The authors would like to thank the reviewers for their constructive criticism and their valuable comments and suggestions, and the editor for editing the manuscript entitled "Vascular Endothelial Growth Factor (VEGF) for the treatment of femoral head osteonecrosis. An experimental study in canines", with ID 39142.
Please find below the answers to all the comments. All changes are marked in the revised manuscript with blue color.

A. 02444711

1. Abstract is not clear, esp. grouping. I cannot get the full picture of the study after reading it.
The abstract was re-written with emphasis in the description of the different groups and our actions per group.

2. What is the hypothesis of this study? I cannot find the hypothesis in Introduction.
We introduced a clearer formulation of our study hypothesis in the last sentence of the introduction.

3. The first sentence of Method states the ethical approval. Please provide the reference number, if available.
The reference number of the IACUC (Institutional Animal Care And Use Committee) of Duke University is A317-00-06-03.

4. The animals were sacrificed at 12 weeks. Why choose this time point? Any literature support to select this time point?
We chose this time point for the canines to be euthanized according to the findings of our previous study (ref. 26) in which we observed that osteonecrosis was well established in a time period of 12 weeks, using specific radiographic and histologic signs.

5. I am a bit confused of the sample allocation. There were 6 groups with n=5 in each group. They were cut half, which one half underwent decalcification, while another half of two canines per group were subjected to undecalcification. Then how about the another half of the remaining 3 samples? Also, it specifies "the exception of the t-NS group, why? I am confused with these and please clarify.
The t-NS group (group with local injection of normal saline) was the first group of beagles receiving any kind of local infusion and at that time we had not made the decision yet to perform additional non-decalcified tissue processing. Once we made this decision all femoral heads of the VEGF treatment groups were cut in half in the frontal level and each half underwent decalcified and non-decalcified tissue processing. For financial reasons we only used 2 samples (randomly selected) per group to undergo non-decalcified tissue processing.

6. I wonder for all the animals, any one of them showed bone collapse?
We didn't observe bone collapse in any sample.

7. On page 11, last paragraph, it mentions "In the present study the influence of the VEGF on angiogenesis and osteogenesis …" but this study did not examine anything

about angiogenesis?! Please clarify. This is also related to the keyword selection of this study.
We would like to thank the reviewer for the useful observation. We removed the word “angiogenesis” from the keywords and we rephrased the meaning of the last paragraph on page 11 according to your suggestion.

8. Discussion should have a part to discuss study limitations.
According to your suggestion, we added a brief statement in the last paragraph of the discussion regarding the limitations of our study.

9. This study only demonstrated the general effect on bone without any in-depth mechanistic work done.
Indeed, the objective of this experimental study in canines is the evaluation of the effect of VEGF in femoral head osteonecrosis and the potential enhancement of bone formation and not the identification of the molecular pathways that determine the events that occur in the femoral head of the canines. Future studies should further investigate, in a variety of experimental conditions, the role of VEGF as a key molecule and essential player for therapeutic strategies targeting bone reconstruction, so that an even transition to clinical trials may be achieved. After the suggestion of one of the reviewers, a diagram summarizing potential interactions of VEGF related to bone tissue healing in femoral head osteonecrosis was included as a separate figure in the manuscript (Fig. 8).

B. 00505434

Abstract:
1. Change "femoral head osteonecrosis" to "osteonecrosis of the femoral head (ONFH)".
We changed the phrase “femoral head osteonecrosis – FHON” to “osteonecrosis of the femoral head – ONFH” throughout the abstract and the text.
2. Chang "FHON" to "ONFH" as indicated above.
We changed the phrase “femoral head osteonecrosis – FHON” to “osteonecrosis of the femoral head – ONFH” throughout the abstract and the text.
3. Clear state how many groups, and how many animals in each group in method section
We made a detailed and well-defined - we hope - description of the groups and the specific treatment per group.

Introduction:
Change the first sentence to "osteonecrosis (ON), also known as avascular necroiss (AVN)...". use ON to replace AVN throughout the entire manuscript since ARCO recommended to use the new term ON to replace the old term AVN.
We replaced the term AVN with ON throughout the introduction and the entire manuscript so that we correspond to the latest ARCO recommendations.

Materials and methods:
1. Briefly state the surgical techniques, including anesthesia, sterile techniques, and pain management after surgery, as well as weight bearing status.

We made a short but comprehensive statement about surgical/sterile techniques, and anesthesia/pain management in the beginning of this section.

2. Clearly state how the specimens were fixed? in what solutions?

A clear description of the methods of specimen preparation and fixation is included in the Materials and methods section of the manuscript.

3. How did the uncalcified specimens were sectioned?

One half of the retrieved FH was processed without decalcification, dehydrated, embedded in methylmethacrylate and sectioned with Polycut Model microtome (Leica, Heidelberg, Germany). This is clearly stated in the Materials and methods section of the manuscript.

Discussion:

1. Change "Avascular necrosis (AVN) of the FH" to "Osteonecrosis of the femoral head (ONFH)"

Done.

2. Again, eliminate the term "AVN" from the entire manuscript.

Done.

C. 00058340

The authors demonstrated in this study that local administration of Vascular Endothelial Growth Factor (VEGF) is effective treatment of femoral head osteonecrosis in a canine model.

Comments

1) The study is reasonably performed, however it does not provide in depth insight into the mechanism of VEGF action. Is it the therapeutic effect of VEGF due to stimulation of angiogenesis, stimulation of osteoblats and/or activation of progenitor cells. Answer to these questions will definitely enhanced this manuscript. The authors have the histologic specimens, so they can use them to evaluate above factors.

The purpose of the present work is to evaluate that use of VEGF as a growth factor for the treatment of femoral head osteonecrosis. Unfortunately, the existing histologic specimens -prepared and stained to evaluate new bone formation- can not provide these answers. The deeper study of the mechanisms, including identification of the molecular pathways that determine the specific pathways enhancing angiogenesis and osteogenesis, will be the subject of future works, where the potential role of VEGF as a key molecule and essential player for therapeutic strategies targeting bone reconstruction will be evaluated. However, a separate figure (Fig. 8), summarizing the potential interactions of VEGF related to bone tissue healing in femoral head osteonecrosis, was added to the manuscript in relation to your comment #5.

2) Comparative boxplot of the TbTh values. Please explain what does it mean.

The graph shows the distribution of trabecular thickness (TbTh) values across the different groups. The lowest whisker represents the low 95% confidence interval value observed and the upper whisker the high 95% confidence interval value. The upper and lower part of the box represent the 3rd and 1st quartile of values respectively and the thick line represents the median. The dots and asterisks show the most extreme values within each group.

In the manuscript we added a brief additional description in figures 5 and 7.

3) Tables 1 and 2 contain numbers e.g., 29,437 etc Do you mean 29.437 ???
The values on tables 1 and 2 contain three decimal digits and for this reason the numbers appear as 29,497 and not 29.497.

4) Histologic figures should be labelled with arrowhead, asterics and letters so the readers, who are not expert pathologists can fully understand them.
Histologic figures have been labeled with symbols in order to become more familiar and comprehensible to any reader.

5) A diagram summarizing potential mechanisms of VEGF action on femoral head osteonecrosis healing would be very useful.
A diagram summarizing potential interactions of VEGF related to bone tissue healing in femoral head osteonecrosis was included as a separate figure in the manuscript (Fig. 8).

6) Manuscript requires linguistic improvements, e.g. “apart from” should be replaced with except “analogous differences” should be replaced with similar differences, “were sacrificed” should be replaced with were euthanized, etc, etc.
All requested changes were done.

7) In the reference related to VEGF and angiogenesis the authors should cite paper of Naploeone Ferrara, who identified, sequenced and named VEGF.
The reference was added.

8) The authors may consider adding below reference Tannast M1,2, Wolfer N2, Ryan MK3, Nuss KM2, von Rechenberg B2, Steppacher SD1.The Vascular Supply of the Femoral Head in Sheep - Implications for the Ovine Femoroacetabular Impingement Model. J Orthop Res. 2018 Mar 25. doi: 10.1002/jor.23897 Xu T, et al Administration of erythropoietin prevents bone loss in osteonecrosis of the femoral head in mice. Mol Med Rep. 2017 Dec;16(6):8755-8762. doi: 10.3892/mmr.2017.7735. PMID: 29039481
The reference “Administration of erythropoietin prevents bone loss in osteonecrosis of the femoral head in mice. Mol Med Rep. 2017 Dec;16(6):8755-8762. doi: 10.3892/mmr.2017.7735. PMID: 29039481” was added.