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The Effect of FGF2 Antibody and Mature Adipocytes on Bovine Vascular Endothelial Cells Morphology

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Abstract

Fibroblast growth factor 2 (FGF2) is a crucial signaling molecule in tissue development and angiogenesis and is particularly important in adipose tissue, which has unlimited growth potential and is highly vascular (Monaco, Corrine. et al, 2023). The intricate relationship between angiogenesis and adipose tissue underscores the importance of understanding how FGF2 influences vascular endothelial cells (VEC)(Ali, Tariq. et al, 2013). This study investigates the impact of FGF2 and adipocytes on vascular endothelial cell (VEC) morphology, hypothesizing that blocking adipocyte FGF2 availability will alter the progression of VEC morphology. Adipose tissue was collected from 3 mature, Brahman influenced cows to harvest cells for culture. Employing a range of FGF2 antibody dilutions (control 0:0, 1:50, and 1:100) we aimed to observe the effects of FGF2 inhibition on VEC morphology in stromal vascular fraction (SVF) cells cultured with mature adipocytes. Cell well plates were incubated for a total of 48 hours. Morphology of VEC in the SVF only cultures were recorded at 24 hr after which adipocytes and treatment were added to the SVF cells and cultured for an additional 24 hr. All morphology was recorded using a high-resolution microscope. The study documented morphological changes in VEC, including elongation, sprouting, nascent tubes, and vessel tube formation. The data implies treatment induced patterns in VEC morphology, which warrants further investigation. It is anticipated that further study will strengthen our understanding of the complex interplay between FGF2, adipocytes, and VEC, and potentially the broader implications for angiogenesis, tissue regeneration, and wound healing processes.

PURPOSE

The purpose of this study is to investigate the effect of the availability of adipocyte produced FGF2 on the morphology of bovine vascular endothelial cells.

METHODS

Animals

- 3 bred cattle

Tissue Collection

- Subcutaneous adipose tissue (~3 g) was collected from the tailhead region and transported in Hanks media at 37ºC for processing.

Cell Preparation

- Adipose tissue was mechanically minced and enzymatically digested in phenol-free Dulbecco's Modified Eagle's Medium Nutrient Mixture (DMEM) with Type II Collagenase (1.5 mg/ml; Sigma) in a shaking water bath at 37˚ C for 90 min.
- Cell separation: Stromal vascular fraction (SVF: VEC, fibroblasts, pericytes, smooth muscle cells, preadipocytes) were separated from mature adipocytes (MA, floating cells) for pre-treatment culture.

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 ºC in an atmosphere of 5% CO_2 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 ºC in an atmosphere of 5% CO_2 for 24 hr to acclimate cells to culture.

METHODS, cont.

Treatment Plate Cultures

- Attachment media was removed from SVF cultures and replaced with standard media. MA cells were added to SVF cultures with or without treatment: 1.) 0M (culture media only), 2.) anti-FGF2 antibody (1:50 dilution M), 3) anti- FGF2 antibody (1:100 dilution). 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.

RESULTS

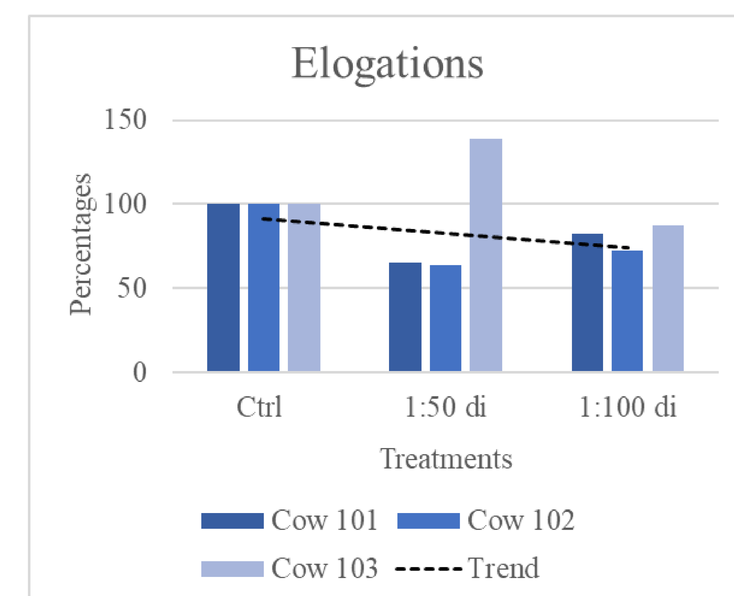

Fig1a– Percentage of VEC elongating

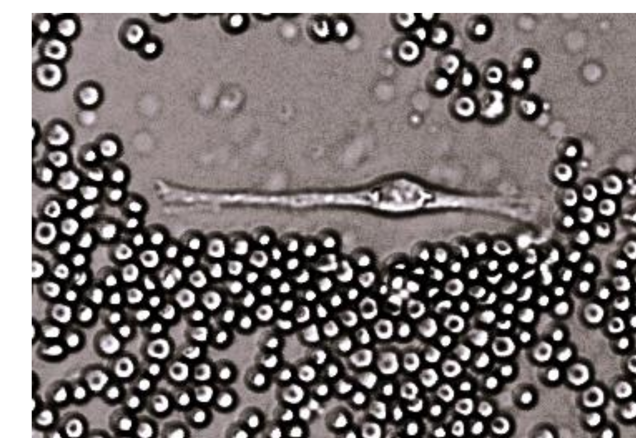

Fig1b– Example of an Elongation

Figure 1. 1a: The treatment groups (Control: Ctrl; 1:50 antibody dilution: 1:50 dil; and 1:100 antibody dilution: 1:100 dil) and their effect on VEC elongation morphology. Each cow exhibited a unique response to the treatments, with the graph showing the percentage of elongation compared to the control group (no antibody treatment). Numerically, it appears that blocking FGF2 reduces the number of cells elongating, with the exception of cow 103 in the higher concentration of antibody where it appears to compensate for the reduction of FGF2. 1b: example of a VEC elongating.

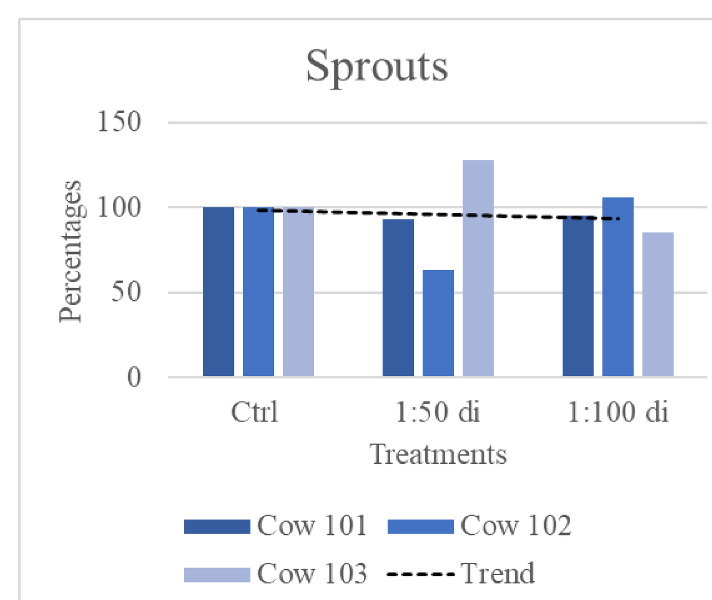

Figure 2a –Percentage of VEC sprouting

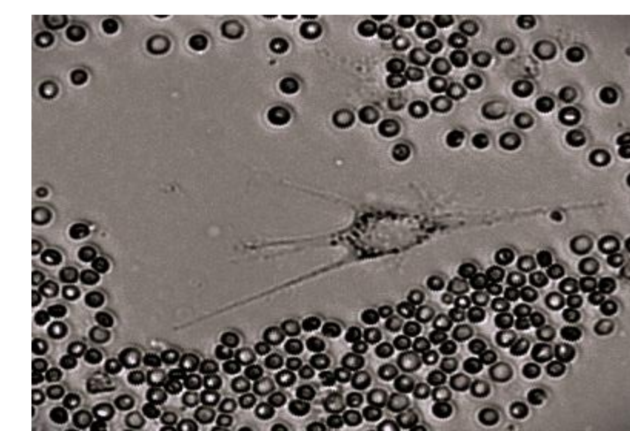

Figure 2b – Example of a Sprout

Figure 2. 2a: The treatment groups (Ctrl; 1:50 dil; and 1:100 dil) and their effect on VEC sprouting morphology. Each cow exhibited a unique response to the treatments, with the graph showing the percentage of elongation compared to the control group (no antibody treatment). Numerically, it appears that blocking FGF2 reduces the number of cells sprouting, with the exception of cow 103 in the higher concentration of antibody where it appears to compensate for the reduction of FGF2. 2b: example of a VEC sprouting

RESULTS, cont.

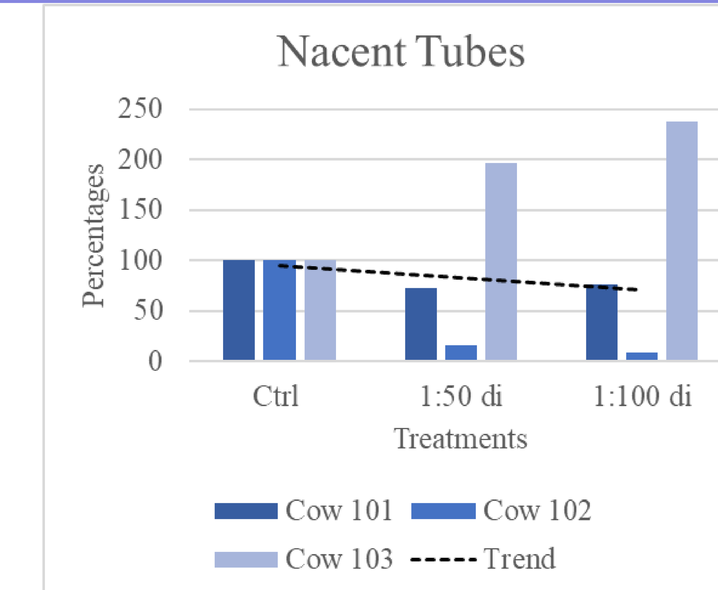

Figure 3a – Example of a Nascent Tube

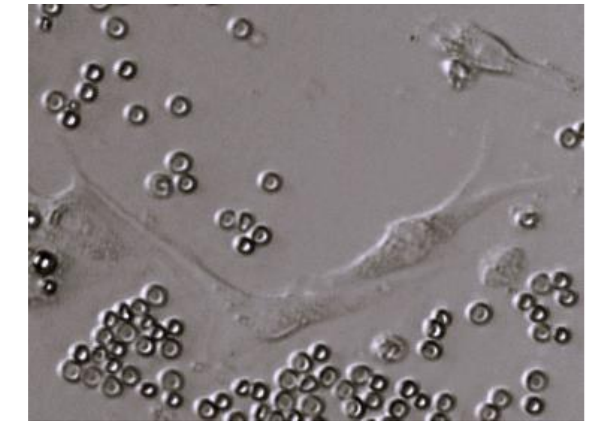

Figure 3b – Example of a Nascent Tube

Figure 3. 3a: The treatment groups (Ctrl; 1:50 dil; and 1:100 dil) and their effect on VEC nascent tube morphology. Numerically, it appears that blocking FGF2 reduces the number of cells forming tubes, with the exception of cow 103 where it appears to compensate for the reduction of FGF2. 3b: example of a VEC nascent tube.

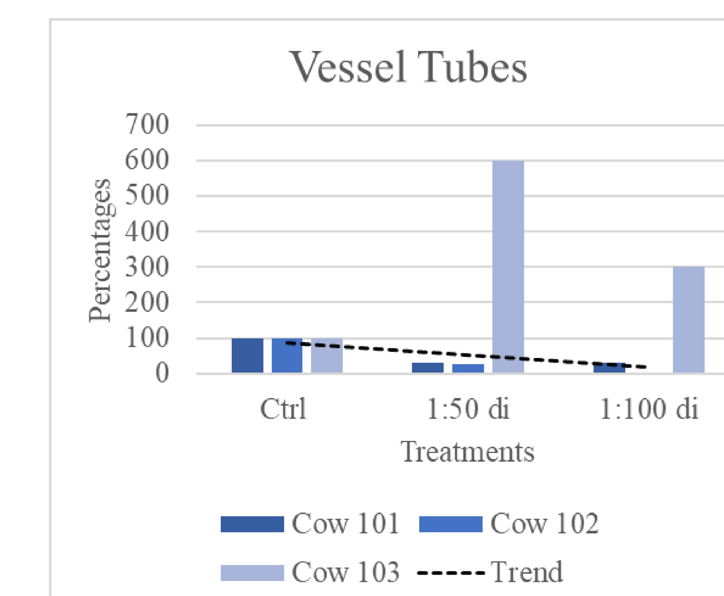

Figure 4a – Percentage of Vessel Tubes

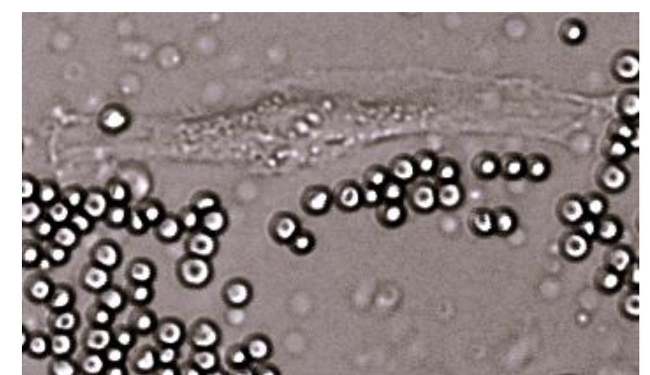

Figure 4 – Example of a Vessel Tube

Figure 4. 4a: The treatment groups (Ctrl; 1:50 dil; and 1:100 dil) and their effect on VEC vessel tube morphology. Numerically, it appears that blocking FGF2 reduces the number of cells forming tubes, with the exception of cow 103 where it appears to compensate for the reduction of FGF2. 4b: example of a VEC vessel tube.

CONCLUSIONS

This study investigated the effects of blocking FGF2 on vascular endothelial cell (VEC) morphology in three cows. There was a consistent trend in morphological changes in the preliminary data, with 2/3 animals exhibiting a reduction in VEC morphology and 1/3 exhibiting an increase in treatment cultures compared to control.

- Blocking FGF2 may delay the progression of VEC; however, there could be a compensatory increase in FGF2 production if bioavailability is altered which would enhance changes in VEC morphology.

Future investigation will clarify the role FGF2 in VEC morphology.

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