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Christopher Morrison

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Christopher D. Morrison
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Animal Science Department
Pennington Biomedical Research Center
Louisiana State University
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Introduction

In livestock, adipose stores, or body condition, is a governing factor in the relationship between nutrition and reproductive performance. Although many studies have illustrated the reproductive impairment that occurs due to loss of body condition, the actual metabolic and reproductive mediators of this connection are still only vaguely known. The recent discovery and study of the obesity gene, leptin, and the leptin receptor have provided an opportunity for progress toward the understanding of these interactions. While leptin has the potential to influence a broad range of physiological systems, its impact on reproduction has only recently begun to emerge. Early research has confirmed an influence of leptin and the presence of its receptor in several reproductive tissues: most notably the hypothalamus, pituitary, and gonads. Leptin presents the possibility for both the expansion of our knowledge of a fundamental area of reproduction, as well as a potential means for improving reproductive efficiency and thus profitability in livestock production. We therefore attempted first to determine if a leptin receptor exists within the bovine corpus luteum (CL) and granulosa cells. Second, we attempted to determine if leptin influences steroid production. And third, we attempted to determine if receptor mRNA is differentially expressed within these cells.

Literature Review

Reproductive efficiency is an extremely important component in livestock production. In beef cattle, time until puberty and length of the postpartum interval have significant economic impacts. Early in the study of these reproductive parameters, it became evident that nutrition and body condition had profound effects on subsequent reproductive performance. Studies of the influence of nutrition on reproduction show that nutritional status affects age at puberty in heifers (Perry et al., 1991). Cows that are on restricted feed intake have an increased time until first postpartum estrus (Wiltbank et al., 1964). It has also become evident that body condition is a major factor influencing

the effects of nutrition on reproduction. Body condition, especially at calving, is a dominating factor influencing the initiation of ovarian activity following the postpartum anestrous period (Dunn and Kaltenbach, 1980; Richards et al., 1986; Selk et al., 1988). Cows that have a body condition score of five or greater at calving will return to estrus earlier than those with a score of four or less, despite moderate reductions in postpartum body weight or nutrition (Dunn and Kaltenbach, 1980; Richards et al., 1986).

It is obvious that nutrition, and more specifically body condition, plays a pivotal role in reproduction and fertility. However, the physiological changes that occur during the cessation or delay of ovarian activity, and the signals linking these changes and nutrient status, are less clearly understood. Dramatic endocrine changes occur at puberty and parturition. One interesting event is a gradual rise in progesterone in the days immediately preceding the first postpartum estrus (Donaldson et al., 1970). It is likely that progesterone production by the corpus luteum (CL) and luteinized follicles may be significant in the resumption of reproductive activity. Indeed, when cows are on restricted feed intakes that cause weight loss, luteal activity ceases when a body condition score of 3.5 - 4 is reached, while refeeding causes a resumption of luteal activity at a body condition score of 4.5 (Richards et al., 1989).

It is very clear that restricting food intake and reducing body condition reduces circulating progesterone (Gombe and Hansel, 1973; Villa-Godoy et al., 1988, 1990; Shrick et al., 1992). However the reason for the impairment of CL function is not fully resolved. The principal hypothesis is that reduced intake and body condition affects the hypothalamic-pituitary axis, causing a reduction in luteinizing hormone (LH) secretion. This hypothesis is supported by the experiments of Richards et al. (1989) which showed a reduction in LH following food restriction, and Bishop et al. (1997) which demonstrated an influence of body condition at early weaning on LH pulse frequency. However, conflicting evidence has been provided by others, who showed no change (Villa-Godoy et al., 1990; Shrick et al., 1992) or increases (Gombe and Hansel, 1973) in LH secretion following negative energy balance or food restriction, despite reductions in progesterone secretion. It has also been shown that the CL of cows on restricted feed intakes display no difference in LH receptor numbers, percent bound, or in progesterone

synthesis in response to LH stimulation when compared to controls (Shrick et al., 1992). However, LH stimulated progesterone production in luteal tissue from cows in positive energy balance but not from cows in negative energy balance (Villa-Godoy et al., 1990). In the explanation of these conflicting results, a recurrent idea is the influence of body condition, especially at the onset of food restriction or negative energy balance, on the subsequent results. Villa-Godoy et al. (1990) showed that placing heifers in a negative energy balance reduced serum progesterone concentration, but that progesterone dropped earlier in moderate body condition animals than in fatter, higher body condition animals. In the higher body condition heifers, progesterone did not drop until body condition declined below moderate, despite a continual drop in body weight. Therefore, it is apparent that restricted feed intake and especially reduced body condition decreases progesterone production and has a detrimental effect on reproduction, despite the debate over the mediator of these effects.

Clearly then, adequate adipose mass and body condition is central to efficient reproductive performance. Yet the means with which the body signals body condition status and how these signals are communicated is still not definitively known. A recent discovery has shed some light on these signaling mechanisms. This extremely interesting and promising new discovery is the obesity gene (Zhang et al., 1994), whose protein product is termed leptin. Leptin is produced and secreted by adipocytes (He et al., 1995; Hosoda et al., 1996), and was initially believed to have a target site in the brain and function as a signal for regulating food intake and adipose mass (Collins et al., 1996b). Leptin plays a role in the regulation of body composition by signaling an increase in energy expenditure and thermogenesis in brown adipose tissue (Collins et al., 1996a), affecting food intake (Campfield et al.,1995; Pelleymounter et al., 1995), and reducing body weight (Halaas et al., 1995).

A strain of genetically selected (*ob/ob*) mice, which due to a genetic mutation produce a faulty leptin protein (Zhang et al., 1994), are obese, hyperphagic, and classically infertile (Bray and York, 1979). These obese mice also have reduced levels of LH, FSH, and reproductive steroids (Swerdloff et al., 1976). Recently, however, it has been shown that leptin injections will reduce food intake, reduce body weight, increase

metabolic rate (Pelleymounter et al., 1995) and induce fertility (Chehab et al., 1996; Mounzih et al., 1997) in some of these obese, infertile *ob/ob* mice. It was also shown that this effect was not simply due to weight loss, since *ob/ob* mice pair fed to leptin treated *ob/ob* mice lost weight but had continued infertility (Chehab et al., 1996). These results suggest that leptin is involved in multiple signaling systems throughout the body.

The exact function of circulating leptin is not entirely known. What is known is that serum leptin levels are highly correlated with percent body fat, leading to the hypothesis that leptin is a circulating signal for body adipose reserves (Considine et al., 1996b; Ostlund et al., 1996, Ryan and Elahi, 1996; Grinspoon et al., 1996; Weigle et al., 1997). Leptin expression and secretion increases in response to glucocorticoids, (Murakami et al., 1995; Larrson and Ahren, 1996; Slieker et al., 1996), and estrogen, but not testosterone (Slieker et al., 1996). Insulin may also stimulate leptin expression and secretion (Saladin et al., 1995; Slieker et al., 1996), although others show conflicting responses to it (Cohen et al., 1996; Kolaczynski et al., 1996; Larrson and Ahren, 1996). Fasting, which reduces serum insulin, causes a decrease in leptin levels, while refeeding restores levels to normal (Saladin et al., 1995; Weigle et al., 1997).

Leptin then appears to be a circulated product which is differentially regulated by several known metabolic hormones. It is for this reason that following the discovery of leptin, a high level of attention was given to its use for treatment of human obesity. Although leptin has some promise in this area, studies have shown that it is not necessarily a cure for obesity, and that data produced in the mouse may not be applicable to humans. A mutation similar to the *ob/ob* mutation has presently not been found in humans (Maffei et al., 1996). Consequently, attention has most recently turned to the leptin or Ob-receptor, with the hypothesis that leptin resistance is a cause of obesity.

Another line of genetically obese mice, the diabetic or *db/db* mouse, have a similar phenotype to that shown in ob/ob mice (Bray and York, 1979). Further research shows that these mice express a high level of circulating leptin (Halaas et al., 1995), and that the phenotype arose from a mutated leptin receptor (Chen et al., 1996; Chua et al., 1996; Lee et al., 1996). A similar phenotype exists in rats (the *fa/fa* rat), also resulting from a leptin receptor mutation (Chua et al., 1996).

The leptin receptor appears to exist in multiple alternatively spliced variants, resulting in at least five different cytoplasmic domains (Lee et al., 1996, Cioffi et al., 1996). Initially, only the so called long form was believed to be functional (Baumann et al., 1996; Gainsford et al., 1996), but more recent research indicates that the shorter form may generate a signal (Murakami et al.,1997), or at least modulate (White et al., 1997) the activity of the longer form. The leptin receptor has sequence similarity to the cytokine receptor family, especially the interleukin-6 receptor (Tartaglia et al., 1995; Baumannet al., 1996), stimulates STAT1 and STAT3 signaling mechanisms (Rosenblum et al., 1996), and activates janus kinase 2 (Ghilardi and Skoda, 1997). Another splice variant is thought to be a soluble carrier protein (Lee et al., 1996). This hypothesis is supported by the indication of free and bound leptin in circulation (Sinha et al., 1996). Although no mutations similar to the *db* or *fa* mutation in mice and rats have presently been found in obese humans (Considine et al., 1996A), much research continues on the leptin receptor, especially in the hope for discovery of a new treatment for human obesity.

The leptin receptor is expressed in a variety of tissues (Tartaglia et al., 1995; Cioffi et al., 1996), but its expression in the brain and hypothalamus has been most researched. Expression of receptor mRNA and binding of labeled leptin is detected in the choroid plexus (Tartaglia et al., 1995; Cioffi et al., 1996; Devos et al., 1996), as well as the ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), ventral premamillary nucleus (VPN) (Elmquist et al., 1997), and arcuate nucleus (ARC) (Dyer et al., 1997). Regulation of the leptin receptor also appears to exist, since food restriction was shown to increase receptor expression in the VMH and ARC (Dyer et al., 1997). Several reports have shown the possible transport of peripheral leptin into these brain areas. It has been shown that leptin binds and is internalized by brain capillaries (Golden et al., 1997), and is unidirectionally transported into the brain through a saturable, insulin independent process in the choroid plexus, median eminence and arcuate nucleus (Banks et al., 1996). Taken together these results strongly suggest an action of leptin in the brain, giving support to the hypothesis that leptin is an adipostatic signal.

However, the leptin receptor also exists in tissues outside the brain. Leptin receptors, binding of leptin, or receptor mRNA have been found in the heart, liver, spleen, lung, kidney, adrenal, prostrate, testis and ovary, (Cioffi et al., 1996; Hoggard et al., 1997), as well as in hematopoietic tissues (Cioffi et al., 1996; Gainsford et al., 1996). Functional leptin receptors have been found in hepatic cell lines, where leptin affected Insulin Receptor Substrate-1 (IRS-1) phosphorylation, and GRB2 and PI 3-kinase binding to IRS-1 (Cohen et al., 1996). Leptin receptors also exist within the pancreatic islets, (Keiffer et al., 1996; Emilsson et al., 1997). Leptin administration decreases insulin secretion as well as attenuates the stimulatory effects of glucose on insulin secretion (Emilsson et al., 1997) in cultured pancreatic cells. Leptin impairs several metabolic actions of insulin in isolated rat adipocytes, such as stimulation of glucose transport, lipogenesis, and glycogen synthase (Müller et al., 1997). The presence of leptin receptors and effects of leptin in peripheral tissues is clear evidence of its actions outside the brain.

As has been previously mentioned, mice with mutations in either leptin or its receptor suffer from many abnormalities, including impaired reproductive performance. This fact, as well as the discovery of receptors in the hypothalamus, ovary, and testis (Cioffi et al., 1996; Lee et al., 1996) and the restoration of fertility following leptin administration in *ob/ob* mice (Chehab et al., 1996; Mounzih et al., 1997) leads to the possibility of an effect of leptin on reproduction. Further confirmation of this hypothesis occurred when recent research with the *ob/ob* mouse showed that leptin administration stimulates the reproductive endocrine system, increases gonadal function and blocks a fasting induced reduction in gonadotropins (Barash et al., 1996). Not only can leptin administration restore fertility to infertile *ob/ob* mice, but leptin injections also influenced the onset of puberty in female mice (Chehab et al., 1997; Ahima et al., 1997), and blocked the delay in puberty in female rats on restricted food intake (Cheung et al. 1997). In a longitudinal study of young boys, a rise in serum leptin was detected just prior to the onset of puberty (Mantzoros et al., 1997). These results illustrate the broad range of leptin's influence, and suggest a distinct role for leptin in the reproductive system.

Therefore, if leptin is a circulating signal for adipose reserves or body condition, perhaps it is the missing signal, or at least a part of the signal, that mediates the clear

effect of reductions in intake or body condition on reproduction. But where does leptin exert its effect? One candidate is the hypothalamic-pituitary axis. Leptin stimulates LH, FSH, and prolactin release in incubated anterior pituitary (Yu et al., 1997), and the expression of an anterior pituitary leptin receptor has been confirmed by others (Dyer et al., 1997). Yu et al. (1997) have also shown an effect of leptin on LHRH release in incubated median eminence and arcuate nucleus explants, as well as an increase in plasma LH following third ventricle leptin administration.

Although leptin's action on the hypothalamic-pituitary axis most likely constitutes a significant share of its overall influence on reproduction, the possibility of its action on other reproductive organs should not be ignored. High levels of receptor expression were detected in the human ovary and testis (Cioffi et al., 1996). Along with detection of leptin receptor expression in the ovary, uterus, testis, hypothalamus and pituitary, Zamorano et al. (1997) discovered upregulation of side chain cleavage and 17 alpha-hydroxylase mRNA in the ovaries of *ob/ob* mice treated with leptin. In cultured rat granulosa cells, leptin acted directly to attenuate the synergistic stimulation of IGF-1 and FSH on estrogen production (Zachow and Magoffin, 1997). Although the implications of these findings are unclear, at least a portion of leptin's influence on reproduction may be mediated through the ovary, as well as other tissues outside the hypothalamic-pituitary axis.

We hypothesize that a leptin receptor exists within the bovine ovary, and that some of leptin's reproductive action occurs at the granulosa cells, as well as the corpus luteum. We therefore attempted to determine if a leptin receptor is expressed in the corpus luteum and granulosa cells of the bovine ovary and within various luteal cell subpopulations. We also attempted to determine if receptor mRNA is differentially expressed within luteal cells. Lastly, we attempted to determine if leptin alters ovarian progesterone production.

Materials and Methods

Receptor Cloning and Sequencing

The Access RT- PCR Kit (Promega, Madison, WI) was used to produce a cDNA to a partial coding sequence of the bovine ovarian leptin receptor. Primers (forward primer 5' ggcatatccaattactcc 3'; reverse primer 5' taaggtcataattcttgaaa 3') were developed through a comparison of the partial cDNA sequences for both the ovine (Genbank Accession Number U63719) and porcine (Genbank Accession Number U67739) leptin receptor. After RT-PCR of total ovarian RNA, a 401bp cDNA fragment was produced. This PCR product was then cloned into the pCR 2.1 vector, (Prokaryotic One Shot TA Cloning Kit; Invitrogen, San Diego, CA), and then transformed into E. coli (JM 109) for amplification.

The bacterially amplified plasmid was isolated and then sequenced on a polyacrylimide gel using the Sequenase Version 2.0 DNA Sequencing Kit (Amersham Life Science, Cleveland, OH). The dried gel was then imaged in a phosphoimager (Molecular Dynamics, Sunnyvale, CA) and the sequence read manually in both directions.

Luteal Explants

Initially, bovine luteal explants were used for the determination of receptor expression and progesterone production. Ovaries were obtained from a local abattoir and transported on ice to the laboratory within two hours of removal. After enucleation of the CL, connective tissue was manually removed and the luteal tissue sliced into small pieces. These small pieces were then cultured in DMEM/F-12 with 50,000 units penicillin and 50,000 μg streptomycin per liter for 24 hours at 37°C in 95% air/5% CO_2. Explants were cultured in either a basal medium (control) or with the addition of 1 μg/ml recombinant porcine leptin. Although no RNA could be obtained due to degradation, media were used for progesterone analysis.

Cell Culture

Bovine ovarian luteal cells were cultured according to the protocol described by Alila et al (1988). Ovaries were obtained from a local abattoir and transported on ice to the laboratory within two hours of removal. After enucleation of the corpus luteum, connective tissue was manually removed and the luteal tissue sliced into small pieces. Two 45 minute collagenase digestions (2.5mg/ml collagenase) were used to disperse the cells. The luteal cells were counted after three washes, checked for viability by trypan blue exclusion, and then incubated for 24 hours in DMEM-F/12 with 50,000 units penicillin and 50,000 μg streptomycin per liter for 24 hours at 37°C in 95% air/5% CO_2. Cells were cultured in either a basal medium (control) or supplemented with 1 μg/ml recombinant porcine leptin. After the incubation period, media were collected for progesterone analysis and luteal cells were extracted for total RNA.

In order to determine the difference in receptor expression in various luteal cell subpopulations, cells from a corpus luteum were dispersed as previously described. Following the cell count, unit gravity sedimentation as described by Koos and Hansel (1981) was used to separate the cells into three basic subpopulations; endothelial cells, small luteal cells, and large luteal cells. The cells were allowed to separate within a 3-.3% BSA gradient for approximately 60 minutes. After sedimentation, 10-12 ml fractions were collected, resulting in varying proportions of cell types within each fraction. The cells were then washed, frozen in liquid nitrogen, and stored at -80°C prior to total RNA extraction.

Bovine granulosa cells were isolated as described by Broussard et al.(1995). Follicles from abattoir ovaries were aspirated and the follicular fluid was allowed to stand in 15ml conical centrifuge tubes for ten minutes to allow cumulus-oocyte complexes and aggregated granulosa cells to settle. The supernatant containing primarily individual granulosa cells was then collected, the cells pelleted and washed, and then counted and checked for viability. A third of the granulosa cells were frozen immediately for total RNA extraction. The remaining granulosa cells were cultured in D-EM/F-12 with 10% FBS and 50,000 units penicillin and 50,000 μg streptomycin per liter at 37°C in 95% air/5% CO_2 in 25 cm^2 tissue culture flasks for 4 or 8 days. Media were collected every 48

hours for progesterone analysis, and the cells were collected after their allotted culture period for RNA extraction.

Progesterone Analysis

Media from the cultured cells and explants were analyzed for progesterone concentration. The DSL-3400 I^{125} Radioimmunoassay Kit (Diagnostic Systems Laboratories, Inc., Webster, TX.) was used according to the manufacturer's instructions.

RNA Extraction

Total RNA was extracted from frozen ovarian tissue and cultured or frozen ovarian cells using a modification of the procedure described by Chomczynski and Sacchi (1987). Following acid guanidinium thiocyanate treatment, phenol chloroform extraction, and isopropanol precipitation, the RNA was resuspended in 50% water, 50% formamide. RNA quality was determined by visualization of distinct 28S and 18S rRNA bands following gel electrophoresis in 1% agarose stained with ethydium bromide.

Semi-Quantitative RT-PCR

RT-PCR, as described above, was used to assess leptin receptor expression in various populations of bovine ovarian cells. To evaluate variation in expression both between the cell populations and between luteal cells cultured in the previously described treatments, a semi-quantitative RT-PCR procedure was employed. As a control, primers to the Glyceraldehyde 3-phosphate dehydrogenase (G3PDH) mRNA were used (Clontech, Palo Alto, CA). A logarithmic increase in PCR product was seen as cycle number increased, until a plateau was reached (30 cycles). Amplification of the cDNA was stopped at a point just prior to this plateau. Comparisons of the density of the leptin receptor band were performed and compared against a parallel G3PDH reaction.

Results

Receptor Expression

RT-PCR of total ovarian RNA resulted in a 401 bp PCR product, of which 38 bp consisted of the forward and reverse primer sequences. This product was cloned and sequenced, and the 363 bp sequence appears in Figure 1. Comparison of this sequence with the ovine, porcine, human, and murine leptin receptor sequences showed homology of 95%, 92%, 87%, and 77%, respectively. We therefore conclude that this is a partial coding sequence cDNA for a bovine ovarian leptin receptor mRNA. Through comparison to the human complete coding sequence, we believe our sequence to be near the 5' end of the mRNA, corresponding to an area either in or near the extracellular domain of the receptor. RT-PCR of total RNA in bovine luteal and granulosa cells produced a product similar to that mentioned above (Fig. 2), indicating that the receptor is expressed within both granulosa and luteal cells.

Progesterone Analysis

Radioimmunoassay for progesterone production was conducted on media from incubations of dispersed luteal cells and luteal explants. In 9 of the 11 cultures leptin decreased progesterone production (Fig. 3). Leptin decreased progesterone by at least a third in 6 of these cultures.A paired t-test was used for statistical analysis (basal medium vs. Leptin treated) to counter the extreme variation between cultures. A trend towards a decrease in medium progesterone concentration was detected ($p<.07$). Analysis of the explant cultures indicated that leptin exposure resulted in significant decrease in medium progesterone concentration ($p<.05$), but progesterone was not significantly different in the luteal cell cultures.

Differential Leptin Receptor Expression

We have unfortunately been unable to examine the effect of various treatments on leptin receptor expression, due to complications in the development of a Semi-Quantitative RT-PCR procedure. Initially, Northen Blot was used to detect alterations in receptor expression, but binding of the probe was undetecteacble, indicating either low

expression or problems within the procedure. We consequently moved to the development of a Semi-Quantitative RT-PCR procedure. We have demonstrated logarithmic increases in our control and receptor cDNA amplification , but have some form of DNA contamination within our RNA preparations which prevents accurate quantification. However, upon resolution of this problem we are optimistic of future success in this area and are currently working to refine our protocol.

Discussion

These results are, to our knowledge, the first evidence for the presence of a leptin receptor within the bovine ovary, the first evidence for a leptin receptor within the corpus luteum of any species, and the first to show an effect of leptin on progesterone production. Our data are compatable with the reports of Zachow et al.(1997) and Zamorano et al.(1997) who show direct effects of leptin on other ovarian functions. The presence of a leptin receptor in the bovine CL and leptin's influence on progesterone suggest leptin may have a significant role in reproduction. These data suggest that leptin exerts an effect outside the hypothalamic-pituitary axis, although it is doubtful that leptin's influence occurs solely in the ovary (Yu et al., 1997). The interrelationship between leptin's action on the hypothalamus, pituitary, gonads, and other reproductive organs needs study.

Because our sequence appears to be near the extracellular region, we are unable to determine if this sequence translates to the so called long or short forms that exist in the human and mouse (Cioffi et al., 1996; Lee et al., 1996). Although we cannot rule out the possibility that this receptor is a nonfunctional short form (Baumann et al., 1996; Gainsford et al., 1996), the studies showing activity in short forms (Murakami et al., 1997, White et al., 1997) and our results showing an influence of leptin on progesterone production lead us to believe that this is a receptor with at least partial functionality. However, it is also remotely possible that leptin is acting through another receptor (ie, a cytokine receptor).

The finding that leptin may reduce progesterone production in luteal cells is exciting as well as confusing. The initial concept was that leptin would support

progesterone production. If leptin is a circulating signal of adipose mass (Considine et al., 1996b; Ostlund et al., 1996, Ryan and Elahi, 1996; Grinspoon et al., 1996; Weigle et al., 1997), and if a minimum amount of body condition is needed for cyclicity (Richards et al., 1989), one would anticipate that a minimum amount of leptin would be needed for progesterone production. One would also hypothesize that increasing amounts of leptin would support increases in progesterone secretion. Our results cast doubt on this theory. However, stringent application of these *in vitro* results to *in vivo* actions is not recommended for two reasons. First, *in vitro* results are not always good indicators of *in vivo* actions, and second, the dosage of leptin used was a pharmacological and not physiological one. Although the serum leptin concentration range in the bovine is unknown, serum leptin in humans has been shown to range from less than 5 ng/ml to over 90 ng/ml (Considine et al., 1996; Ostlund et al., 1996) depending on percent body fat. Therefore our dose of 1μg/ml is likely to be much higher than the physiological level, and could possibly cause divergent activity from that seen at lower concentrations. Zachow et al. (1997) saw an effect of leptin on granulosa cell estrogen production at concentrations of 3 ng/ml to 100 ng/ml.

If leptin indeed does decrease progesterone production in luteal cells as our work suggests, then the idea of a inhibitory effect of leptin must be addressed. The attenuation of the synergistic stimulation of IGF-1 and FSH on estrogen production in granulosa cells (Zachow and Magoffin,1997) also leads credence to this possibility. Elevated serum leptin levels were discovered in women with polycystic ovarian syndrome (PCOS), with no association between leptin levels and serum FSH or LH (Brezechffa et al., 1996). Although it appears that depressed leptin is detrimental, our results together with those of Zachow et al. (1997) and Brezechffa et al. (1997) suggest an association beween elevated leptin levels and depressed or abnormal ovarian function. Whether the mechanisms involved in these various effects are similar or divergent, it is apparent that leptin does influence reproduction. The presence of leptin receptors in granulosa and luteal cells, as well as the hypothalamus and pituitary, and the actions of leptin in these various tissues have been demonstrated by several studies. Whether leptin is involved directly in puberty, pregnancy, postpartum anestrus, or general reproductive function in the bovine is

unknown, but the future looks bright for at least a better understanding of the interactions between nutrition, body condition, reproduction and leptin.

Conclusion

In these results we have detected the presence of a leptin receptor mRNA within the bovine ovary, specifically in the corpus luteum and granulosa cells. Our data also show a possible reduction in progesterone production following culture with leptin in luteal tissue. Although these results are moderately surprising, they are supported by the work of others who associate elevated leptin levels and depressed or abnormal ovarian function. Although further research will be necessary to more clearly define the range of leptin's influence, it is clear that leptin can affect reproduction through mechanisms outside the brain, hypothalamus, and pituitary.

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   tatttaagaa tcc 3'
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Figure 1. Partial coding sequence for a bovine ovarian leptin receptor cDNA. RT-PCR was conducted on total ovarian RNA using primers based on comparisons between homologous regions within the ovine and porcine hypothalamic leptin receptor partial coding sequences. Homology between our sequence and the published ovine, porcine, human, and mouse leptin receptor sequences is 95%, 92%, 87%, and 77%, respectively.

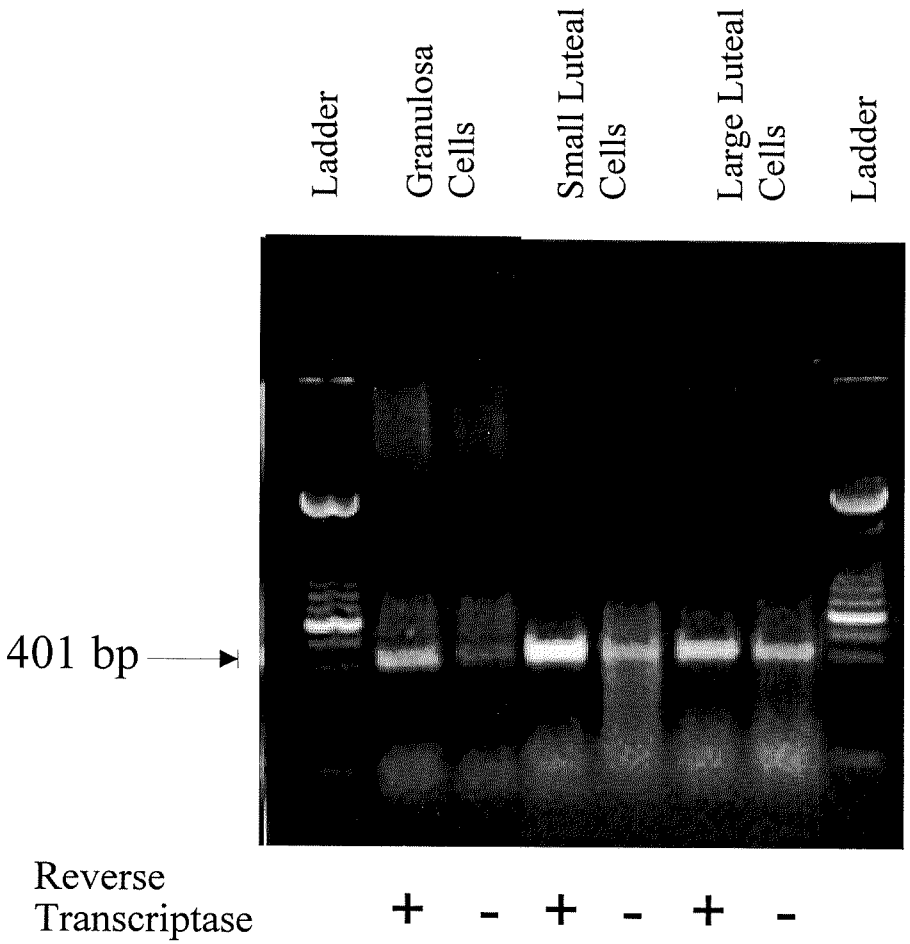

Figure 2. RT-PCR analysis of leptin receptor gene expression within various cells of the bovine ovary. RNA extracted from isolated granulosa, small and large luteal cells was used for RT-PCR. Reactions were run with (+) and without (-) reverse transcriptase to adjust for possible DNA contamination. The 401 bp PCR products were run on a 2% agarose gel stained with ethydium bromide. Expression of a leptin receptor gene was detected in both granulosa and small luteal cells. Expression also appears to exist in large luteal cells, although the high level of DNA contamination makes analysis difficult.

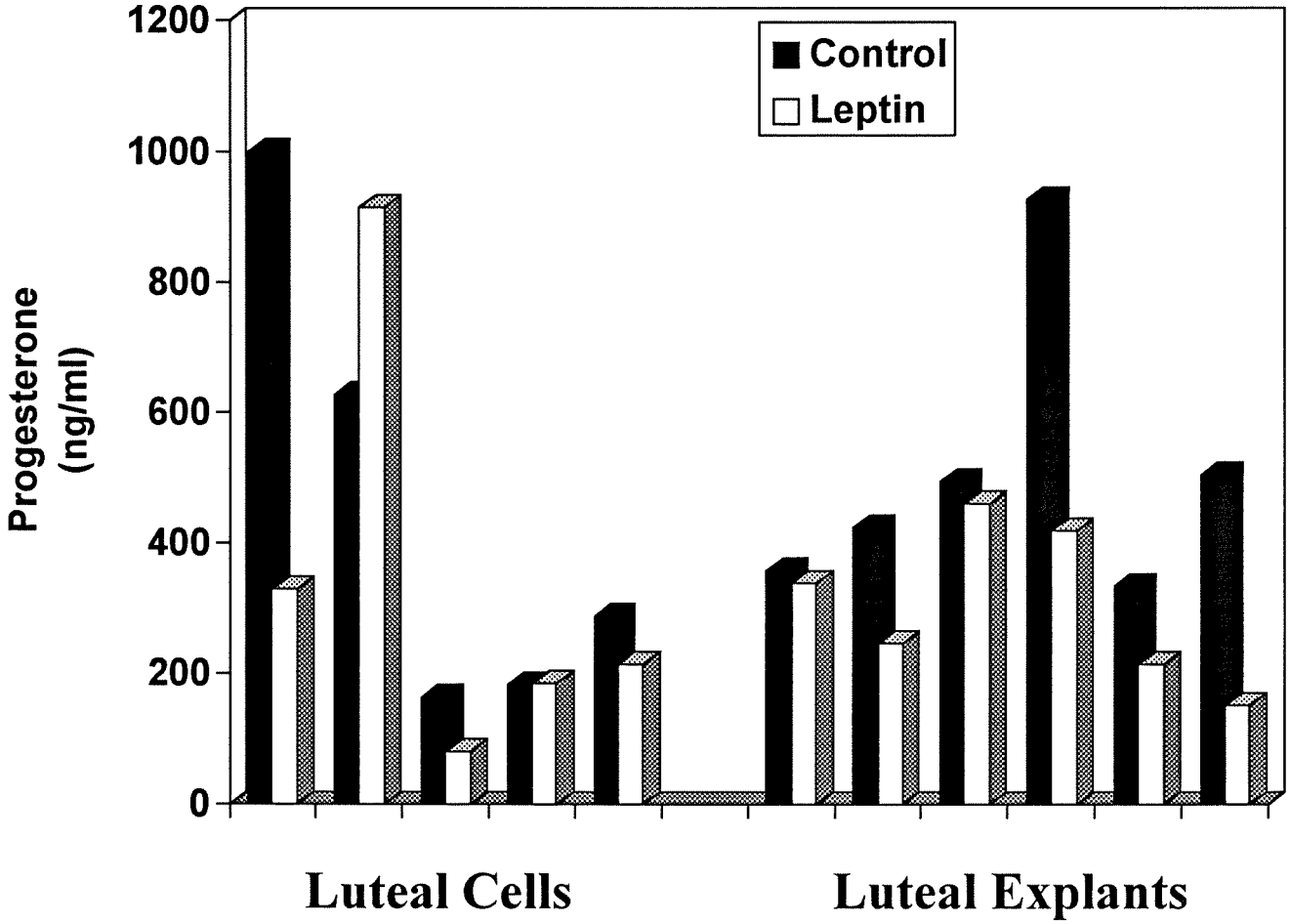

Figure 3. Effect of leptin on progesterone production in the corpus luteum. Luteal cells and luteal explants were cultured for 24 hours with basal media (Control) or with media + 1μg/ml leptin (Leptin). Leptin depressed progesterone production in 9 of the 11 cultures, while progesterone decreased by at least one-third in 6. Leptin decreased progesterone production by an average of 28.3%.

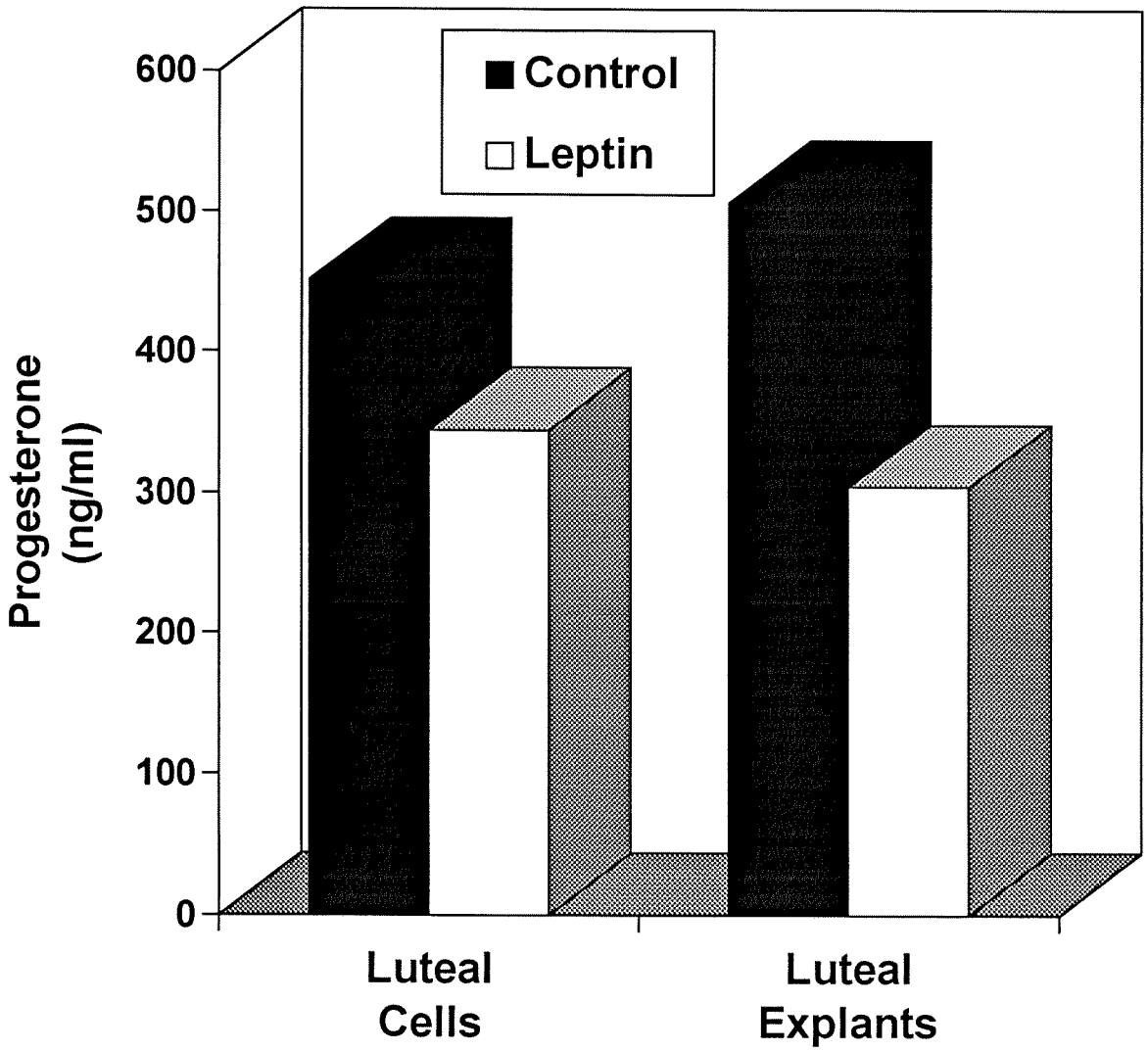

Figure 4. Effect of leptin on mean progesterone production. Luteal cells and explants were cultured for 24 hours with basal medium (Control) or with media + 1μg/ml leptin (Leptin). Leptin tended to decrease progesterone in cultured luteal cells. Leptin significantly decreased progesterone production in cultured luteal explants ($p<.05$), based on a paired t-test.