

# Proof of concept 3D ultrasound strain imaging measuring effect of a ring pessary on avulsed puborectal muscle

Corresponding Author: Dr Shreya Das

**This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.**

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Effect of a ring pessary on avulsed puborectal muscle through ultrasound strain imaging

Thank you for the opportunity to review this article. The authors describe a novel technique of ultrasound imaging to assess puborectal muscle function in women with a diagnosis of POP, pre and post pessary fitting.

I am not that familiar with the ultrasound technique described, however I am very familiar with TPUS and associated POP, thus have a few comments as regard the overall study.

1. The authors acknowledge that this is only a proof of concept study, but it would have been very helpful if they had included a qualitative assessment of the pessary use, as well as trying to quantify any 'functional' change.
2. Using the strain imaging of the PR muscle, I am assuming excludes the other the muscles of the levator ani complex, due to their variances in fibre orientation. Can the authors please comment, and how they would account for the contribution of these muscles to the functional component of the LA complex in the development of POP.
3. It is also unclear if the results were from 'projected strain values' or actual strain values.
4. Were the patients instructed to perform any PF muscle exercises prior to, or after pessary fitting?
5. Did the patients remove and reinsert their pessaries during the three month period and if so, was this captured at all?
6. Was there any information on the stage of prolapse for these patients prior to fitting – and associated symptom scores?
7. It is well recognised that prolapse occurs with intact LA muscles as well as those who have suffered avulsion. Do the authors see a use for this technique in a more general population of women?
8. Can the authors comment as to why an older cohort of women were chosen for this study. Whilst it is still highly possible that symptoms of POP are associated with a historical avulsion, there are likely other confounding factors that would contribute to the prolapse (hormonal changes, age etc.). How do the authors explain the results of 'improved function' after three months – with an injury that is likely to be > 20yrs with no added muscle rehabilitation?
9. The authors compare their results to normalised strain values obtained from a previous study, where the imaging was acquired from women an 'intact' PRM. The data from this study is acting as a 'control'. Please clarify the demographics of this cohort.
10. Avulsion is often diagnosed after childbirth with many younger women also experiencing symptoms of prolapse. Could the authors comment on the validity of US strain imaging in younger women?

Overall the manuscript is well written ( I have included a few minor comments in the pdf).

This may indeed add to our knowledge on the functional component of the pelvic floor muscle following avulsion, or indeed just in the development of prolapse. However, I would highly recommend that any future studies must include PROM's –

otherwise the exercise is merely academic.

Reviewer #2

(Remarks to the Author)

Summary

=====

In this paper, the authors describe the application of 3D strain imaging for assessing functional changes in women with pelvic organ prolapse who are treated with a ring pessary. The hypothesis that underpins this research is that the normalized strain ratio after three months of pessary usage will change to levels obtained in women with intact puborectal muscle (PRM).

## 2 Overall Evaluation

=====

This is an important and well-written paper by an excellent group of researchers. The study was conducted in a highly repeatable fashion and results show differences between the normalized strain before and after pessary; however, the size of the study cohort is too small to reach a meaningful conclusion. Yes, I agree that the results look very promising but I would be more excited if more women, but controls and women with POP, was included in the study. Another limitation, it would be nice to conduct measurements at other time points besides 3 months to get better insights into temporal changes of normalized strain. Also, there is no data to support the author's claim that after 3 months of pessary usage, the normalized strain levels are identical to those of women with intact (PRM). Another limitation is the lack of a goal standard to compare the results or even to get a sense of the maximum and minimum exerted by an individual during the study. Given these limitations, I recommend this article should not be accepted in its current form.

## 3 Specific Comments

=====

1. Page 2 line 1, why measure 3 months after placement and not other time points. It would be interesting to see how the strain patterns change 1, 3, 9, and 12 months after placements.
2. Page 2, Data acquisition. The maximum contraction will vary from woman to woman. Since the resulting strain will vary based on the contraction force, how was the normalization performed?
3. Page 6, it would also be useful to provide a representative strain image of a healthy volunteer
4. Page 10, limitation of work. Since no studies were performed on women with intact PRM there is no way to corroborate the hypothesis made at the outset of this work.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I would like to acknowledge the authors comprehensive response to my comments on their paper. I am delighted to see that they have now included PROM's as part of this study - and from my perspective a very important part!

I have a small further comment - I would suggest the authors consider modifying the title of the paper to reflect that this was indeed a small study. Including the term 'a pilot study' or 'proof of concept study' would better reflect the nature of the paper.

Otherwise I have no further comments. The authors have addressed all my other comments and concerns in this manuscript adequately, and I would recommend publication.

Good luck and I hope to this methodology expanded in the future.

## Reviewer #3

### (Remarks to the Author)

Thank you for allowing me to review this paper. It is a very interesting area of development in the urogynaecology field.

"The hypothesis of this study is that the normalized strain ratio after three months of pessary usage will change to levels obtained in women with intact PRMs as compared to the normalized strain ratio measured prior to pessary placement" – this should come at the end of the intro section.

"In this work, PGI-I as well as PGI-severity (PGI-S) have been reported as measures of condition of the PF [26-27]. We find that the NSR scores of these women also match to the PGI-I scale as reported by the women after three months of pessary use." – this should not be in the introduction, as it is a result.

My main concern is about the clinical usefulness of the study because the pessary was "Removed twenty minutes prior to data acquisition" – line 80. This is a very short period prior to measurement, the effect of the pessary will last for days if not weeks. I think this is a limitation as the true strain on the muscles will be masked. What this study is measuring is the short term therapeutic effect of the pessary being in place rather than the long term effect on the muscles. You say "In other words, effects were observed which were more than just the mechanical effect due to the presence of pessary." I would disagree with that as the mechanical effect will remain for some time after pessary removal. I think the authors recognise this when they say in line 281 "it cannot be yet said whether the partial recovery of the damaged end due to pessary would be present always."? In a current study we are asking women to remove their pessary 2 weeks before we collect symptom data.

Women perform a "Voluntarily contract" – line 64 - What instructions were the women given? Eg. to achieve maximal contraction? It is important to know a strong contraction was achieved.

There should be a fuller description of the POP-Q, PGI-S and PGI-I in the methods section (stages, response options etc). There is no title or footnote in Table 3 to explain what the POP-Q numbers relate to eg. anterior, posterior, apical stage? Usually use I, II, III, IV. Or what time point the data relate to.

Re ethical approval - line 84 - I am surprised that no ethical approval was required as the participants were having additional research data collected about them. Was there hospital or university ethical approval?

"As a first step, median values of the strain values were obtained for the three regions for the women." – line 134 – is this a median of multiple measurements for the same woman? Is this detail explained anywhere?  
Were questionnaires completed pre and post? If so, pre scores could be presented too.

### Minor changes

1. Generally I feel the English in the paper should be checked as the meaning of what is written in not always clear.
2. Normalized strain ratios – introduce the abbreviation on first use.
3. "...can be quantified by US-based strain imaging whereas the woman voluntarily contracts her PRM [8]." Check wording in this sentence in line 34
4. "patients' perspective" – move apostrophe in line 44 to patient's
5. Patient Specific Outcome Measures (PROMs) – line 46, change to Patient Reported Outcome Measures
6. PROMs allow the clinician to better understand the patient symptoms as POP-quantification (POP-Q) score allow the understanding of the anatomy of PF support [29]. – line 46-48, correct the wording here.
7. In this work, PGI-I as well as PGI-severity (PGI-S) have been reported as measures of condition of the PF – these are measures of women's perceptions of the severity / improvement in their condition – line 50
8. These characteristics are listed as indicators of the prolapse and the condition of their PF muscles. – line 161 – this is not accurate, POP-Q, PGI-S and PGI-I do not relate to the muscles.
9. "These scores, obtained from these women are detailed in table 3" – move to results - line 82
10. The reference numbering needs re-done now that new references have been added.
11. "However, since the muscle fiber direction is dependent on the location, "– location of what? - line 101
12. The normalized strain ratio (NSR) was calculated for all women, before pessary placement and three months after using a pessary. – line 147 – add "with the pessary taken out 20 minutes before"?
13. The developed method have been applied – line 153 – "methods" plural.
14. Basal Metabolic Index (BMI) – table 1, change to body mass index
15. Tables 1 and 2 could be combined.
16. ...the presented analysis might turn out to be a parameter. – line 335 – I don't understand what this means
17. Future extensions of this work could also be extended to the whole LA muscles, apart from PRM – line 344 – "...as well as PRM" This sentence is repeated again in line 349, delete.
18. "Table 2 shows the demographic characteristics of the women with intact PRMs, such as median of age and body mass index (BMI) and parity. This table, from our previous publication is included here for readability." – I don't think this fits in the Discussion section.

#### Reviewer #4

(Remarks to the Author)

Thank you for the opportunity to review this article. The authors introduce a novel technique utilizing ultrasound imaging to assess puborectal muscle function in women diagnosed with pelvic organ prolapse (POP), both prior to and following pessary fitting. Given my extensive experience managing over 700 cases of POP, I am well-acquainted with transperineal pelvic ultrasound (TPUS), POP, and the application of vaginal pessaries. I would like to offer several comments regarding the study's overall approach and the responses provided by the authors.

1. The authors appropriately identify this study as a proof-of-concept; however, the qualitative assessment of pessary use lacks a direct correlation to the anticipated quantification of functional change based on ultrasound imaging of puborectal muscles (PRMs). The reliance on the prolapse score (POP-Q) and the Patient Global Impression of Severity (PGI-S) and Improvement (PGI-I) scales is insufficient without a clear link to the ultrasound findings.
2. The precise function of the PRMs and the entire LA complex remains inadequately understood. This research can be more convincing if utilize ultrasound imaging of the PRMs to elucidate the impact of the complete LA complex on the development of POP.
3. The authors' comparison of their findings with normalized strain values derived from a previous study, which utilized data from women with an 'intact' PRM, lacks validity due to notable demographic differences between the cohorts. To enhance the rigor of this study, it is essential to incorporate matched comparisons with appropriate control groups.
4. All reviewers have adequately pointed out the critical issue of the small cohort size, which limits the ability to draw meaningful conclusions. While the initial results appear promising, the inclusion of both control subjects and women with POP is imperative. Furthermore, the study's inclusion and exclusion criteria were insufficiently defined, resulting in significant variability among patient conditions. Consequently, the clinical applicability of the 3D volumetric ultrasound imaging of the PRMs is not convincingly established. A more stringent prospective cohort design, along with estimated case numbers for improved predictive power, is necessary to enhance the significance of this study.

Version 2:

Reviewer comments:

#### Reviewer #3

(Remarks to the Author)

I am happy with the responses to my comments provided by the authors, and the additions to the manuscript.

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## Reviewer #1

### Effect of a ring pessary on avulsed puborectal muscle through ultrasound strain imaging

Thank you for the opportunity to review this article. The authors describe a novel technique of ultrasound imaging to assess puborectal muscle function in women with a diagnosis of POP, pre and post pessary fitting.

I am not that familiar with the ultrasound technique described, however I am very familiar with TPUS and associated POP, thus have a few comments as regard the overall study.

1. The authors acknowledge that this is only a proof of concept study, but it would have been very helpful if they had included a qualitative assessment of the pessary use, as well as trying to quantify any 'functional' change.

- We would like to thank the reviewer for the comment. Indeed this would be an excellent addition to the manuscript, to include functional as well as qualitative assessment. The functional changes have been quantified using the normalised strain ratio (NSR), in the manuscript. Now, we have included this additional information on the pessary usage in the form of the below table, as qualitative assessment. This table (Table 3 in the revised manuscript) shows the prolapse score or POP-Q score as well as the PGI-S (Patient Global Impression of Severity) and PGI-I (Patient Global Impression of Improvement) scales [26-27].

Table 3:

| Patient No. | Avulsion type | Age (yrs) | BMI (Kg/m <sup>2</sup> ) | Parity (no. of vaginal deliveries) | POP-Q score | PGI-S scale | PGI-I scale      |
|-------------|---------------|-----------|--------------------------|------------------------------------|-------------|-------------|------------------|
| 1           | Unilateral    | 71        | 23.43                    | 2                                  | 3,3,3       | Moderate    | Very much better |
| 2           | Unilateral    | 64        | 23.14                    | 2                                  | 3,2,2       | Mild        | Much better      |
| 3           | Unilateral    | 66        | 25.69                    | 2                                  | 3,0,1       | Severe      | Much better      |
| 4           | Unilateral    | 39        | 24.16                    | 2                                  | 2,0,2       | Moderate    | Much better      |
| 5           | Unilateral    | 35        | 20.45                    | 3                                  | 2,0,1       | Moderate    | Much better      |
| 6           | Unilateral    | 79        | 23.51                    | 4                                  | 3,3,1       | Severe      | Much better      |
|             |               |           |                          |                                    |             |             |                  |
| 7           | Bilateral     | 67        | 19.38                    | 2                                  | 3,2,2       | Moderate    | Much better      |
| 8           | Bilateral     | 71        | 25.51                    | 2                                  | 3,1,1       | Moderate    | Much better      |
| 9           | Bilateral     | 65        | 23.88                    | 2                                  | 2,1,1       | Moderate    | Much better      |

(POP-Q score: Anterior compartment stadium, Central compartment stadium, Posterior compartment stadium) (BP: Before pessary, AP: After pessary)

Please note that the red parts in the above table are not included in table 3 of the manuscript, since table 1 in the manuscript already mentions the medians of age, BMI and parity.

- All these women also reported betterment or improvement of their experience after pessary use of three months, as shown in table 3. POP-Q scores of these women show that all these women had some levels of anterior and posterior vaginal wall prolapse. Thereafter, after pessary use, improvement shown by the PGI-I scores show that the changes in NSR is also reflected by these scores. Review of current literature shows that PROMs like PGI-I are

essential in assessing the outcome of a treatment equal to anatomic measurements [29]. Thus, NSR provides an additional quantitative measure of the success of pessary use after three months along with the qualitative PROM.

- The above explanation has been now included in the revised manuscript, in the Discussion section.

**2. Using the strain imaging of the PR muscle, I am assuming excludes the other the muscles of the levator ani complex, due to their variances in fibre orientation. Can the authors please comment, and how they would account for the contribution of these muscles to the functional component of the LA complex in the development of POP.**

- As is well known, levator ani (LA) muscle is a muscle complex consisting of PRM as well as pubococcygeous and iliococcygeous muscles [17-18]. The support provided by the LA is combination of the function of all the constituent muscles as well as connective tissues and smooth muscle [1]. It is thus very difficult to assess the functional component of the entire LA complex without at least measuring strain also from all the constituent muscles. Furthermore, as several studies already show that the contributing factors of the components, be it the constituent muscles of the LA or the connective tissues or the injuries to the muscles have to be considered through studying their biomechanics [22, 28]. Thus, for our current study, since we focussed only on the function of PRM, we feel unable to provide an explanation about the influence of whole LA complex on the development of POP.
- For this work, we needed a way to distinguish only PRM from the other muscles of LA complex. We used US volumes, thus we could distinguish this muscle using first segmentation of PRM at rest and then fibre orientation to calculate the strain in correct directions, as the reviewer already deduced.

**3. It is also unclear if the results were from 'projected strain values' or actual strain values.**

- The results shown in the article are all actual strain values projected in the fibre direction or orientation, using vector projection. In mathematical representation, these are least square strain estimation (LSQSE) values, aligned with the fibre orientation [14].

**4. Were the patients instructed to perform any PF muscle exercises prior to, or after pessary fitting?**

- These women were not instructed to do any exercise throughout the time of before to three months after pessary fitting. Thus there was no instructed pelvic floor muscle therapy (PFMT) and the women also reported that they had not exercised themselves.
- This information is now added to the manuscript, in Data Acquisition section.

**5. Did the patients remove and reinsert their pessaries during the three month period and if so, was this captured at all?**

- These women did not remove the pessary. At the visit after three months the pessary was removed and cleaned for the first time after insertion.
- This information is now added to the manuscript, in Data Acquisition section.

**6. Was there any information on the stage of prolapse for these patients prior to fitting – and associated symptom scores?**

- The stage and area of prolapse regarding the anterior, central and posterior compartment is available as the POP-Q score (and now given in Table 3). This information shows the stage of prolapse prior to pessary fitting.
- Table 3 (as shown in the response to comment 1) also mentions the PGI-S scale for these women. These are the associated symptom scores.
- This information is now added to the revised manuscript.

**7. It is well recognised that prolapse occurs with intact LA muscles as well as those who have suffered avulsion. Do the authors see a use for this technique in a more general population of women?**

- We would like to thank the reviewer for this comment as the response gives us an opportunity to explain about the future use of this technique.

It is indeed well recognised that prolapse occurs for both intact as well as avulsed LA muscles. Moreover, childbirth is one of the main causes of damage to one of the LA muscles, i.e., the PRM. Thus, for a more general population of women, such as women who would like to be pregnant, women at different time points during pregnancy, and women immediately after delivery could benefit from strain imaging. For women who would like to become pregnant and during pregnancy, strain imaging would already determine the health of their PRMs. And for women just after delivery, health of the PRM could be checked irrespective of the presence of absence of avulsion. The health of the PRM would then enable the clinician to choose timely and relevant treatment.

Furthermore, this technique could also be extended to the whole LA muscles, apart from PRM. Strain imaging could in principle be performed for all the LA muscles, if segmentation of those muscles are available. Thus the entire LA muscles could be monitored by means of strain imaging. Also, for monitoring the health of the LA muscles, this technique could be applied for woman of any age, including young women.

- This information is now added to the revised manuscript in Limitations and future work section.

**8. Can the authors comment as to why an older cohort of women were chosen for this study. Whilst it is still highly possible that symptoms of POP are associated with a historical avulsion, there are likely other confounding factors that would contribute to the prolapse (hormonal changes, age etc.,). How do the authors explain the results of 'improved function' after three months – with an injury that is likely to be > 20yrs with no added muscle rehabilitation?**

- The women who were included in this study came to the clinic with prolapse and were diagnosed with unilateral or bilateral avulsion. Typically, these women were of older age. These nine women were randomly chosen for this study, without considering their age. Thus, the inclusions happen to belong to an older cohort. Choice of an older cohort was not deliberate.

- This being a proof of concept study with small dataset, likely confounding factors like age, hormonal changes or others could not be considered. The effects of these factors can only be considered in further studies with datasets consisting of appropriate inclusions.
- We would agree with the reviewer's observation and comment that it is not appropriate to use the term 'improved function' after only three months of pessary use for a likely old injury, without any muscle rehabilitation. Thus this term is now removed and a possible explanation is provided for the observed change in NSR.
- A possible explanation for the observed reduction in NSR after three months of pessary use can be the 'contraction hypothesis' as explained in literature [23]. In this hypothesis, it is explained that women with POP try to relieve their symptoms by contracting the PRM, which counteracts the abnormal pressure gradient originating during POP development. The presence of vaginal pessaries support the POP and thus the need for this constant contraction is nullified. This would lead to the ability of the PRM to relax. Since strain is a measure of contraction of the muscle, the less contraction would also lead to less strain.
- The PRM is a striated muscle fibre and also from our results we find that the contraction is happening in the mid-region of the muscle. In case of women with avulsion, less contraction and less strain would mean also less strain in the mid-region of the PRM. This would lead to the lowering of the NSR after pessary use of three months. Thus in presence of the support provided by pessary, the constant contraction of the PRM was reduced.
- We have added the term 'contraction hypothesis' in the revised manuscript, in the Discussions section.

**9. The authors compare their results to normalised strain values obtained from a previous study, where the imaging was acquired from women an 'intact' PRM. The data from this study is acting as a 'control'. Please clarify the demographics of this cohort.**

- The normalized strain values are indeed from a previous study in which women had intact PRMs [20]. The demographics of this cohort is mentioned below.

Table 2:

|                                    | Women with intact PRMs<br>(n=8) |
|------------------------------------|---------------------------------|
| Median of age (yrs)                | 34                              |
| Median of BMI (Kg/m <sup>2</sup> ) | 20.9                            |
| Parity                             | 0                               |

- This table and brief explanation is now added to the revised manuscript as Table 2.

**10. Avulsion is often diagnosed after childbirth with many younger women also experiencing symptoms of prolapse. Could the authors comment on the validity of US strain imaging in younger women?**

- We would like to state that US strain imaging would also be feasible in younger women, there is no age effect in imaging the PRM. So also young women with symptoms would benefit since

the contractile function of their PRMs could be monitored and potentially be treated accordingly. However, additional studies have to be performed to demonstrate the efficacy in all these different populations.

- Please also see the response of comment 7.

**Overall the manuscript is well written (I have included a few minor comments in the pdf).**

- We would like to thank the reviewer for the encouraging comment. Changes have been made in the manuscript according to the comments.
- **Other comments by Reviewer #1 (mentioned in the manuscript):**
  - Comment 1:** The indicated typo in Section 2.3 of the manuscript has been removed.
  - Comment 2:** The text in question in Section 3 of the manuscript has been modified.
  - Comment 3:** The explanation as asked by the reviewer in Section 3.1 of the manuscript has been provided in the Discussion, in Section 4.2.
  - Comment 4:** Figure 5 of the manuscript is improved according to the comment.

**This may indeed add to our knowledge on the functional component of the pelvic floor muscle following avulsion, or indeed just in the development of prolapse. However, I would highly recommend that any future studies must include PROM's – otherwise the exercise is merely academic.**

- We would like to thank the reviewer for this comment. We have now included PROMs and it is indeed our objective in future to be able to investigate the state of the pelvic floor muscles and the development of prolapse through strain imaging in larger cohorts. Also, we agree that future studies should also be done keeping in mind clinical relevance.
- We also completely agree with the reviewer that including PROMs is essential. Thus, PGI-I and PGI-S scales are mentioned in Table 3, to include PROM. It is also now included in the revised manuscript.

## Reviewer #2 (Remarks to the Author):

### Summary

=====

In this paper, the authors describe the application of 3D strain imaging for assessing functional changes in women with pelvic organ prolapse who are treated with a ring pessary. The hypothesis that underpins this research is that the normalized strain ratio after three months of pessary usage will change to levels obtained in women with intact puborectal muscle (PRM).

### 2 Overall Evaluation

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- **This is an important and well-written paper by an excellent group of researchers. The study was conducted in a highly repeatable fashion and results show differences between the normalized strain before and after pessary; however, the size of the study cohort is too small to reach a meaningful conclusion. Yes, I agree that the results look very promising but I would be more excited if more women, but controls and women with POP, was included in the study.**
  - We would like to thank the reviewer for an encouraging comment.
  - Indeed the number of included women with POP is small. But since we looked at the difference before and after pessary placement, the women serve as their own control and in all women a decrease of the NSR is observed (except one in which only a very small increase was observed close to the measurement error). These findings are in accordance with a previous study in women with intact PRMs and women with avulsed PRMs [20]. Thus, we were convinced of the results obtained for the current study to include women before and after pessary use.
- **Another limitation, it would be nice to conduct measurements at other time points besides 3 months to get better insights into temporal changes of normalized strain.**
  - The objective of this pilot study was to determine whether there could be any difference in strain from before to after pessary usage of three months. The three months' time point is clinically relevant since in the Netherlands women get their first check after pessary placement at this time point. We agree that the change of strain pattern could be valuable information regarding capturing the gradual and temporal changes in the PRM due to the presence of pessary over a longer time. But that would warrant an additional study with relevant time points.
- **Also, there is no data to support the author's claim that after 3 months of pessary usage, the normalized strain levels are identical to those of women with intact (PRM).**
  - Indeed, the NSR values are not identical to those of women with intact PRMs. But in a previous study it has been demonstrated that even in women with intact PRMs, not all NSR values are identical. It has been seen that NSR value close to 1 or below 1 are found in women with intact PRMs, while values above one are observed in women with avulsions [20]. The findings in the current study are fully in line with these previous findings. We like to emphasize that the main finding in this study is that all NSR values reduce to values closer to one with respect to the NSR before pessary

placement. Therefore, we did not mention that the normalized strain levels are identical to those in women with intact PRM, rather that they are in the same range.

- **Another limitation is the lack of a goal standard to compare the results or even to get a sense of the maximum and minimum exerted by an individual during the study. Given these limitations, I recommend this article should not be accepted in its current form.**
  - In previous studies, it has been demonstrated that NSR values provide a good representation of the function of the PRM. Although tissue strain analysis has proven to be very effective in improving diagnosis in cardiac imaging, breast cancer detection and some other applications, quantitative values of strain imaging in skeletal muscles remains scarce. To the best of our knowledge, it is not possible to directly measure strain in an individual muscle from a group of muscles in which the individual muscles are situated very close to each other. The individual muscles can only be distinguished by their function and fibre orientation. Thus we have also used fibre orientation in developing our method. Currently, there is no gold standard available in current literature about strain in pelvic floor muscles. Therefore, there is also not a standard range of values which shows maximum or minimum contraction in the PRM. Thus, we have used NSR as a means to normalize with the amount of contraction that the woman is exerting. This is then inherently independent of any standard value of maximum contraction.

### **3 Specific Comments**

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**1. Page 2 line 1, why measure 3 months after placement and not other time points. It would be interesting to see how the strain patterns change 1, 3, 9, and 12 months after placements.**

- We would like to thank the reviewer for this comment as it would be useful to check the PRM and changes in strain throughout different time points. The data acquired from the women included in this study were indeed for only two time points; before pessary fitting and after three months of pessary usage. Since, this was a pilot study and we could only acquire data from these women according to the time points which were clinically relevant. But indeed, it could be beneficial to get more information about the process of changes in the PRM due to pessary, through strain, over time. Moreover, to include the extra time points, data acquisition needs to be more than the current and usual clinical convention. Additional data acquisitions would also involve additional requests for permission from ethics committee. Thus, we are convinced that this warrants an additional study.

**2. Page 2, Data acquisition. The maximum contraction will vary from woman to woman. Since the resulting strain will vary based on the contraction force, how was the normalization performed?**

- This is an important point and exactly the reason why we developed a normalised strain value by calculating the NSR that inherently compensates for the variation in maximum contraction.
- Please see also the response to the last comment in Overall Evaluation.

**3. Page 6, it would also be useful to provide a representative strain image of a healthy volunteer.**

- An example of an intact PRM is mentioned in Figure 2a of the manuscript.

**4. Page 10, limitation of work. Since no studies were performed on women with intact PRM there is no way to corroborate the hypothesis made at the onset of this work.**

- Projected strain has been calculated in previous publication, which we refer to in this article [20]. So, a previous study was performed in women with intact PRMs.

#### **References:**

**(Note: Reference numbers are identical to the reference numbers in the manuscript. The newly added references are shown in bold.)**

[1] J. O. L. DeLancey et al., "Comparison of levator ani muscle defects and function in women with and without pelvic organ prolapse," *Obstetrics and Gynecology*, vol. 109, no. 2 PART 1, pp. 295–302, 2007.

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[18] M. Heit, J. T. Benson, B. Russell, and L. Brubaker, "Levator ani muscle in women with genitourinary prolapse: Indirect assessment by muscle histopathology," *Neurourology and Urodynamics: Official Journal of the International Continence Society*, vol. 15, no. 1, pp. 17–29, 1996.

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**Reviewers' comments:**

**Reviewer #1 (Remarks to the Author):**

I would like to acknowledge the authors comprehensive response to my comments on their paper. I am delighted to see that they have now included PROM's as part of this study - and from my perspective a very important part!

I have a small further comment - I would suggest the authors consider modifying the title of the paper to reflect that this was indeed a small study. Including the term ' a pilot study' or 'proof of concept study' would better reflect the nature of the paper.

Otherwise I have no further comments. The authors have addressed all my other comments and concerns in this manuscript adequately, and I would recommend publication.

**Good luck and I hope to this methodology expanded in the future.**

- We would like to thank the reviewer for his/her encouragement and also agree that the title of the article should include the phrase 'a proof of concept study'.

**Reviewer #3 (Remarks to the Author):**

Thank you for allowing me to review this paper. It is a very interesting area of development in the urogynaecology field.

"The hypothesis of this study is that the normalized strain ratio after three months of pessary usage will change to levels obtained in women with intact PRMs as compared to the normalized strain ratio measured prior to pessary placement" – this should come at the end of the intro section.

"In this work, PGI-I as well as PGI-severity (PGI-S) have been reported as measures of condition of the PF [26-27]. We find that the NSR scores of these women also match to the PGI-I scale as reported by the women after three months of pessary use." – this should not be in the introduction, as it is a result.

- We would like to thank the reviewer for the recognising the fact that this area of development in the urogynaecology field is an interesting one and also for providing the comments.
- The minor comments mentioned above have now been incorporated in the manuscript.

My main concern is about the clinical usefulness of the study because the pessary was "Removed twenty minutes prior to data acquisition" – line 80. This is a very short period prior to measurement, the effect of the pessary will last for days if not weeks. I think this is a limitation as the true strain on the muscles will be masked. What this study is measuring is the short term therapeutic effect of the pessary being in place rather than the long term effect on the muscles. You say "In other words, effects were observed which were more than just the mechanical effect due to the presence of pessary." I would disagree with that as the mechanical effect will remain for some time after pessary removal. I think the authors recognise this when they say in line 281 "it cannot be yet said whether the partial recovery of the damaged end due to pessary would be present always."? In a current study we are asking women to remove their pessary 2 weeks before we collect symptom data.

- Indeed, we have measured the short term effect of pessary use of three months, through strain. It is our understanding that if the pessary use of three months had no lasting effect on the muscle due to its presence, then the muscle would immediately revert back to the condition which was before pessary use. When we mention 'mechanical effect of the pessary', what we mean is the support that is provided when pessary is in use.
- This lasting effect in this case, is the difference in strain measured after pessary use from strain measured before pessary use. We observed that strain had changed and the only difference was the pessary use. Thus this change is concluded as the effect of pessary. As is already mentioned, how long this effect would remain, cannot be determined from these strains only. It would indeed need further and longer measurements, as the reviewer has mentioned as an example, that symptom data are being collected 2 weeks after pessary removal.
- In our understanding, measuring the short term effect would be the first step before determining any long term effect. To our knowledge, there has been no study which has measured this short term effect of pessary use on the puborectal muscle through strain from 3D ultrasound volumes. Thus this is the contribution of our study. Further steps would be indeed more inclusions and data acquisitions after longer time after pessary removal.
- The summary of the above points have been included in the Discussion of the article.

**Women perform a “Voluntarily contract” – line 64 - What instructions were the women given? Eg. to achieve maximal contraction? It is important to know a strong contraction was achieved.**

- The presence of PRM contraction in these women were ensured by a positive biofeedback while the clinician observed the muscle contraction in the US machine during data acquisition. If the contraction was not considered adequate by the clinician, the women was asked to contract again.
- We have now mentioned the last sentence in the article, in the Data Acquisition sub-section.

**There should be a fuller description of the POP-Q, PGI-S and PGI-I in the methods section (stages, response options etc). There is no title or footnote in Table 3 to explain what the POP-Q numbers relate to eg. anterior, posterior, apical stage? Usually use I, II, III, IV. Or what time point the data relate to.**

- PGI-I and PGI-S have now been briefly explained in the Introduction. Stages and response options have been included in table 1 and mentioned in the Data Acquisition sub-section of Methods.

Table 1 (from [27]):

| <b>Patient Global Impression of Severity (PGI-S) scale</b> | <b>Patient Global Impression of Improvement (PGI-I) scale</b> |
|------------------------------------------------------------|---------------------------------------------------------------|
| Very much better                                           | Normal                                                        |
| Much better                                                | Mild                                                          |
| A little better                                            | Moderate                                                      |
| No change                                                  | Severe                                                        |
| A little worse                                             | -                                                             |
| Much worse                                                 | -                                                             |
| Very much worse                                            | -                                                             |

- The reference for POP-Q has been mentioned in the Introduction. The footnote is now added below in Table 3. It is also added to the manuscript.

Table 3:

| <b>Patient No.</b> | <b>Avulsion type</b> | <b>POP-Q score<sup>*</sup></b> | <b>PGI-S scale</b> | <b>PGI-I scale</b> |
|--------------------|----------------------|--------------------------------|--------------------|--------------------|
| 1                  | Unilateral           | 3,3,3                          | Moderate           | Very much better   |
| 2                  | Unilateral           | 3,2,2                          | Mild               | Much better        |
| 3                  | Unilateral           | 3,0,1                          | Severe             | Much better        |
| 4                  | Unilateral           | 2,0,2                          | Moderate           | Much better        |
| 5                  | Unilateral           | 2,0,1                          | Moderate           | Much better        |
| 6                  | Unilateral           | 3,3,1                          | Severe             | Much better        |
|                    |                      |                                |                    |                    |
| 7                  | Bilateral            | 3,2,2                          | Moderate           | Much better        |
| 8                  | Bilateral            | 3,1,1                          | Moderate           | Much better        |
| 9                  | Bilateral            | 2,1,1                          | Moderate           | Much better        |

(\*POP-Q score: Anterior compartment stadium, Central compartment stadium, Posterior compartment stadium)

**Re ethical approval - line 84 - I am surprised that no ethical approval was required as the participants were having additional research data collected about them. Was there hospital or university ethical approval?**

- No additional research data were collected from the participants other than what was required for the routine diagnostic procedures. That is why, the Medical Research Ethics Committee of UMC Utrecht exempted the project from approval. There were no further hospital or university ethical approval.
- We have used the same US volumes for our purposes that the clinician has used for her diagnosis. Research consent forms were also signed by each of the women.
- We could have publications in other journals with the same ethical approval, such as,
  - o Ultrasound in Medicine and Biology (UMB)
  - o International Urogynecology Journal (IUJ)
  - o Insights in Imaging

**“As a first step, median values of the strain values were obtained for the three regions for the women.” – line 134 – is this a median of multiple measurements for the same woman? Is this detail explained anywhere?**

- The strain which is calculated using the developed method, is strain for every 3D index in the muscle. These indices are actually positions of the muscle in the acquired 3D ultrasound volume. Since we obtain strain for every position or indices, median of all those strain values in a certain region (mid, right or left) have been taken for the strain ratio calculation.
- Thus it is not median of multiple measurements for the same woman.
- The method in details is explained in articles as given in [8] and [20].

**Were questionnaires completed pre and post? If so, pre scores could be presented too.**

- We did not ask the patients to fill out pre-questionnaires in this study, so consequently those cannot be included.

#### **Minor changes**

1. Generally I feel the English in the paper should be checked as the meaning of what is written in not always clear.
2. Normalized strain ratios – introduce the abbreviation on first use.
3. “...can be quantified by US-based strain imaging whereas the woman voluntarily contracts her PRM [8].” Check wording in this sentence in line 34.
4. “patients' perspective” – move apostrophe in line 44 to patient’s.
5. Patient Specific Outcome Measures (PROMs) – line 46, change to Patient Reported Outcome Measures.
6. PROMs allow the clinician to better understand the patient symptoms as POP-quantification (POP-Q) score allow the understanding of the anatomy of PF support [29]. – line 46-48, correct the wording here.
7. In this work, PGI-I as well as PGI-severity (PGI-S) have been reported as measures of condition of the PF – these are measures of women’s perceptions of the severity / improvement in their condition – line 50.

8. These characteristics are listed as indicators of the prolapse and the condition of their PF muscles. – line 161 – this is not accurate, POP-Q, PGI-S and PGI-I do not relate to the muscles.

9. “These scores, obtained from these women are detailed in table 3” – move to results - line 82.

10. The reference numbering needs re-done now that new references have been added.

11. “However, since the muscle fiber direction is dependent on the location, “– location of what? - line 101.

12. The normalized strain ratio (NSR) was calculated for all women, before pessary placement and three months after using a pessary. – line 147 – add “with the pessary taken out 20 minutes before”?

13. The developed method have been applied – line 153 – “methods” plural.

14. Basal Metabolic Index (BMI) – table 1, change to body mass index.

15. Tables 1 and 2 could be combined.

16. ...the presented analysis might turn out to be a parameter. – line 335 – I don’t understand what this means

17. Future extensions of this work could also be extended to the whole LA muscles, apart from PRM – line 344 – “...as well as PRM” This sentence is repeated again in line 349, delete.

18. “Table 2 shows the demographic characteristics of the women with intact PRMs, such as median of age and body mass index (BMI) and parity. This table, from our previous publication is included here for readability.” – I don’t think this fits in the Discussion section.

- We would like to thank the reviewer for checking and drawing our attention to all these errors in the manuscript and we agree to all these changes. These changes ( as suggested in point 1-14 and 16-18) have now been implemented in the manuscript.
- The tables 1&2 are combined along with mentioning that the last column of the table is also published in a previous publication (as suggested in point 15).

**Reviewer #4 (Remarks to the Author):**

Thank you for the opportunity to review this article. The authors introduce a novel technique utilizing ultrasound imaging to assess puborectal muscle function in women diagnosed with pelvic organ prolapse (POP), both prior to and following pessary fitting. Given my extensive experience managing over 700 cases of POP, I am well-acquainted with transperineal pelvic ultrasound (TPUS), POP, and the application of vaginal pessaries. I would like to offer several comments regarding the study's overall approach and the responses provided by the authors.

- We would like to thank the reviewer for the comments provided.

**1. The authors appropriately identify this study as a proof-of-concept; however, the qualitative assessment of pessary use lacks a direct correlation to the anticipated quantification of functional change based on ultrasound imaging of puborectal muscles (PRMs). The reliance on the prolapse score (POP-Q) and the Patient Global Impression of Severity (PGI-S) and Improvement (PGI-I) scales is insufficient without a clear link to the ultrasound findings.**

- We would like to mention that the ultrasound findings or images also show the presence of avulsion when data was acquired before pessary fitting. Thereafter, as PGI-I score showed improvement after pessary use of three months, corresponding reduction in strain (through reduction in normalized strain ratio (NSR) could also be observed.
- To the best of our knowledge there is no qualitative assessment of the pessary that can be linked to the ultrasound findings apart from the fact that patients were satisfied with the pessary which translates to the PGI-I score. One way to add more quantitative data would be to investigate muscle functionality by electromyograms (EMGs). However, this is not feasible to incorporate in this study anymore.

**2. The precise function of the PRMs and the entire LA complex remains inadequately understood. This research can be more convincing if utilize ultrasound imaging of the PRMs to elucidate the impact of the complete LA complex on the development of POP.**

- The reviewer has already mentioned in the first comment that it is indeed correct to term this study as a proof of concept study. We believe strain analysis of PRM as one of the muscles of the LA complex is the first step towards strain analysis of the entire LA complex on the development of POP. We have also stated this in the manuscript as future extensions of this proof of concept.

**3. The authors' comparison of their findings with normalized strain values derived from a previous study, which utilized data from women with an 'intact' PRM, lacks validity due to notable demographic differences between the cohorts. To enhance the rigor of this study, it is essential to incorporate matched comparisons with appropriate control groups.**

- We would agree with the reviewer that matched comparisons with appropriate control groups would be ideal. However, as we mentioned that this is a proof of concept study, control groups could not be matched. We only had a small group of women for our study. The group of women with 'intact' PRM has been considered as a control group only because their PRMs were intact and they are completely or vaginally nulliparous women. Whereas, all women with avulsed PRMs were with parity 2 or 3. Thus, although the difference in median of age between the two groups exist, the group with 'intact' PRMs could still be considered as a control group.
- We do agree, that in future studies with more number of inclusions, it would be essential to incorporate matched comparisons with appropriate control groups.

- The above points have been included in the Future works in the manuscript.

**4. All reviewers have adequately pointed out the critical issue of the small cohort size, which limits the ability to draw meaningful conclusions. While the initial results appear promising, the inclusion of both control subjects and women with POP is imperative. Furthermore, the study's inclusion and exclusion criteria were insufficiently defined, resulting in significant variability among patient conditions. Consequently, the clinical applicability of the 3D volumetric ultrasound imaging of the PRMs is not convincingly established. A more stringent prospective cohort design, along with estimated case numbers for improved predictive power, is necessary to enhance the significance of this study.**

- We agree once again, that this has been a small scale proof of concept study only. The title of the article now includes the term 'proof of concept study'. Thus it is not possible to make far reaching clinical conclusions based only on the results obtained from this study. We would like to emphasize that this is a precursor to a longer and large scale study with appropriately matched patients and controls.

#### **References (numbered same as in the manuscript)**

- [8] S. Das et al., "3D ultrasound strain imaging of puborectalis muscle," *Ultrasound in Medicine & Biology*, vol. 47, no. 3, pp. 569–581, 2021.
- [20] S. Das et al., "3D ultrasound strain imaging of puborectal muscle with and without unilateral avulsion," *International urogynecology journal*, vol. 34, no. 9, pp. 2225–2233, 2023.
- [27] I. Yalcin, R. C. Bump RC, "Validation of two global impression questionnaires for incontinence," *Am J Obstet Gynecol*;189(1):98-101, 2003.