Author’s response to reviews

Title: Longitudinal Study of Electrical, Functional and Structural Remodelling in an Equine Model of Atrial Fibrillation

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Please see the response to each reviewer comment below the individual comments (response provided in italics). Changes in the manuscript are made using track changes

Reviewer #1:

Agnieszka Noszczyk - Nowak (Reviewer 1): This is not a new model, tachypacing is a known-model in animals (dogs, pigs and rats).
We agree that atrial tachypacing is not a new model. On the contrary, we have been very inspired by the existing literature when planning this project and have referred to major previous works. However, we do believe that this horse model are relevant in AF research as none of the existing animal models fully recapitulate the human phenotype. As in humans, horses spontaneously develop AF. They have a natural substrate for AF, which is enriched by exercise, and therefore a high inducibility of AF. This makes the horse interesting as a surrogate for the human phenotype. But chronic AF experimentation in horses is a still a fairly new field. Hence, for future studies, it is important to assess the degree of remodeling over time in this tachy-induced AF horse model.

But we agree that using the word “new” is stretching it a bit. Therefore, we have deleted the “new” in front of model (line 363)

Base-apex lead is insufficient to assess electrical remodeling un AF.

As described in detail below (#4) we acknowledge that AFR only provides a summary of the atrial activity and that the lack of invasive electrophysiological measurement is a limitation of this study. We have therefore included the following in the limitations section (L356-357): “Technical errors prevented us from assessing local changes in AFCL and atrial refractoriness”.

Reviewer #2: Annelies Decloedt, PhD

This manuscript describes the use of horses as a large animal model for chronic atrial fibrillation. The horse has been previously described as a potential large animal model for atrial fibrillation, however, most studies only described short term AFib and did not include ion channel expression and myocardial tissue fibrosis analysis. Therefore, this study could be a useful addition to the existing literature.

Specific comments:

1. My main remark is the power/statistical analysis of this study. Was a power calculation performed a priori or how was the sample size selected? The control group only consists of 3 horses, and the AF group of 6 horses. For example, the heart rate increases clearly during the study period but this is not significant, could this be due to lack of power? For the echocardiographic results, several measurements are missing which further decreases the sample size. This should be clarified by the authors.

This following section holds the answer to comment 1 and 2:

We acknowledge that our rather small sample size is a limitation to this study. The study was designed as a longitudinal study to facilitate repeated measurements and increase power. However, both AF induction and restoration of SR was more difficult than anticipated which
resulted in several missing values. We have elaborated on this in the limitations section. Line 348-355: “This study is limited by the relatively low number of animals included. The small sample size is strengthened by the longitudinal design of the study which facilitates repeated measurements, however, both the AF and SR measurements holds several missing values. The missing AF values are attributed to the prolonged AF induction, which could have been avoided by continuous burst-pacing. Sinus rhythm measurements were limited by the attenuated ability of flecainide to cardiovert longstanding AF. Future studies should consider either a more potent drug than flecainide or electrical cardioversion in order to obtain SR measurements.”

The small sample size and the missing values are also the reason for the very simple statistic applied, where longitudinal analysis only was performed on the echocardiographic examinations. For the ECG analyses (including AFR) and fibrosis analysis, t-tests were performed between baseline and day 55 (ECG) or between AF and control group (fibrosis). We believe that this is the most transparent statistic we could do with this dataset.

2. The statistical analysis should also be described in more detail. As measurements over different timepoints are being assessed, longitudinal analysis seems like the most logic choice of statistical method, however, the authors also mention t-tests. It is not clear which test was used for which comparison.

We agree that this could be described in greater detail, why the following changes have been made (L174-178): Changes in AFR and other ECG parameters between baseline and day 55, were assessed using student’s t-tests where paired analysis were performed when appropriate. Difference in fibrosis and ion channel expression between AF and control groups were also assessed using student’s t-test.

3. Second, the AF burden seems to differ largely between the horses, making this animal perhaps less interesting as a large animal model in an experimental study? The discussion should go more into detail about this, and about possible solutions for this problem.

We believe that the AF induction protocol was responsible for the difference in AF burden. Continuous burst-pacing are used in other models and in hind sight we should have done the same. In a project carried out by our group after this study we have used a higher pacing rate which resulted in much faster and more uniform AF induction (about 1 week of pacing for sustained AF). We therefore believe that the horse is a very interesting model but that a different AF induction protocol should be used in future studies.

This have been elaborated in the discussion (L 269-273): The pacemaker ensured a constant rate between 170-340 min-1 in the atria, however this did not lead to sufficient capture nor the development of self-sustained AF. Burst-pacing was therefore introduced and combined with pacing from the pacemaker it proved feasible for inducing self-sustained AF in horses. Other animal studies (8, 20) have used neurostimulators to automatically burst-pace the atria which is
desirable as it is less time-consuming for the operators and ensures a uniform and a most likely faster AF induction.

4. The AFR was measured from the surface ECG. As the horses were instrumented with leads in the right atrium anyway, wasn't it possible to measure the atrial fibrillation rate invasively from the intracardiac electrogram? Similarly, no electrophysiological measurements were included in the study, this is a major limitation.

We agree that this is a limitation and raises questions as both aEGM and aERP recordings were possible from the pacemaker leads. Both measurements were originally included in the protocol however; we chose to exclude these data as the results of both measurements contained several missing values (both due to recordings errors and poor quality (AFCL) and due to decreased cardioversion success rate (aERP)). As AFR derived from surface ECG is a valid measure of electrical remodeling, we decided to use this.

In addition, I have some other questions and suggestions:

Line 100: How long was manual burst pacing performed? As this was done manually, I presume that it was not during hours?

During AF induction we almost constantly had a person in the stable to manually start burst-pacing every time the horses cardioverted to SR. This resulted in 1-8 hours in total of burst-pacing per day for each horse which have been added in the manuscript (line 100). As discussed above this was quite time consuming and probably contribution to the prolonged and uneven AF induction

Line 117: Was flecainide also administered to horses that were not in AFib? Why? Why was it also administered to the control group? Was this done to assess the effect of flecainide on the echocardiographic measurements? Were measurements in sinus rhythm in the control group also performed after flecainide administration as in the AFib group? This is not entirely clear from the manuscript. Why was the pacemaker turned off 30 minutes before flecainide treatment?

All horses underwent the exact same protocol regardless of group (except for AF induction in the control group) and rhythm. That resulted in both control horses and horses in the AF group in SR were administered with flecainide. This was mainly due to the possible effect of flecainide on electrophysiology and less in respect to echocardiography.

The pacemaker was turned off 30 min before flecainide treatment to try to distinguish between spontaneous cardioversion and “flecainide cardioversion”. To clarify we have added ”. to avoid spontaneous cardioversion was attributed to the effect of flecainide”(line 118-119)

Line 236: did some horses develop mild valvular insufficiency during the study period?
In the AF group, one horse developed a “mild” aortic valvular insufficiency at day 55 compared to baseline where it was classified as “clinical insignificant”. In the control group one horse developed a “mild” mitral valvular insufficiency at day 55 compared to baseline. The valvular insufficiencies were graded into categories by one observer (all echocardiographic examinations, from which valvular insufficiencies were determined, were randomized and blinded) which is quite subjective and depend on how the images are obtained.

Line 238-239: is it correct that the difference between SR/baseline in SR and AF was not assessed? It is not entirely clear from these sentences that there was no evolution of LV function or size throughout the AF period, however, there may have been a difference compared to the baseline in SR.

We have previously shown that AF causes poor performance (Buhl 2018, J Vet Intern Med) so we would expect the LV function to decrease between AF and SR. However, as the aim of this study was to characterize the functional remodelling due to AF over time, we have only compared AF measurements to AF measurements and SR measurements to SR measurements, to show that horses do not go into heart failure as other animal models do. We have in line 242-243 included: To study ventricular remodelling and monitor signs of heart failure, LV size and function were in each group assessed during the study.

The ion channel expression methodology is included in the supplementary information but this is not clear from the manuscript. Furthermore, information is lacking about the reason why these ion channels specifically were chosen and whether changes have been demonstrated in horses in previous studies.

We thank the reviewer for bringing this to our attention. We have clarified the matter both in the manuscript (L159) and in the supporting material (L 50-51) “We investigated the most prominent calcium, sodium and potassium ion channels responsible for the cardiac action potential”

Fig. 3 is interesting but shows individual data of each horse and might therefore be replaced by a figure combining data of all horses.

This was also our initially thought; however, we feel that combining all the data might not give the full picture of the difference in AF burden. We therefore prefer to leave it as it is, but will naturally change it if important to the reviewer.

Fig. 6: from the figure legend, the difference between A/B and D/E is not clear.

We have been more specific in the figure legend (line 450-451, 454-455)

Supplementary Info:
How do you explain the different results for LAAmx in the AF group when measured in SR vs AF? In SR, the area does not change at all, while during AF the area increases significantly.

Although we do not see an increase in LAD, we suspect that there is a dilation of the atria. The LAD is a one-dimensional cross-section where the LAA is 2-dimensional and holds more information. At day 55 only two horses cardioverted on flecainide. It is possible that these two horses were less affected by remodeled/dilated atria, whereas the four horse that were unable to cardiovert on Day 55 had more dilated atria (hence affecting the mean values of LAAmx in the AF group measured during AF).

How do you explain the decrease of LAAa during the AF period?

Data could suggest some degree of diastolic dysfunction after cardioversion with reduced passive filling of the ventricle. However, with the limited number of horses and the lack of significance other than on Day 3, this remains speculations.

To our knowledge, this has not been described before. LAAa is measured just before the onset of the P wave where the atria are most relaxed/dilated. We know that there is an overload of both intra- and extra cellular calcium why it could be reasonable to suggest that the atria are in a somewhat contracted state shortly after cardioversion. We are currently using more advanced echocardiographic protocols shortly after spontaneous cardioversion and will for sure look into this.

Finally, the text contains several typos or grammatical errors (mainly verbs that are plural when they should be singular or vice versa). Extra proofreading is recommended.

We thank the reviewer for this very valuable feedback! We have proof read the manuscript and all changes can be found in the manuscript.