
NEB	Negative Energy Balance
PEB	Positive Energy Balance
$PGF_{2\alpha}$	Prostaglandin F2 alpha
RIA	Radioimmunoassay
RNA	Ribonucleic Acid

INTRODUCTION

Fertility is the ability of females to produce offspring (Hensyl, 1987). Fertility is determined in dairy cows by detection of estrus, conception and survival of embryos. Interestingly, the previous determinants of fertility in dairy cows are correlated positively with decreased serum concentrations of progesterone (Rosenberg et al., 1977; Erb et al., 1976; Folman et al., 1973; Hill et al., 1970). Therefore, it is important to investigate luteal development and function.

Serum concentrations of progesterone are decreased in negative energy balance (NEB) heifers compared with positive energy balance (PEB) heifers (Villa-Godoy et al., 1990; Harrison and Randel, 1986). Negative energy balance occurs when calories needed to support body functions exceed calories ingested. During NEB, body fat is mobilized, causing decreased body weight (Villa-Godoy et al., 1989). During PEB, calories ingested exceed calories needed to support body functions. Thus, nutrients are stored and body weight increases (Villa-Godoy et al., 1989).

During NEB in heifers, serum concentrations of progesterone and lutyeal weight are decreased (Villa-Godoy et

al., 1990; Harrison and Randel, 1986). Eighty-one to 92% of dairy cows experience NEB during early lactation (Villa-Godoy et al., 1989; Reid et al., 1966). Because of the high preveance of NEB and its negative effects on luteal development and function, defining mechanisms of NEB induced retarted luteal function are merited.

During NEB in heifers, decreased serum concentrations of progesterone and decreased luteal weight are associated with decreased postprandial concentrations of insulin (Villa-Godoy et al., 1990; Harrison and Randel, 1986). Exogenous insulin increased luteal content of progesterone and luteal weight in NEB but not PEB heifers (Harrison and Randel, 1986). Therefore, reduced luteal development and function may be due to low postprandial secretion of insulin, but these data were confounded by NEB.

Serum concentrations of IGF-I are less during early lactation than during late lactation (Ronge and Blum, 1988). Since a majority of dairy cattle experience NEB during early lactation, decreased serum concentrations of IGF-I are coincident with decreased luteal development and function. Insulin-like growth factor I increases basal secretion of progesterone from cultured bovine luteal cells (McArdle and Holtorf, 1989). But, IGF-I did not affect basal or LH-induced secretion of progesterone from bovine luteal cells in other studies (Leavitt and Condon, 1989; Schams et al., 1988).

These disceptancies may be due to differences in dose of IGF-I.

Knowledge of development and function of corpora lutea and effects of insulin and IGF-I on bovine corpora lutea are presented in this review of literature. Current knowledge does not answer several questions. Does low postprandial concentrations of insulin decrease serum concentrations of progesterone in heifers maintaining energy balance? Also, does IGF-I increase basal and LH-induced secretion of progesterone and number of cells from cultured bovine luteal cells? These questions will be addressed in this thesis.

REVIEW OF LITERATURE

DEVELOPMENT AND FUNCTION OF CORPORA LUTEA

FUNCTION OF CORPORA LUTEA

Length of the bovine estrous cycle averages 21d. One corpus luteum develops from cells of each ovulated follicle and is maintained for an average of 17d (Kaltenbach and Dunn, 1980). Progesterone is secreted from corpora lutea. Immunoneutralization of progesterone caused precocious estrus and ovulation in heifers (Roche, 1976). Therefore, secretion of progesterone from corpora lutea maintains length of estrous cycle in cattle.

In cattle, if pregnancy is not recognized by the uterus from d16 to d18 postestrus, uterine secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) increases (Kaltenbach and Dunn, 1980). In combination with estradiol, $PGF_{2\alpha}$ decreases secretion of progesterone from corpora lutea, causing luteal regression (Kaltenbach and Dunn, 1980). If pregnancy is recognized by the uterus during this time, uterine secretion of $PGF_{2\alpha}$ is not increased and corpora lutea are maintained throughout pregnancy (Jainuden and Hafez, 1980). Thus, secretion of progesterone from corpora lutea also maintains pregnancy in cattle.

A putative mechanism for maintenance of estrous cycle length and pregnancy by the secretion of progesterone is by

inhibition of ovulation. A peri-ovulatory increase in serum concentrations of LH is essential for ovulation in cattle (Kaltenbach and Dunn, 1980). In ovariectomized heifers, administration of progesterone decreases frequency of LH pulses (Price and Webb, 1988). Consequently, spontaneous ovulation is inhibited by luteal secretion of progesterone (Price and Webb, 1988).

DEVELOPMENT AND MAINTENANCE OF CORPORA LUTEA

The preovulatory surge of LH causes ovulation and initiates differentiation of granulosal and thecal cells into luteal cells (Donaldson and Hansel, 1965). During ovulation, cytologic changes and vascularization of follicular tissue are characteristic of luteinization (Donaldson and Hansel, 1965). After ovulation, luteal weight in cattle (Ireland et al., 1973) and sheep (Farin et al., 1986; Schwall et al., 1986; Parry et al., 1980) and steroidogenic cell numbers in sheep (Farin et al., 1986; Schwall et al., 1986; Parry et al., 1980) increase until d8 to d12 postestrus.

Luteinizing hormone maintains secretion of progesterone from corpora lutea. Hypophysectomy in ewes (Kaltenbach et al., 1968) or immunoneutralization of LH in heifers (Hoffman et al., 1974) causes luteal regression. Exogenous LH maintains serum concentrations of progesterone in hypophysectomized ewes (Kaltenbach et al., 1968). Additionally, LH maintains epithelial morphology

(Gospodarowicz and Gospodarowicz, 1974) and increases secretion of progesterone (Rodgers et al., 1988) from cultured bovine luteal cells.

Luteinizing hormone may mediate decreased secretion of progesterone during NEB since concentrations of LH in serum are decreased in NEB cows (Rutter and Manns, 1987). However, in other studies, serum concentrations of LH did not differ between NEB and PEB heifers (Villa-Godoy et al., 1990; Knutson and Allrich, 1988). Thus, factors other than LH also mediate decreased luteal function during NEB.

BOVINE LUTEAL CELLS IN VITRO

In vitro, bovine luteal cells collected at ≥d5 postestrus respond to LH (Rodgers et al., 1988). In addition, LH maintained epithelial morphology of cultured bovine luteal cells (Gospodarowicz and Gospodarowicz, 1974). At d8 to d12 postestrus, basal and LH-induced secretion of progesterone from bovine luteal cells decrease with increased duration of culture (Pate and Condon, 1982). Also, luteal cells collected at d8 to d12 postestrus are responsive to LH from 0 to 48h and from 5 to 7d in culture but not between 3 to 4d in culture (Pate and Condon, 1982). Conversely, basal secretion of progesterone from bovine luteal cells collected at d2 to d4 postestrus is maintained for up to 5d in culture (McArdle and Holtorf, 1989). McArdle and Holtorf (1989) purified luteal cells to remove red blood cells and cellular debris. It is

not known whether sustained secretion of progesterone from cultured bovine luteal cells is dependent on corpora lutea age or purity of cell preparation. Investigation of basal and LH-induced secretion of progesterone from non-purified bovine luteal cells is limited to luteal cells collected at ≥ d5 postestrus and cultured for 0 to 48h or 5 to 7d.

SUMMARY

Secretion of progesterone from corpora lutea maintains length of the estrous cycle and pregnancy in cattle (Kaltenbach and Dunn, 1980; Jainuden and Hafez, 1980). Decreased concentrations of progesterone and luteal weight during NEB are not due only to decreased concentrations of LH (Villa-Godoy et al., 1990; Knutson and Allrich, 1988). Additional mediators of decreased luteal development and function during NEB are not defined.

In vitro, bovine luteal cells are responsive to LH from 0 to 48h and 5 to 7d in culture (Pate and Condon, 1982). Also, bovine luteal cells from corpora lutea ≥ d5 postestrus respond to LH in culture (Rodger et al., 1988). Thus several constraints preside when investigating luteal development and function in vitro.

INSULIN

STRUCTURE AND GENERAL FUNCTION

In the early 20th century, the Islets of Langerhans were determined to secrete a product that prevented diabetes (Orci et al., 1988). In 1921, the protein was isolated and named insulin, from the latin "insulae" (islands) (Orci et al., 1988).

Insulin consists of two polypeptide chains connected by disulfide bonds and labelled as A and B chains (Orci et al., 1988). Insulin is a glucoregulatory hormone which increases glucose, amino acid and lipid uptake into adipose, muscle and hepatic tissues (Froesch et al., 1986). Insulin also has stimulatory effects on ovarian tissue (Adashi et al., 1988a; Maruo et al., 1988).

CONTROL OF INSULIN SECRETION

Glucose stimulates secretion of insulin from beta cells of the Islets of Langerhans in nonruminants (Orci et al., 1988) and ruminants (Manns and Boda, 1967). But, most glucose is metabolized during ruminal digestion so butyrate, propionate and acetate are main ruminal products used for calories. In sheep, acetate does not affect secretion of insulin (Manns and Boda, 1967). But, propionate and butyrate increases beta cell secretion of insulin (Brockman and

Laarveld, 1986) and increases serum concentrations of insulin (Trenkle, 1971; Manns and Boda, 1967).

Serum concentrations of insulin can be altered by qualatative nature of diet. Diet affects the molar proportion of volatile fatty acids from rumen digestion. For example, grain diets increase rumen production of propionate and butyrate, but hay diets decrease propionate and butyrate production from the rumen (Trenkle, 1971). Production of acetate is not affected by diet (Trenkle, 1971). In cattle (McAtee and Trenkle, 1971) and sheep (Trenkle, 1971) postprandial concentrations of insulin increase with feeding grain and hay but does not increase with feeding only hay. Consequently, diets with high starch increase postprandial concentrations of insulin. Whereas, diets with high fiber and low starch do not increase postprandial insulin.

In NEB heifers, postprandial concentrations of insulin are decreased compared with PEB heifers (Villa-Godoy et al., 1990; Harrison and Randel, 1986). Likewise, serum concentrations of insulin are lower during early lactation than during late lactation (Smith et al., 1976; Koprowski and Tucker, 1973). Consequently, insulin secretion is altered by NEB.

ACTION OF INSULIN

Effects of Insulin

Insulin increases uptake of glucose, lipids and/or amino acids in every tissue investigated to date. However, actions of insulin have been studied mostly in adipose, skeletal muscle and hepatic tissues.

Insulin increases number of intracellular and membrane bound transporters for glucose in adipose and muscle tissue (Simpson and Cushman, 1986). In adipocytes, synthesis of fatty acids from glucose (Jungas, 1975; Simpson and Cushman, 1986) and acetate (Brockman and Laarveld, 1986) are increased by insulin. In skeletal muscle, insulin increases uptake of amino acids and reduces protein breakdown (Fukagawa et al., 1985). Insulin also increased glycogen synthesis in hepatocytes (Brockman and Laarveld, 1986). Consequently, insulin facilitates transfer and storage of nutrients and precursors from blood into tissues in adipose, muscle and hepatic tissues.

Mechanism of insulin action

Insulin acts by binding to receptors on target cells (Carpentier, 1989; Czech, 1985). Receptors for insulin are protein complexes with two extracellular (α) and two transmembrane (β) subunits. Membrane receptors are arranged in a β-α-α-β configuration by disulfide bonds (Carpentier, 1989; Czech, 1985). Insulin binds to alpha subunits of receptors, causing phosphorylation of tyrosine and methionine

residues on beta subunits (Czech et al., 1988; Czech, 1985). After binding, insulin and receptor are internalized, insulin is degraded and receptors are either recycled or degraded (Carpentier, 1989).

Tyrosine kinase action and hormone-receptor internalization provide post-receptor mechanisms for actions of insulin (Carpentier, 1989; Caro et al., 1988). During insulin binding, various enzymes, including glycogen synthase (Sheorain et al., 1982), pyruvate dehydrogenase (Hughes et al., 1980) and acetyl CoA carboxylase (Witters, 1981) are phosphorylated-dephosphorylated to active forms. Tyrosine kinase action may be one of the kinase promotors involved with enzyme activation (Carpentier, 1989; Czech et al., 1988; Czech, 1985), but only coincidental evidence supports this hypothesis. Internalization of insulin-receptor complex is not essential for acute intracellular responses (Carpentier, 1989). However, with large doses of insulin, receptor internalization increases and causes decreased number of membrane insulin receptors (Carpentier, 1989). Internalization of receptors may reduce sensitivity of tissues to insulin, but it is not certain whether internalization of receptors occurs <u>in vivo</u> in response to endogenous concentrations of insulin.

Several intracellular compounds are associated with actions of insulin (e.g. decreased cAMP, calcium influx), suggesting involvement of second messengers (Saltiel and

Cuatrecass, 1988; Cheng and Larner, 1985). But, there is no evidence that these compounds are endogenous post-receptor mediators for actions of insulin.

EFFECTS OF INSULIN ON OVARIAN CELLS

Diabetes in humans is associated with decreased fertility. For example, exogenous insulin, given to diabetics, increases fertility (Poretsky and Kalin, 1987). In addition, hyperinsulinemia is associated with polycystic ovarian disease in humans (Poretsky and Kalin, 1987). Thus both, low and high concentrations of insulin decrease fertility.

Granulosal-thecal cells

Receptors for insulin are in human ovarian tissue (Poretsky et al., 1988) and porcine granulosal cells (Rein and Schomberg, 1982). Insulin increases basal and LH- or follicle-stimulating hormone- (FSH) induced secretion of progesterone from cultured porcine granulosal cells (Veldhuis and Kolp, 1985; May and Schomberg, 1981; Channing et al., 1976). Insulin also increases LH-induced secretion of androgens from rat thecal cells (Cara and Rosenfield, 1988; Magoffin and Erickson, 1988). Insulin, therefore, increases steroidogenesis in granulosal and thecal cells.

Positive steroidogenic effects of insulin on granulosal cells are caused by multiple actions of insulin. Insulin increases numbers of receptors for LH from porcine granulosal

cells (Maruo et al., 1988) and rat leydig cells (Charreau et al., 1978). Insulin also increases number of cultured bovine and ovine granulosal cells (Peluso and Hirshel, 1987) and sustained morphology of porcine granulosal cells (May and Schomberg, 1981; Channing et al., 1976). Therefore, insulin is both steroidogenic and mitotic in cultured granulosal cells.

Luteal cells

Receptors for insulin are in rat luteal cells (Ladenheim et al., 1984). Insulin increases basal (O'Shaughnessy and Wathes, 1985) and LH-induced (Leavitt and Condon, 1989; Poff et al., 1988; O'Shaughnessy and Wathes, 1985) secretion of progesterone from cultured bovine luteal cells. Mechanisms for luteotropic actions of insulin have not been determined.

In vivo, decreased postprandial secretion of insulin is coincident with decreased serum concentrations of progesterone in NEB heifers compared with PEB heifers (Villa-Godoy et al., 1990; Harrison and Randel, 1986). In addition, exogenous insulin increases luteal weight and luteal content of progesterone in NEB, but not PEB, heifers (Harrison and Randel, 1986). Therefore, postprandial concentrations of insulin may stimulate serum concentrations of progesterone in vivo. However, it is not certain why postprandial insulin affects luteal development and function during NEB, but not PEB. Perhaps exogenous insulin is therapeutic only during NEB.

SUMMARY

Insulin is a glucoregulatory hormone and increases uptake of nutrients and precursors into adipose, muscle and liver. Serum concentrations of insulin are controlled positively by propionate and butyrate absorbed from the rumen (Trenkle, 1971; Manns and Boda, 1967). Serum concentrations of insulin are low during NEB (Villa-Godoy et al., 1990; Harrison and Randel, 1986) and early lactation (Koprowski and Tucker, 1973) compared with PEB or later lactation. Consequently, concentrations of insulin are dependent on diet and metabolic/physiologic state.

Insulin also increases basal and LH- or FSH-induced secretion progesterone from cultured granulosal (Veldhuis and Kolp, 1985; May and Schomberg, 1981; Channing et al.,1976) and luteal cells (Leavitt and Condon, 1989; Poff et al., 1988; O'Shaunessy and Whathes, 1985). In vivo, exogenous insulin increases luteal function in NEB, but not PEB, heifers (Harrison and Randel, 1986). It is not known whether insulin affects luteal development or function during PEB or if insulin is therapeutic only during NEB.

INSULIN-LIKE GROWTH FACTOR I

STRUCTURE AND GENERAL FUNCTION

Insulin-like growth factor I is named for its hypoglycemic effects (Froesch et al., 1986). Because IGF-I "mediates" effects of growth hormone (GH) on bone growth (Salmon and Daughaday, 1957), IGF-I is also been termed somatomedin-C. Insulin-like growth factor I is a 7.5 kilodalton (kd) protein (Rechler, 1989) that consists of four domains (A,B,C,D) arranged B-C-A-D from the amino terminus (Baxter, 1988). Amino acid sequence of the A and B domains of IGF-I are 40% homologous to the A and B chains of insulin, respectively (Baxter, 1988), which may explain some of the insulin-like actions of IGF-I.

CONTROL OF INSULIN-LIKE GROWTH FACTOR I SECRETION

In this review, IGF-I will be called somatomedin activity when quantified by bioassay and will be called IGF-I when quantified by RIA.

Growth hormone regulates hepatic secretion of IGF-I. Exogenous GH increases hepatic content and serum concentrations of IGF-I in rats and sheep (Clemmons, 1989; Davis, 1989). Pituitary secretion of GH is regulated by negative feedback of IGF-I on somatotropes (Clemmons, 1989; Mathews et al., 1988; Phillips, 1986; Tannenbaum et al.,

1983). Therefore IGF-I, in addition to GH, controls secretion of IGF-I from liver.

During certain metabolic/physiologic states, GH appears to have less influence on concentrations of IGF-I in serum. Serum concentrations of GH increases during early lactation compared with late lactation (Koprowski and Tucker, 1973). But serum concentrations of IGF-I (Ronge and Blum, 1988) and somatomedin activity (Falconer et al., 1980) decrease during early versus late lactation. Malnutrition or diabetes in humans also increases concentrations of GH but somatomedin activity decreases (Winter et al., 1979; Grant et al., 1973). Number of GH receptors are decreased in hepatocytes from diabetic rats (Maes et al., 1983), suggesting a mechanism for decreased GH-induced secretion of IGF-I in hepatocytes during diabetes and other metabolic/physiologic states.

Ninety percent of serum IGF-I is derived from liver (Baxter, 1988; Scott et al., 1985). All serum IGF-I is bound to binding proteins (Baxter, 1988). Most IGF-I in serum is bound to 150 kd binding proteins (Baxter and Martin, 1987; Furnanetto, 1980). However some IGF-I is complexed to 34 kd binding proteins (Baxter, 1988). Both binding proteins are produced by hepatocytes (Baxter, 1988; Scott et al., 1985; Walton and Etherton, 1989). Secretion of the 150 kd, but not the 34kd, species is stimulated by GH in hepatocytes (Baxter, 1988). Production and release of both binding proteins is independent of IGF-I production and release and does not

affect secretion of IGF-I (Baxter, 1988; Schwander et al., 1983). Binding proteins for IGF-I decrease rate of IGF-I clearance from blood (Cascieri et al., 1988; Hodgkinson et al., 1987). Consequently, binding proteins facilitate retention of IGF-I in blood.

ACTIONS OF INSULIN-LIKE GROWTH FACTOR I

Effects of IGF-I

Most actions of IGF-I mimic actions of insulin or promote growth. In adipose tissue, IGF-I increases numbers of glucose transporters (Zapf et al., 1978), increases glucose conversion to fatty acids, increases lipid synthesis and decreases lipolysis (Froesch et al., 1986). However, 50- to 100-fold more IGF-I (moles) than insulin is needed to stimulate insulin-like responses in adipocytes. Insulin-like growth factor I also causes differentiation of preadipocytes to adipocytes (Smith et al., 1988). Increased serum concentrations of GH do not accelerate body weight gain in rats until serum concentrations of IGF-I increases after two weeks of age (Mathews et al., 1988). In addition, IGF-I increases cell replication in cultured smooth muscle (Froesch et al., 1986), satellite cells (Dodson et al., 1985) and chick fibroblasts (Zapf et al., 1978). Therefore, IGF-I causes insulin-like effects in adipose tissue and mitosis in muscle and conceptus.

Both the 150 and 34 kd binding proteins inhibit IGF-I

action in adipocytes, chondrocytes and uterine endothelium (Walton et al., 1989; Ritvos et al., 1988; Rutanen et al., 1988). But, IGF-I-induced fibroblastic growth increases with addition of 34 kd binding proteins (Blum et al., 1989; Elgin et al., 1987). Although not defined, inhibitory/stimulatory effects of binding proteins for IGF-I on actions of IGF-I may be tissue dependent.

Mechanism of IGF-I action

Both serum and tissue IGF-I bind to type I and II IGF receptors and insulin receptors (Czech et al., 1983; Rechler and Nissley, 1985). Type I IGF receptors bind IGF-I with highest affinity but also bind IGF-II and insulin with 50 to 100 times less affinity than IGF-I (Rechler, 1989; Rechler and Nissley, 1985; Czech et al., 1983). Structure of type I IGF receptors, like receptors for insulin, have four subunits, arranged in a β-α-α-β configuration and linked by disulfide bonds (Rechler and Nissley, 1985; Czech et al., 1983). Type I IGF receptors also have tyrosine kinase activity on their beta subunits like receptors for insulin (Rechler, 1989; Jacobs et al., 1983; Zick et al., 1984). Consequently, although not defined, post-receptor mechanisms of type I IGF receptors may be similar to mechanisms of insulin receptors.

Type II IGF receptors bind IGF-I and IGF-II with high affinity (Rechler, 1989; Rechler and Nissley, 1985; Czech et al., 1983) but have no detectable affinity for insulin (Rechler and Nissley, 1985). Type II IGF receptors are single

polypeptide chains (Rechler, 1989; Czech et al., 1983). Post-receptor mechanisms of type II receptors are not defined.

Receptors for insulin also bind IGF-I and IGF-II with 50 to 100 times less affinity than insulin (Rechler and Nissley, 1985). Therefore, observed insulin-like effects of IGF-I may be due in part to binding of IGF-I to insulin receptors (Froesh et al., 1986; Zapf et al., 1978). Likewise, growth promoting effects of insulin may be due in part to binding of insulin to type I IGF receptors (Fukagawa et al., 1985).

EFFECTS OF INSULIN-LIKE GROWTH FACTOR I ON OVARIAN TISSUE

Granulosal-thecal cells

Insulin-like growth factor I increases basal (Schams et al., 1988) and FSH-induced secretion of progesterone (Maruo et al., 1988; Adashi et al., 1988a) from cultured rat and ovine granulosal cells. Luteinizing hormone-induced secretion of androgens from rat thecal cells *in vitro* are also increased by IGF-I (Cara and Rosenfield, 1988). Thus, IGF-I, like insulin, stimulates steroidogenesis from cultured granulosal and thecal cells.

Type I IGF receptors are in rat and ovine granulosal cells (Monget et al., 1989; Adashi et al., 1988b). Insulin-like growth factor I increases number of LH and FSH receptors in swine (Maruo et al., 1988) and increases number of LH but not FSH receptors in rat granulosal cells (Adashi et al., 1988b). Adenylate cyclase activity is also increased

with IGF-I in rat granulosal cells (Adashi et al., 1988a). In swine granulosal cells, IGF-I increases low density lipoprotein (LDL) receptors, LDL internalization and subsequent cellular content of cholesterol (Veldhuis et al., 1987). Aromatase activity, induced by FSH, is also increased by IGF-I in human granulosal cells (Erickson et al., 1989). Consequently, positive steroidogenic effects of IGF-I in granulosal cells are well defined in vitro.

Ovarian production of IGF-I has also been observed. Messenger RNA for IGF-I is present in rat and porcine granulosal cells (Hernandez et al., 1989; Mondschein and Hammond, 1988). Granulosal cell secretion of IGF-I is increased by GH (Hsu and Hammond, 1987a; Davoren and Hsueh, 1986) estradiol, FSH and LH (Hsu and Hammond, 1987b). Immunoneutralization of IGF-I from porcine granulosal cells decrease FSH-induced secretion of progesterone in the presence and absence of exogenous IGF-I (Mondschein et al., 1989). Consequently, IGF-I also has autocrine/paracrine modes of action on secretion of progesterone from cultured granulosal cells.

Luteal cells

In 48h cultures of bovine luteal cells collected at d2 to d4 postestrus, IGF-I increases basal secretion of progesterone (McArdle and Holtorf, 1989). However IGF-I does not affect basal or LH-induced secretion of progesterone from bovine luteal cells cultured for 4h (Schams et al., 1988) or 8d

(Leavitt and Condon, 1989). These bovine luteal cells are derived from corpora lutea at d8 to d12 postestrus. Therefore age of corpora lutea may affect IGF-I action.

SUMMARY

Hepatic secretion and subsequent serum concentrations of IGF-I are regulated mainly by GH (Clemmons, 1989; Davis, 1989). Serum concentrations of IGF-I decrease with diabetes although serum concentrations of GH are increased (Winter et al., 1979; Grant et al., 1973); most likely because of decreased number of hepatic GH receptors (Maes et al., 1983). Serum concentrations of IGF-I (Ronge and Blum, 1988) and somatomedin activity (Falconer et al., 1980) are lower during early compared with late lactation. Therefore decreased concentrations of IGF-I are coincident with NEB in cattle.

Structure of IGF-I is similar to insulin (Baxter, 1988) and IGF-I cross-reacts with receptors for insulin (Rechler, 1989; Rechler and Nissey, 1985). Basal and FSH-induced secretion of progesterone from granulosal cells is increased with IGF-I (Adashi et al., 1988a; Maruo et al., 1988; Schams et al., 1988). Purified bovine luteal cells collected at d2 to d4 postestrus increase basal secretion of progesterone in response to IGF-I (McArdle and Holtorf, 1989). However, IGF-I does not affect basal or LH-induced secretion of progesterone from bovine luteal cells collected at d8 to d12 postestrus (Leavitt and Condon, 1989; Schams, et al., 1988).

Hence, steroidogenic effects of IGF-I *in vitro* may be dependent on age of corpora lutea and/or purity of luteal cell preparations.

GENERAL SUMMARY

After parturition, 81 to 92% of dairy cows experience NEB (Villa-Godoy et al., 1989; Reid et al., 1966). Serum concentrations of progesterone and luteal weight are decreased in NEB heifers compared with PEB heifers (Villa-Godoy et al., 1990; Harrison and Randel, 1986). Therefore, the coincidiental observations of decreased luteal development and function with infertility of dairy cows during NEB raises the question of whether decreased luteal function causes infertility of dairy cows.

After ovulation in sheep and cattle, serum concentrations of progesterone (Villa-Godoy et al., 1990), luteal weight (Ireland et al., 1973) and numbers of steroidogenic cells (Farin et al., 1986) increase. Corpora lutea are comprised of two steroidogenic cell types (Alila and Hansel, 1984), which differ in morphology (O'Shea et al., 1979), number (Schwall et al., 1986) and basal and LH-induced secretion of progesterone (Fitz et al., 1982).

Decreased serum concentrations of progesterone and decreased luteal weight are coincident with decreased postprandial concentrations of insulin in NEB heifers (Villa-Godoy et al., 1990; Harrison and Randel, 1986). In NEB, but not PEB heifers, exogenous insulin increases luteal content of progesterone and luteal weight (Harrison and Randel, 1986). However, it is not known whether postprandial

insulin affects luteal development and function during PEB or if exogenous insulin affects corpora lutea only during NEB. The first objective of this thesis was to determine effects of postprandial concentrations of insulin on serum concentrations of progesterone in heifers maintaining energy balance.

In early lactation, serum concentrations of IGF-I were decreased compared with later lactation (Ronge and Blum, 1988). Therefore, decreased serum concentrations of IGF-I are coincident with decreased luteal development and function during NEB. Basal secretion of progesterone is increased with IGF-I from bovine luteal cells cultured at d2 to d4 postestrus (McArdle and Holtorf, 1989). But, basal or LH-induced secretion of progesterone from bovine luteal cells cultured at d8 to d12 postestrus, is not affected by IGF-I (Leavitt and Condon, 1989; Schams et al., 1985). Therefore, corpora lutea age may affect IGF-I actions on cultured bovine luteal cells. Also, increases in number of bovine luteal cells are associated positively with duration of culture (O'Shaughnessy and Wathes, 1985). Since IGF-I stimulates mitotic activity in other tissues, IGF-I may also increase number of luteal cells during culture. The second set of objectives were to determine the effects of IGF-I on 1) basal and LH-induced secretion of progesterone, 2) number of cells after culture and 3) steroidogenic and mitotic response of cultured bovine corpora lutea at different stages of development.

EXPERIMENT I:

POSTPRANDIAL INSULIN DOES NOT AFFECT SERUM CONCENTRATIONS OF PROGESTERONE IN HEIFERS.

INTRODUCTION

Decreased concentrations of progesterone in cattle are associated with decreased rate of conception (Erb et al., 1976; Folman et al., 1973) and decreased embryo survival (Hill et al., 1970). In NEB heifers, decreased postprandial concentrations of insulin are coincident with decreased serum concentrations of progesterone (Villa-Godoy et al., 1990).

Positive steroidogenic effects of insulin have been demonstrated in granulosal (Lino et al., 1985; Veldhuis and Kolp, 1985; May and Schomberg, 1981) and luteal cells (Leavitt and Condon, 1989; O'Shaunessy and Wathes, 1985) in vitro. Administration of insulin also alleviates secondary amenorrhea in diabetic women (Poretsky and Kalin, 1987). Decreased serum concentrations of progesterone and decreased luteal weight are coincident with decreased postprandial concentrations of insulin in NEB heifers (Villa-Godoy et al., 1990; Harrison and Randel, 1986). In NEB but not PEB heifers, exogenous insulin increased luteal weight and luteal content of progesterone (Harrison and Randel, 1986). It is not known whether postprandial secretion of insulin is luteotropic in cattle during PEB. My objective was to determine the effect of low

postprandial concentrations of insulin on serum concentrations of progesterone in heifers maintaining energy balance.

METHODS

Sixteen postpubertal Holstein heifers were blocked according to body weight and assigned to a diet of 100% hay or 90% corn silage:10% soybean meal (CS). Both diets were formulated to support 0.45 kg body weight gain/d (NRC, 1978) and were presented to heifers at 0700 and 1900h daily.

Each heifer was fed the same diet for the duration of the experiment (44 days) which included a 21d adjustment period and a 23d experimental period (d0 to d22). Heifers were weighed on two consecutive days every two weeks throughout the experiment. The adjustment period was included to allow ruminal acclimation to diets. Prostaglandin F2 alpha[1] (25 mg) was injected intramuscularly 13 and 3d before d0 to synchronize estrus for d0 in all heifers. After $PGF_{2\alpha}$, estrus of heifers was judged to be synchronized if serum concentrations of progesterone were below 1.0 ng/ml for at least three days. From 21 to 8 days before d0 heifers were housed and fed in groups (n=8) and from 7 days before and throughout the experimental period heifers were housed and fed in individual stalls.

During the experimental period (d0 to d22), feed offered and orts were measured to determine daily intake of diet. Orts were discarded immediately before the subsequent meal.

Daily consumption and nutritive estimates of diets (NRC, 1978) were used to estimate caloric intake. Heifers that lost body weight or failed to consume calories required for maintenance were excluded from all analyses.

A cannula was inserted into a jugular vein of each heifer on the last day of the adjustment period. On d0, 7, 14 and 21 blood was sampled every 20 min from 0500h to 1300h to determine basal (2h preprandial) and postprandial (6h) concentrations of insulin in serum. Jugular blood was also sampled daily at 0630h and 1830h from d0 to d22 and hourly from 0800 to 1200 h on d7 and d14 to quantify progesterone in serum. Blood was allowed to clot at room temperature (12 to 20 C) and then was stored at 4 C overnight. Serum was harvested by centrifugation (2000 rpm for 25 min) and stored at -20 C until analyzed.

A previously validated double antibody radioimmunoassay (RIA) was used to quantify insulin in serum (Villa-Godoy et al., 1990). The intra-assay coefficient of variation from several dilutions of standard sera for the insulin RIA was 5.7%. Progesterone was also quantified by RIA (Spicer et al., 1981). Mean extraction efficiency was 93.0 ± 1.3%. Intra and interassay coefficients of variation were 9.3 and 12.3% for standard sera, respectively.

Data were examined by split plot analysis of variance with repeated measurements (Gill, 1986) and specific contrasts were by Bonferroni's T test.

RESULTS

One heifer fed CS lost weight during the experimental period and was excluded from analysis. Mean body weight (Figure 1) of heifers fed hay (n=8) or CS (n=7) did not change throughout the adjustment or experimental periods. Based on estimates of caloric requirements for heifers (NRC, 1978), feed consumed by heifers on both diets satisfied or exceeded maintenance requirements for energy (data not shown).

Basal concentrations of insulin (2h preprandial) at d0 and d14 in heifers fed hay were less ($P<.05$) than in heifers fed CS (Figure 2). In heifers fed hay, area of periprandial serum insulin profiles (2h before to 6h after feeding) were decreased ($P<.05$) at d0, 7, 14 and 21 compared with heifers fed CS (Figure 2). Concentrations of insulin from feeding to 6h after feeding increased ($P<.05$) in heifers fed CS but not in heifers fed hay on d7, 14 and 21 (Figure 2). Consequently, we examined the association of low (hay diet) or increased (CS diet) postprandial concentrations of insulin on secretion of progesterone in heifers maintaining energy balance. Heifers will now be classified as heifers with low insulin (hay diet) or normal insulin (CS diet).

Based on patterns of progesterone in serum (Figure 3), estrus was synchronized in all heifers within a period of 3d (d0 to d2). Total area of progesterone from d0 to d22, mean concentrations of progesterone from d0 to d22, peak concentrations of progesterone and interval from nadir to peak

progesterone did not differ in heifers with low or normal insulin (Figure 3). Within 5h after feeding, when differences in concentrations of insulin were greatest, mean concentrations of progesterone on d7 or d14 were not different between heifers with low or normal insulin (Figure 4).

Figure 1. Body weight of heifers fed hay (●——●) or corn silage (O——O). Heifers were weighed on two consecutive days every other week during the experiment. Pooled standard error was 2.8 kg.

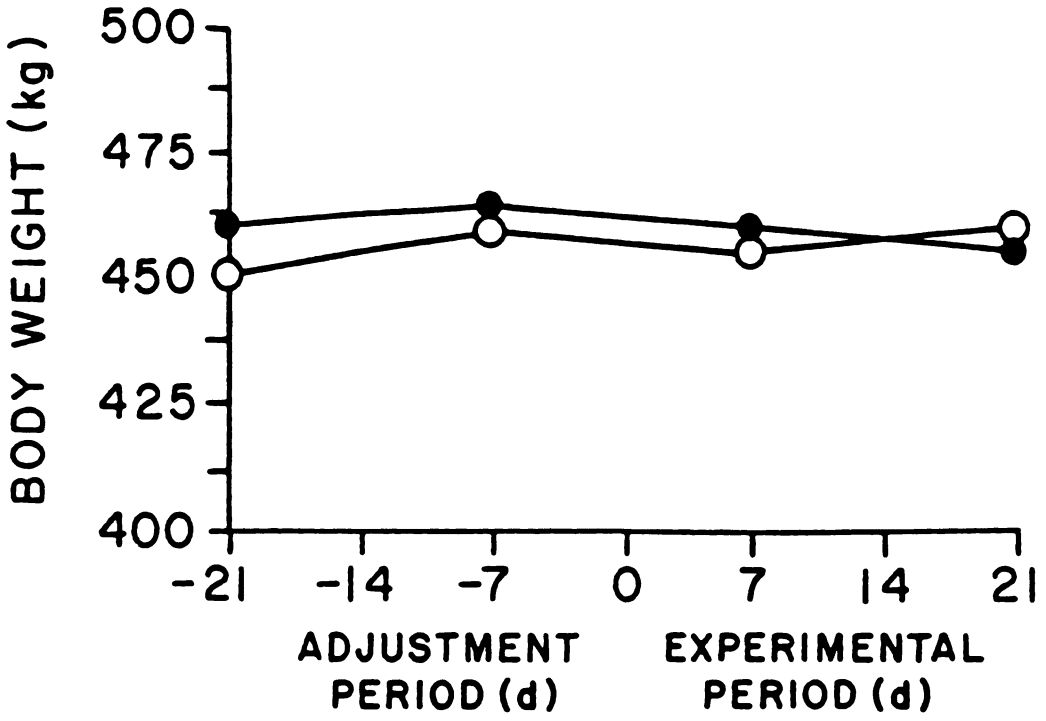

BODY WEIGHT (kg)
500
475
450
425
400
-21
-14
-7
0
7
14
21
ADJUSTMENT PERIOD (d)
EXPERIMENTAL PERIOD (d)

Figure 2. Effect of diet on serum concentrations of insulin in heifers from 2h before to 6h after feeding. Jugular blood was sampled every 20 min in heifers fed hay (●——●) or corn silage (O——O). Concentrations of insulin were measured on d0, 7, 14 and 21. Arrow indicates presentation of feed. Pooled standard error of insulin area was 25.97 $ng \cdot ml^{-1} \cdot min^{-1}$ of serum.

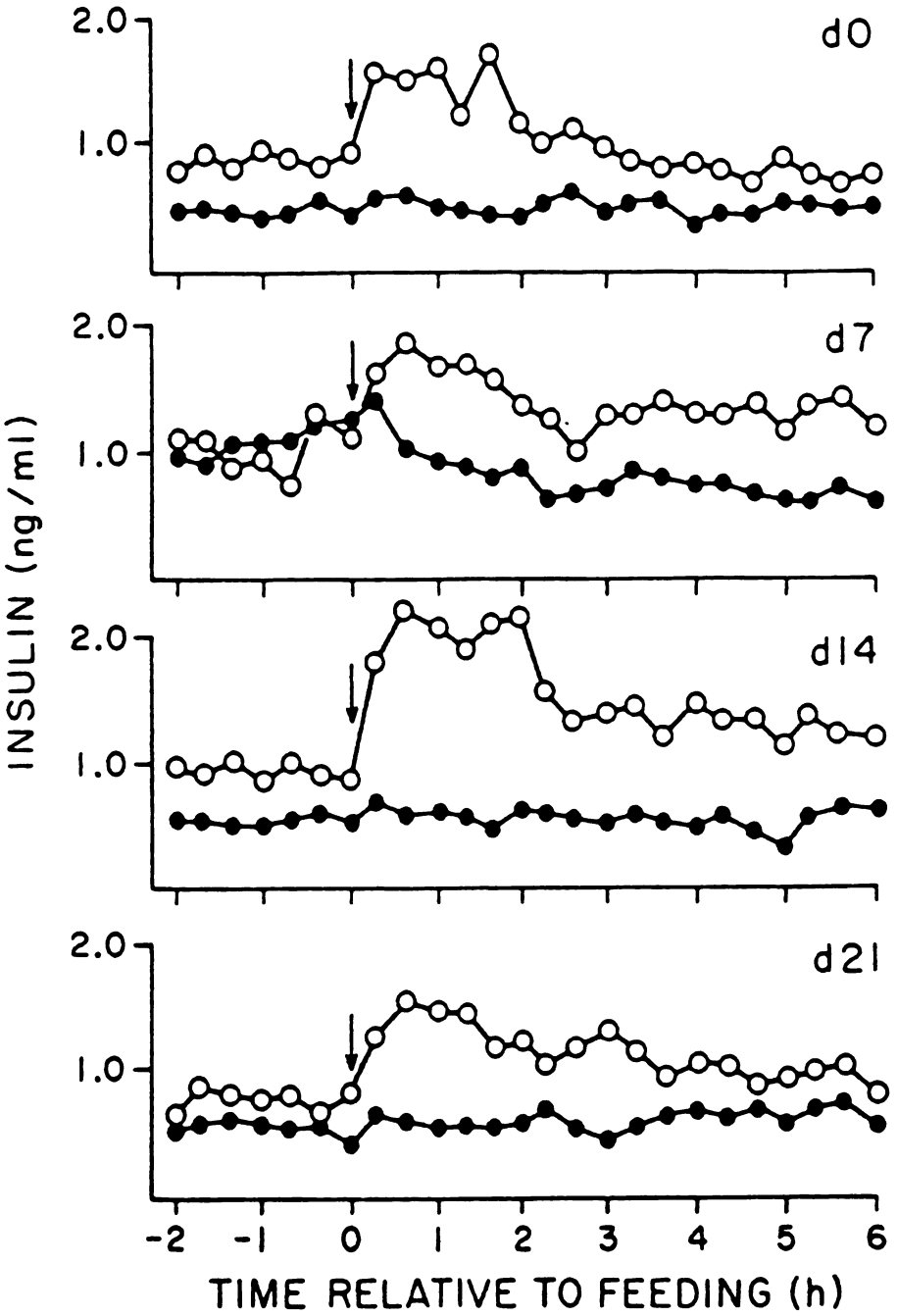

2.0
1.0
d0
d7
d14
d21
INSULIN (ng/ml)
-2
-1
0
1
2
3
4
5
6
TIME RELATIVE TO FEEDING (h)

Figure 3. Effect of postprandial insulin on serum concentrations of progesterone. Blood was sampled twice daily from d0 to d22 in heifers with low (●——●) or normal (O——O) insulin. Pooled standard error was .16 ng/ml of serum.

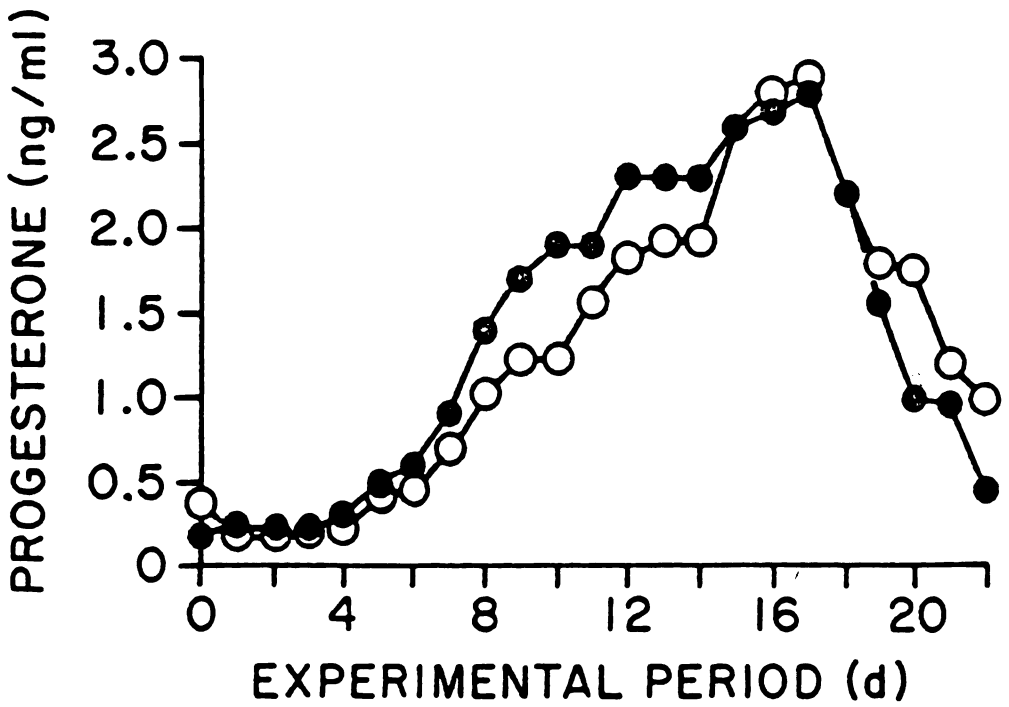

PROGESTERONE (ng/ml)
3.0
2.5
2.0
1.5
1.0
0.5
0
0
4
8
12
16
20
EXPERIMENTAL PERIOD (d)

Figure 3. Effect of postprandial insulin on serum concentrations of progesterone. Blood was sampled twice daily from d0 to d22 in heifers with low (●——●) or normal (O——O) insulin. Pooled standard error was .16 ng/ml of serum.

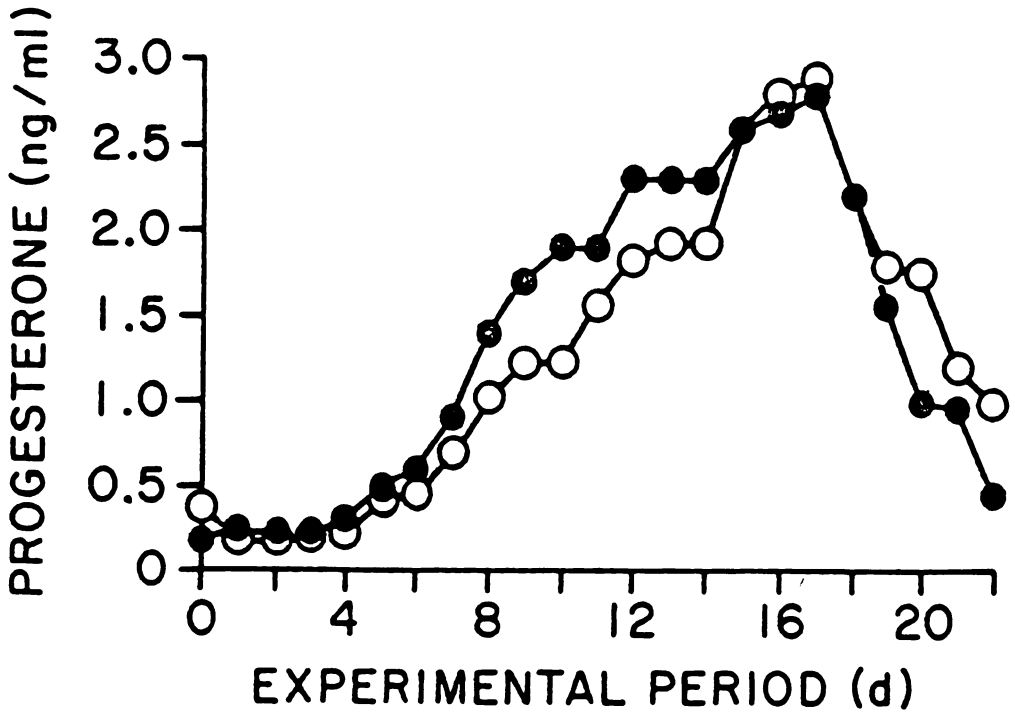
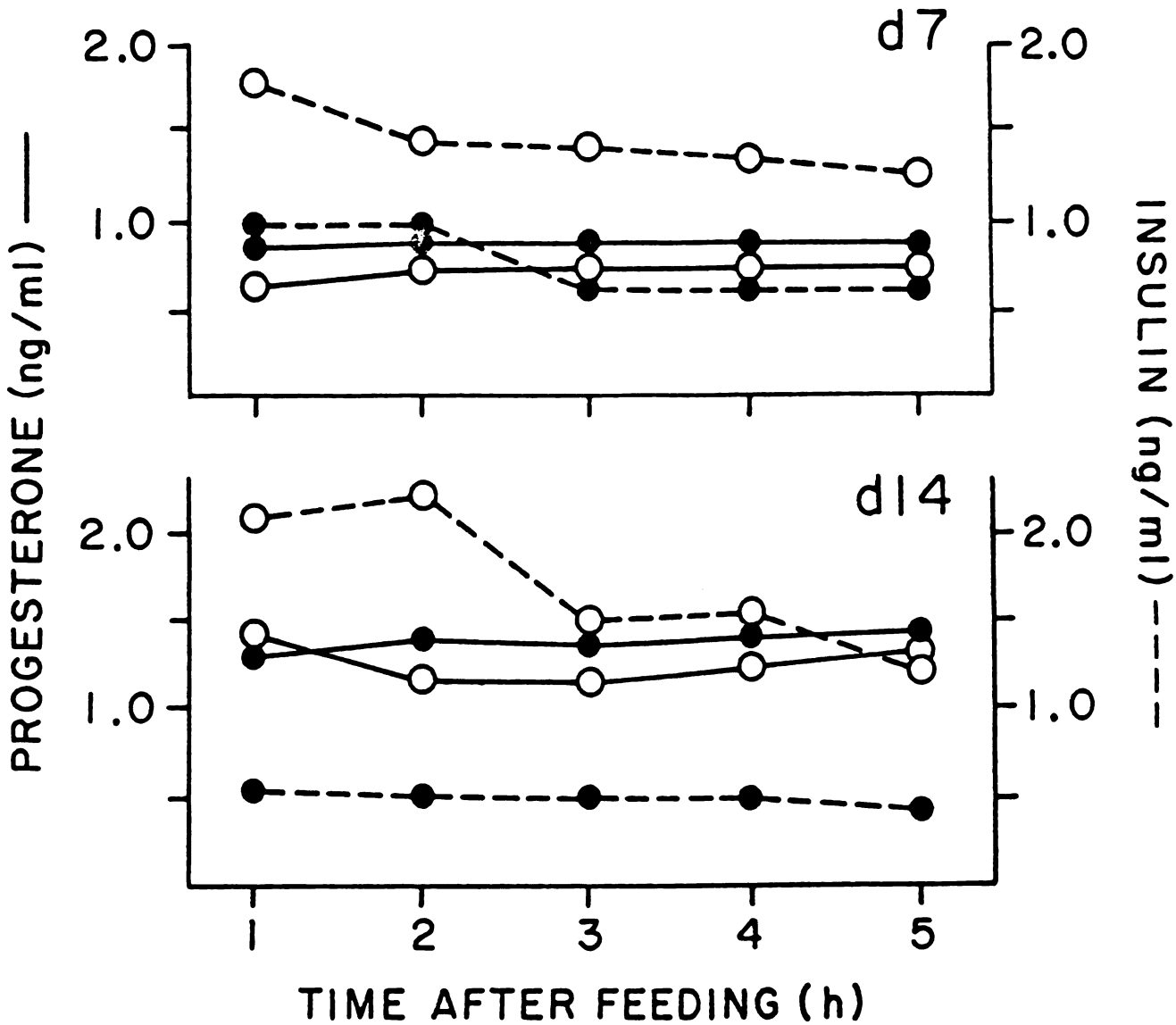

PROGESTERONE (ng/ml)
3.0
2.5
2.0
1.5
1.0
0.5
0
0
4
8
12
16
20
EXPERIMENTAL PERIOD (d)

Figure 4. The acute effect of postprandial insulin with progesterone in heifers. Jugular blood was sampled hourly for the first 5h after feeding. Serum concentrations of progesterone in heifers with low (●——●) or normal (O——O) insulin were compared with serum concentrations of insulin in heifers with low (●- -●) or normal (O- - -O) insulin. Pooled standard error was .36 ng/ml for progesterone and .35 ng/ml for insulin.


d7
d14
2.0
1.0
PROGESTERONE (ng/ml) ——
---- INSULIN (ng/ml)
1
2
3
4
5
TIME AFTER FEEDING (h)

DISCUSSION

Calories ingested were adequate for maintenance (NRC, 1978). Furthermore, body weight of heifers fed diets of hay or CS did not decrease during the trial. Therefore heifers were at energy balance, which allowed us to determine effects of insulin on serum concentrations of progesterone without confounding by NEB.

Diets clearly affected postprandial concentrations of insulin in heifers. In ruminants, secretion of insulin is controlled primarily by propionate and butyrate, not by glucose or acetate (Trenkle, 1971; Manns and Boda, 1967). Ruminal digestion of diets high in starch (CS diet) produce more propionate and butyrate. Conversely, diets with high fiber and low starch (hay diet) produce less propionate and butyrate (Trenkle, 1971; Manns and Boda, 1967). In addition, it took longer for heifers to consume a hay diet than a CS diet, which may also affect patterns of serum concentrations of insulin.

Insulin increases secretion of progesterone from granulosal (Lino et al., 1985; Veldhuis and Kolp, 1985; May and Schomberg, 1981) and luteal cells (Leavitt and Condon, 1989; O'Shaunessy and Wathes, 1985) <u>in vitro</u> and in corpora lutea <u>in vivo</u> (Harrison and Randel, 1986). Receptors for insulin are present in rat luteal tissue (Ladenheim et al., 1984). Additionally, diabetes in humans decreases fertility and exogenous insulin is therapeutic (Poretsky and Kalin,

1987). Thus, there is evidence that insulin is gonadotropic. But, in the present study, normal postprandial concentrations of insulin had no effect on serum concentrations of progesterone.

Decreased postprandial concentrations of insulin are coincident with decreased serum concentrations of progesterone in NEB heifers (Villa-Godoy et al., 1990; Harrison and Randel, 1986). In NEB but not PEB heifers, exogenous insulin increases luteal weight and luteal content of progesterone (Harrison and Randel, 1986). From my data, reduced postprandial concentrations of insulin did not alter luteal development or function in heifers in energy balance. However, in previous studies of heifers in NEB, serum concentrations of progesterone were not decreased until ≥two estrous cycles after inducing NEB (Villa-Godoy et al., 1990; Harrison and Randel, 1986). Consequently, if low postprandial insulin is necessary for reduced luteal development and/or function, absence of increased postprandial insulin for one estrous cycle may not be of sufficient duration to detect effects.

In summary, all heifers fed hay or CS maintained energy balance during the experiment. Heifers fed CS, but not heifers fed hay, had increased postprandial concentrations of insulin. But absence of postprandial insulin did not affect secretion of progesterone during one estrous cycle or within 5h after feeding. I conclude that low postprandial

concentrations of insulin during energy balance are adequate for sustained secretion of progesterone in heifers maintaining energy balance. An implication of these data is that low postprandial insulin may not mediate adverse effects of NEB on secretion of progesterone.

EXPERIMENT II:

THE EFFECT OF INSULIN-LIKE GROWTH FACTOR I ON SECRETION OF PROGESTERONE FROM BOVINE LUTEAL CELLS

INTRODUCTION

Serum concentrations of IGF-I are lower during early lactation compared with later lactation in dairy cows (Ronge and Blum, 1988; Falconer et al., 1980). During early lactation, 81 to 92% of dairy cows are in negative energy balance (Villa-Godoy et al., 1989; Reid et al., 1966), which is associated with decreased serum concentrations of progesterone and luteal weight (Villa-Godoy et al., 1990; Harrison and Randel, 1986). Therefore, decreased serum concentrations of IGF-I coincide with decreased luteal development and function during NEB.

Perhaps IGF-I affects luteal development and function. Insulin-like growth factor I increases basal secretion of progesterone from cultured bovine luteal cells (McArdle and Holtorf, 1989) in one study but not others (Leavitt and Condon, 1989 ;Schams et al., 1988). Detection of positive steroidogenic effects of IGF-I may be dependent on dose of IGF-I administered. Also, since IGF-I effects were not observed until after 48h of culture (McArdle and Holtorf, 1989), duration of culture may also affect detection of IGF-I effects from bovine luteal cells.

Increases in bovine luteal cell numbers are associated

positively with duration of culture (O'Shaughnessy and Wathes, 1985). Insulin-like growth factor I increases replication of cells from smooth muscle cells (Froesch et al., 1986), satellite cells (Dodson et al., 1985) and embryonic chick fibroblasts (Zapf et al., 1978). Mitotic activity of cultured bovine luteal cells may also be stimulated by IGF-I. Our objectives were to determine effects of IGF-I on basal and LH-induced secretion of progesterone and number of bovine luteal cells during culture.

MATERIALS AND METHODS

Ten postpubertal Holstein heifers were fed corn silage and hay to gain .6 kg body weight daily (NRC, 1978). Body weights were measured weekly. Heifers were observed for estrus three times daily. Estrus was determined by two observations of a heifer standing to be mounted by another heifer within a 30 min detection peroid.

On d5 (n=5) or d10 (n=5) postestrus (estrus=d0), Luteal tissue was removed and dissociated enzymatically as described previously (Villa-Godoy et al., 1990). Following dissociation, cells were washed and centrifuged four times with Hams F12 media[2] containing 25 mM Hepes[2], 14 mM sodium bicarbonate[2], .1 g/L streptomycin sulfate[2] and .07 g/L potassium penicillin[2] (pH 7.35). Viability of cells was estimated by exclusion of trypan blue. Media (1 ml Hams F12) containing 5 x 10^5 cells were placed in 24 well-plates[3] and

incubated at 37 C in 95% oxygen: 5% carbon dioxide for 24h. Before culture, some plated cells and media were harvested, centrifuged (5 min at 1000 rpm), media were aspirated and cells were resuspended with .05 M phosphate buffered saline containing 2.0 M sodium chloride[2] and .003 M EDTA[2] (PBS-EDTA; pH 7.35) and stored at -20 C until DNA was quantified. These cells were used to estimate content of DNA before culture.

Immediately before culturing, cells and media were treated with LH[4] (0, .1 or 1 ng) and IGF-I[5] (0 or 500 ng). Luteinizing hormone was solubilized in Hanks buffered saline solution (pH 7.0), frozen on dry ice and stored at -70 C until use. One ng LH increased secretion of progesterone from bovine luteal cells in preliminary experiments and was used in this experiment to determine effects of IGF-I on LH-induced secretion of progesterone. Treatment of cells and media with .1 ng LH was also included to determine if any synergistic or additive effects between LH and IGF-I occur when the dose of LH does not stimulate secretion of progesterone.

Insulin-like growth factor I was reconstituted with 10 mM sodium acetate[2] (pH 5.5) and frozen at -70 C until use. Treatment with 500 ng IGF-I was chosen because it is between the range of doses used in previous experiments (Leavitt and Condon, 1989; McArdle and Holtorf, 1989; Schams et al., 1988). Also, 500 ng IGF-I increased FSH-induced secretion of progesterone from cultured granulosal cells (Maruo et al., 1988).

After 24h, cells and media were collected and centrifuged (5 min at 1000 rpm). Media were aspirated and stored at -20 C until progesterone and DNA were quantified. Cells were resuspended with PBS-EDTA and stored at -20 C until DNA was quantified.

Quantification of progesterone and DNA: Progesterone in media was quantified as described previously (Spicer et al., 1981). Efficiency of progesterone extraction averaged 94.1%. Intra and interassay coefficients of variation were 5.9 and 14.6% from pooled media, respectively. Quantification of DNA in cells and media were described previously (Labarca and Paigen, 1980). Intra and interassay coefficients of variation were 5.4 and 10.9% from pooled cells and 7.7 and 5.9% from pooled media, respectively.

Statistical analysis: Data were examined by split-plot analysis of variance with repeat measurements (Gill, 1986). Specific contrasts were by Bonferroni's T test.

RESULTS

All heifers gained body weight before lutectomy. Viability of cells was >92.0% following dissociation and >86.0% after 24h of culture. Cell viability after 24h was not affected by age of corpora lutea or by presence of LH or IGF-I.

Percent DNA recovered after 24h did not differ between luteal cells cultured at d5 (82.1 ± 12.8%) and d10 (95.8 ±

6.77%) postestrus. In luteal cells collected at d5 and d10 postestrus, 1 ng LH increased ($P<.05$) percent DNA after 24h compared with basal or .1 ng LH (Figure 5). Percent DNA after 24h did not differ between basal and .1 ng LH at either day postestrus. Percent DNA at 24h was not affected by IGF-I and there were no interactions of IGF-I with LH or day postestrus (Figure 5).

Concentrations of progesterone in media from luteal cells collected on d5 postestrus did not differ from that of luteal cells collected on d10 postestrus. In luteal cells collected on d5 and d10 postestrus, 1 ng LH increased ($P<.01$) concentrations of progesterone in media compared with basal or .1 ng LH (Figure 6). Basal concentrations of progesterone in media did not differ from .1 ng LH at either day postestrus. Insulin-like growth factor I did not affect basal or LH-induced concentrations of progesterone in media from luteal cells cultured at d5 or d10 postestrus (Figure 6).

Figure 5. Effect of LH and IGF-I on DNA in media and cells at 24h. DNA was expressed as percent of DNA in cells before culture. Pooled standard error was 1.78%.

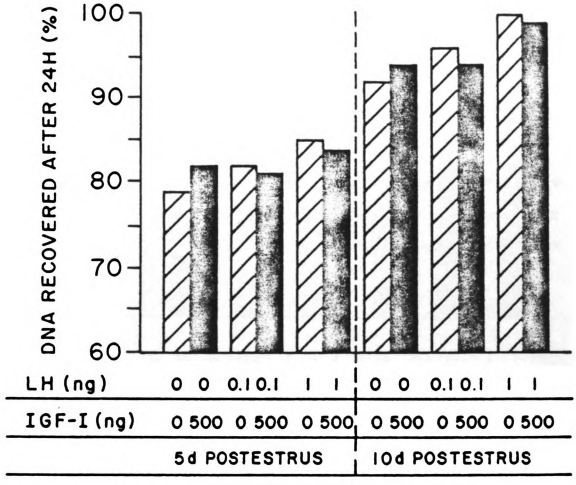
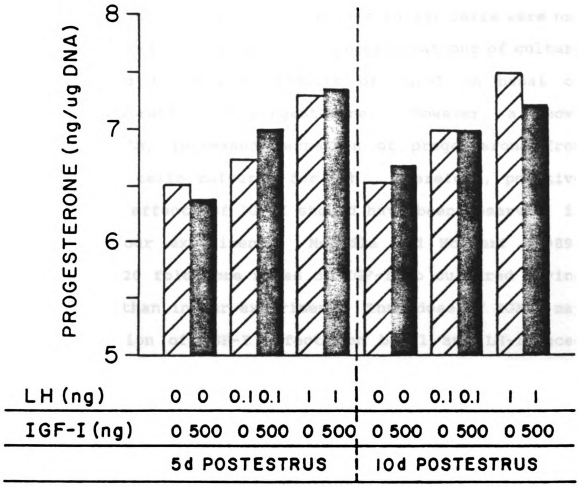

DNA RECOVERED AFTER 24H (%)
100
90
80
70
60
LH (ng)
0 0 0.1 0.1 1 1 0 0 0.1 0.1 1 1
IGF-I (ng)
0 500 0 500 0 500 0 500 0 500 0 500
5d POSTESTRUS
10d POSTESTRUS

Figure 6. Effect of LH and IGF-I on secretion of progesterone from bovine luteal cells cultured at d5 or d10 postestrus. Pooled standard error was .26 ng/ug DNA.


PROGESTERONE (ng/ug DNA)
8
7
6
5
LH (ng)
0 0 0.1 0.1 1 1 0 0 0.1 0.1 1 1
IGF-I (ng)
0 500 0 500 0 500 0 500 0 500 0 500
5d POSTESTRUS
10d POSTESTRUS

DISCUSSION

In this experiment, heifers gained body weight before lutectomy, so data were not confounded by NEB. After 48h of culture, positive steroidogenic effects of IGF-I from bovine luteal cells have been observed (McArdle and Holtorf, 1989). In our experiment, basal and LH-induced secretion of progesterone from 24h cultures of bovine luteal cells were not affected by IGF-I. Consequently, longer durations of culture may be needed to observe effects of IGF-I on basal or LH-induced secretion of progesterone. However, a known luteotropin, LH, increased secretion of progesterone from bovine luteal cells cultured for 24h. Therefore, positive steroidogenic effects of IGF-I should have been observed, if present, in our experiment. McArdle and Holtorf (1989) administered 20 fold more moles of IGF-I to cultured bovine luteal cells than in our experiment. Thus dose of IGF-I may effect detection of IGF-I effects on basal and LH-induced secretion of progesterone from bovine luteal cells.

Number of cells in culture did were not affected by IGF-I in our experiment. Duration of culture may not have been adequate for detection of IGF-I effects on mitotic activity since 48 to 96h of culture are needed to observe a 2 fold increase in number of luteal cells (O'Shaugnessy and Wathes, 1985). However, LH, but not IGF-I, increased maintenance of luteal cell numbers after 24h of culture. Thus IGF-I does not

affect maintenance of luteal cell numbers after 24h of culture.

In summary, LH increased secretion of progesterone from luteal cells collected on d5 and d10 postestrus. Also, LH increased maintenance of luteal cell numbers. But, IGF-I did not affect basal or LH-induced secretion of progesterone or maintenance of luteal cell numbers at either day postestrus. Perhaps greater doses of IGF-I are needed to detect effects of IGF-I on steroidogenic and mitotic activity in bovine luteal cells. In conclusion, 500 ng of IGF-I does not increase basal or LH-induced secretion of progesterone or number of cells from cultured bovine luteal cells.

FOOTNOTES

[1] Lutylase, (25 mg); Upjohn Co., Kalamazoo, Michigan

[2] Sigma Chemical Company; St. Louis, Missuori.

[3] Costar; Cambridge, Massachusetts

[4] NIH-bLH4; NIH, Beltsville, Maryland

[5] IMCERA; Terre Haute, Indiana

SUMMARY AND CONCLUSIONS

In the first experiment, low postprandial concentrations of insulin had no effect of serum concentrations of progesterone in heifers maintaining energy balance. Conversely, during NEB, low postprandial insulin was coincident with decreased serum concentrations of progesterone and luteal weight (Villa-Godoy et al., 1990; Harrison and Randel, 1986). Additionally, exogenous insulin increased luteal content of progesterone and luteal weight in NEB but not PEB heifers (Harrison and Randel, 1986). However, low postprandial insulin was not associated with decreased serum concentrations of progesterone until after the second estrous cycle that heifers experienced NEB (Villa-Godoy et al., 1990). Therefore , it is not known whether low postprandial insulin decreased luteal function only during NEB or if low postprandial insulin must exist for ≥ two estrous cycles to decrease luteal function.

In the second experiment IGF-I did not affect basal or LH-induced secretion of progesterone or number of cells from 24h cultures of bovine luteal cells collected on d5 or d10 postestrus. However, 20 fold more (moles) IGF-I increased basal secretion of progesterone from bovine luteal cells

(McArdle and Holtorf, 1989). Thus, IGF-I effects on steroidogenesis of bovine luteal cells may be dependent on dose of IGF-I. Bovine luteal cells double in number from 48 to 96h of culture (O'Shaunessy and Wathes, 1985). Therfore, my culture duration may have been inadequate to detect mitotic activity of IGF-I on bovine luteal cells.

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