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**AAO Foundation Final Report Form**
**(a/o 1/3/2018)**

*In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:*

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Please prepare a report that addresses the following:

Type of Award, e.g., Orthodontic Faculty Development Fellowship Award, Postdoctoral Fellowship Award, Biomedical Research Award, Center Award, Educational Innovation Award, Program Award, Research Aid Award

Name(s) of Principal Investigator(s): Dr. Shariq Khan

Institution: Stony Brook School of Dental Medicine

Title of Project

Period of AAOF Support (e.g. 07-01-18 to 06-30-19):

Amount of Funding: $5000

Summary/Abstract

Orthodontic tooth movement relies on effective bone remodeling of the alveolar process. Bone remodeling is a highly dynamic process that interacts with a wide array of cells and tissues. Homeostatic balance of bone removal and replacement are orchestrated by osteoblasts and osteoclasts. Similarly, orthodontic tooth movement is characterized by mechanical loading induction of compression and tension sides in the PDL, with a repeated process of net bone resorption on the pressure side and net new bone formation on the tension side. Although the exact mechanisms of bone remodeling during orthodontic tooth movement are not clearly known, a number of cytokines including vascular endothelial growth factor (VEGF), which is a potent angiogenic factor, have reported be able to accelerate tooth movement by enhancing the bone remodeling process in animal studies. Osteoblasts have two types of VEGF-A receptors, VEGFR-1 and -2. In this study, we aimed to identify which receptor(s) in the osteoblasts mediate VEGF-A-induced osteoclast formation with the hypothesis that both receptors are responsible. Osteoblastic MC-4 cells were incubated with specific

antagonist or agonist for the receptors for 4, 7, 14, and 21 days. The conditioned media were then used to treat pre-osteoblastic RAW 264.7 cells and assess osteoclast formation and function with TRAP staining and mineral resorption analysis, respectively. We found that specific agonist of VEGFR-1 or 2 significantly stimulated the formation and function of osteoclasts through the osteoblastic cells similar to VEGF-A. On the other hand, specific VEGFR-1 or -2 antagonist significantly inhibited VEGF-A-induced osteoclast formation and function through the osteoblasts. We concluded that both VEGF-A receptors contribute to the full effects of VEGF-A-induced osteoclast formation.

Detailed results and inferences:

1. If the work has been published please attach a pdf of manuscript OR
2. Describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis and interpretation of results should be included.

These results will be presented in our future publication.

Conditioned media from specific VEGF agonists or antagonists -treated MC-4 cells were used to culture pre-osteoclastic RAW246.7 cells. The conditioned media from osteoblastic cultures treated with PIFG, the specific VEGFR-1 agonist, significantly stimulated the formation of osteoclast at all time points on days 4, 7, 14 and 21. (Figure 1). The effect was similar with conditioned media treated with VEGF-A (Figure 1). The induction of osteoclasts was more potent at earlier time points than later time points (Figure 1). The functional assay with mineral resorption pit formation measurements showed the similar results on days 4, 7, 14, 21 (Figure 2), indicating that the osteoclasts formed from the stimulation of the conditioned media were capable of bone resorption. As for the specific VEGFR-2 agonist VEGF-E, we found similar results with PIGF and VEGF-A for both osteoclast formation on days 4, 7, 14, 21 (Figure 1) and function on days (Figure 2). The numbers for osteoclast formation and resorption pit measurements can be found in Tables 1 and 2, indicating that both specific agonists of VEGFR-1 and -2 significantly induced osteoclast formation and function via osteoblastic cells, similar to VEGF-A These results indicated that specific VEGFR-1 or -2 agonist has similar effect on osteoclast formation and function via osteoblasts.
The presence of specific VEGFR-1 antibody in the osteoblastic culture treated with VEGF-A significantly inhibited osteoclast formation induced by the conditioned media compared with those from the VEGF-A-treated cultures collected on days 4, 7, and 14 and 21 (Figure 3). The functional assay showed similar results (Figure 4). The presence of specific VEGFR-2 antibody had similar effects on osteoclast formation (Figure 3) and function (Figure 4). The numbers for osteoclast formation and resorption pit measurements can be found in Tables 3 and 4, indicating that blocking either VEGFR-1 or -2 significantly inhibited VEGF-A-induced osteoclast formation and function through osteoblasts. The effects of specific VEGFR-1 and -2 antagonists were different from the effects of specific agonists (i.e. effects of specific VEGFR-1 agonist was different from that of VEGF-A plus VEGFR-2 antagonist, and the effects of specific VEGFR-2 agonist was different from that of VEGF-A plus VEGFR-1 antagonist). The significance and possible explanations will be presented in the discussion.

Figure 1. VEGF induced osteoclast formation treated with specific agonists. a, significant for the effect of agonists when compared with control and b for the effect of time. Greatest effect of time was seen on the osteoclast formation was seen when days 4, 7, 14 compared with day 21 ($p<0.01$).

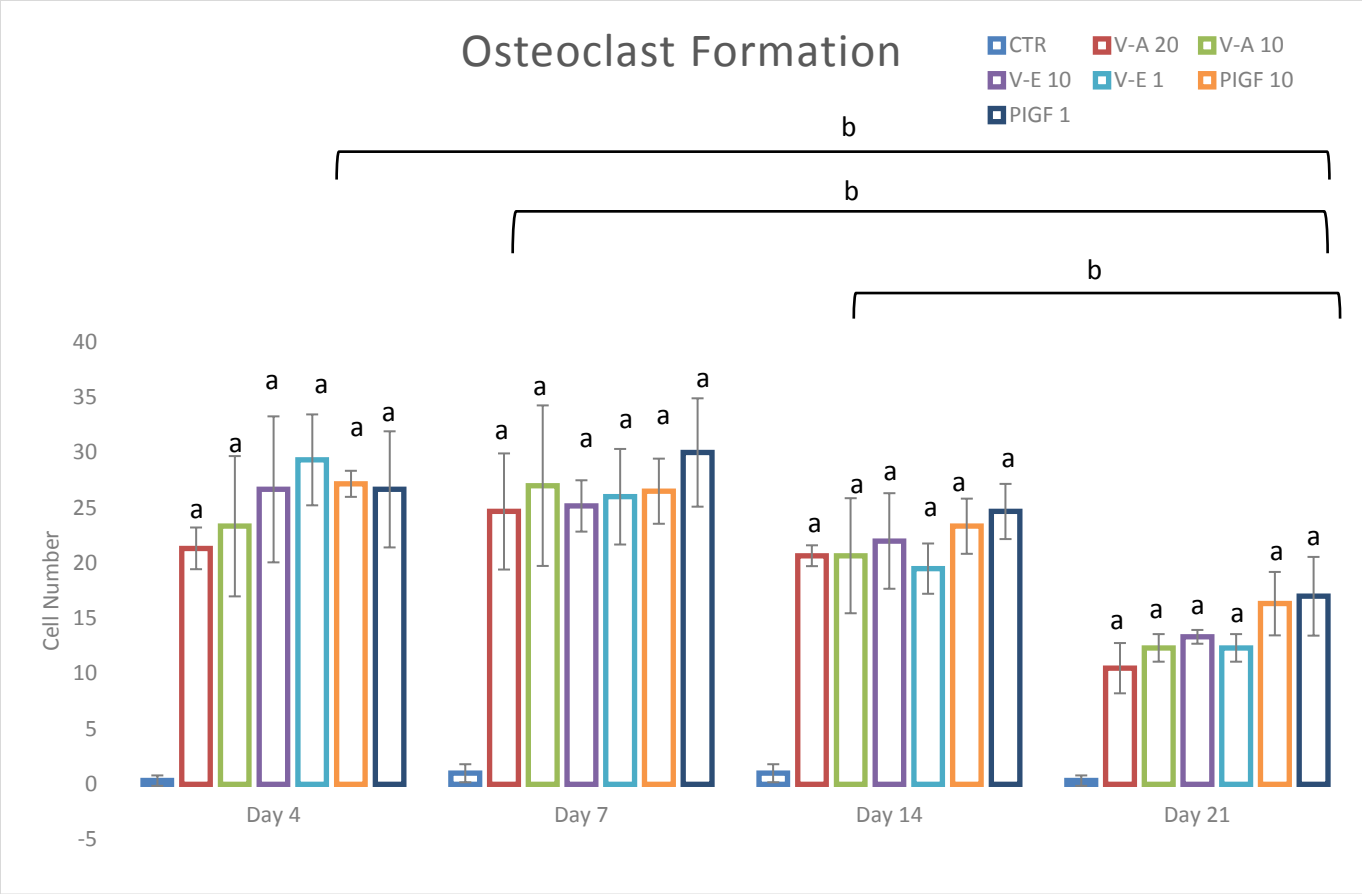


**Table 1.** Osteoclast formation in RAW 264.7 cell cultures treated with conditioned media in the presence of VEGF agonists

| | Day 4 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **Control** | 0.33±0.47 | 1.0±0.82 | 1.0±0.82 | 0.33±0.47 |
| **VEGF-A 20 ng/ml** | 21.33±1.89[a] | 24.67±5.25 [a] | 20.67±0.94[a] | 10.50±2.27[a] |
| **VEGF-A 10 ng/ml** | 23.33±6.34[a] | 27.0±7.26[a] | 20.67±5.20[a] | 12.33±1.25[a] |
| **VEGF-E 10 ng/ml** | 26.67±6.60[a] | 25.17±2.32[a] | 22.0±4.32[a] | 13.33±0.62[a] |
| **VEGF-E 1 ng/ml** | 29.33±4.11[a] | 26.0±4.32[a] | 19.5±2.27[a] | 12.33±1.25[a] |
| **PIGF 10 ng/ml** | 27.17±1.18[a] | 26.5±2.94[a] | 23.33±2.49[a] | 16.33±2.87[a] |
| **PIGF 1 ng/ml** | 26.67±5.25[a] | 30.0±4.90[a] | 24.67±2.50[a] | 17.0±3.60[a] |

Data are presented as means and_standard deviations.

[a]Significant difference from control ($p<0.01$).

Figure 2. VEGF induced osteoclast pit formation treated with specific agonists. a, significant for the effect of agonists when compared with control and b for the effect of time. Greatest effect of time was seen on the osteoclast pit formation was seen when days 4, 7, 14 compared with day 21 ($p<0.01$).

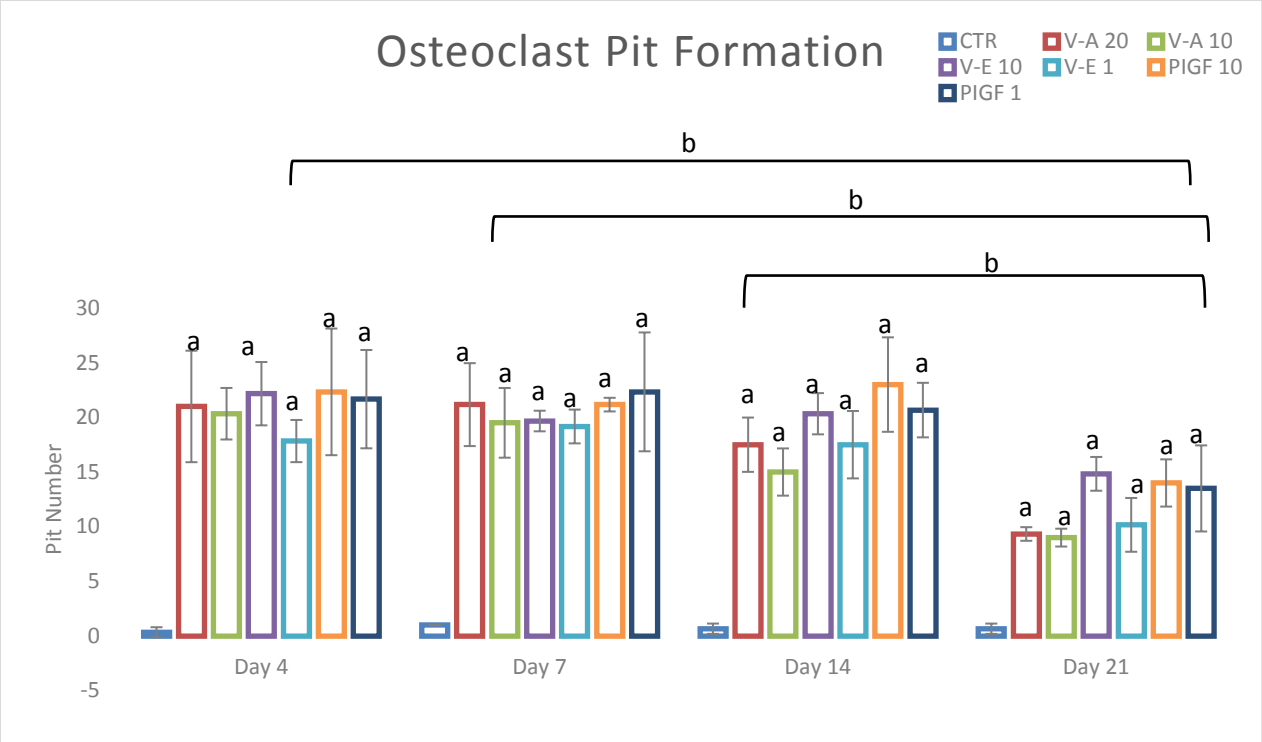


**Table 2.** Osteoclast pit formation in RAW 264.7 cell cultures treated with conditioned media in the presence of VEGF agonists

| | Day 4 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **Control** | 0.33±0.47 | 1.0±0 | 0.67±0.47 | 0.67±0.47 |
| **VEGF-A 20 ng/ml** | 21.0±5.10[a] | 21.17±3.80[a] | 17.5±2.48[a] | 9.33±0.62[a] |
| **VEGF-A 10 ng/ml** | 20.33±2.36[a] | 19.5±3.19[a] | 15.0±2.16[a] | 9.0±0.82[a] |
| **VEGF-E 10 ng/ml** | 22.17±2.90[a] | 19.67±0.94[a] | 20.33±1.89[a] | 14.83±1.54[a] |
| **VEGF-E 1 ng/ml** | 17.83±1.93[a] | 19.17±1.55[a] | 17.5±3.08[a] | 10.17±2.46[a] |
| **PIGF 10 ng/ml** | 22.33±5.79[a] | 21.17±0.62[a] | 23.0±4.32[a] | 14.0±2.16[a] |
| **PIGF 1 ng/ml** | 21.67±4.49[a] | 22.33±5.43[a] | 20.67±2.49[a] | 13.5±3.94[a] |

Data are presented as means and standard deviations.

[a]Significant difference from control ($p<0.01$).

Figure 3. VEGF-A induced osteoclast formation treated with specific antagonists. a, significant for the effect of factor of control a1, factor of VEGF-A, a2; and b for the effect of time. Greatest effect of time was seen on the osteoclast formation was seen on days 4, 7, and 14 ($p<0.01$)

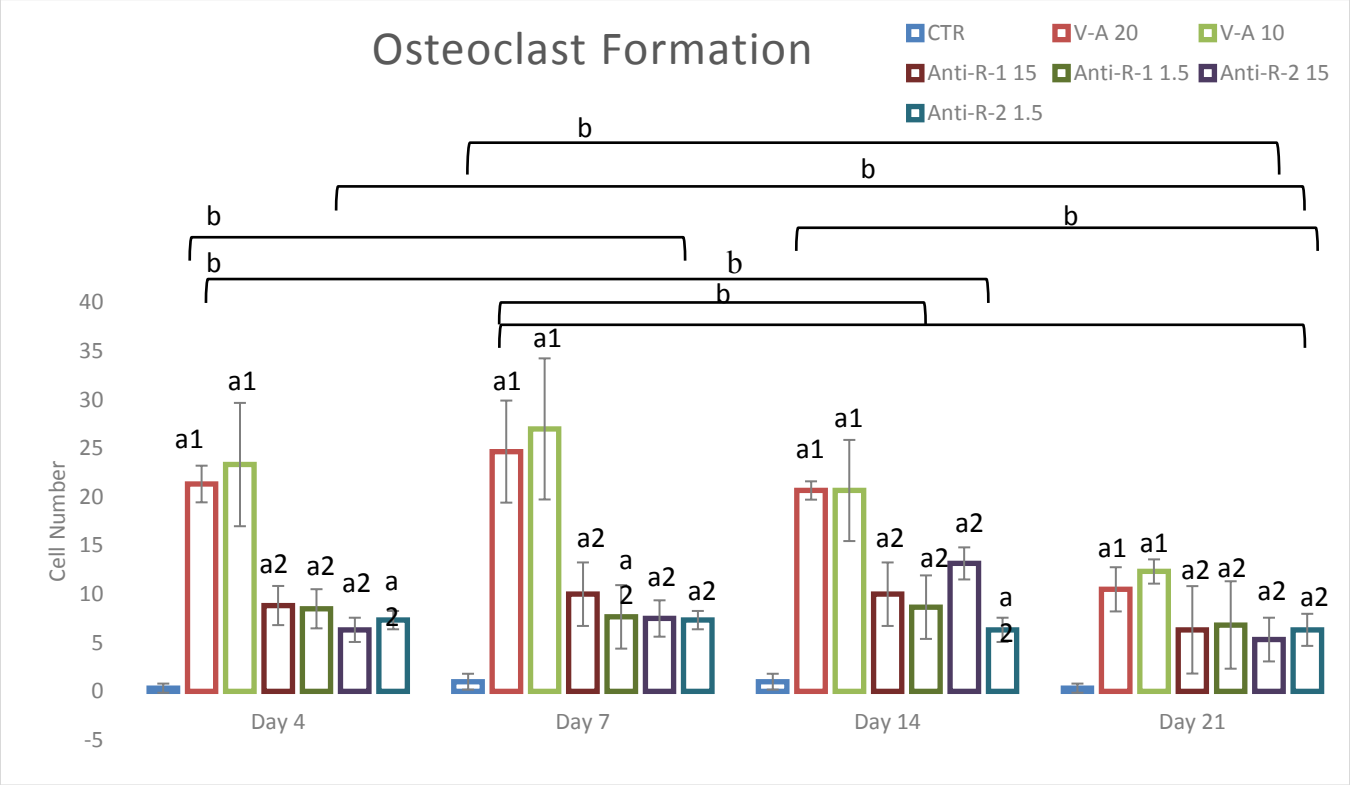


**Table 3.** Osteoclast formation in RAW 264.7 cell cultures induced with VEGF-A

| | Day 4 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **Control** | 0.33±0.47 | 1.0±0.82 | 1.0±0.82 | 0.33±0.47 |
| **VEGF-A 20 ng/ml** | 21.33±1.89$^{a1}$ | 24.67±5.25$^{a1}$ | 20.67±0.94$^{a1}$ | 10.50±2.27$^{a1}$ |
| **VEGF-A 10 ng/ml** | 23.33±6.34$^{a1}$ | 27.0±7.26$^{a1}$ | 20.67±5.20$^{a1}$ | 12.33±1.25$^{a1}$ |
| **Anti-VEGFR-1 15µg/ml** | 8.83±2.01$^{a2}$ | 10.0±3.27$^{a2}$ | 10.0±3.27$^{a2}$ | 6.33±4.50$^{a2}$ |
| **Anti-VEGFR-1 1.5µg/ml** | 8.5±3.49$^{a2}$ | 7.67±2.32$^{a2}$ | 8.67±0.94$^{a2}$ | 6.83±2.32$^{a2}$ |
| **Anti-VEGFR-2 15µg/ml** | 6.33±1.25$^{a2}$ | 7.5±1.87$^{a2}$ | 13.17±1.65 | 5.33±2.25$^{a2}$ |
| **Anti-VEGFR-2 1.5µg/ml** | 7.33±0.94$^{a2}$ | 7.33±0.94$^{a2}$ | 6.33±1.25$^{a2}$ | 6.33±1.65$^{a2}$ |

Data are presented as means and standard deviations.

$^{a1}$Significant difference from control ($p<0.01$).

$^{a2}$Significant difference from VEGF-A ($p<0.01$).

Figure 4. VEGF-A induced osteoclast pit formation treated with specific antagonists. a1 significant for the effect for control, a2 for the effect of VEGF-A, b for the effect of time. Greatest effect of time was seen on the osteoclast pit formation was seen on days 4, 7, and 14.

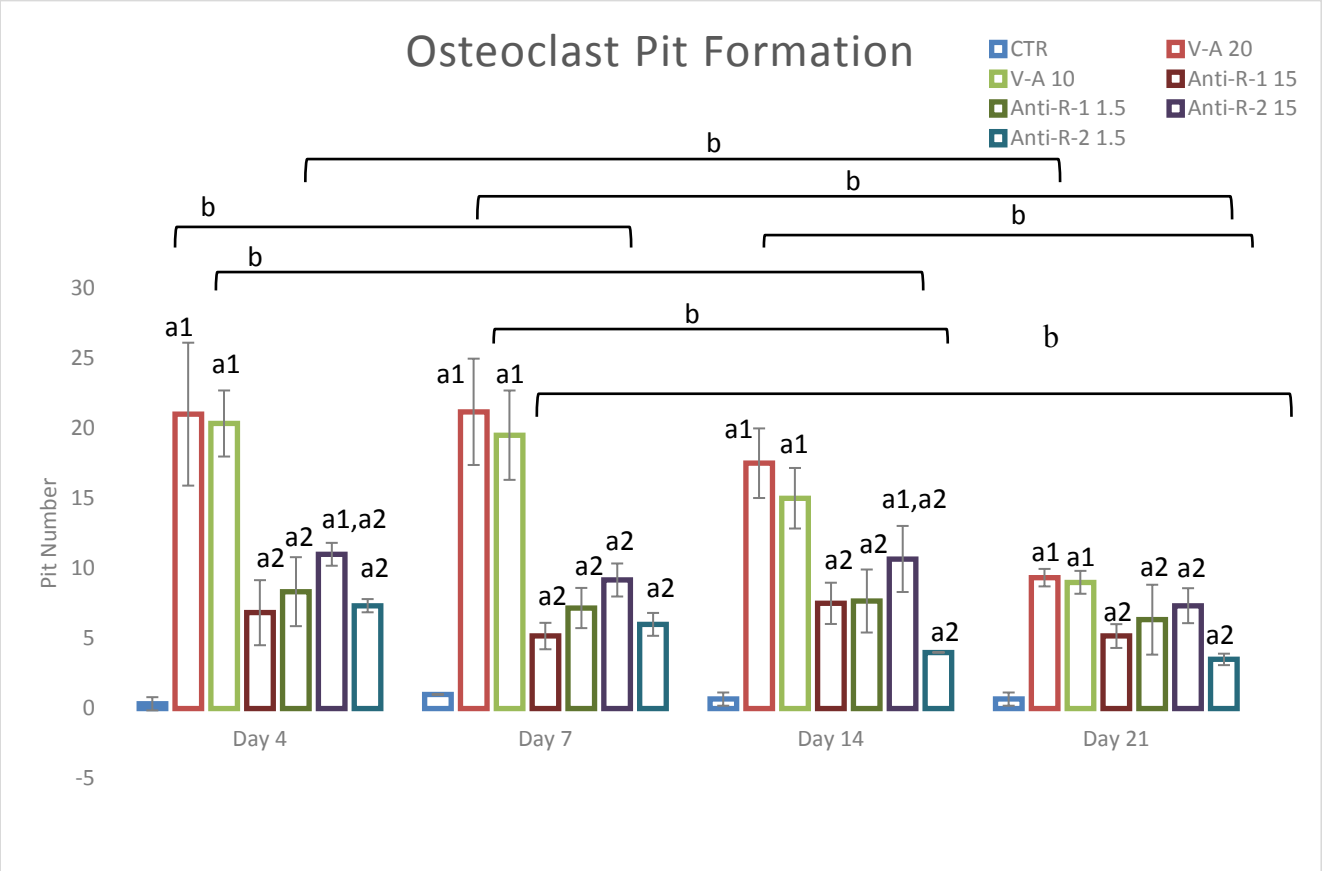


**Table 4.** Osteoclast formation in RAW 264.7 cell cultures induced with VEGF-A

| | Day 4 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **Control** | 0.33±0.47 | 1.0±0 | 0.67±0.47 | 0.67±0.47 |
| **VEGF-A 20 ng/ml** | 21.0±5.10$^{a1}$ | 21.17±3.80$^{a1}$ | 17.5±2.48$^{a1}$ | 9.33±0.62$^{a1}$ |
| **VEGF-A 10 ng/ml** | 20.33±2.36$^{a1}$ | 19.5±3.19$^{a1}$ | 15.0±2.16$^{a1}$ | 9.0±0.82$^{a1}$ |
| **Anti-VEGFR-1 15µg/ml** | 6.83±2.32$^{a2}$ | 5.17±0.94$^{a2}$ | 7.5±1.47$^{a2}$ | 5.17±0.84$^{a2}$ |
| **Anti-VEGFR-1 1.5µg/ml** | 8.33±2.46$^{a2}$ | 7.17±1.43$^{a2}$ | 7.67±2.25$^{a2}$ | 6.33±2.49$^{a2}$ |
| **Anti-VEGFR-2 15µg/ml** | 11.0±0.82$^{a1,2}$ | 9.17±1.18$^{a2}$ | 10.67±2.36$^{a1,2}$ | 7.33±1.25$^{a2}$ |
| **Anti-VEGFR-2 1.5µg/ml** | 7.33±0.47$^{a2}$ | 6.0±0.82$^{a2}$ | 4.0±0$^{a2}$ | 3.5±0.41$^{a2}$ |

Data are presented as means and standard deviations.

$^{a1}$Significant difference from control ($p<0.01$)

$^{a2}$Significant difference from VEGF-A ($p<0.01$)

Response to the following questions:

1. Were the original, specific aims of the proposal realized? Yes
2. Were the results published?
    a. If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers
    b. Was AAOF support acknowledged?
    c. If not, are there plans to publish? If not, why not? Not yet, were are currently working on preparing a manuscript for AJODO.
3. Have the results of this proposal been presented?
    a. If so, list titles, author or co-authors of these presentation/s, year and locations
    b. Was AAOF support acknowledged?
    c. If not, are there plans to do so? If not, why not? We intended to present the findings to AAO meeting but the program was cancelled.
3. To what extent have you used, or how do you intend to use, AAOF funding to further your career?
    AAOF funding has been very helpful in the completion of this project. This AAOF support is vital for my career and to further advance my interest as an independent researcher and work as a academician.