

The Effect of Blocking Endogenous FGF2 on Leptin Gene Expression and Total Morphological Occurrences of Vascular Endothelial Cells in Enzymatically Dispersed Adipose Tissue of Bovine in the Angiogenic Process

C. Cantu, F.E. Escamilla, D.V. Ruiz, M.R. Garcia Ph.D.
Department of Animal Science and Veterinary Technology

ABSTRACT

The role of leptin gene expression and its effect on biological processes such as tissue repair and wound healing, lacks better understanding. Although the relationship between fibroblast growth factors (FGFs) and leptin's capability of promoting reproductive development, angiogenic processes, and metabolism has been studied before, the specific cellular mechanisms that enable these actions are not clear. This study aims to evaluate how FGF2, produced by adipocytes, works with leptin to regulate angiogenic processes that promote tissue repair and wound healing. Furthermore, it is hypothesized that blocking FGF2 will alter leptin gene expression and vascular endothelial cell (VEC) morphology. To investigate this statement, adipose tissue samples were randomly collected from three steers, enzymatically dispersed, and separated into stromal vascular cells (SV) and mature adipocytes (MA) to determine the effect of blocking MA production of FGF2 or leptin gene expression. Quantitative data was analyzed using quantitative polymerase chain reaction (PCR) to evaluate gene activity and provide a more precise interpretation and comparison. The results showed that blocking endogenous FGF2 at a higher concentration (1:50 FGF2 antibody dilution) led to a decrease in leptin gene expression, while a lower concentration (1:100 FGF2 antibody dilution) resulted in increased leptin gene expression compared to the control group (0 FGF2 antibody dilution). VEC morphology, analyzed using the SAS PROC MIXED procedure, showed a 50-58% total reduction of morphological occurrences in antibody-treated cultures compared to control cultures that included MA and unblocked endogenous FGF2. This suggests that FGF2 signaling may play a role in regulating leptin gene expression in bovine adipose tissue and influences early stages of VEC development.

PURPOSE

This study's purpose is to analyze how mature adipocytes and stromal vascular cells (SV) co-cultures, regulate endothelial cell morphology through adipocytes to produce FGF2, which will work with leptin to regulate angiogenic processes.

METHODS

➤ Animals

• Three Brangus steers of similar age and weight were utilized for this study (IACUC approval #2023-06-23).

➤ Cell Culture:

❑ SVF Attachment cultures:

• SVF were cultured in triplicate for 24 hr in attachment media [DMEM, 10% charcoal stripped fetal bovine serum (CFBS, Hyclone), 0.1 mg/ml streptomycin, 100 U/ml penicillin, 2.5 mM L-glutamine (Hyclone)] at 37 °C in an atmosphere of 5% CO₂ in air with 95% humidity to promote cellular aggregation and attachment. Following 24 hrs cultures were analyzed for evidence of angiogenic progression.

❑ MA Acclimation culture:

• MA were cultured in triplicate in standard non-attachment media [DMEM, 0.1 mg/ml streptomycin, 100 U/ml penicillin, 2% CFBS, 1.5% bovine serum albumin, 2.5 mM L-glutamine] at 37 °C in an atmosphere of 5% CO₂ for 24 hr to acclimate cells to culture.

METHODS, cont.

❑ Treatment Cultures:

• Attachment media was removed from SVF cultures and replaced with standard media. MA cells were added to SVF cultures with and without treatment. Treatments were 1.) 0M (control, media), 2.) 1:50 dilution of FGF2 polyclonal antibody or 3) 1:100 FGF2 polyclonal antibody. Antibody treatment was utilized to inhibit endogenous FGF2 to confirm that MA influenced angiogenesis through FGF2. Cells were incubated for 24 hr under the same incubation conditions. After 24 hr time period, the cells were collected and terminated with denaturing solution. Angiogenic progression was documented via microscope.

➤ Gene Expression Analysis

❑ Real-time RT-PCR:

• Gene expression was analyzed by relative real-time RT-PCR using a C1000 Touchtm thermal cycler CFX96 Real-Time system (Bio-Rad). Total RNA was reverse transcribed with MMLV (Promega). Real-time PCR was conducted on RT products using Takara SYBR® Green kit (Madison, WI). Leptin, FGF2, FGF receptor (FGFR1&2), and cyclophilin primers were synthesized by IDT (Coraville, IA) using a specific temperature sequence, respective to the primers used. Relative values of leptin gene expression were quantified from a relative standard curve (1 ug to 10 pg of total RNA) generated from cyclophilin mRNA of the steer SV cells. Values were transformed to Log10 and normalized with cylophilin (housekeeping gene).

RESULTS

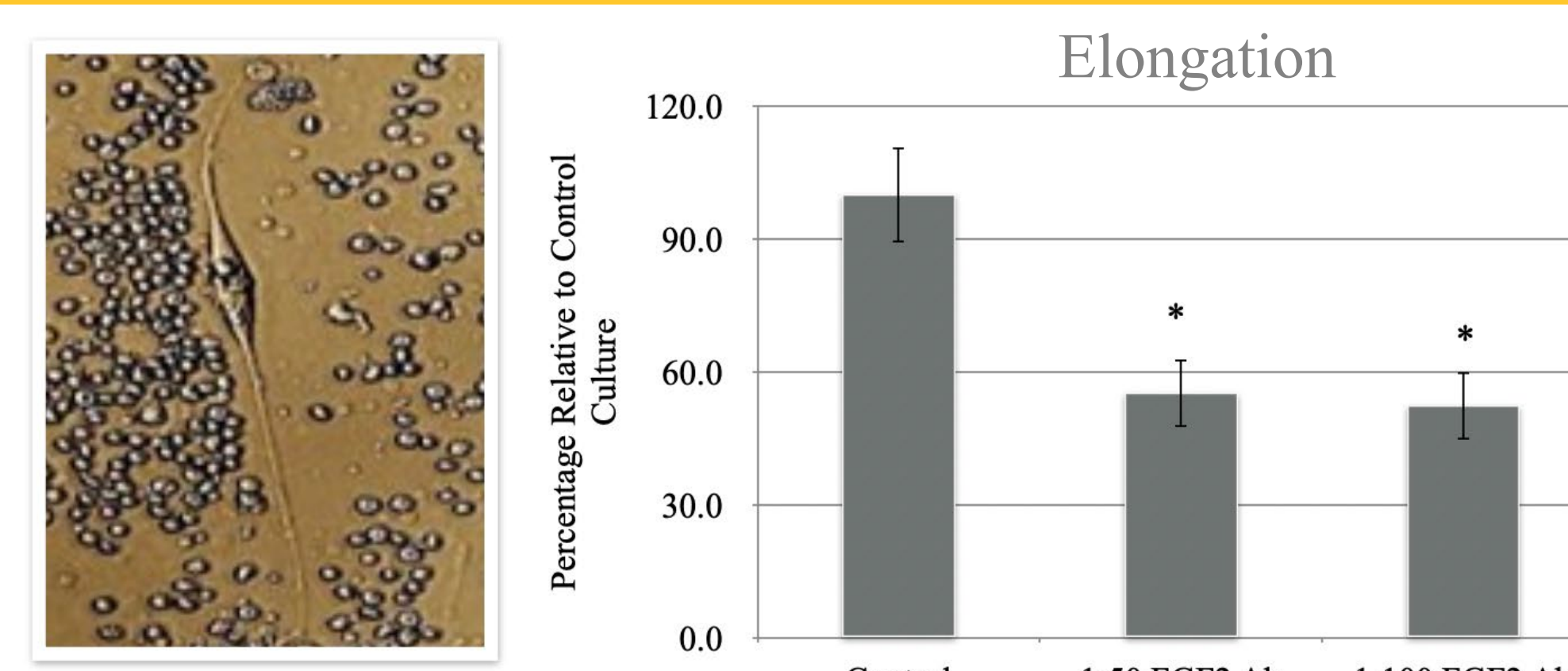


Figure 1 - Elongation: Blocking endogenous FGF2 with FGF2 polyclonal antibody (FGF2 Ab; 1:50 or 1:100 dilution) decreased (*P=0.01) the number of VEC elongating relative to control.

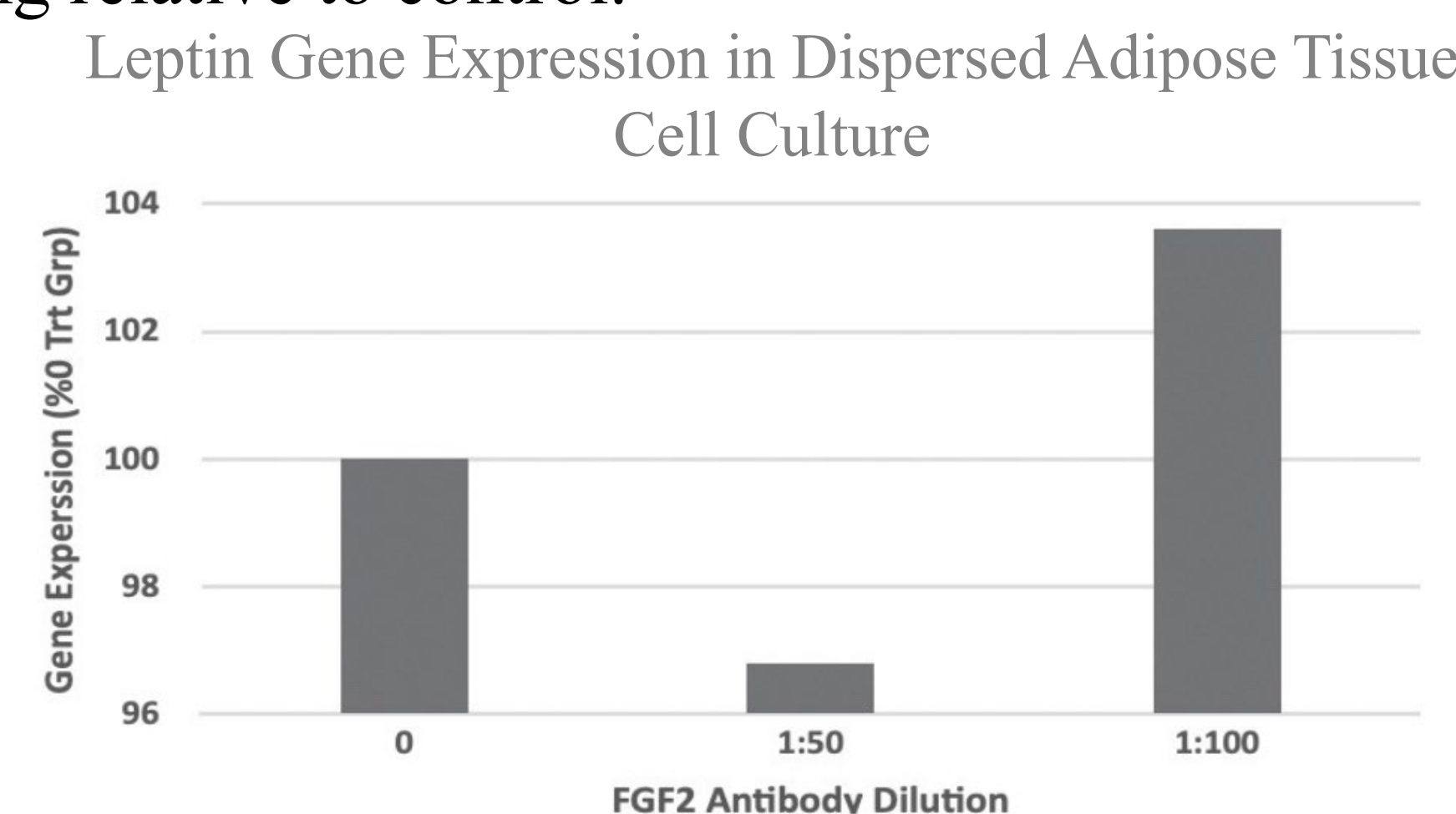
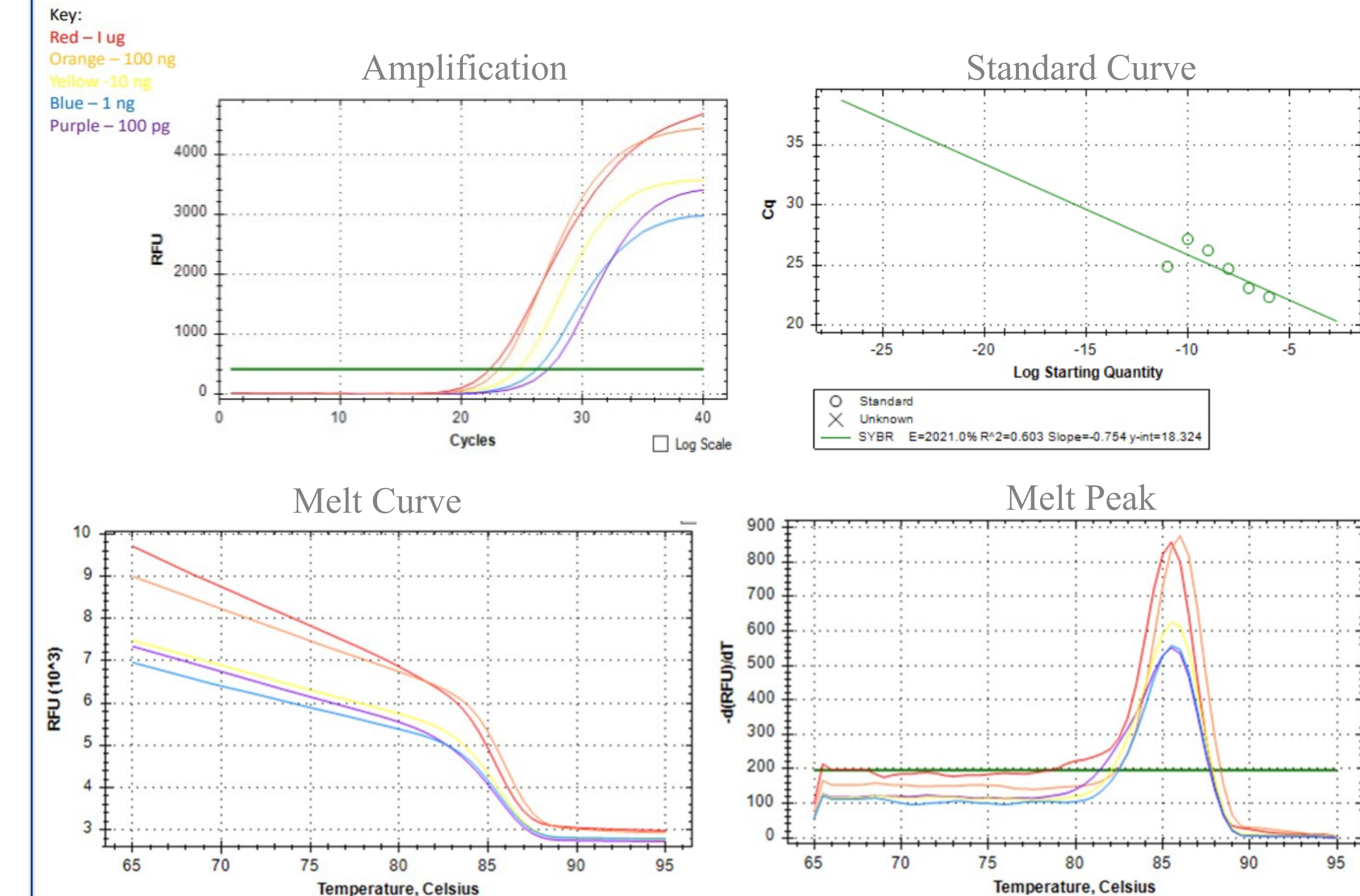


Figure 2: Blocking endogenous FGF2 at a higher concentration (1:50 FGF2 antibody dilution) led to a decrease in leptin gene expression, while a lower concentration (1:100 FGF2 antibody dilution) resulted in increased leptin gene expression compared to the control group (0 FGF2 antibody dilution).

RESULTS, cont.



Figures 3-6: PCR quantification results relative to the standard curve (green)

DISCUSSION

❑ The results suggest that the absence of FGF2 in co-cultures of mature adipocytes and stromal vascular (SV) cells regulates vascular endothelial cell (VEC) morphology. Particularly, treatment with FGF2 antibodies significantly reduced VEC elongation during the growth stage, suggesting reduced activity of angiogenesis.

❑ PCR analysis revealed that leptin gene expression may be influenced by FGF2 signaling in bovine adipose tissue. This finding supports the hypothesis that FGF2 appears to be associated with the regulatory role in leptin expression.

❑ Collectively, these results suggest that leptin is closely involved in adipocyte activity and contributes to the regulation of angiogenesis within bovine adipose tissue.

CONCLUSIONS

Leptin, a hormone produced by fat cells, regulates metabolism, hormones, and immune function, and works with FGF2 to support tissue repair and wound healing. In this study, a higher FGF2 antibody concentration (1:50) reduced leptin expression, while a lower concentration (1:100) increased it compared to the control. These findings suggest that FGF2 plays a role in leptin gene expression, with potential implications for veterinary and biomedical research.

REFERENCES

1. Farooq, M., Khan, A. W., Kim, M. S., & Choi, S. (2021). The role of fibroblast growth factor (FGF) signaling in tissue repair and regeneration. *Cells*, 10(11), 3242. doi: <https://doi.org/10.3390/cells10113242>
2. Münzberg, H., & Heymsfield, S. B. (2019). New insights into the regulation of leptin gene expression. *Cell metabolism*, 29(5), 1013-1014. doi: <https://doi.org/10.1016/j.cmet.2019.04.005>
3. Ramos-Lobo, A. M., & Donato Jr, J. (2017). The role of leptin in health and disease. *Temperature*, 4(3), 258-291. doi: <https://doi.org/10.1080/23328940.2017.1327003>
4. Dudley, A. C., & Griffioen, A. W. (2023). Pathological angiogenesis: mechanisms and therapeutic strategies. *Angiogenesis*, 26(3), 313-347. doi: <https://doi.org/10.1007/s10456-023-09876-7>
5. Krüger-Genge, A., Blocki, A., Franke, R. P., & Jung, F. (2019). Vascular endothelial cell biology: an update. *International journal of molecular sciences*, 20(18), 4411. doi: <https://doi.org/10.3390/ijms20184411>