SciMedCentral

JSM Invitro Fertilization

Research Article

The Effect of Leptin on Luteal Angiogenic Factors in the Developing Porcine Corpus Luteum

Robin A. Katchko[1], Jessica R. Wiles[1], Martha A. Ramirez[1], Luis Ayala[1], Fang Xie[1], Chad W. O'Gorman[1], Duane H. Keisler[2], Randy L. Stanko[1,3], Michelle R. Garcia[1]*, and Elizabeth A. Benavides[4]

[1]*Department of Animal, Rangeland & Wildlife Science, Texas A&M University-Kingsville, USA*

[2]*Division of Animal Sciences, University of Missouri, USA*

[3]*Animal Reproduction Laboratory, Texas A&M University, USA*

[4]*Department of Agriculture,Texas State University, USA*

***Corresponding author**

Michelle R Garcia, Department of Animal, Texas A&M University-Kingsville, Rangeland & Wildlife Sciences, Kleberg Ag. Bldg. Rm 133, Kingsville, TX 78363, USA, Tel: 1-361-593-3197; Fax: 1-361-593-3788; Email: michelle.garcia@tamuk.edu

Submitted: 18 September 2017

Accepted: 11 October 2017

Published: 13 October 2017

Keywords

• Leptin angiogenesis development CL

Abstract

Defects or abnormalities in a CL are believed to account for approximately 65% of recurrent miscarriages. Abnormalities can be associated with incomplete angiogenesis, which leads to a decrease in progesterone production and potential loss of a conceptus. Luteal angiogenesis is regulated by factors, such as fibroblast growth factor-2 (FGF2), vascular endothelial growth factor (VEGF), and angiopoietin-1 (Ang1). Leptin can influence the expression of VEGF, Ang1, and FGF2 in a caprine CL. Therefore, it is hypothesized that leptin is involved in luteal angiogenesis during CL development in the porcine species. Twenty-four mature crossbred gilts were allocated to one of four groups relative to day of the estrous cycle in preparation for CL collection: day (D) 4, 5, 6, or 7 of the estrous cycle (n = 6 gilts/D). Luteal tissue was divided and either frozen in liquid nitrogen for RNA analysis or enzymatically dispersed for cell culture. Gene expression for VEGF, Ang1, FGF2, leptin (tissue only), and leptin receptor (ObRb, tissue only) in CL tissue and cell cultures was determined by qPCR. Dispersed cells were cultured with or without leptin (0, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8} M; n = 3 wells/dose/gilt) for 24 hours. The expression of FGF2 in luteal tissue significantly ($P<0.0003$) varied relative to D and Ang1 tended ($P=0.1$) to be highest in D 6 CL. Leptin increased ($P<0.05$) Ang1 in D4 cultured dispersed lutea and decreased ($P<0.03$) FGF2 in D7 cultured dispersed lutea. Collectively, leptin appears to be involved in the angiogenic process in the developing CL.

ABBREVIATIONS

VEGF: Vascular Endothelial Growth Factor; FGF2: Fibroblast Growth Factor; Ang1: Angiopoietin 1; D: Day; CL: Corpus Luteum; IGF-1: Insulin-Like Growth Factor 1

INTRODUCTION

Abnormal vasculature of the CL is a defect that leads to abnormal development, decreased progesterone production, and subsequent loss of a conceptus [1,2]. Identifying and understanding the underlying mechanisms of luteal angiogenesis may lead to a reduction in luteal-related infertility and alternate methodology for regulation of reproductive cycles. The luteal neo-vascularization process is attributed to the biological activity of fibroblast growth factor 2 (FGF2), angiopoietin 1 (Ang1), and vascular endothelial growth factor (VEGF). Both VEGF and FGF2 promote capillary membrane destabilization, endothelial cell differentiation, proliferation, migration, and vascular tube formation in human, bovine, and ovine luteal tissue [3,4]. Maturation and stabilization of nascent vessels is then promoted by Ang1 through the recruitment of stromal support cells, including pericytes and smooth muscle cells [5]. Each of these angiogenic regulatory factors is regulated by leptin in both non-ovarian and ovarian tissues [6-8].

Both leptin and its receptor, ObRb, have been identified in normal and polycystic ovaries of various species [9-12], particularly in steroidogenically active follicles and luteal tissue where it has been reported to influence the synthesis and secretion of progesterone [8,13]. However, in order to substantially modify steroid production cells are usually incubated with leptin in the presence of an additional growth promotant, such as IGF-I [13-17], which suggests that a potential ancillary role for leptin in ovarian tissue, such as angiogenesis, may exist. It has recently been reported that leptin is present in luteal vascular endothelial cells and in small and large luteal cells of an early stage CL [8]. Furthermore, leptin upregulates the expression of angiogenic factors in the early caprine CL [8]. Collectively, the evidence supports the supposition that leptin may be involved in the development of a CL, which may include proper vascularization of the tissue. Understanding the mechanisms of the development of luteal tissue may lead to a future reduction in the occurrence of luteal-associated infertility. Therefore, it is hypothesized that leptin is involved in luteal angiogenesis during CL development in the porcine species. The study herein characterizes the primary angiogenic growth promotants VEGF, Ang1, FGF2, leptin, ObRb in developing lutea and investigates the effect of leptin on the production of the angiogenic factors throughout early luteal development.

MATERIALS AND METHODS

Animals and management

Twenty-four, mature crossbred (Yorkshire x Landrace) gilts, *Sus scrofa*, of similar age and weight, from the Texas A&M University-Kingsville (TAMUK) Farm were utilized. Gilts were randomly allocated to one of four groups relative to day of the estrous cycle in preparation for CL collection: day (D) 4, 5, 6, or 7 of the estrous cycle (n = 6 gilts/D). Ovulation in the porcine species occurs between 10 and 85 hours after the onset of estrus [18], therefore, CL collection began on D4 of the estrous cycle to allow for completion of the ovulation process. Animals were housed in concrete pens (7 m x 13 m) in an outdoor, sheltered facility and had *ad libitum* access to water and a corn-soy based diet [19]. Females were checked twice daily (30 minutes) for classical, behavioral estrus using an intact boar. Tissue collection occurred on the third cycle after animals exhibited two consistent consecutive cycles. On the day of CL tissue collection, blood samples were collected, via jugular venipuncture, for analysis of serum progesterone for confirmation of normal luteal function.

Corpus luteum collection

Feed was removed from the gilts for a period of 24 hours prior to tissue collection. On the day of CL tissue collection, body weight was recorded and the gilts were transported to a surgical facility located on the TAMUK farm. Gilts were pre-anesthetized with sodium thiopental (2.5%) administered intravenously (ear vein) using a butterfly infusion set (25G x 1.65 cm needle, 30.48 cm tubing; Abbott Laboratories, Chicago, IL). For a surgical plane of anesthetization, the inhalation agent, isoflurane, was utilized. Gilts received isoflurane gas from an isoflurane vaporizer that was set to 3-4%. Corpora luteal were harvested from the ovaries and placed in ice-cold Hank's Balanced Salt Solution (1X) (HyQ HBSS; HyClone, Logan, UT) for transportation. Harvested tissue was divided and processed for RNA extraction, or enzymatically digested and dispersed for cell culture.

Serum analyses

Serum and culture media were analyzed for progesterone concentration by radioimmunoassay (Coat-A-Count®; Diagnostics Product Corporation, Los Angeles, CA) [20,21] to confirm a functional CL at the time of tissue harvest, cell viability during culture process, and response to culture treatment. Serum concentrations of leptin were determined using ovine specific leptin RIA validated for porcine serum [19,22]. Inter- and intra-assay coefficients of variation for progesterone and leptin assays were < 10% as previously reported [19,23].

Dispersed CL cell cultures

Luteal tissue (n=6 animals/per day assignment) was processed as previously reported [8]. Briefly, tissue was mechanically minced and enzymatically dissociated in digestion media (Type IA collagenase, 1.27 mg/ml, 0.5% BSA in Dulbecco's Modified Eagle's Medium; DMEM) for 90 minutes at 37°C with gentle agitation in a shaking water bath. Cells were filtered through a metal mesh (80 µm) and centrifuged twice at 150 X *g* for 4 minutes and the enzymatic supernatant was removed. Cells were re-suspended and the numbers of viable cells were determined using the Trypan Blue Exclusion Test. Cell viability was greater than 85% for each cell culture. The dispersed cells were seeded in a treated polystyrene 24-well cluster dishes (Sigma) at 3 X 10^6 cells/well in attachment serum media [1 ml/well; DMEM supplemented with 10% (v/v) charcoal stripped fetal calf serum and 5,000 µg/mL penicillin/streptomycin] and incubated for 24 hours in a humidified atmosphere of 5% CO_2 in air at 37°C to equilibrate cells to culture condition, allow aggregation, and promote adherence. Following the 24 hour incubation, attachment serum media was aspirated and replaced with culture media (1 ml/well; DMEM, 0.1% (w/v) BSA, 2.0% (v/v) charcoal stripped fetal calf serum, 0.5 mM ascorbic acid, and 5,000 µg/mL penicillin/streptomycin) containing recombinant ovine leptin (0, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8} M; Cell Sciences) in triplicate (3 wells/dose/gilt) for 24 hours in a humidified atmospere of 5% CO_2 in air at 37°C. Doses of leptin were based on previous studies examining the *in vitro* effect of leptin in metabolically active cells [8,24] and conditions where leptin stimulates the MAPK signal transduction cascade [25]. Biologically active ovine leptin protein, which is >92% identical porcine leptin (UniProt access. #Q29406 and # G8BLB2), was substituted due to availability. After 24 hours, the cell culture media was aspirated and stored at -80°C for measurement of secreted progesterone as a marker for cell responsiveness to leptin *in vitro* [8,26,27]. Denaturing solution [1 ml/well; 25 mM sodium citrate (pH 7.0), 0.5% (w/v) N-laurylsarcosine, 4 M guanidine thiocyanate with 0.7% (v/v) 2-mercaptoethanol] was added to cells, aspirated, pooled by dose, and stored at -80°C for total RNA extraction and mRNA analysis of VEGF, Ang1, and FGF2.

RNA extraction and qPCR

Total RNA was extracted from CL tissue (~20 mg) and denatured cells using a phenol: chloroform based procedure [8,19,28]. Total RNA was quantified using a spectrophotometer (640 UV/Visible Beckmann) at UV absorbencies (A) of 260 to 280. Purity of the extracted RNA was evaluated by determining the A260/ A280 ratio. The extracted total RNA was then analyzed for VEGF, Ang1, FGF2, leptin (tissue only), and ObRb (tissue only) mRNA using quantitative polymerase chain reaction (qPCR) with the DNA Engine Opticon II (MJ Research, Inc., Miami, FL). Two µg of extracted total RNA was DNase (RQ1 DNase) (Promega, Madison, WI) treated and reverse transcribed using moloney murine leukemia virus reverse transcriptase (M-MLV RT; Promega). Briefly, total RNA was incubated at 37°C for 30 minutes for DNase 1 treatment, followed by 75°C incubation for 8 minutes to terminate DNase 1 activity. Samples were placed on ice and oligo dt15 (Promega) and M-MLV RT (Promega) were added. Samples were incubated at 37°C for 1 hour to RT mRNA into cDNA. Samples were then incubated at 85°C for 5 minutes to terminate the RT.

Four µl of cDNA were amplified by qPCR using 25 pmol of VEGF, Ang1, FGF2, leptin, ObRb, and cyclophilin primers previously published [8], all synthesized by Integrated DNA Technologies Inc. (Coralville, IA), using a SYBR® green kit (Takara Mirus Bio®, Madison, WI) and diethyl pyrocarbonate (Sigma) treated water in a 50 µl reaction. The PCR cycles were as follows: a 10 minute hotstart at 94°C, then 94°C for 1 minute, anneal temperature of 50°C for 30 seconds, 72°C for 1 minute for 35 cycles followed by

a 10 minute extension at 72°C that was conducted at the end of the PCR. To control for potential primer-dimer formation, control tubes containing everything except total RNA were subjected to the same PCR conditions. Relative quantities of VEGF, Ang1, FGF2, leptin, and ObRb, and cyclophilin (housekeeping gene) were quantitated from a relative standard curve (1 µg to 10 pg of total RNA) obtained from qPCR of cyclophilin mRNA in porcine liver tissue [8,19,23,29]. Each qPCR was performed in duplicate to reduce experimental errors. Cycle threshold (C_t), the cycle at which the amplification curve crosses threshold, was set to standardize each PCR run. Relative values obtained from the relative standard curve were transformed to $\log_{10}$ and normalized with cyclophilin. Cyclophilin mRNA was used to account for variability in sample preparation and procedural variability to normalize targeted amplicons. Dispersed luteal cell values are represented as a % of the control (0M) as previously reported [8]. PCR products were then separated on a 2% agarose gel by electrophoresis and visualized with ethidium bromide staining for estimated size verification.

Statistical analyses

qPCR values of VEGF, Ang1, FGF2, leptin, and ObRb gene expression in luteal tissue were transformed to $\log_{10}$ and analyzed using the PROC MIXED procedure of SAS with day of development as the source of variation. qPCR values of VEGF, Ang1, and FGF2 obtained in cultured cells were transformed to $\log_{10}$ and expressed as a percentage of 0M treatment dose. The effect of leptin on each of the angiogenic factors *in vitro* was analyzed using the PROC MIXED procedure of SAS. Leptin dose was the source of variation. Animal was used as the subject to account for correlated variation within all MIXED analysis procedure. The PDIFF option of least square mean (LS Mean) values was conducted to compare means where significance of the model was detected to determine the specific day (tissue and serum analyses) or doses (cell culture) that were significantly different than 0M. The values are represented as LS Means ± standard error of the mean.

RESULTS

Neither serum concentrations of progesterone or leptin (Figure 1) significantly differed relative to day as the CL developed over the 4 day sampling period. However, serum progesterone values are indicative of a normal, functional CL at the time of tissue collection.

Vascular endothelial growth factor, ObRb, and leptin gene expression did not significantly differ by day of developing luteal tissue (Figure 2). However, FGF2 varied significantly (P<0.0003) by day, decreasing between D4 and D5, increasing between D5 and D6, and decreasing on D7 to concentrations detected in D4 tissue. Angiopoietin 1 tended (P=0.1) to differ relative to day increasing on D6 and, similar to FGF2, decreasing on D7 to concentrations detected in D4 tissue.

Leptin treatment (Figure 3) stimulated (P=0.05) Ang1 gene expression in D4 dispersed cell cultures at the -11M dose compared to all other doses, but did not upregulate VEGF or FGF2 at any dose. Leptin did not significantly influence gene expression of VEGF, Ang1, or FGF2 in the D5 or D6 cell cultures; however, leptin did significantly reduce (P<0.03) FGF2 in D7 cell cultures across all doses compared to 0M. Leptin influenced (P≤0.05) progesterone secretion in D4, 5, 6, and 7 cell cultures (Figure 4) verifying cellular responsiveness to leptin as previously reported [8,27].

DISCUSSION

Gene expression of VEGF, leptin, and ObRb did not differ by day in luteal tissue, which is not attributed to abnormal CL function since circulating concentrations of progesterone were normal for each day of the porcine estrous cycle [23] and serum leptin remained constant. Insignificant changes during the 4 days of early development does not necessarily imply that each factor would not have differed prior to or after the collection period as previously reported in the caprine [8], porcine [10] and human [30] CL. Vascular endothelial growth factor is reported to increase and remain elevated throughout the luteal lifespan to support angiogenic processes [3,4] and maintain vascular viability [31]. The expression of leptin and ObRb has also been reported to vary throughout the luteal phase in the caprine [8] and porcine [10] CL. However, leptin only appears to influence angiogenic factor expression in the early stage caprine CL [8], which implies leptin may potentially have an alternate role in the mature CL. Both Ang1 and FGF2 exhibited a similar pattern of expression in CL tissue where D7 expression appeared to be lower than in D6 CL.

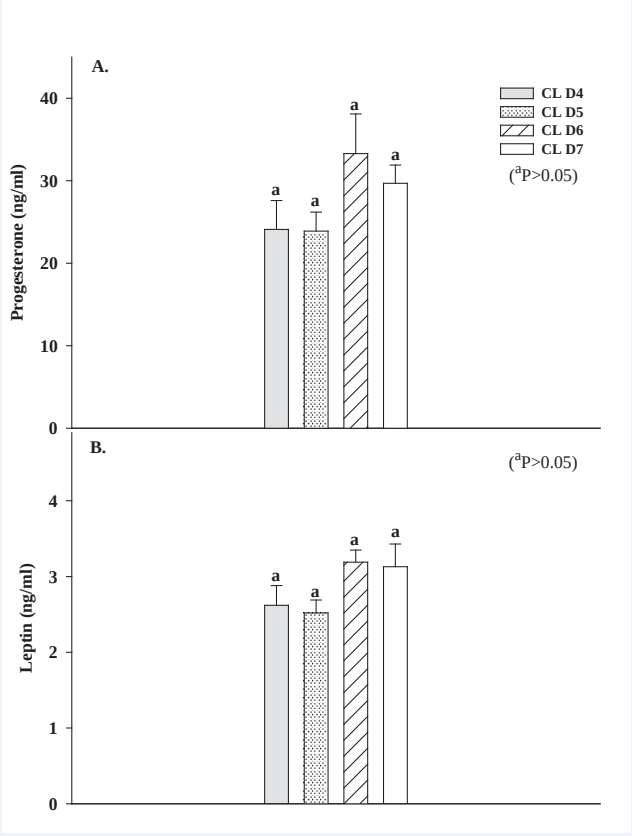

Figure 1 Serum concentrations of progesterone (Panel A) and leptin (Panel B) by day (D) in mature gilts (n=6 gilt/D). Blood samples were collected at the time of CL harvest on D4, 5, 6, and 7 of the estrous cycle. Serum concentrations of progesterone and leptin did not significantly differ by D. Similar superscripts represent LS means that are not statistically different (P>0.05).

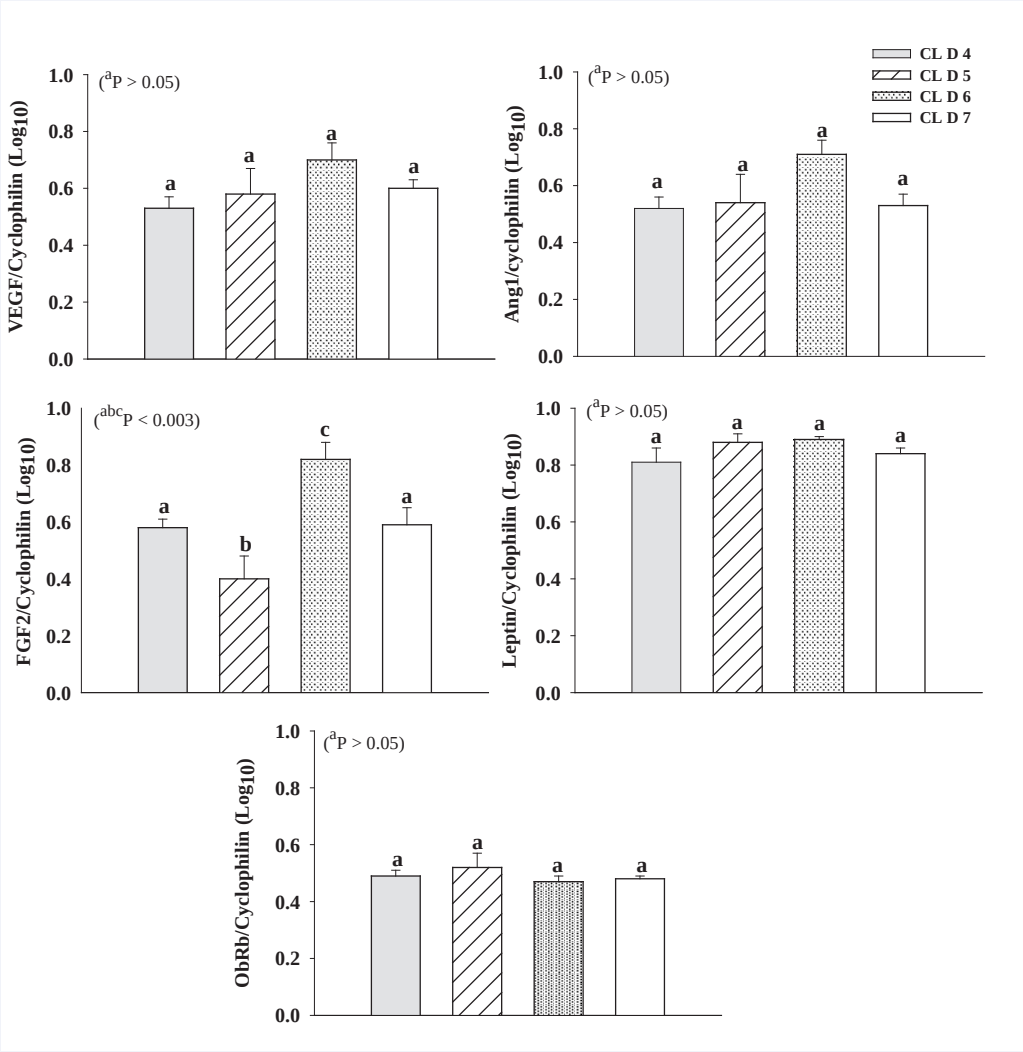

Figure 2 The gene expression of VEGF, Ang1, FGF2, leptin, and ObRb in CL tissue relative to day (D) from mature gilts (n=6 gilts/D). Total RNA was extracted and relatively quantitated using qPCR. Relative quantities of PCR amplicons were transformed to $\log_{10}$ and normalized with cyclophilin. No differences in VEGF, leptin, or ObRb gene expression was detected between D of the developing CL. FGF2 varied significantly (P<0.003) as the CL developed and Ang1 tended (P=0.1) to vary by D decreasing between D6 and 7. [abc]Different superscript represent LS means that significantly differ ($P \leq 0.05$) within target gene by D.

Angiopoietin 1 is important in the stabilization of nascent vessels [5], therefore, a tendency towards a decrease in Ang1 availability in D7 tissue may imply a vascular stabilization event in D6 tissue. The variation in FGF2 during the 4-day window of CL development suggests that as development occurs the requirement for FGF2 may alter in availability. Luteal FGF2 is believed to be a primary regulator of luteal angiogenesis during CL development [4,32,33]. However, FGF2 can mitigate cellular apoptosis in the ovary [34-36], which may support the supposition that FGF2 is important for luteal angiogenic processes as well as the maintenance of forming luteal tissue. Therefore, the required availability of FGF2 may vary daily depending on the functional requirements of the hormone. Interestingly, leptin reduced FGF2 in D7 dispersed cell cultures rather than increase it, as observed for Ang1 in D4 cultures. A previous report in the caprine CL indicated that leptin stimulated VEGF, Ang1, and FGF2 in the early stage CL [8]. However, the development of the caprine CL is a slower process than in the porcine species [37]. Therefore, D4 in the current study may have been too late for detecting the stimulatory affect of leptin on all angiogenic factors in the porcine CL. The reduction of FGF2 in D7 cultures may explicate, in part, the decrease in FGF2 in D7 CL tissue, but this does not explicate the inhibitory rather than a stimulatory affect *in vitro* as previously reported [8]. This inhibitory and stimulatory variation was also observed in secreted progesterone in cell culture media where leptin increased progesterone secretion in dispersed cell cultures in the study herein, which is in contrast to previous reports. Leptin decreases progesterone secretion in luteinized bovine granulosa cells [14] and in dispersed caprine lutea [8], however, leptin can both increase or decrease progesterone secretion in cultured porcine luteinized granulosa cells [27]. The direction and magnitude through which leptin influences progesterone secretion can be altered by dose of leptin and/or the addition of growth promoting hormones such as IGF-I [18,27]. Collectively,

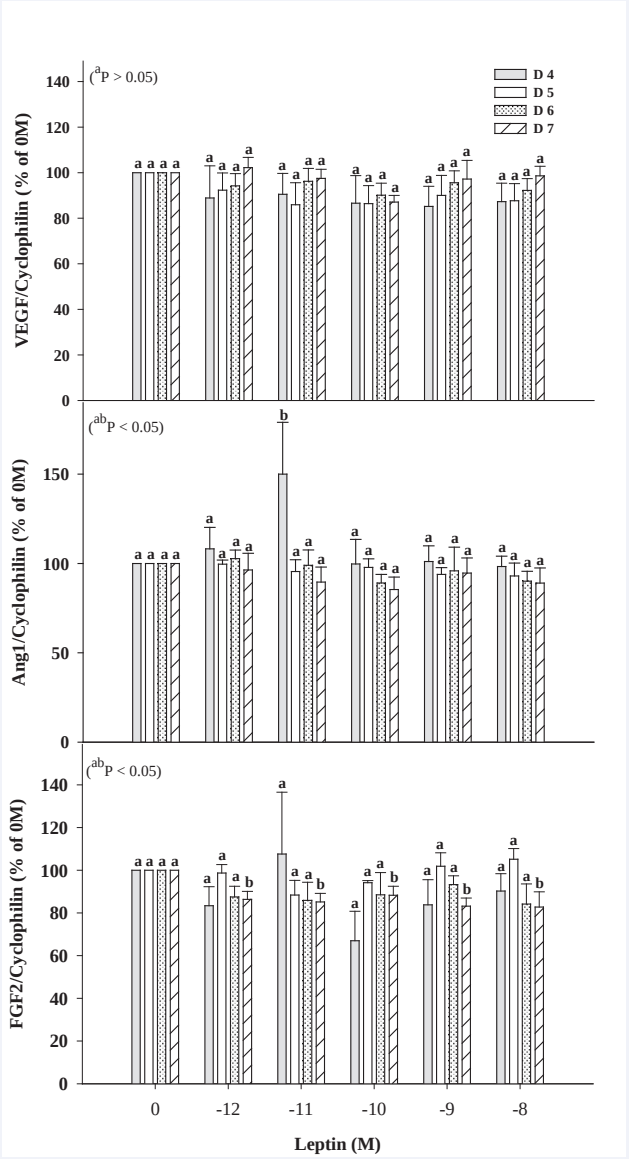

Figure 3 The gene expression of VEGF, Ang1, and FGF2 gene in cultured dispersed lutea from mature gilts on days (D) 4, 5, 6, and 7 of the estrous cycle (n=6 gilts/D). Cells were incubated with or without leptin (0, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8} M) for 24 hours. Leptin increased (P=0.05) Ang1 in the -11M dose compared to all other doses in the D4 cells and significantly decreased (P<0.03) FGF2 in D7 cultures across all doses compared to 0M. [abc]Different superscript represent LS means that significantly differ ($P \leq 0.05$) from control dose (0M).

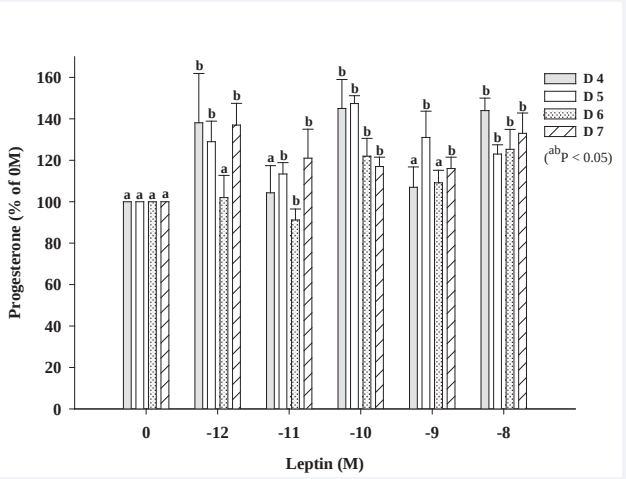

Figure 4 Concentrations of progesterone in aspirated cultured media. Leptin increased (P≤0.05) progesterone secretion in D4, 5, 6, and 7 cell cultures. [ab]Different superscript represent LS means that significantly differ ($P \leq 0.05$) from control dose (0M).

leptin influences progesterone secretion in luteinized cells, which may be influenced by various factors as the CL experiences the daily biochemical and biophysical modifications that occur as a CL develops [38]. Therefore, variations in endogenous parameters that influence the affect of leptin on progesterone secretion may also influence the affect of leptin on luteal angiogenic factor expression.

CONCLUSION

Leptin influences the expression of angiogenic factors during early luteal development supporting a potential role for leptin in the luteal angiogenic process of the porcine CL. Further investigation into the functional role of leptin in early luteal development is needed to definitively determine how leptin influences angiogenic processes in the developing CL.

ACKNOWLEDGEMENTS

We acknowledge with gratitude the TAMUK farm personnel for the use, care, and maintenance of the animals used on the project reported herein.

REFERENCES

1. Glock JL, Brumsted JR. Color flow pulsed Doppler ultrasound in diagnosing luteal phase defect. Fertility Sterility. 1995; 64: 500-504.
2. Hazzard TM, Stuffer RL. Angiogenesis in ovarian follicular and luteal development. Baillier Best Practices in Research and Clinical Obstetric Gynecology. 2000; 14: 883-900.
3. Susuki T, Sasano H, Takaya R, Fukaya T, Yajima A, Nagura H. Cyclic changes of vasculature and vascular phenotypes in normal human ovaries. Hum Reprod. 1998; 13: 953-959.
4. Reynolds LP, Redmer DA. Expression of the angiogenic factors, basic fibroblast growth factor and vascular endothelial growth factor, in the ovary. American Society of Animal Science. 1998; 76: 1671-1681.
5. Shalaby F, Rossant J, Yamaguchi TP, GertsensteinM, Wu X-F, Breitman ML, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature. 1995; 376: 62-66.
6. Cao R, Brakenhielm E, Wahlestedt C, Thyberg J, Cao Y. Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF. Proc Natl Acad Sci USA. 2001; 98: 6390-6395.
7. Aleffi S, Petrai I, Bertolani C, Parola M, Colombatto S, Novo E, et al. Upregulation of proinflammatory and proangiogenic cytokines in human hepatic stellate cells. Hepatology. 2005; 42: 1339-1348.
8. Wiles JR, Katchko RA, Aguirre EA, O'Gorman CW, Escudero JM, Keisler DH, et al. The effect of leptin on luteal angiogenic factors during the luteal phase of the estrous cycle in goats. Anim Reprod Sci. 2014; 148: 121-129.
9. Loffler S, Aust G, Kohler U, Spanel-Borowski K. Evidence of leptin

expression in normal and polycystic ovaries. Mol Hum Reprod. 2001; 7: 1143-1149.

10. Ruiz-Cortes ZT, Men T, Palin MF, Downey BR, Lacroix DA, Murphy BD. Porcine leptin receptor: molecular structure and expression in the ovary. Mol Reprod Dev. 2000; 56: 465-474.

11. Ryan NK, Van Der Hoek KH, Robertson SA, Norman RJ. Leptin and leptin receptor expression in the rat ovary. J Endocrinol. 2003; 144: 5006-5013.

12. Munoz-Gutierrez M, Findlay PA, Adam CL, Wax G, Campbell BK, Kendall NR, et al. The ovarian expression of mRNAs for aromatase, IFG-I receptor, IGF-binding protein-2, -4 and -5, leptin and leptin receptor in cycling ewes after three days of leptin infusion. Reproduction. 2005; 130: 869-881.

13. Spicer LJ, Francisco CC. The adipose obese gene product, leptin: evidence of a direct inhibitory role in ovarian function. Endocrinol. 1997; 138: 3374-3379.

14. Spicer LJ, Francisco CC. Adipose tissue obese gene product, leptin inhibits bovine ovarian thecal cell steroidogenesis. Biolo Reprod. 1998; 58: 207-212.

15. Zachow RJ, Magoffin DA. Direct intraovarian effects of leptin: impairment of the synergistic action of insulin-like growth factor-I on follicle-stimulating hormone-dependent estradiol-17beta production by rat ovarian granulosa cells. Endocrinol. 1997; 138: 847-850.

16. Karlsson C, Lindell K, Svensson E, Bergh C, Lind P, Billig H, et al. Expression of functional leptin receptors in the human ovary. J Clinic Endocrinol Metab. 1997; 82: 4144-4148.

17. Brannian J, Zhao Y, McElroy M. Leptin inhibits gonadotropin-stimulated granulosa cell progesterone production by antagonizing insulin action. Hum Reprod. 1999; 14: 1445-1448.

18. Soede NM, Kemp B. Expression of oestrus and timing of ovulation in pigs. J Reprod Fertil Suppl. 1997; 52: 91-103.

19. O'Gorman CW, Matsumoto Y, Stanko RL, Keisler DH, Garcia MR. Effects of acute fasting and age on leptin and peroxisome proliferator-activated receptor gamma production relative to fat depot in pigs. J Anim Physiol Anim Nutr (Berl). 2010; 94: e266-e276.

20. Novak S, Almeida FR, Cosgrove JR, Dixon WT, Foxcroft GR. Effect of pre- and postmating nutritional manipulation on plasma progesterone, blastocyst development, and the oviductal environment during early pregnancy in gilts. J Anim Sci. 2003; 81: 772-783.

21. Mao J, Tracy BK, Almeida FRCL, Novak S, Dixon WT, Fozcroft GR. Feed restriction and insulin treatment affect subsequent luteal function in the immediate postovulatory period in pigs: progesterone production in vitro and messenger ribonucleic acid expression for key steroidogenic enzymes. Biology of Reproduction. 2001; 64: 359-367.

22. Berg EP, McFadin EL, Maddock KR, Goodwin RN, Baas TJ, Keisler DH. Serum concentrations of leptin in six genetic lines of swine and relationship with growth and carcass characteristics. J Anim Sci. 2003; 81: 167-171.

23. Matsumoto Y, O'Gorman CW, Gonzales E, Keisler DH, Stanko RL, Garcia MR. Effects of development, the estrous cycle and 17β-estradiol on adipocyte leptin, ERα and ERβ gene expression in gilts. Journal of Animal and Veterinary Advances. 2006; 5: 762-770.

24. Bates SH, Gardiner JV, Jones RB, Bloom SR, Bailey CJ. Acute stimulation of glucose uptake by leptin in L6 muscle cells. Horm Metab Res. 2002; 34: 111-115.

25. van den Brink GR, O'Toole T, Hardwick JCH, van den Boogaardt DEM, Versteeg HH, van Deventer SJH, et al. Leptin signaling in human peripheral blood mononuclear cells, activation of p38 and p42/44 mitogen-activated protein (MAP) kinase and p70 S6 kinase. Mol Cell Biol Res Communi. 2000; 4: 144-150.

26. Nicklin LT, Glister C, Robinson RS, Marsters P, Campbell BK, Knight PG, et al. Leptin in the bovine corpus luteum – receptor expression and effects on progesterone production. Mol Reprod Dev. 2007; 74: 724-729.

27. Ruiz-Cortes ZT, Martel-Kennes Y, Gevry NY, Downey BR, Palin M-F, Murphy BD. Biphasic effects of leptin in porcine granulosa cells. Biol Reprod. 2003; 68: 789-796.

28. Garcia MR, Amstalden M, Williams SW, Stanko RL, Morrison CD, Keisler DH, et al. Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. J Anim Sci. 2002; 80: 2158-2167.

29. Bustin SA. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J Mol Endocrinol. 2000; 25: 169-193.

30. Wulff C, Wilson H, Largue P, Duncan WC, Armstrong DG, Fraser HM. Angiogenesis in the human corpus luteum: localization and changes in angiopoietins, tie-2, and vascular endothelial growth factor messenger ribonucleic acid. J Clin Endocrinol Metab. 2000; 85: 4302-4309.

31. Gerber H-P, Dixit V, Ferrara N. Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. J Biol Chem. 1998; 273: 13313-13316.

32. Neuvians TP, Berisha B, Schams D. Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) expression during induced luteolysis in the bovine corpus luteum. Mol Reprod Dev. 2004; 67: 389-395.

33. Robinson RS, Nicklin LT, Hammond AJ, Schams D, Hunter MG, Mann GE. Fibroblast growth factor 2 is more dynamic than vascular endothelial growth factor A during the follicle-luteal transition in the cow. Biol Reprod. 2007; 77: 28-36.

34. Tilly JL, Billig H, Kowalski KI, Hsueh AJW. Epidermal growth factor and basic fibroblast growth factor suppress the spontaneous onset of apoptosis in cultured rat ovarian granulosa cells and follicles by a tyrosine kinase-dependent mechanism. Molecular Endocrinology. 1992; 6: 1942-1950.

35. Florkiewicz RZ, Majack RA, Buechler RD, Florkiewicz E. Quantitative export of FGF-2 occurs through an alternative, energy-dependent, non-ER/Golgi pathway. J Cell Physiol. 1995; 162: 388-399.

36. Florkiewicz RZ, Anchin J, Baird A. The inhibition of fibroblast growth factor-2 export by cardenolides implies a novel function for the catalytic subunit of Na+, K+-ATPase. J Biol Chem. 1998: 273: 544-551.

37. Simões J, Almeida JC, Baril G, Azevedo J, Fontes P, Mascarenhas R. Assessment of luteal function by ultrasonographic appearance and measurement of corpora lutea in goats. Anim Reprod Sci. 2007; 97: 36-46.

38. Stocco C, Telleria C, Gibori G. The molecular control of corpus luteum formation, function, and regression. Endocr Rev. 2007; 28: 117-149.

Cite this article

Katchko RA, Wiles JR, Benavides EA, Ramirez MA, Ayala1 L, et al. (2017) The Effect of Leptin on Luteal Angiogenic Factors in the Developing Porcine Corpus Luteum. JSM Invitro Fertil 2(3): 1020.