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Quantification of levator ani hiatus enlargement by Magnetic Resonance Imaging in males and females with pelvic organ prolapse

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Cover letter

Dear Editor,

Enclosed please find the revised version of the paper entitled **"**Quantification of levator ani hiatus enlargement by Magnetic Resonance Imaging in males and females with pelvic organ prolapse" by V. Piloni *et al*. The present version has been modified according to the constructive criticisms of the reviewers.

I'm sending it to you in the hope that you'll consider it suitable for publication in your Journal.

Thank you for your interest in this matter

Sincerely yours

Vittorio Piloni, md

Vittorio Piloni

TITLE:

Quantification of Levator Ani Hiatus Enlargement by Magnetic Resonance Imaging in Males and Females with Pelvic Organ Prolapse

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KEYWORDS:
Levator ani hiatus, pelvic organ prolapse, magnetic resonance imaging, pelvic examination, transperineal ultrasonography, MR defecography, endopelvic fascia, hiatus size measurement

SUMMARY:
Here we present a protocol to standardize the measurement of the levator ani hiatus size by magnetic resonance imaging. The purpose is to extract biomechanical inferences from image analysis by comparing resting and straining values in patients of both sexes with pelvic prolapse, using consistent anatomical bony landmarks.

ABSTRACT:
Here we present a protocol to examine the levator ani hiatus in males and females with pelvic organ prolapse, during the Valsalva maneuver and while evacuating acoustic gel, using a horizontally oriented 1.5 T magnetic resonance (MR) scanner. On midsagittal images, the vertical distance of pelvic organs is measured in millimeters relative to the hymen plane (female) and to the lower border of the symphysis pubis (male), preceded by - (above) or + (below) signs. On axial images, the levator ani area is calculated in square centimeters with a free-hand tracing method from three key images, passing through the midsymphysis (level I), tangential to the lower border of the symphysis (level II), and at the maximal anterior rectal wall bulging (level III). Areas at rest and strained are compared to find evidence of a percentage of increase. The purpose is to provide objective evidence of the maximal extent of pelvic organs descent and hiatus enlargement without the interference of foreign objects or the examiner's proximity, so as to overcome the limitations of pelvic examination and transperineal sonography (i.e., subjectivity and sex-related limitations [female only]).

INTRODUCTION:

Pelvic organ prolapse (POP) develops when the forces acting inside the boundaries of levator ani hiatus are no longer counteracted by those outside, leading to abnormal enlargement and organ impingement. Several factors are responsible for the disease, including ligaments, fascia, or muscular tonic activity. Whatever the mechanism involved, the increased hiatus size has been credited with a reliable index to assess the inability to keep it closed. Usually, the status of pelvic support is determined in women during a pelvic examination[1] by observing the location of the cervix, vaginal apex, and vaginal walls during the Valsalva maneuver. However, the inaccuracy of the method, combined with a failure to identify all involved sites[2,3] and sex-related constrictions (female only), has led clinicians and researchers to seek alternative methods, namely diagnostic imaging.

Current methods for determining hiatus size include transperineal ultrasonography (TPUS)[4,5] and, more recently, magnetic resonance imaging (MRI). Unfortunately, existing methods of performing the examination and measurement of individual parameters vary greatly among researchers[6–13], making a comparison of study results difficult. Moreover, significant differences still exist in the definition and terminology of the most common pelvic descent processes, as well as in the classification and quantification of the adopted systems[14,15].

This study highlights the advantages of MRI over other methods and describes the technical details and diagnostic criteria for the quantification of POP in patients of both sexes. In particular, the description focuses on the quantification of pelvic organs descent and levator ani hiatus enlargement when straining, with the patient supine, to demonstrate that the lack of a vertically oriented MR system[16,17] (i.e., gravity will not adversely affect the detection of various changes associated with POP).

PROTOCOL:

Procedures involving human subjects have been performed according to the National Guidelines of the Italian Radiological Society

1. Patient preparation

1.1. Help patients to fill in a form which provides information on their history, current symptoms, treatments (either medical or surgical), and prior medical records, if any.

1.2. Obtain each patient's written consent before beginning the examination.

1.3. Clearly explain in advance the characteristics and purpose of the procedure, including the performance of various maneuvers such as squeezing, straining, and rectal emptying.

1.4. Give information on the duration (average time: 25 min) of the procedure and the need for the insertion of a small catheter into the anal canal for contrast administration (acoustic gel).

1.5. Ask the patients to empty their bladder in the toilet just before starting the examination.

NOTE: On the basis of the patients' histories and presenting symptoms, tailor the procedure to each single case with regard to the amount of contrast administered and the number of scan planes and pulse sequences used.

2. Diagnostic room and facilities

2.1. Keep a trolley inside the area of the diagnostic room, equipped with all necessary instruments and supplies, including gloves, syringes, a catheter, lubricant jelly, acoustic gel, etc. (see **Table of Materials**).

[Place **Figure 1** here]

2.2. Ask the patient to lay on the diagnostic table of the MR scanner in the left lateral position (Sims' position). Gently insert the catheter into the rectum and administer the contrast administration (acoustic coupling gel) until the patient experiences a characteristic desire to evacuate (the average amount is 250 mL). Turn the patient in the supine position afterward.

2.3. Adjust the absorbent pad beneath the buttocks, and wrap around the patient's pelvis a surface phased-array coil for the image acquisition.

NOTE: In case of anticipated sensation, urgency, discomfort, or involuntary leakage, interrupt the injection and register the total volume injected before the leakage, as well as the volume leaked.

3. Technique and image acquisition

3.1. Acquire a localizer scout scan in the coronal, axial, and sagittal planes (TFE T1 pulse sequence, TR of 8 ms, TE of 5 ms, flip angle (FA) of 25°, thickness of 15 mm, and the number of images: 5–11) to mark the boundaries of the region of interest (ROI).

3.2. Then, obtain three subsequent dynamic series in the midsagittal plane (see **Table 1**, series 1: TR/TE of 2.7/1.3 ms; FA of 45°) centered over the anorectal junction, with the patient at rest, squeezing his/her anal sphincter, and straining (10 s each).

3.3. Thereafter, instruct the patient to start—at will—the movement of rectal emptying, and notice when it starts (by acoustic device) to allow the simulatenous acquisition of images over an entire time cycle of 58 s (see **Table 1**, series 1: TR/TE of 2.7/1.3 ms; FA of 45°; thickness of 35 mm; acquisition time of 58 s).

NOTE: If necessary, repeat the series until obtaining an adequate stream of contrast.

3.4. Repeat the latter sequence in the coronal plane (see **Table 1**, series 2: TR/TE of 2.8/1.3 ms; FA of 45°; thickness of 35 mm; acquisition time of 58 s) while the patient is expelling the residual rectal contrast.

3.5. Then, instruct the patient to perform a steady-state Valsalva maneuver without interruption for 9 s.

3.6. Taking the sagittal images acquired during the rectal emptying as a reference, select three horizontal planes in the axial plane (see **Figure 3**) to image the levator hiatus as follows: the first at the midsymphysis (level I), the second tangent to the inferior border of the symphysis (level II), and the third at the point of the maximal bulging of the anterior rectal wall (level III).

NOTE: The reason for the above is to include most relevant anatomical areas inside and outside the hiatus boundaries of both sexes in the ROI: bladder base, urethra, vagina, cervix, perineal body, anorectal junction, endopelvic fascia, and fat recesses (female), or bladder base, retropubic space, prostate, seminal vesicles, Denonvilliers' fascia, anorectal junction, mesorectal fascia, and presacral space (male).

3.7. Acquire a horizontal 1 cm-thick section in the axial plane (see **Table 1**, series 3: TR/TE of 3/1.5 ms; FA of 45°; thickness of 10 mm; acquisition time of 9 s) from each level during the Valsalva maneuver, leaving the patient a 60 s interval between two subsequent maneuvers to relax.

3.8. Finally, acquire static T2-weighted images when at rest in the axial, sagittal, and coronal planes (see **Table 1**, series 4, 5, and 6: TR/TE of 3649–4656/100; FA of 90°; thickness/gap of 4/0.4 mm; acquisition time of 3:00–3:44 min) to provide a complete evaluation of the pelvic anatomy.

NOTE: Refer to **Table 1** for the technical settings.

[Place **Table 1** here]

4. Image analysis and measurements

4.1. To measure the position of the pelvic organs when at rest and while straining, from midsagittal dynamic MR images in the analysis software, go to the list of toolbar options positioned at the top of the screen and hover over **Annotation Tools**.

4.2. Then, click on the arrow and select **Ruler** to obtain a linear measurement in millimeters of the vertical distance of the bladder neck, uterine cervix, prostate base, seminal vesicles, and rectal floor from two reference lines, as follows: express the distances by negative (proximal) or positive (distal) numbers relative to the hymen plane (female) or to a horizontal line drawn tangent to the inferior border of the symphysis pubis (male).

[Place **Figure 2** here]

4.3. To measure the hiatal anterior/posterior and transverse diameter (in millimeters) from the axial static and dynamic images, repeat the same selection of linear measurements described in steps 4.1–4.2 and calculate the distance from the pubic symphysis to the anterior margin of the puborectalis sling and the distance between the medial borders of the levator ani muscle.

4.4. To measure the hiatal area (in square centimeters) when at rest and during maximum strain, click again on **Annotation Tools** and choose **Free-ROI** to select a free-hand contour-tracing technique (see **Figure 3**).

4.5. Depict the internal area of the levator ani muscle and express the differences between resting and straining measurements as absolute values and an increase in percentage from sections of the same level identified by the recognition of bony landmarks, namely the symphysis pubis and the ischial tuberosities (level 2).

NOTE: Register any impingement of the organs within the levator ani hiatus and refer, for organ prolapse definition and grading, to the standards recommended by the international committee on pelvic organ prolapse[14,15] and to the traditional "HMO" MRI classification system of pelvic organ prolapse described by Comiter et al.[9].

[Place **Figure 3** here]

REPRESENTATIVE RESULTS:

Between 2012 and 2018, this protocol has been successfully adopted in three different diagnostic centers in Italy at an average cumulative rate of 30 ± 4 exams per month, using the same 1.5 T MR scanner model and technical settings (see **Table 1** and **Table of Materials**). During this period, over 2,000 examinations have been performed in patients of both sexes for the following three main disease categories: pelvic organ prolapse and evacuation disturbances (Group 1), ano-perianal fistula (Group 2), and chronic pelvic pain from known or suspected pudendal neuropathy (Group 3). Most relevant demographic characteristics of the patient population were: a mean age of 48 ± 3.4 years and a range of 25–82 years in males and of 51 ± 4.2 years and 34–88 years in females (Group 1); a mean age of 36 ± 3.2 years and a range of 31–82 years in males and 54 ± 2.5 years and 35–78 years in females (Group 2); a mean age of 43 ± 3.3 years and a range of 27–78 years in males and 41 ± 4.4 years and 28–78 years in females (Group 3). Each disease category led to specific variants of the MR imaging examination. Over time, the huge amount of examinations helped to obtain an almost uniform reproducibility of the protocol in subjects referred for POP and evacuation dysfunctions, until reaching the standard described herein.

The most relevant results can be summarized as follows: pelvic organ prolapse also occurs in males (see **Figure 2B**), although it is more common in females[18–20]. In addition, regardless of sex, levator ani hiatus ballooning when straining (see **Figure 4**) has emerged as the most reliable index of the disease. Its area can easily be quantified with axial dynamic MR pelvic imaging and graded as normal (≤20 cm^2), as a prolapse of the first or second degree (20–40 cm^2), or as a prolapse of the third or fourth degree (≥40 cm^2), or it can be described as an increase of >100% compared to the size when at rest. Interestingly, the actual enlargement of the hiatus when straining cannot be predicted based on its size when at rest, as demonstrated in a previous study in nulliparous and parous women[18] whose values when at rest did not correlate with those during the Valsalva maneuver (see **Figure 5**). This, in turn, emphasizes the value of the present protocol. Finally, the evacuation of rectal contrast in the horizontal position is virtually coterminous with the standardization of the maximal Valsalva maneuver. More precisely, the position reached by pelvic organs under its effect can be assumed as representative of the descent reaching the maximal potential extent.

[Place **Figure 4** here]

[Place **Figure 5** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Supplies. This picture shows (**A**) a trolley with supplies for the MR examination and (**B**) the dilution of acoustic gel with water (50/30 mL per syringe) in the area adjacent to the diagnostic room before the administration.

Figure 2: Reference lines for pelvic organ descent on midsagittal MR images. (**A**) A 61-year-old woman with a rectal descent of >10 cm below the hymen plane (yellow line) and sigmoidocele. (**B**) A 42-year-old man with rectal intussusception and a descent of >3 cm below the lower border of the symphysis pubis (yellow line). bl = bladder; sp = symphysis pubis; ut = uterus; r = rectum; p = prostate.

Figure 3: Method for levator ani hiatus imaging and area measurement. (**A**) A selection of three axial scan sections from a midsagittal image taken relative to the midsymphysis pubis (level 1), tangent to its lower border (level 2), and at the maximal bulging of the anterior rectal wall (level 3) during rectal emptying. (**B**) An example of an asymmetric area measured when at rest from level 2 with the free-hand contour-tracing method in a 52-year-old woman with a focal defect of the right pubococcygeus muscle (arrow). s = symphysis pubis; bl = bladder; r = rectum. The left panel = a sagittal view; the right panel = a coronal view. The area values are expressed in square centimeters. 1 = first level; 2 = second level; 3 = third level.

Figure 4: The ballooning of the levator ani hiatus. Depicted in the steady-state Valsalva maneuver, as seen (**A**) in a woman and (**B**) in a man with rectal prolapse (i.e., the same patients as depicted in **Figure 2**). S = symphysis pubis; IT = ischial tuberosity; ut = uterus; sc = sigmoid colon; sv = seminal vesicles; r = rectum.

Figure 5: Results of the hiatus area measurement. (**A**) The graph shows an overlap of the hiatus area when at rest in nulliparous and parous women who delivered vaginally or via a cesarean section. (**B**) Moreover, the lack of correlation between the values when at rest and when straining indicates the inability to predict the actual enlargement in singular cases. This picture has been reproduced from Piloni et al.[18] with permission.

Figure 6: Effect of vector forces on solid materials. (**A**) A T2-weighted MR image showing a childbirth focal defect (red empty arrow) of the levator ani muscle at rest; (**B**) when straining, despite their equal intensity, the vector forces from the intra-abdominal pressure produce a different geometrical deformity on the hiatus boundaries (thicker red arrow) and an asymmetric

hiatus ballooning. (**C**) The same vector force of wind (yellow empty arrows) bends trees in different ways, depending on their cross-section diameter, structure, and elastic properties. S = symphysis pubis; u = urethra; v = vagina; a = anus; IT = ischial tuberosity.

Table 1: Technical settings for MR defecography, using a 1.5 T scanner and an external coil.

DISCUSSION:

This method has an overt advantage over pelvic examinations that are limited to the assessment of the urogenital hiatus in only females. In contrast, the method presented here examines the entire levator ani hiatus in both sexes. Moreover, although easily examined by palpation by the gynecologist, the female hiatus can be calculated only approximately with a ruler, to produce the area of an oval[1]. Similarly, an advantage does exist over 2- and 3-D TPUS[4,5], which are not suitable for male patients. However, a more important advantage of this protocol is the unique ability to document the rectal emptying function, which is peculiar to MR defecography and is done with no interference due to the proximity of the examiner or the ultrasonographic probe held close to the labia, both of which may prevent maximal pelvic organ descent. A limitation of this MR protocol, which requires the patient to be in a horizontal position, is that the absence of gravity may lead to the patient's inability to adequately empty their rectum and to a potential underestimation in the depiction of various abnormalities, due to the fact that the examination is not physiologic. It can be argued, however, that the extraordinary effort of the patient to relieve themselves of the rectal contrast in a horizontal position helps to standardize the maximal Valsalva maneuver, eliminating the uncertainty which always remains after pelvic examinations and TPUS.

For a successful performance of this protocol, the acoustic gel contrast in the syringe is diluted with water (see **Table 1**), added just before the administration in the area adjacent the diagnostic room, as shown in **Figure 1B**. In addition, it is also mandatory to follow the next recommendations. (a) Coach the patient carefully on how to produce his/her maximal pelvic strain without moving the position of the pelvis relative to the diagnostic table and without interrupting the Valsalva maneuver until they are told to breathe again and relax. (b) Select the axial MR sections relative to consistent bony landmarks (i.e., the symphysis pubis) without tilting the scan planes. This will prevent the addition of undesired factors of variability when measuring various parameters of biometry. (c) Compare the values of the hiatus diameters and the areas when at rest and when straining on axial sections identified on the basis of the same bony landmarks. This strategy is crucial for the exact spatial localization of a given change and for biomechanical inferences (see **Figure 6**). (d) Use the hymen plane (female) and the tangent to the lower border of the symphysis pubis (male) as reference lines on sagittal MR images, instead of the more traditional pubococcygeal line (PCL)[9]. This will avoid discrepancies between radiologists and clinicians who are unaware of the PCL during pelvic examinations[14,15].

Another limitation of the present protocol comes from the comparison with 3-D computational models [21,22], which are overtly superior in depicting the pelvic floor anatomy in its entirety. However, 3-D imaging results in an overload of image analysis and interpretation which cannot be sustained equally by all examiners. Although admitting the inferiority of the present 2-D protocol due to a lack of anatomical information, we emphasize the greater ease associated with its use. As a matter of fact, it can be applied to routine assessments of the levator ani hiatus performed by all radiologists in the clinical practice, even if said radiologists are not familiar with the use of sophisticated and expensive software applications or complex mathematical formulas.

The hard limitation of this protocol for calculating the levator hiatus area remains the excessive intra-observer variation of the free-hand contour-tracing method, as shown by the fact that it is almost impossible to obtain the same result in two subsequent measurements by the same examiner. Referring to the hiatal area, the so-called "20-40-60 cm^2" has been developed to discriminate patients with normal pelvic support (20 cm^2 or less) from those with prolapse (between 20 and 40 cm^2) and those with recurrence after prolapse repair (between 40 and 60 cm^2). Rather than relying on such absolute area values, its percentage increase when straining, relative to resting values, is recommended as a more reliable index of the hiatal defective function. The most striking result obtained by this protocol is the levator ani hiatus ballooning and the pelvic organ impingement that could be demonstrated equally in nulliparous and multiparous women with pelvic prolapse and also in those who delivered their child by cesarean section. This observation partially contradicts the role of birth injury and the theory that the lateral separation of hiatal structures mainly occurs due to abnormal laxity in the vagina or its supporting ligaments, leading to organ prolapse.

This conviction is reinforced by the fact that a similar enlargement, combined with an excessive descent of the prostate base and seminal vesicles, has been demonstrated in men[20]. This indicates the need to consider several other factors, such as the combination of fat and the pelvic connective network as a whole, which might be seen as a sort of "interlock mechanism" responsible for the reciprocal cohesion among organs.

Conceptually, it may be hypothesized that the overcoming of such cohesive forces will lead to excessive slide motion and pelvic organ descent due to repetitive load. Under certain aspects, the quantitative analysis of the geometrical and structural deformity of hiatus boundaries, as seen with this protocol, closely reflects the action of a vector force from above which determines the displacement of pelvic organs. In the case of a decreased resistance of hard (bone) and soft tissues (skin, tendon, muscle, and fat), the application of the present protocol might contribute to a better understanding of the pathophysiology of pelvic organ prolapse.

[Place **Figure 6** here]

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DISCLOSURES:
The authors have nothing to disclose.

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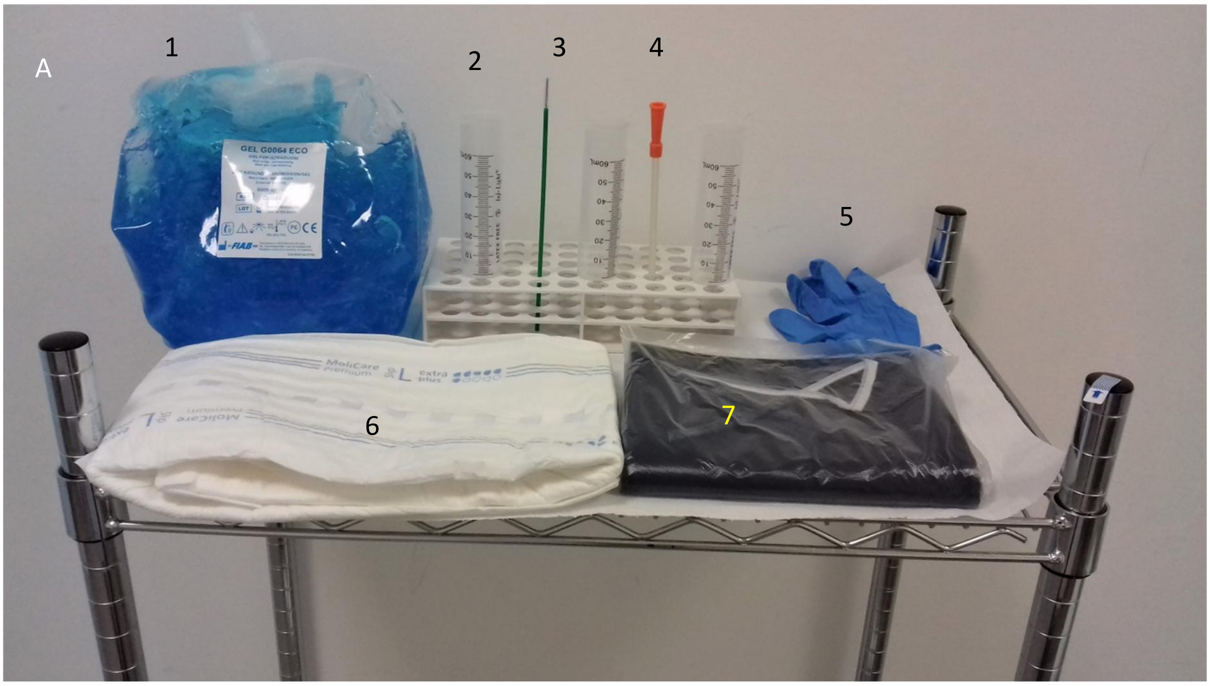
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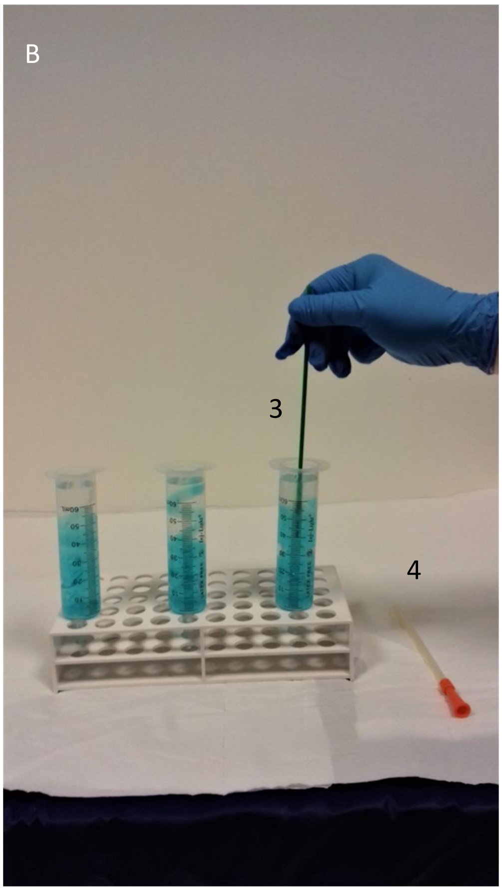
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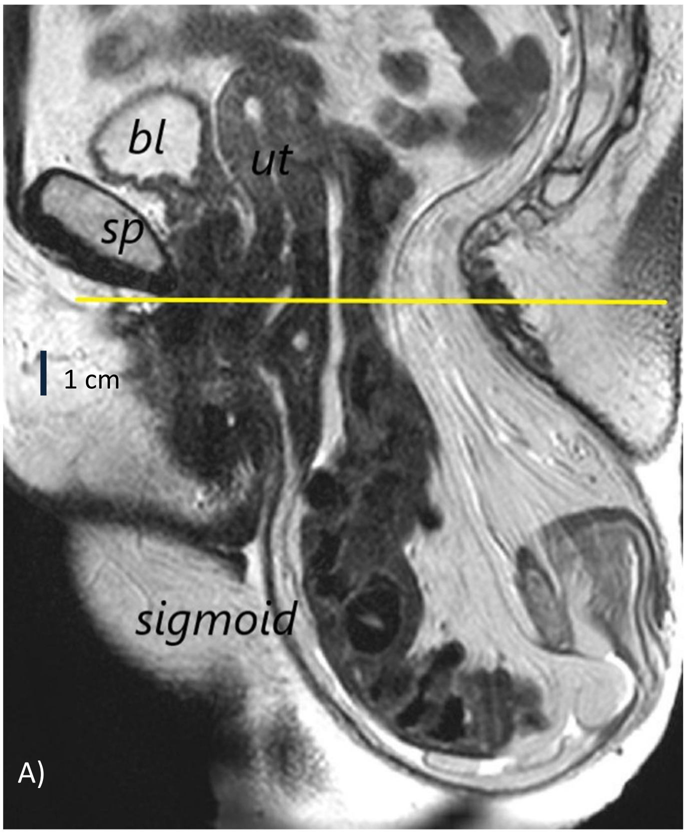
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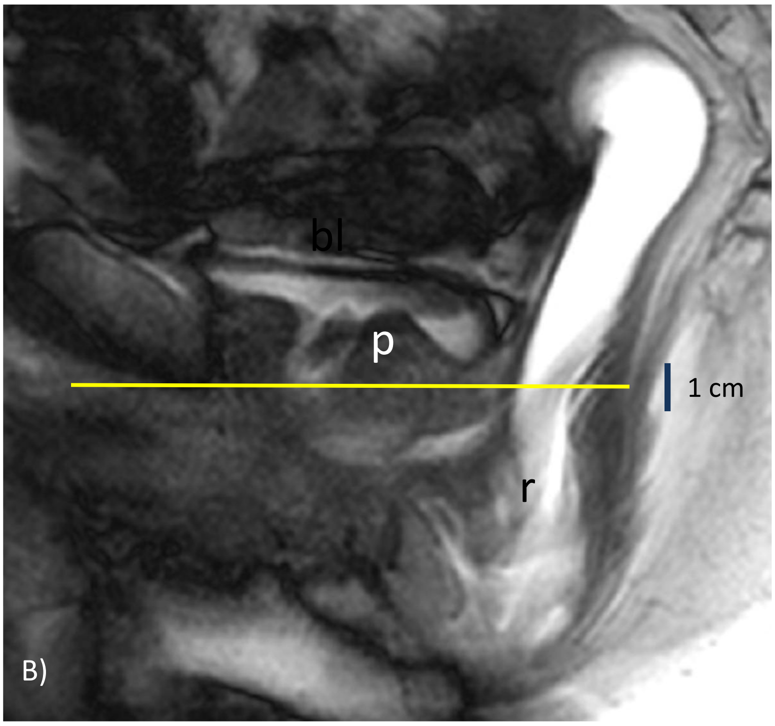
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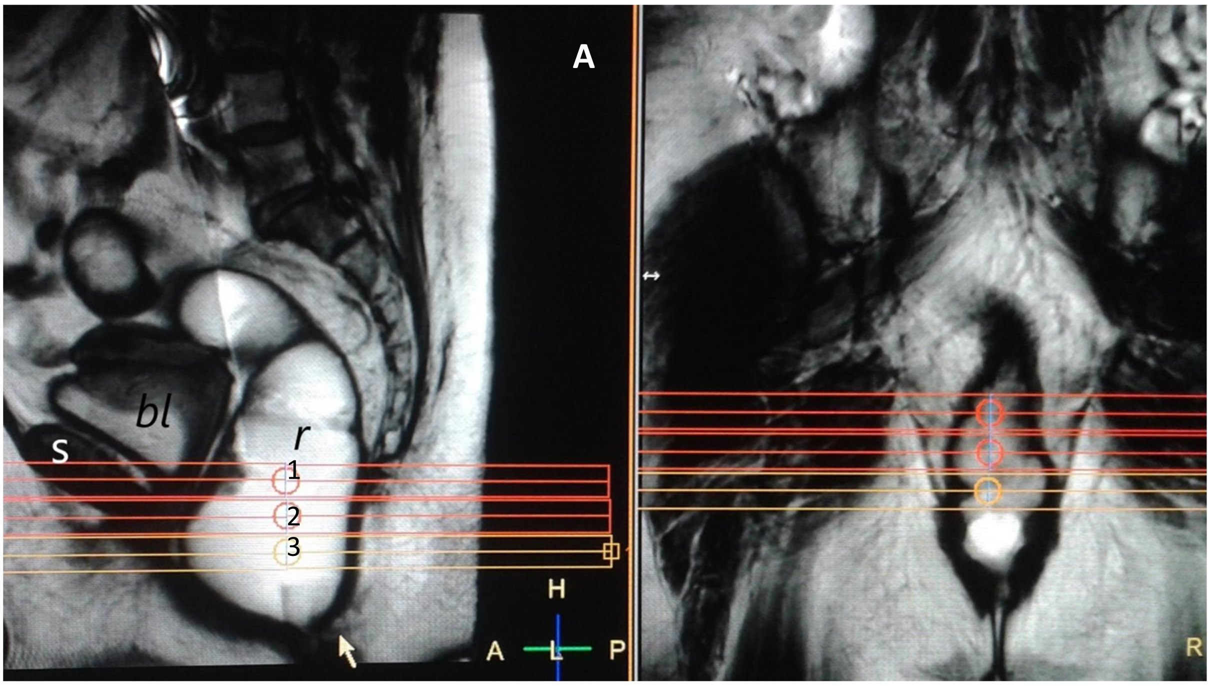
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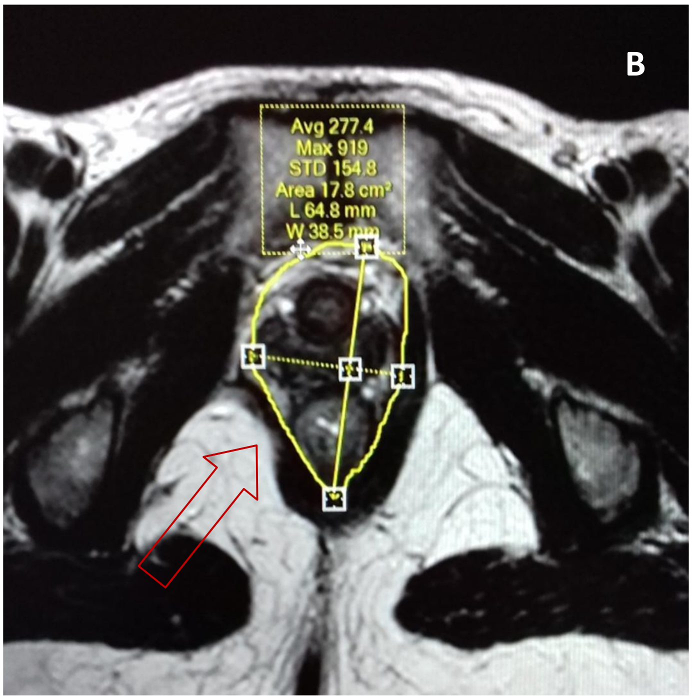
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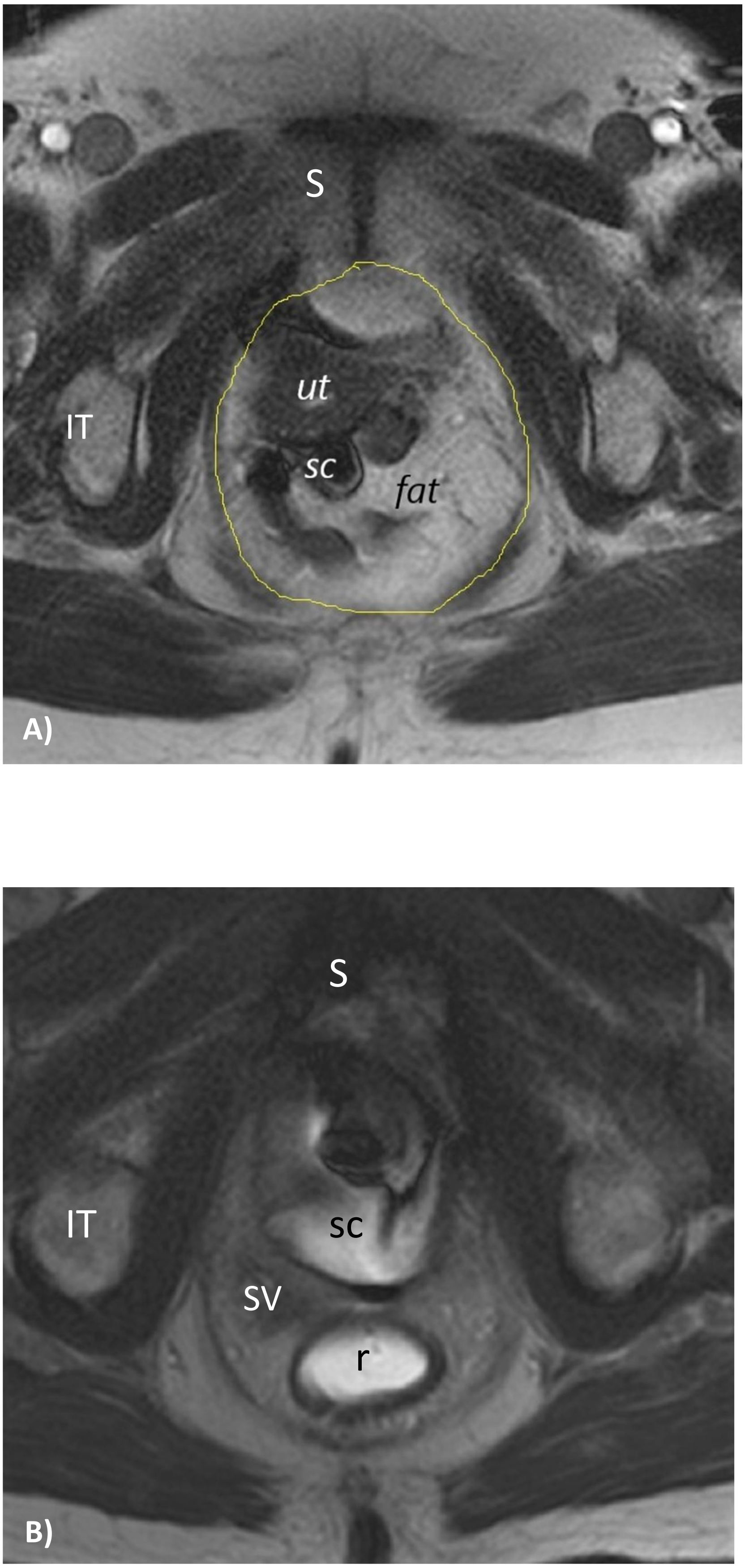

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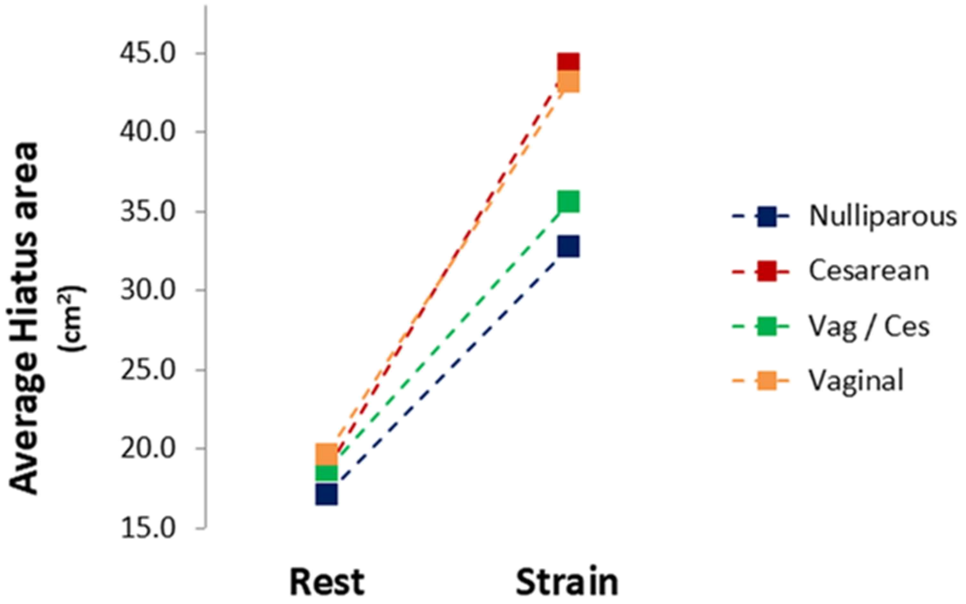
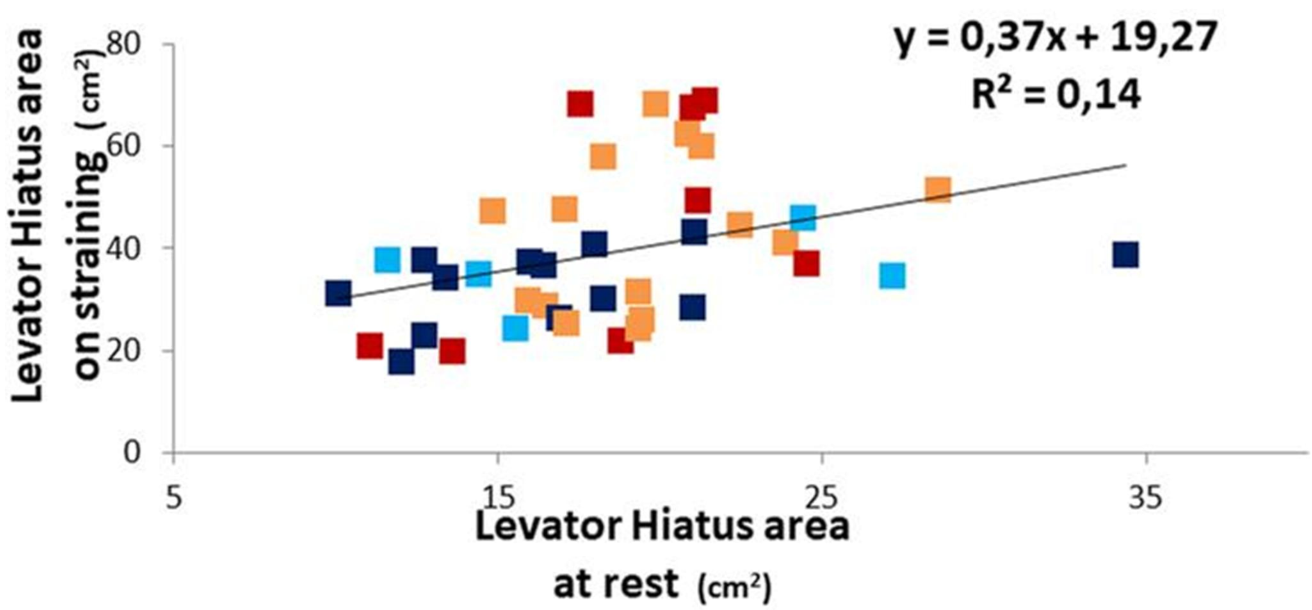
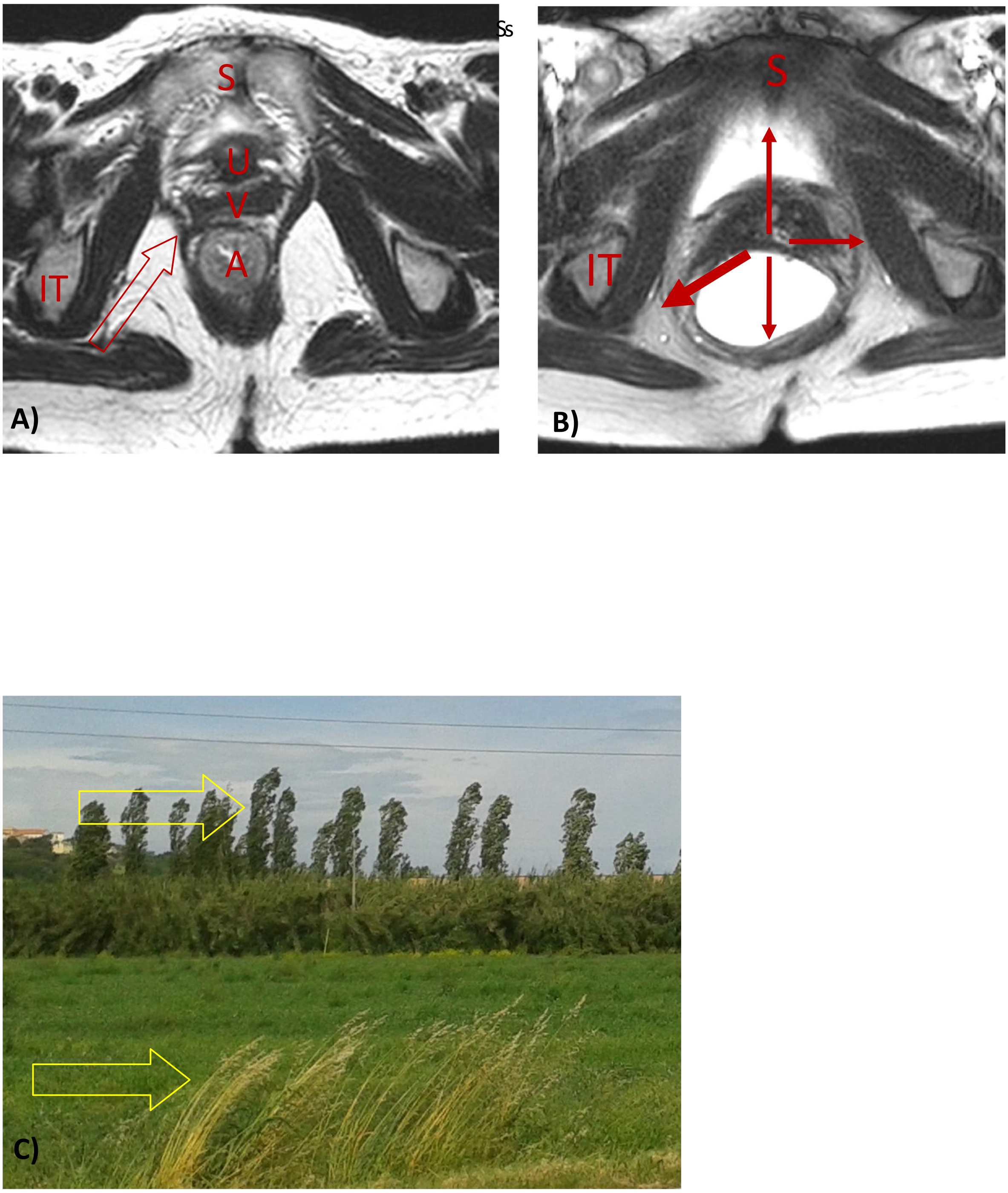

45.0
40.0
35.0
30.0
25.0
20.0
15.0
Average Hiatus area
(cm²)
Rest
Strain
Nulliparous
Cesarean
Vag / Ces
Vaginal

B


Levator Hiatus area
on straining (cm²)
80
60
40
20
0
5
15
25
35
Levator Hiatus area
at rest (cm²)
y = 0,37x + 19,27
R² = 0,14


S
U
V
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IT
A)
Ss
S
IT
B)
C)

Parameter	Series 1	Series 2	Series 3	Series 4	Series 5	Series 6
Pulse Sequence	BTFE	BTFE	BTFE	FSE T2-W	FSE T2-W	FSE T2-W
Plane	Sagittal	Coronal	Axial	Axial	Sagittal	Coronal
TR (ms)	2.7	2.8	3	4656	3865	3649
TE (ms)	1.3	1.3	1.5	100	100	100
ETL	16	16	16	16	16	16
NEX	2	2	2	4	3	3
FOV	300	300	300	350	280	280
Matrix	256	256	256	576	576	576
Slice thick/gap (mm)	35	35	10	4/0.4	4 / 0.4	4/0.4
Flip angle (°)	45	45	45	90	90	90
Scan time (min)	0.58	0.58	0.09	3:37	3:44	3:00
Slices (n°)	60	60	3	35	35	35

Notes: Series 1-3 = Dynamic; Series 4-6 = Static; BTFE = balance turbo spin echo ; FSE = fast spin echo; T2-W= T2-weighted; TR = repetition time; TE= echo time; FOV = field of view; ETL = echo train length; NEX= number of excitations

Name of Material/ Equipment	Company	Catalog Number
MR scanner	Philips Medical Systems, High Tech Campus 37, 5656 AE, Eindhoven, The Netherlands	
Catheter	Convatek ltd, First avenue Deeside, Flintshire CH5 2NU UK	
Holder	Kartell Plastilab, Artiglas Srl, Via Carrara Padua, Italy	
Syringe	Pikdare Srl, Via Saldarini Catelli 10 , 22070 Casnate con Bernate (Como) Italy	
Contrast	Ceracarta SpA, Via Secondo Casadei 14 47122 Forlì Italy	
Mixer device	Kaltek Srl, Via del Progresso 2 Padua Italy	

Pad	Fater SpA, Via A. Volta 10, 65129 Pescara Italy	
Lubricant	Molteni farmaceutici, Località Granatieri Scandicci (Florence) Italy	
Apron	Mediberg Srl, via Vezze 16/18 Calcinate 24050 (Bergamo) Italy	
Gloves	Gardening Srl, Via B. Bosco 15/10 16121 Genova Italy	

Comments/Description
1.5 T horizontally oriented, Multiva model, SENSE XL Torso coil; Position the patient in the left lateral decubitus on the diagnostic table with the coil warpped around the pelvis
Sterile vaginal catheter, 16 ch,180 mm long, 3 mm wide; Gently insert the lubricated tip inside the anal canal for contrast administration with patients in the left side position
Universal test-tube holder with multiple 13-mm holes; Put 3 empty syringes vertically inside the holes with the outlet cone down
Sterile, latex free,60 mL graduated transparent cylinder, catheter cone; Fill with contrast, adjust the plunger and connect to the catheter
Eco supergel not irritant, water soluble, salt free; Dilute the content of each syringe adding 30 mL of tap water to 50 mL of acustic gel
Kito-Brush for endovaginal sampling; Rotate one full turn 10-20 times until obtaining an homogeneous gel

Pad for incontinent subjects; Wrap around patient's pelvis to collect any material and prevent diagnostic table contamination
Luan gel 1%; Apply on the tip of catheter before insertion
Kimono; Put on counteriwise (opening back) to maintain patient's dignity
Nitrile, latex free, no talcum powdered; Wear during contrast preparation and catheter insertion; change regularly to prevent cross contamination

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Title of Article: Quantification of levator ani hiatus enlargement by Magnetic Resonance Imaging in males and females with pelvic organ prolapse

Author(s): Vittorio Piloni[1], Mattia Bergamasco[2], Andrea Chiapperin[3]

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CORRESPONDING AUTHOR

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Department: Diagnostic Imaging

Institution: *Iniziativa Medica spa, via Rialto 14, 35043 Monselice (Padua), Italy*

Title: Quantification of levator ani hiatus enlargement by Magnetic Resonance

Dott. Vittorio Piloni

Signature:

Date:October 17,2018

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Rebuttal Document

Editorial comments:

1. **Please take this opportunity to thoroughly proofread** the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Reply: the manuscript has be revised by an english mather language teacher .

2**. Please revise lines 59-62, 182-185, and 260-262 to avoid previously published text**.

Reply: all lines mentioned above have been revised and cancelled

3. **Please obtain explicit copyright permission** to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

Reply: explicit copyright permission to reuse Figure 4 and 5 from previous publication [see reference 18] is enclosed (see file “permission”)

4. **Figures 4 and 5: Please combine all panels** of one figure into a single image file.

Reply: all panels of one single figure have been combined into a single image file. Please kindly note that the entire list and number of pictures have been modified due to inclusion of new figures, according to the suggestions of reviewer 3.

5**. Figures: Please define all labels** (ut, sc, bl, sv, etc.) in the figure legend.

Reply: all labels in the figure legends have been defined.

6. **Please provide a title and a description of Table 1** in Figure legends after the Representative Results of the manuscript text.

Reply: the title of Table 1 has been provided and renumbered as Table 2, due to inclusion of a new Table 1.

7. **Please revise the title to be more concise**.

Reply: the title has been shortened.

8. **Please rephrase the Summary** to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

Reply: the short abstract now includes the protocol and its application

9. **Abstract should include a statement about the purpose of the method.** A more detailed overview of the method and a summary of its advantages, limitations, and applications is appropriate. Please revise the Abstract accordingly.

Reply: the abstract has been modified according to the suggestions of the editor.

10. **Please include an ethics statement** before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.

Reply: the ethics statement has been included.

11. **Please revise the protocol text** to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Reply: the use of personal pronouns has been eliminated.

12. Please revise the Protocol steps so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. For example, 3.2, 4.4, etc.

Reply: the description of the Protocol steps now follows the suggestions of the editor

13. **Please ensure that the highlighted steps (2.75 pages or less) form a cohesive narrative** with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

Reply: the highlighted portions of the protocolos now are coherent and cohesive and written in imperative tense.

14. Discussion: **Please also discuss any limitations** of the technique.

Reply: the limitations of the technique have been included

15. JoVE article **does not have a Conclusion section**. Please move information in the Conclusion section to Results or Discussion section.

Reply: the "Conclusion" section has been cancelled

16. Please **include an Acknowledgements section**, containing any acknowledgments and all funding sources for this work

Reply: an Acknowledgements section has been included. No need to cite any funding sources-

.

17. References: Please **do not abbreviate journal titles**. Please include volume and issue numbers for all references.

Reply: The References now are written according to the Editor's guidelines.

18. Please **upload a table of the essential supplies**, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

Reply: a Table of the essential supplies (numbered as Table 1) has been included

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
This manuscript describes pelvic organ prolapse evaluation on dynamic MRI sequences. Manuscript also describes data acquisition and how to perform standard measurement in both man and women. Method is well described and figures seems to be represent clinical conditions.

Major Concerns:
none

Minor Concerns:
none

Reviewer #2:
Manuscript Summary:
The article presents a new way of measuring the levator ani hiatus size using multiple MRI images. Also, the changes due to resting and straining were quantified. Several techniques are listed based on the prolapse quantification standard for extracting area calculation information from images. Also, on MR images, levator ani defect including thinning, discontinuity, geometrical deformity were characterized. While this method may serve as a possibly better standard over crude pelvic examination or transperineal sonography as stated, it may have several flaws with respect to the accuracy of information obtained using 2D images.

Major Concerns:
The very reason MRI image segmentation based 3D computational models of the pelvic system are developed is to obtain anatomical information of organs which are missing in 2D. Thus **the limitations of this work should be clearly acknowledged**. Also, it is recommended **to include and cite the following articles:**
i) Rostaminia, Ghazaleh, and Steven Abramowitch. "Finite element modeling in female pelvic floor medicine: a literature review." Current Obstetrics and Gynecology Reports 4.2 (2015): 125-131.
ii) Chanda, Arnab, et al. "Computational modeling of the female pelvic support structures and organs to understand the mechanism of pelvic organ prolapse: a review." Applied Mechanics Reviews 67.4 (2015): 040801.

Reply: the limitation and the inferiority of the protocol, compared to 3D computational model have been mentioned in the text. At the same time, the two articles mentioned above have been included in the list of references.

What is **the basis of selecting the MRI images** captured in each plane for analysis? There can be thousands of images captured on each plane, with different quantity of anatomical information. Why specific images were selected should be explained.

Reply: reasons for selection of scan planes have been included in the text.

Minor Concerns:
Significant differences have been observed in previous studies using 2D images acquired from the the same patient and during the study of the same physiological process across patients. **Limitations with respect to errors and variability across measurements** should be discussed.

Reply: the issue of limitation of intraobserver variation has been mentioned in the text.

The wording in several sections such as the abstract and protocol section should be mainly in third person (i.e. use "was" or "were") and not as instructions which should be followed in general.

Reply: the choice of imperative tense follows specific indications of “authors guidelines”

Reviewer #3:
Manuscript Summary:
I believe this is a good work, and might allow radiologists to approach a field that is considered quite complex. Also, male pelvic floor failure is seldom described in literature but is not uncommon in clinical practice.

Major Concerns:
I think **putting more images** might be useful, displaying normal structures, i.e. pelvic floor muscles, signs of fascial integrity/disruption and more examples of slight-moderate pelvic floor failure. This would be in keeping with the assumption of an integrated mechanism of pelvic floor insufficiency and tribology.

Reply: more images have been included

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Padova September 26th 2018

Dear Dr. Piloni,

Thank you for your interest in the journal Pelviperineology.

Concerning your article entitled “Quantification of abnormal levator ani hiatus enlargement in males and females by Magnetic Resonance Imaging “, you are allowed to reproduce the Figures 4 and 5 from the paper “ Quantification of levator ani (LA) hiatus enlargement and pelvic organs impingement on Valsalva maneuver in parous and nulliparous women with obstructed defecation syndrome (ODS): a biomechanical perspective“ by Piloni V et al, published in the Journal Pelviperineology 2015; 35: 25-31, including the full reference to the paper.

Thank you for your collaboration

prof. Giuseppe Dodi

Managing Editor Pelviperineology

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