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« In vivo characterization of women's pelvic floor muscles viscoelastic properties during pregnancy »

« Caractérisation in vivo des propriétés viscoélastiques du plancher pelvien de la femme au cours de la grossesse»

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In vivo characterization of women's pelvic floor muscles viscoelastic properties during pregnancy

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Caractérisation in vivo des propriétés viscoélastiques du plancher pelvien de la femme au cours de la grossesse

Résumé substantiel en Français

Introduction

L'accouchement est un évènement spécial dans la vie d'une femme, bien évidemment du fait de ses aspects émotionnels mais également sur le plan physiologique. En effet, durant la progression du fœtus au sein du plancher pelvien maternel, les muscles du périnée sont étirés de 300 % [1, 2]. Il existe des modifications des propriétés biomécaniques intrinsèques des tissus au cours de la grossesse qui pourraient avoir pour but de leur permettre de supporter une telle contrainte. Malgré cela, un traumatisme périnéal grave peut survenir dans 5 à 20% des cas sous la forme d'une lésion obstétricale du sphincter anal et/ou d'une désinsertion des muscles *levator ani* [3, 4]. Ces complications impactent de manière importante la vie des femmes puisque 50% d'entre elles resteront symptomatiques définitivement de ces lésions. Des facteurs de risques sont bien décrits pour la survenue de ce type de complication (premier accouchement, accouchement instrumental, poids important de l'enfant) [4, 5]. Néanmoins, les stratégies de prédiction du risque existantes à ce jour restent décevantes [6, 7]. Nous pensons que la prise en compte caractéristiques biomécaniques intrinsèques des tissus et notamment les propriétés élastiques du plancher pelvien pourrait nous permettre d'améliorer les possibilités de prédiction de ce risque.

Etude 1 : Pourquoi et comment prendre en compte le comportement biomécanique des muscles du plancher pelvien de la femme dans la prédiction du traumatisme périnéal obstétrical ?

Considérations anatomiques

Le plancher pelvien féminin est un ensemble musculo-ligamentaire complexe dont les éléments musculaires principaux sont les muscles *levator ani* et le muscle sphincter anal externe. Le muscle *levator ani* est un muscle bilatéral composé de trois faisceaux, qui vient s'insérer au niveau de la symphyse pubienne en avant et dont les fibres rejoignent celles du sphincter anal externe en arrière [8]. Les muscles droit et gauche forment le hiatus des releveurs de l'anus qui correspond à un espace au travers duquel le fœtus va devoir progresser durant la phase d'expulsion de l'accouchement. Les lésions du muscle *levator ani* sont étroitement associées à l'existence d'un prolapsus génital. Le muscle sphincter anal externe vient lui réaliser un manchon concentrique autour de la muqueuse digestive du canal anal et du sphincter anal interne [9]. Ce muscle est lui associé à l'existence d'une incontinence anale.

Accouchement normal et dystocique

Pour permettre un accouchement par voie vaginale, la tête fœtale doit avoir progressé au-delà d'un plan passant par les épines ischiatiques. Le fœtus se présente le plus souvent en variété antérieure, c'est-à-dire avec son occiput orienté vers la symphyse pubienne maternelle autorisant ainsi une flexion maximale de la tête fœtale et donc l'obtention de son diamètre minimal [10]. L'enfant doit ensuite progresser au sein du plancher pelvien maternel, plus précisément au sein du hiatus des releveurs de l'anus, et c'est à ce moment que survient une distension majeure (jusqu'à 300 %) des muscles *levator ani.* Une fois cette progression achevée, la tête de l'enfant va devoir se dégager du plancher pelvien maternel par un mécanisme de déflexion occasionnant un étirement majeur des tissus situés entre l'anus et le vagin (le périnée), avec donc un étirement important du sphincter anal. Cette progression de l'enfant et son dégagement vont être permis par l'action combinée des contractions utérines et des efforts maternels de poussée [10]. Une déchirure du périnée survient dans 50% des accouchements mais il s'agit majoritairement de déchirures « superficielles » (1er degré : épithélium vaginal, peau ; 2ème degré : muscle superficiels du périnée) [11]. Il peut cependant survenir des déchirures plus importantes (3ème et 4ème degré), correspondant à une Lésion Obstétricale du Sphincter Anal (LOSA) et/ou une désinsertion du *levator ani.* La survenue de ce type de lésions est plus fréquente en cas de mauvaise flexion de la tête fœtale et donc de diamètre plus important (variété postérieure), d'un poids de naissance plus important, de l'utilisation d'un instrument (surtout si forceps) et en cas de mauvais contrôle manuel du dégagement de la tête fœtale (l'obstétricien doit freiner la sortie de la tête d'une main et soutenir le périnée maternel de l'autre) [4, 5, 12, 13].

Epidémiologie du traumatisme périnéal obstétrical

La prévalence des LOSA est estimée entre 0,25% et 6% dans la littérature [4]. L'amplitude de cette estimation est liée aux difficultés diagnostiques de cet évènement et à des différences de classification entre les équipes cliniques. Les principaux facteurs de risque sont la nulliparité, l'accouchement instrumental (surtout en cas de forceps), un poids de naissance important, un antécédent de LOSA [4, 5, 14]. Les principales complications sont le risque d'incontinence anale, la douleur périnéale de la fonction sexuelle et la dépression postnatale [15-17]. La prévalence des désinsertions du muscle *levator ani* est estimée à 15% des accouchements spontanés et jusqu'à 52% en cas de forceps [3]. Il s'agit d'une désinsertion au niveau de son insertion sur le pubis. Les facteurs de risque sont les mêmes que ceux décrits pour les LOSA, les deux évènements partageant probablement une physiopathologie commune [3, 18, 19]. Ces désinsertions sont associées à une augmentation de surface du hiatus des releveurs de l'anus et à une augmentation du risque de prolapsus génital [20].

Modifications des caractéristiques biomécaniques de la femme pendant la grossesse

Au cours de la grossesse, il est bien décrit une augmentation de mobilité articulaire concernant aussi bien les articulations des membres supérieurs que celles des membres inférieurs [21-26]. Ces modifications ont été interprétées comme une préparation en vue de l'accouchement par voie vaginale (articulations du pelvis) et comme étant le reflet d'un changement global des propriétés biomécaniques des tissus mous [23]. Cette hypothèse semble confirmée devant l'observation d'une augmentation de la mobilité du plancher pelvien de la femme au cours de la grossesse [21, 23]. Sur le plan clinique, il a été rapporté une mobilité plus importante de différents points et un allongements de différents segments anatomiques du plancher pelvien au cours de la grossesse [21-23, 27, 28]. De la même manière, des travaux échographiques ont mis en évidence une mobilité du col vésical et une surface du hiatus des muscles releveurs de l'anus progressivement plus importantes avec l'avancée de la grossesse [21-23, 29, 30]. Là encore, ces modifications ont été interprétées comme un mécanisme de préparation du plancher pelvien au cours de la grossesse en vue de pouvoir accepter les contraintes massives qui lui sont appliquées lors de l'accouchement [23]. Les mécanismes physiologiques impliqués sont discutés, notamment le rôle de la relaxine et des hormones sexuelles pour lesquelles les résultats sont discordants [23, 31, 32]. Le substrat physiopathologique semble être un remodelage du tissu conjonctif et en particulier du collagène au profit d'un type de collagène ayant des propriétés élastiques plus importantes. Il n'existe actuellement aucune donnée humaine *in vivo* concernant une évaluation directe des propriétés élastiques des muscles du plancher pelvien.

Données animales sur le comportement biomécanique du plancher pelvien pendant la grossesse et l'accouchement

Il a été démontré chez le rat qu'il existait, au cours de la grossesse, un allongement des fibres musculaires des muscles du plancher pelvien en lien avec une augmentation du nombre de sarcomères en série [33-35]. Parallèlement il semble également exister une augmentation de raideur de ces muscles au cours de la grossesse en lien avec une augmentation de la quantité totale de collagène [33-35]. Cette modification est interprétée comme un mécanisme de protection vis-à-vis du risque de rupture musculaire à l'accouchement. Il est intéressant de noter que ces modifications ne concernaient que les muscles du plancher pelvien et pas les muscles périphériques, suggérant l'impact de l'environnement local (sollicitation de plus en plus importante par le poids de l'utérus gravide) plutôt que de l'environnement hormonal [33-36].

Association entre caractéristiques biomécaniques de la femme et traumatisme périnéal obstétrical

Il existe peu de données sur ce point. Néanmoins une étude prospective portant sur 300 femmes a mis en évidence une association entre une mobilité articulaire élevée (articulation métacarpo-phalangienne) en fin de grossesse et la survenue d'une LOSA à l'accouchement [37]. Ces données sont limitées car le site anatomique évalué était bien loin du plancher pelvien. Toutefois, cette étude supporte l'hypothèse d'une association entre propriétés mécaniques des tissus et risque périnéal à l'accouchement [23].

Méthodes innovantes pour mesurer les propriétés élastiques des muscles du plancher pelvien de la femme.

La technique d'élastographie par onde de cisaillement [38] est une technique non invasive permettant de mesurer les propriétés élastiques des muscles et présente un potentiel intéressant pour l'évaluation du plancher pelvien de la femme. Elle est la seule à permettre actuellement une mesure directe, focalisée sur le muscle, quantitative, *in vivo*, et de manière non invasive [23, 39-41]. Cette méthode est basée sur la mesure de la vitesse de propagation d'une onde ultrasonore (onde de cisaillement) au sein d'un tissus donnée permettant ainsi de calculer les propriétés élastiques d'un tissus [38, 42]. L'onde se propage d'autant plus rapidement que le tissu est rigide. Cette méthode permet une mesure du module de Young qui est ensuite divisé par un facteur 3 pour obtenir une mesure du module de cisaillement qui est plus approprié à l'étude de tissus anisotropes tels que les muscles [43, 44]. Cette technique présente l'avantage d'avoir déjà été utilisée sur les muscles périphériques avec une très bonne reproductibilité [45]. Elle a également été utilisée pendant la grossesse sur d'autres tissus que les muscles du plancher pelvien, sans évènement indésirable, garantissant son innocuité au cours de cette période [46-48]. Enfin, cette mesure est intégrée dans un échographe, et les échographies sont réalisées de manière régulière pour le suivi de la grossesse. Les mesures d'élasticité des muscles du plancher pelvien seraient donc faciles à réaliser si elles présentent un facteur prédictif d'événement indésirables à l'accouchement.

Vers une approche individuelle de la prédiction du risque de traumatisme périnéal

Il existe actuellement des algorithmes prédictifs vis-à-vis du risque de traumatisme périnéal obstétrical, en particulier du risque de LOSA [6, 7]. Ces algorithmes ont des performances décevantes et leur utilisation dans ces conditions risquerait de conclure à tort à un haut ou bas risque et donc d'exposer la femme enceinte à des interventions non justifiées et potentiellement morbides [23]. Nous pensons que les performances modérées de ces algorithmes pourraient être en lien avec le manque de considération pour les caractéristiques intrinsèques des tissus. Nous faisons l'hypothèse que leurs performances pourraient être significativement améliorées en y incluant des données sur les propriétés tissulaires des muscles du plancher pelvien qui pourraient être obtenues grâce à la technique d'élastographie par onde de cisaillement [23]. Ceci pourrait nous permettre d'évoluer vers une prédiction individuelle du risque autorisant ainsi une information personnalisée des femmes et la mise en place de stratégies individuelles de prévention[23].

Etude 2 : Faisabilité d'une mesure *in vivo* des propriétés élastiques du muscle *levator ani* chez la femme [49]

Objectifs

La première étape expérimentale consistait à évaluer la faisabilité d'une mesure des propriétés élastiques du muscle *levator ani* chez la femme en élastographie par onde de cisaillement. L'objectif secondaire était de rechercher s'il existait un changement concernant ces propriétés élastiques entre la position de repos et un étirement induit par une manœuvre de Valsalva.

Méthodes

Il s'agissait d'une étude prospective monocentrique concernant des femmes non enceintes, sans troubles périnéaux ni troubles articulaires. Une seule visite était prévue au protocole au cours de laquelle les propriétés élastiques du muscles *levator ani* étaient mesurées en élastographie par onde de cisaillement. Nous avons collecté les caractéristiques socio-démographiques et anthropométriques des femmes. Les mesures en élastographie étaient réalisées à l'aide d'un appareil Aixplorer ® V11 (Supersonic Imagine, France), chez des femmes installées en position gynécologique, vessie vide. Nous utilisions la voie d'abord transpérinéale pour visualiser l'insertion pubienne du muscle en échographie 2D et, une fois le muscle repéré, nous procédions aux mesures en élastographie [50]. La sonde était placée de manière sagittale avec une inclinaison latérale de 10°, permettant de visualiser le muscle à son insertion pubienne. La mesure au repos consistait en un seul cliché au sein duquel la zone d'intérêt correspondant au muscle était délimitée manuellement avec une mesure du module de cisaillement au sein de celle-ci. Pour les mesures en Valsalva, nous procédions à l'acquisition d'un clip vidéo de 5s au sein duquel les propriétés élastiques étaient mesurées au sein d'une zone délimitée manuellement image par image. La moyenne des mesures réalisées sur chaque image était retenue pour l'analyse. La co-activation des muscles *levator ani* était contrôlée par une manœuvre de biofeedback [51]. La même procédure était utilisée du côté droit et du côté gauche. Les propriétés élastiques du muscle étaient rapportées sous la forme du module de cisaillement, en kPa. Nous avons d'abord décrit les caractéristiques de notre population. Nous avons ensuite rapporté le nombre de procédures réussies (possibilité de voir le muscle et d'obtenir une valeur de module de cisaillement). Nous avons rapporté les valeurs de module de cisaillement au repos et en Valsalva pour chaque côté. Nous avons recherché une différence entre le côté droit et le côté gauche ainsi qu'entre la position de repos et la manœuvre de Valsalva à l'aide d'un test de Wilcoxon. Le seuil de significativité était fixé pour $p<0,05$.

Résultats

Douze femmes ont été incluses dans cette étude. L'âge moyen était de 31 ans, l'indice de masse corporelle moyen de 28 $kg.m^{-2}$, la parité moyenne était de deux enfant avec un délai moyen depuis le dernier accouchement de 14 mois. Toutes les mesures au repos ont été réalisées avec succès alors que nous rapportons 2 échecs en manœuvre de Valsalva survenus chez les femmes ayant les indices de masse corporelle les plus hauts (supérieur à 35 $kg.m^{-2}$). A droite, le module de cisaillement mesure sur le *levator ani* était de 16,0 (6,9) kPa au repos versus 35,4 (13,9) en Valsalva ($p<0,005$). A gauche, il était de 17,1 (7,6) kPa au repos versus

37 ,6 (13,1) en Valsalva ($p<0,005$). Le module de cisaillement augmentait donc d'un facteur 2 entre la position de repos et la manœuvre de Valsalva. Il n'y avait pas de différences entre le côté droit et le côté gauche.

Conclusion

Il apparaît faisable de mesurer les propriétés élastiques du *muscle levator ani in vivo* en utilisant la technique d'élastographie par onde de cisaillement chez la femme. Il s'agit de la première description d'une telle mesure en utilisant une technologie non invasive basée sur les ultrasons. La prochaine étape est d'évaluer la reproductibilité de cette mesure ainsi que de s'assurer de la concordance entre les propriétés élastiques mesurées et la distension objectivée au niveau du plancher pelvien, avant d'envisager son utilisation en pratique clinique.

Etude 3 : Reproductibilité d'une mesure des propriétés élastiques du muscle *levator ani* en élastographie par onde de cisaillement chez la femme [52]

Objectifs

L'objectif principal de cette étude était d'évaluer la reproductibilité inter session, intra opérateur d'une mesure des propriétés élastiques du muscle *levator ani* en élastographie par onde de cisaillement. L'objectif secondaire était de comparer la reproductibilité pour ce muscle avec celles mesurées pour les muscles périphériques : *biceps brachii* et *gastrocnemius medialis.*

Méthodes

Il s'agissait d'une étude prospective monocentrique, comprenant deux visites espacées au minium de 12 heures et au maximum de 7 jours. Les participantes étaient des femmes non enceintes, nullipares, sans antécédent de pathologie périnéale et/ou musculaire, avec un indice de masse corporelle de moins de $3Kg.m^{-2}$.

Lors de la première visite, nous collections les caractéristiques socio-démographiques et anthropométriques. Le contenu des deux visites était ensuite identique : mesure des propriétés élastiques du muscle *levator ani* (repos, Valsalva, contraction), mesure des propriétés des muscles *biceps brachii* et *gastrocnemius medialis* (repos, étirement, contraction).

Concernant les mesures au niveau du muscle *levator ani*, le protocole d'acquisition des données était comparable à celui décrit dans l'étude précédente. Seul le muscle du côté droit était évalué. Nous réalisions 3 acquisitions au repos, puis 3 acquisitions en Valsalva et 3 acquisitions en contraction, chacune sous la forme d'un clip vidéo de 5s.

Concernant les mesures au niveau du *biceps brachii*, elles étaient réalisées au niveau du muscle droit chez un sujet assis avec le bras en position fléchi (90° de flexion au niveau du coude), à la même hauteur que l'épaule, l'avant-bras reposant sur un support plan et le muscle *biceps brachii* libre de tout appui. Là encore 3 acquisitions étaient réalisées au repos puis trois en étirement (même position mais extension du bras avec 180° au niveau du coude) et 3 en contraction, chacune sous la forme d'un clip vidéo de 5s.

Concernant les mesures au niveau du *gastrocnemius medialis* celles-ci étaient réalisées au niveau du muscle droit chez un sujet en décubitus latéral gauche, jambe gauche fléchie. Trois acquisitions étaient réalisées au repos avec le genou tendu et la cheville en position neutre. Trois acquisitions étaient réalisées avec le genou tendu et la cheville reposant sur un plan incliné de 20°. Trois acquisitions étaient réalisées en contraction maximale, dans la même position que pour les mesures au repos. Toutes les acquisitions étaient sous la forme d'un clip vidéo de 5s.

Toutes les mesures étaient réalisées avec un appareil Aixplorer® V12 (Supersonic Imagine, France) et la sonde linéaire SL 18-5 (5-18MHz). Les mesures étaient toutes réalisées par un seul opérateur. Les propriétés élastiques étaient rapportées sous la forme de la valeur du module de cisaillement (en kPa), comme évoqué dans les chapitres précédents. Pour les mesures au repos et en étirement/Valsalva nous considérions la moyenne de la valeur du module de cisaillement au sein de l'acquisition complète. Pour les mesures en contraction, nous retenions la valeur maximale mesurée au sein de l'acquisition. Nous avons retenu pour l'analyse la moyenne des 3 mesures pour chaque temps (repos, étirement/Valsalva, contraction).

Nous avons d'abord décrit les caractéristiques de notre population. Nous avons ensuite rapporté les indices de reproductibilité pour chacun des muscles et des temps étudiés à l'aide des indicateurs suivants : coefficient de corrélation intra classe (CCI), erreur standard de mesure (ESM, en kPa) et coefficient de variation (CV, en %).

Résultats

Vingt femmes ont été incluses, pour un âge moyen de 23 ans, un indice de masse corporelle moyen de 22,6 $kg.m^{-2}$. Le délai moyen entre les deux visites était de 46,6 heures. Toutes les femmes ont suivi l'intégralité du protocole. Les résultats sont exposés dans le tableau ci-dessous (Tableau A). La reproductibilité était excellente pour le *levator ani* au repos et en manouvre de Valsalva alors qu'elle était faible en contraction. Concernant le muscle *biceps brachii*, la reproductibilité était bonne au repos et en étirement mais faible en contraction. Pour le *gastrocnemius medialis*, seule la reproductibilité en étirement était bonne.

Tableau A : Reproductibilité intra opérateur inter session au niveau du *levator ani*, du biceps brachii et du gastrocnemius medialis

	Module de cisaillement moyen à V1, en kPa (écart-type)	**Module de cisaillement moyen à V2, en kPa (écart-type)**	**CCI [95%CI]**	**CV, en %**	**ESM, en kPa**
	Reproductibilité inter session intra opérateur pour le *levator ani*				
Repos	22.8 (8.0)	21.9 (6.8)	0.90 [0.80-0.95]	15.7	3.5
Valsalva	44.5 (13.1)	46.5 (14.2)	0.94 [0.88-0.97]	10.6	4.8
Contraction	59.3 (11.8)	55.1 (15.7)	0.43 [0.07-0.69]	25.1	14.8
	Reproductibilité inter session intra opérateur pour le *biceps brachii*				
Repos	5.1 (1.1)	5.1 (1.4)	0.77 [0.56-0.89]	17.6	0.9
Etirement	21.6 (5.4)	22.0 (5.0)	0.75 [0.52-0.87]	17.9	3.9
Contraction	83.4 (28.4)	87.2 (22.3)	0.56 [0.25-0.77]	28.6	24.4
	Reproductibilité inter session intra opérateur pour le *gastrocnemius medialis*				
Repos	4.7 (1.2)	5.1 (1.3)	0.49 [0.15-0.73]	24.5	1.2
Etirement	25.4 (11.4)	23.7 (8.3)	0.70 [0.45-0.85]	32.6	8.0
Contraction	82.3 (30.6)	77.9 (32.1)	0.56 [0.24-0.77]	37.8	30.3

V1 : première visite V2 : deuxième visite

Conclusion

L'élastographie par onde de cisaillement apparaît comme un outil reproductible pour l'évaluation des propriétés élastiques du muscle *levator ani* chez la femme au repos et en manœuvre de Valsalva. Les mesures réalisées sur ce muscle en contraction n'étaient pas reproductibles. Les résultats étaient plus décevants concernant les muscles périphériques : bon à acceptable pour le *biceps brachii* et modéré à faible pour le *gastrocnemius medialis.* Cette technologie pourrait être utile pour améliorer notre connaissance de la physiopathologie du traumatise périnéal obstétrical.

Etude 4 : Reproductibilité et acceptabilité d'une mesure des propriétés élastiques du muscle sphincter anal externe en élastographie par onde de cisaillement, chez la femme enceinte à terme

Objectif

L'objectif principal de cette étude était de venir évaluer la reproductibilité intra opérateur inter session ainsi que la reproductibilité inter opérateur intra session d'une mesure des propriétés élastiques du muscle sphincter anal externe en élastographie par onde de cisaillement chez la femme enceinte à terme. L'objectif secondaire était d'apprécier l'acceptabilité de cette mesure dans la population étudiée.

Méthodes

Il s'agissait d'une étude monocentrique, prospective comprenant deux visites espacées au minium de 12 heures et au maximum de 7 jours. Les femmes éligibles étaient les femmes majeures, nullipares, avec un fœtus unique en présentation céphalique, avec une grossesse de déroulement normal. L'inclusion était possible dans l'étude à partir de 37 semaines d'aménorrhée (à terme).

Lors de la première visite, nous collections les caractéristiques socio-démographiques et anthropométriques. Nous réalisions également une mesure des propriétés élastiques du muscle sphincter anal externe, toujours par le même opérateur pour chacune des participantes. Les mesures étaient réalisées chez une patiente en position gynécologique avec la vessie vide selon les mêmes modalités que dans l'étude précédente. Le muscle sphincter anal externe était visualisé en échographie 2D en utilisant la voie transpérinéale. Une fois le muscle visualisé nous procédions aux acquisitions sous la forme de clips vidéo de 5 s : 3 au repos, 3 en manœuvre de Valsalva et 3 en contraction périnéale.

Lors de la seconde visite nous procédions à une nouvelle évaluation des propriétés élastiques du muscle sphincter anal externe par deux opérateurs aveugles l'un de l'autre. Le premier était systématiquement le même que celui de la première visite (reproductibilité inter session intra opérateur), le second opérateur était systématiquement le même pour toutes les femmes (reproductibilité intra session inter opérateur).

Toutes les mesures étaient réalisées avec un appareil Aixplorer® V12 (Supersonic Imagine, France) et la sonde linéaire SL 18-5 (5-18MHz). Les mesures étaient toutes réalisées par un seul opérateur. Les propriétés élastiques étaient rapportées sous la forme de la valeur du module de cisaillement (en kPa), comme évoqué dans les chapitres précédents. Au sein de chaque acquisition, la région d'intérêt était identifiée manuellement. Pour les mesures au repos et en Valsalva nous considérions la moyenne de la valeur du module de cisaillement au sein de l'acquisition complète. Pour les mesures en contraction, nous retenions la valeur maximale mesurée au sein de l'acquisition. Nous avons retenu pour l'analyse la moyenne des 3 mesures pour chaque temps (repos, étirement/Valsalva, contraction).

En fin de deuxième visite nous évaluions l'acceptabilité à l'aide de la question suivante : « Si cet examen vous été proposé dans le cadre de votre suivi de grossesse pour prédire votre risque de lésions du sphincter anal à l'accouchement, le réaliseriez-vous ? Merci de répondre sur une échelle de 0 (certainement pas) à 10 (oui c'est certain) ».

Nous avons rapporté les indices de reproductibilité pour la reproductibilité inter sessions intra opérateur puis la reproductibilité inter opérateur intra session à l'aide des indicateurs suivants : coefficient de corrélation intra classe (CCI), erreur standard de mesure (ESM, en kPa) et coefficient de variation (CV, en %). L'acceptabilité était évaluée par le score moyen obtenu à la question posée. L'acceptabilité était jugée excellente en cas de score supérieur à 8/10.

Résultats

Trente-sept femmes ont été considérées pour l'analyse pour un âge moyen de 29 ans, un indice de masse corporelle moyen de 23,2 $kg.m^{-2}$ et un terme moyen à l'inclusion de 37 semaines d'aménorrhée. Le délai moyen entre les deux visites était de 42,3 heures. Les données de reproductibilité sont présentées dans le tableau ci-dessous (Tableau B). La reproductibilité était excellente en intra opérateur au repos et bonne en Valsalva et contraction. Pour la reproductibilité inter opérateur, elle était bonne au repos et Valsalva et modérée en contraction. L'acceptabilité était excellente avec un score moyen de 9,6/10 et aucune note inférieure à 9/10.

Tableau B : Reproductibilité intra opérateur inter session et inter opérateur intra session d'une mesure des propriétés élastiques du sphincter anal externe chez la femme enceinte à terme

	Module de cisaillement moyen à V1, en kPa (écart-type)	**Module de cisaillement moyen à V2, en kPa (écart-type)**	**CCI [95% IC]**	**CV, en %**	**ESM, en kPa**
	Reproductibilité intra opérateur inter session				
Repos	10.0 (4.4)	10.1 (3.9)	0.91 [0.84-0.95]	18.8	1.9
Valsalva	16.2 (6.6)	17.6 (7.0)	0.83 [0.72-0.90]	23.7	4.0
Contraction	34.6 (11.8)	37.5 (14.0)	0.85 [0.75-0.91]	20.5	7.4
	Reproductibilité inter opérateur intra session				
Repos	10.1 (3.9)	10.3 (4.0)	0.79 [0.66-0.87]	25.5	2.6
Valsalva	17.6 (7.0)	18.6 (8.0)	0.84 [0.73-0.90]	23.9	4.4
Contraction	37.5 (14.0)	35.4 (13.9)	0.70 [0.53-0.82]	30.2	11.0

V1 : première visite   V2 : deuxième visite

Conclusion

Il s'agit de la première description d'une mesure quantitative *in vivo* des propriétés élastiques du muscle sphincter anal externe. Cet examen apparaît acceptable pour les femmes et fiable en termes de reproductibilité. Cette technique pourrait nous permettre d'améliorer notre appréciation du risque individuel de rupture sphinctérienne lors de l'accouchement et ainsi d'offrir une information personnalisée aux femmes enceintes.

Etude 5 : Modifications des propriétés élastiques des muscles du plancher pelvien de la femme au cours de la grossesse

Objectifs

L'objectif principal était de décrire l'évolution des propriétés élastiques des muscles du plancher pelvien (*levator ani*, sphincter anal externe) et des muscles périphériques (*biceps brachii* et *gastrocnemius medialis*) au cours de la grossesse. L'objectif secondaire était de rechercher si les propriétés élastiques des muscles du plancher pelvien à terme étaient associées au risque de déchirures périnéales à l'accouchement, en cas d'accouchement par voie vaginale.

Méthodes

Il s'agissait d'une étude prospective, monocentrique, longitudinale. Les femmes éligibles étaient enceintes, nullipares, avec une grossesse de déroulement normale, sans pathologie périnéales ou articulaire pré existantes, avec un indice de masse corporelle inférieur à 35 $kg.m^{-2}$. Trois visites étaient prévues au protocole : entre 14 et 18 semaines puis entre 24 et 28 semaine set enfin entre 34 et 38 semaines.

Lors de la première consultation, les données socio démographiques et anthropométriques étaient collectées. A l'issue de l'accouchement, les données concernant celui-ci étaient collectées dans le dossier médical. Chacune des trois visites comportait une mesure des propriétés élastiques des muscles suivant : *levator ani* (repos, Valsalva, contraction), sphincter anal externe (repos, Valsalva, contraction), *biceps brachii* (repos, étirement, contraction), *gastrocnemius medialis* (repos, étirement, contraction). Le protocole utilisé était exactement identique à celui décrit pour les études 3 et 4 avec exactement le même matériel.

Nous avons d'abord décrit les caractéristiques de notre population. Puis sous avons décrit l'évolution des propriétés élastiques des muscles étudiés au cours de la grossesse en utilisant une analyse one-way ANOVA pour mesures répétées. Nous avons ensuite comparé les propriétés élastiques des muscles du plancher pelvien au troisième trimestre entre les femmes ayant eu une déchirure périnéale (quelle que soit la gravité) et celle ayant un périnée intact en utilisant un test de Student.

Résultats

Quarante-sept femmes ont été considérées pour l'analyse avec un âge moyen de 28 ans, un indice de masse corporelle moyen de 22,1 $kg.m^{-2}$. Dix femmes (21,3%) ont nécessité une aide instrumentale à la naissance (2 césariennes, 8 accouchement instrumentaux par voie basse). Parmi les femmes ayant accouché par voie vaginale, 38 (80,1%) ont eu une déchirure périnéale. Une seule femme (2,1%) a eu une LOSA. L'évolution des propriétés élastiques des muscles étudiés au cours de la grossesse est décrite dans le tableau ci-dessous (Tableau C), il n'existait pas de modification des propriétés des muscles du plancher pelvien concernant les mesures les plus reproductibles (repos, Valsalva).

Tableau C : Evolution des propriétés élastiques des muscles du plancher pelvien ainsi que des muscles périphériques au cours de la grossesse

		Module de cisaillement moyen à V1 (écart-type), en kPa	Module de cisaillement moyen à V2 (écart-type), en kPa	Module de cisaillement moyen à V3 (écart-type), en kPa	p
Biceps brachii					
	Repos	5.4 (0.4)	5.0 (0.3)	5.3 (0.4)	0.48
	Etirement	22.7 (1.1)	21.7 (1.0)	21.5 (1.0)	0.53
	Contraction	84.1 (4.6)	94.1 (4.2)	97.1 (4.2)	0.003
Gastrocnemius medialis					
	Repos	4.1 (0.2)	4.0 (0.2)	3.4 (0.2)	0.004
	Etirement	22.2 (1.5)	21.6 (1.5)	21.3 (1.4)	0.79
	Contraction	70.0 (4.5)	76.9 (4.7)	71.7 (6.7)	0.26
Levator ani muscle					
	Repos	25.8 (1.7)	25.4 (1.6)	27.4 (1.3)	0.43
	Valsalva	43.5 (1.8)	42.8 (1.8)	43.4 (2.0)	0.93
	Contraction	54.8 (2.0)	56.6 (1.6)	57.9 (2.2)	0.40
Sphincter anal externe					
	Repos	9.6 (0.7)	9.4 (0.6)	10.5 (0.6)	0.15
	Valsalva	18.7 (1.5)	19.2 (1.4)	19.6 (1.4)	0.43
	Contraction	33.4 (1.9)	36.6 (2.1)	37.9 (1.9)	0.003

V1 : première visite V2 : deuxième visite V3 : troisième visite

Les femmes ayant eu une déchirure périnéale lors de leur accouchement par voie vaginale avaient un module de cisaillement mesuré au muscle sphincter anal externe en manœuvre de Valsalva plus faible que celles avec un périnée intact (18,2 kPa versus 27 kPa ; p<0,005). Il n'y avait pas d'autres différences concernant les autres muscles ou conditions étudiées.

Conclusion

Nous n'avons pas observé de modification significative des propriétés élastiques des muscles du plancher pelvien ni des muscles périphériques au cours de la grossesse. Les femmes avec une déchirure périnéale avaient un muscle sphincter anal externe plus souple en manœuvre de Valsalva à terme que les femmes avec un périnée intact, alors qu'il n'y avait pas de différence significative pour le *levator ani*. Ces résultats supportent l'hypothèse d'une association entre propriétés élastiques du plancher pelvien et traumatisme perineal obstétrical. D'autres études sont nécessaires pour étudier l'impact sur le risque de traumatisme grave (LOSA, désinsertion du *levator ani).*

Conclusion générale

Dans ce travail, nous avons défendu et argumenté l'hypothèse d'une association entre les propriétés élastiques des muscles du plancher pelvien de la femme enceinte et le risque de traumatisme périnéal obstétrical. L'élément limitant était l'absence de méthode validée pour étudier les propriétés élastiques de ces muscles de manière directe, quantitative, non invasive et *in vivo*. Nous avons donc rapporté la façon dont nous avons utilisé la technique d'élastographie par onde de cisaillement pour la rendre applicable à l'étude des muscles du plancher pelvien de la femme en dehors et pendant la grossesse. Nos résultats mettent en avant que cette technique permet une étude reproductible des propriétés élastiques de ces muscles. Il s'agit là de résultats particulièrement novateurs puisqu'il s'agissait de la première description de ce type de mesure, là où les outils pré existants utilisaient des techniques indirectes et/ou invasives. Nous avons ensuite directement transposé cet outil dans une étude clinique sur des femmes enceinte qui n'a mis en évidence de modification des propriétés élastiques des muscles du plancher pelvien au cours de la grossesse, contrairement à ce qui était décrits sur modèle animal. Nous avons, en revanche, observé que les femmes avec une déchirure périnéale présentaient un sphincter anal externe moins rigide à terme que les femmes avec un périnée intact. Ce résultat vient supporter les données obtenues dans des travaux précédents sur l'association entre laxité ligamentaire et LOSA ainsi que les résultats obtenus sur modèle animal [23, 33, 34, 37]. L'hypothèse d'une association entre traumatisme périnéal obstétrical et propriétés élastiques des muscles du plancher pelvien est donc partiellement validée. D'autres études, de plus grande ampleur, sont nécessaires afin d'étudier l'impact sur le risque de déchirure grave du périnée (LOSA, désinsertion du *levator ani*) dont la prévalence est plus faible d'où la nécessité de gros effectifs. Il pourrait également être intéressant de réaliser des mesures répétées au cours des différentes phases du travail obstétrical pour mieux comprendre les mécanismes associés à la survenue de ces déchirures graves.

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Short abstract

Obstetric perineal tears occurring at childbirth are negative outcomes that strongly impact women's health (pain, incontinence, sexuality). We hypothesized that considering the intrinsic elastic properties of women's pelvic floor muscles would optimize the efficiency of existing predictive strategies. However, there was no validated method allowing an *in vivo*, quantitative and non-invasive assessment of these elastic properties. We considered the technology of shear wave elastography allowing an *in vivo* assessment of a muscle's elastic properties and applied it, for the first time, to the study of pelvic floor muscles. Therefore, we reported that it is feasible to measure the elastic properties of the *levator ani* muscle and the external anal sphincter muscle and that these assessments were reliable. Then, we used this technology into a longitudinal study investigating any change in the elastic properties of women's pelvic floor muscles through pregnancy. We failed to report any significant changes in these muscles elastic properties during pregnancy. We reported that women suffering from any perineal tear at childbirth had a less stiff external anal sphincter during late pregnancy than those having an intact perineum at childbirth. This result is in accordance with our initial hypothesis and support the implementation of upcoming larger studies in this thematic.

List of abbreviations

- BMI: Body Mass Index
- CV: Coefficient of Variation
- EAS: External anal sphincter
- IAS: Internal anal sphincter
- ICC: Intraclass Correlation Coefficient
- LAM: *Levator ani* muscle
- MRI: Magnetic Resonance Imaging
- OASI: Obstetric anal sphincter injury
- OR: Odd Ratio
- PFDI: Pelvic Floor Distress
- PFM: Pelvic floor muscle
- POP-Q: Pelvic Organ Prolapse Quantification
- SD: Standard Deviation
- SEM: Standard Error of Measurement
- SWE: Shear wave elastography

General Introduction

Vaginal delivery is a unique method of childbirth during a woman's lifetime, not only because of its emotional aspects, but also the physiological aspects. During progression of the fetal body through a woman's pelvic region and the perineum, many tissues are subjected to a massive strain reaching up to 300% for some of them [1, 2]. This is the only event during a woman's lifetime when the body can sustain such an amount of strain.

With respect to vaginal delivery, pregnancy is associated with important changes in the intrinsic biomechanical characteristics of the tissues in women [21]. These changes are necessary as an adaptation to the changes in weight and posture that are induced by the gravid uterus and as a preparative process for a vaginal delivery [21]. This preparative process should achieve two main objectives: (1) allow the progression of the fetus through the pelvic inlet and the perineum and (2) protect the women's perineum from damages associated with childbirth.

Women's pelvic floor muscles (PFMs) can be damaged as a result of vaginal delivery, especially involving the *levator ani* muscle (LAM), the *external anal sphincter* (EAS), and, for the worst, *the internal anal sphincter* (IAS) and the rectal mucosa [14, 53]. These injuries are categorized as LAM avulsion (LAM disinsertion from the pubic bone) and obstetric anal sphincter injuries (OASIs). These negative outcomes occur frequently and strongly affect the women's health, being associated with 5% to >20% of the vaginal deliveries [3, 4, 14, 16, 19]. Further, these disorders are associated with anal incontinence, urinary incontinence, perineal pain, sexual dysfunction, and postnatal depression [15-17, 54], and almost 50% of the women presenting with such complications remain symptomatic for several years [15].

Main risk factors for these injuries are well-reported, such as the first delivery, instrumental vaginal delivery, and large newborn birthweight [4, 5, 14, 53]. Despite these risk factors, predicting perineal trauma at childbirth is challenging. Some recent strategies have been reported but with disappointing results [6, 7, 16]. The current predictive strategies focus mainly on the characteristics of the type of delivery and not, or not enough, on the intrinsic characteristics of the tissues. We strongly believe that the upcoming challenge is to improve the efficiency of these strategies by including individual data of the biomechanical characteristics of the pelvic muscles and the perineal tissues in women, especially in terms of the elastic properties of the PFMs [23].

In this thesis, first, a literature review focused on evaluating the biomechanical behavior of the perineal tissues for establishing predictive approaches in relation to perineal trauma at childbirth is presented. Second, the feasibility of performing shear wave elastography (SWE) for investigating the elastic properties of the LAM in women and the reliability of this technique for PFMs in comparison with that for the peripheral muscles are reported. Third, the feasibility and reliability of SWE for investigating the elastic properties of the EAS in pregnant women is presented. Last, the results of a longitudinal study reporting the data related to the elastic properties of both PFMs and peripheral muscles through pregnancy using SWE is provided. Finally, the prospects offered by these results in a clinical situation for optimizing both predictive and preventive strategies are discussed.

Study 1 – Why and how to consider the tissue biomechanical behavior in women for the risk assessment of perineal trauma at childbirth? A literature review [23]

1 – Anatomical considerations

The pelvic floor of a woman is a complex muscular and ligamentous organization, and PFMs are most commonly affected by perineal trauma at childbirth. The two most important muscular structures, which are affected by childbirth, in a woman's pelvic floor, are the LAM and the anal sphincter complex, which includes the EAS, IAS, and rectal mucosa. Herein, we present the anatomical considerations for these two muscular structures.

1.1 – Levator ani muscle (LAM)

The LAM is a muscular complex composed of three portions [1, 8]. The first one is *the iliococcygeus muscle,* which is constituted by the right and the left muscle parts that join behind the rectum and span the gap from one pelvic bone to another. The second portion is the *pubovisceral muscle* which has three specific parts that originate from the pubic bone, and its muscle fibers attach to the walls of the pelvic organ and the perineal body. These three parts include the following structures:

- *The puboperineal* with its insertion into the perineal body (fibrosis area between the posterior vulvar commissure and the anus).

- The *pubovaginal* with its insertion into the vaginal wall.

- The *puboanal* for which the fibers will take an insertion between the EAS and the IAS.

The third portion is the *puborectal muscle,* which is inserted into the pubic bone, but more laterally than the *pubovisceral muscle,* and forms a sling around and behind the rectum [1, 8].

The three portions of the LAM articulate together to represent a hiatus, the levator hiatus, that acts as a diaphragm to sustain and stabilize the pelvic organs. This hiatus is crossed by the urethra anteriorly, the vagina in the middle, and the rectum posteriorly [1, 8]. This hiatus behaves like the collar of a hernia. In case of an increased levator hiatus area, pelvic organs can slide into the levator hiatus leading to pelvic organ prolapse. A schematic representation of the LAM architecture is provided in Figure 1.

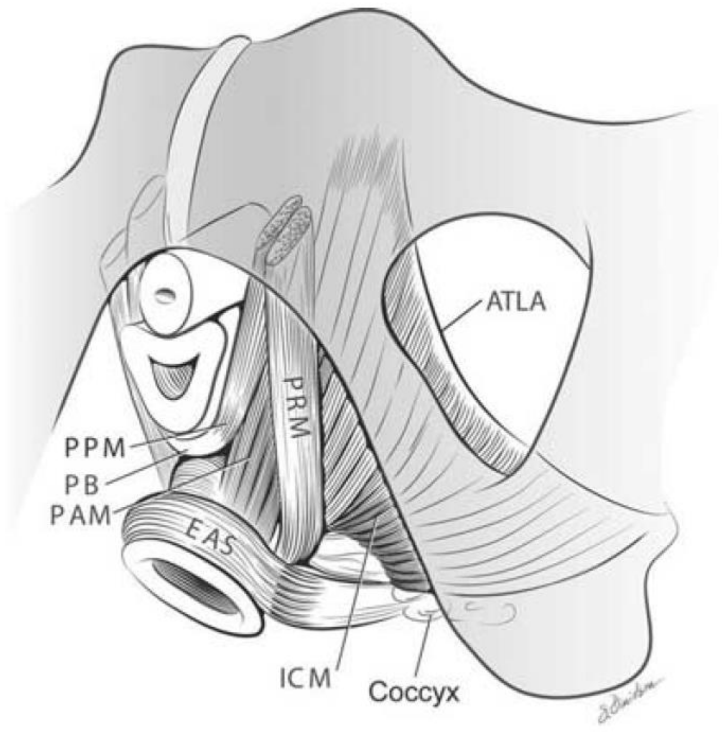


Figure 1: Schematic representation of the *levator ani muscle* architecture [1]

ATLA: arcus tendineus levator ani
EAS: *External anal sphincter*
ICM: *iliococcygeus muscle*
PAM: *puboanal muscle*
PB: perineal body
PPM: *puboperineal muscle*
PRM: *puborectal muscle*

New ultrasound and magnetic resonance imaging (MRI) techniques enable a highly detailed assessment of the LAM's anatomy, especially of the levator hiatus architecture which is well appreciated by the 3D acquisition systems. Figure 2 represents a 3D ultrasound reconstruction of the levator hiatus. The surface of the levator hiatus is associated with the occurrence of a pelvic organ prolapse [20].

LAM is a striated muscle, which is mainly composed of type 1 muscle fibers [8]. The LAM has two main actions. The first is a static or postural one, which is mainly performed by the iliococcygeus muscle [8]. The second main action is the active contraction of the pubovisceral and the puborectal muscles in response to an increased intraabdominal pressure, such as while coughing and impulsion, to avoid an overdistension of the levator hiatus. Moreover, a recent embryological study reports that the medial part of the LAM is mainly composed of smooth muscle cells that are under autonomic nerve influence, and the lateral part comprises the striated muscle cells under somatic nerve influence [55].

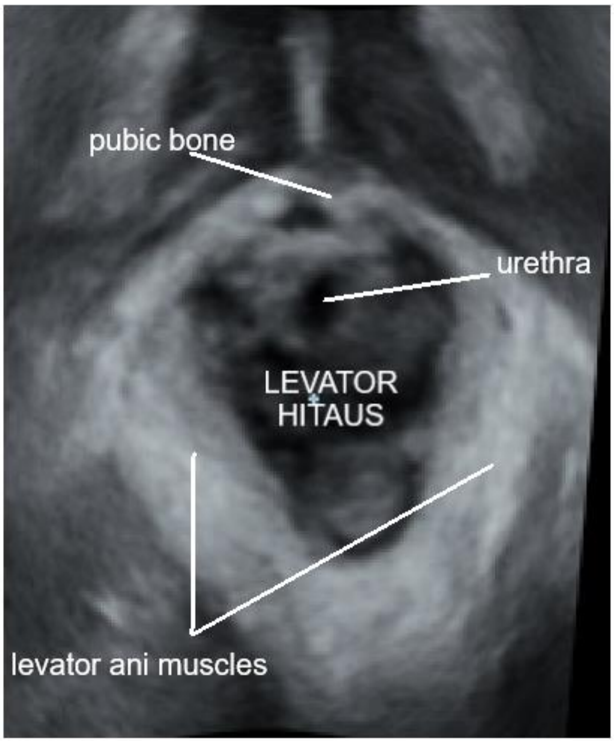


Figure 2: Ultrasound reconstruction of the levator hiatus (personal data)

1.2 – Anal sphincter complex

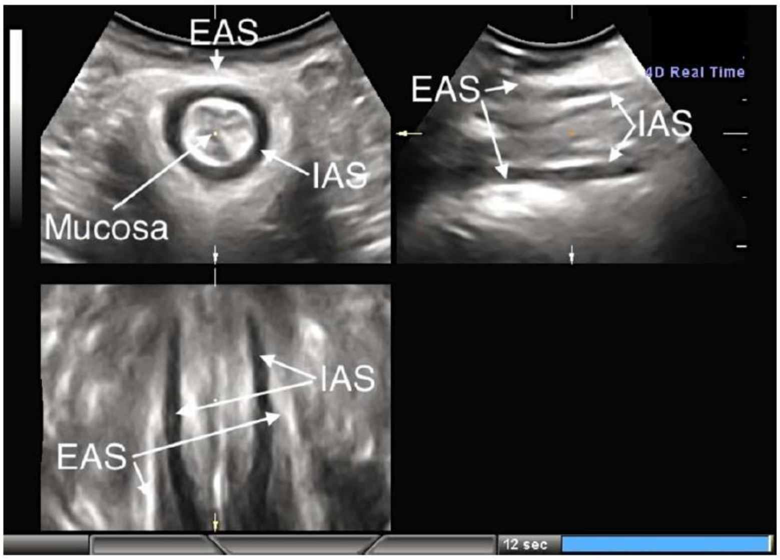
The anal sphincter complex involves three concentric structures represented by (from the outside to inside) the EAS, IAS, and rectal mucosa.

The most superficial part of the anal sphincter complex is the EAS, which is a concentric muscle that inserts into the perineal body and the LAM. It has three parts: a deep part with its insertion into the *puboanalis muscle*, a superficial part with its insertion to the perineal body, and a subcutaneous part, which is the most superficial part of the anal canal [9, 56]. The EAS is mainly composed of striated muscle cells, with type 1 muscle fibers, and receives innervation from a branch of the pudendal nerve. The EAS functions to provide a voluntary control of defecation, which is necessary to avoid anal leakages and promote social continence. Conversely, the relaxation of EAS is necessary to allow for a normal, unobstructed, defecation. Any damage to the EAS induces anal incontinence, especially, the inability to avoid anal leakages (gas and/or stool) in case of urgent need to defecate [9, 56].

Underlying the EAS, the IAS is a thin muscular structure made of smooth muscle cells and receives innervation from the autonomic nervous system. The IAS is responsible for maintaining approximately 70% of the muscle relaxation and mainly functions to permit passive anal continence. Moreover, any damage to the IAS may lead to anal incontinence, especially to passive anal incontinence (gas and/or stool), without feeling the urge to defecate [9, 56].

Finally, the IAS is developed from the rectal mucosa, a mucosal structure that delimits the anal canal, which is the deepest part of the anal sphincter complex and the anal canal. In case of any pelvic floor trauma leading to a damage of the anal sphincter complex with associated damage to the EAS, IAS, or rectal mucosa, there is a direct communication between the vaginal and the anal canal. The rectal mucosa defines the limits of the anorectal-ampulla and ensures anal continence. Any damage/disease in this structure may lead to defecation disorders and/or anal incontinence. [9, 56]

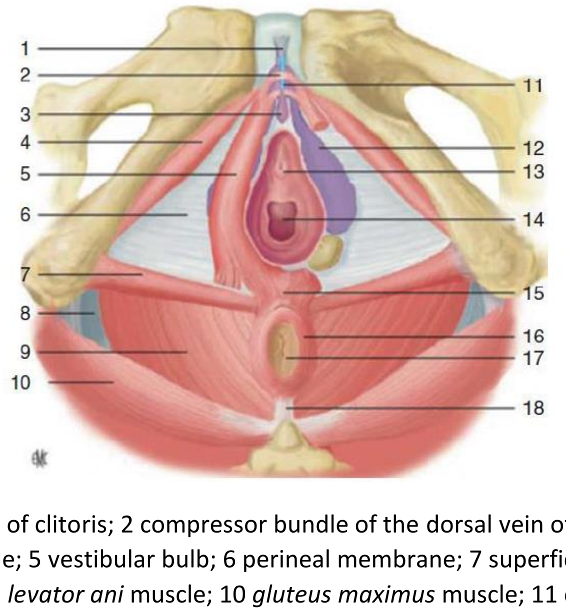
As we reported it for the LAM, new ultrasound and MRI technologies allow a high-quality anatomical assessment of the anal sphincter complex. First, these assessments involved endoanal imaging techniques; however, there are burgeoning data reporting that exoanal transperineal techniques provide results with a quality comparable to the endoanal imaging techniques [57]. Figure 3 provides a reconstruction of the anal sphincter complex using a 3D transperineal ultrasound.



EAS: external anal sphincter IAS: internal anal sphincter

Figure 3: Ultrasound reconstruction of the anal sphincter complex using transperineal ultrasound [58]

As represented in Figure 4, the anal sphincter complex is in immediate continuity with the vaginal opening and the perineal body. Therefore, any damage to the pelvic floor at childbirth involving the posterior part of the perineum can easily extend to the anal sphincter complex.



1 Suspensory ligament of clitoris; 2 compressor bundle of the dorsal vein of the clitoris; 3 clitoris; 4 *ischiocavernosus* muscle; 5 vestibular bulb; 6 perineal membrane; 7 superficial transverse muscle; 8 *sacrotuberosus* ligament; 9 *levator ani* muscle; 10 *gluteus maximus* muscle; 11 dorsal vein of the clitoris; 12 *bulbospongiosus* muscle; 13 urethra; 14 vagina; 15 perineal body; 16 external anal sphincter; 17 anus; 18 anococcygeal ligament

Figure 4: Muscles of the female perineum (perineal view) [9, 59]

2 – Physiology and pathophysiology of vaginal delivery

Exploring the mechanisms of vaginal delivery would lead to the study of mechanisms of labor onset, cervical ripening, and fetal descent into the pelvic inlet and head through the pelvic floor of women. Regarding the topic of interest of this thesis, our discussion is focused on the mechanisms associated with the fetal head expulsion, meaning the last part of the vaginal delivery. Herein, we will present the main principles of the fetal head expulsion that are necessary to consider for understanding the research presented in this thesis.

2.1 – Physiology of fetal head expulsion

Once the fetal head progresses beyond the ischiatic spines, the process of fetal head expulsion starts. The fetal head is defined according to the position of the fetal occiput from the pubic bone of the mother. In 75% of cases, the fetal head presentation is an *anterior occiput*, which means that the fetal occiput lies immediately under the mother's pubic bone. In this position, the flexion of the fetal head is optimal; therefore, the presenting fetal head diameter at the pelvic floor in women is the smallest (9.5cm). In any other positions (posterior or lateral occiput presentation) the fetal head is not correctly flexed; therefore, the presenting fetal head diameter is more important (up to 12-13cm) [10, 60].

The fetal head has to progress through the levator hiatus. At this time of the vaginal delivery, a massive stretch is applied to the LAM for fetal progression. Moreover, at this stage of delivery the LAM is stretched up to 300% to permit the progression of the fetal head, which is a high-risk situation for LAM injury. This fact is supported by a study that developed a finite element model, which showed that this stage of vaginal delivery displays the highest risk of LAM injury. This analysis also reported that the maximal *levator ani* stretch occurred in the anteroinferior aspect of the LAM [61]. The progression of the fetal head is a continuous process and not abrupt, which results in the application of massive loads on PFMs. Indeed, the mother has to push at each contraction (approximately by 3 minutes interval) with an increasing strain exerted on the LAM [60, 62].

Once the fetal head progresses beyond the plane of the levator hiatus, the fetal head must emerge from the perineum. The fetal head is in a maximal flexed position under the mother's pubic bone and has to exert a deflective movement around the pubic symphysis

(Figure 6). At this time, the most important constrain is applied on the posterior perineum which is in immediate continuity with the anal sphincter complex (Figure 4, 5, 6). Therefore, this step of vaginal delivery is the one with the high risk for the occurrence of OASI. During this phase the perineal body length, which is the distance between the anus and the posterior vulval commissure, will be stretched up to three times its initial length. Indeed, an observational clinical study providing measurements of the perineal body length at different stages of the labor reports a mean distance of 3.7 cm in antepartum versus 6.1 cm for the maximal length at this stage of vaginal delivery [63]. During this phase of fetal head emergence, the pelvic floor in a woman is supported by manual protection by the obstetrician to reduce the intensity of the constraint applied to the perineum and facilitating the emergence of the fetal head as a progressive and continuous process to avoid an abrupt progression of the fetal head that could induce severe perineal damage [10]. The importance of the perineal body distension can be appreciated in Figure 5.

Figure 5: Perineal body distension during the progression of the fetal head

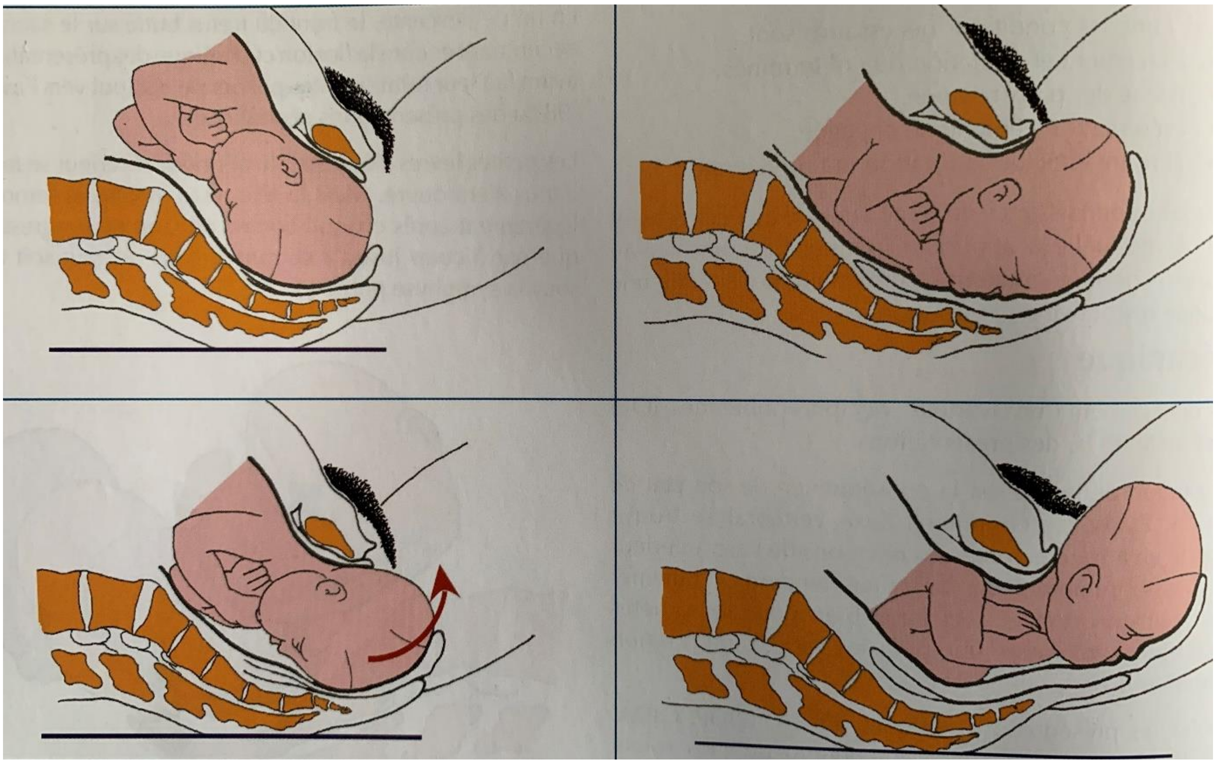


Figure 6: Schematization of the fetal head expulsion [10]

During this phase, a perineal tear often occurs in the perineum (for 50% of women regarding the first vaginal delivery). The tissues mostly affected are the vagina or the superficial perineal muscles, referred to as 1st and 2nd degree perineal tears. In cases with a too high strain and/or pathological situations (see below), the tear can be extended to the anal sphincter complex leading to an OASI.

2.2 – Pathophysiology of fetal head expulsion

2.2.1 – Fetal head position

As previously mentioned, in most cases the fetal head is presents with an anterior occiput presentation, meaning in the smallest head diameter. In 25% of cases, the fetus presents with a posterior or a transverse presentation leading to a deflection of the fetal head and so an increase in the fetal head diameter which presents at the pelvic inlet and the perineum (up to 13 cm compared to 9 cm) [10]. This increase in the fetal head diameter is associated with an increased risk for perineal trauma (OASIs and LAM avulsion) because of the massive strain exerted to the PFMs. This increase in the strain applied to PFMs is well-reported in finite elements studies, suggesting a 3.6 times the strain in case of occiput posterior presentation compared with optimal fetal head presentation [64, 65].

Additionally, in case of occiput posterior presentation, the fetal head expulsion can be difficult and may require instrumental delivery. As we will detail it below, this mode of delivery is the most important risk factor for perineal trauma at childbirth [4, 5, 14].

During a normal vaginal delivery, the fetal head often presents in the occiput posterior presentation for a while with a spontaneous rotation during the labor, which leads to an occiput anterior presentation. In case of persistent occiput presentation, the obstetrician could induce manually a rotation of the fetal head to reduce the fetal head diameter, reduce the risk of instrumental delivery, and probably reduce the risk of perineal trauma [66].

2.2.2 – Prolonged second stage of labor

The second stage of labor is defined as the time lapse between full cervical dilatation and birth. This stage represents the fetal descent into the pelvic inlet, and its expulsion through the pelvic floor. It could be very short; lasting from few minutes, especially in cases of multiparous women, to more than 120 minutes. During this phase, the fetal head pushes on the woman's pelvic floor in a repetitive way. Moreover, at each contraction (approximately one for 3 minutes), the fetal head exerts a strain on the pelvic floor. During the last part of the pushing phase, the strain is higher on the perineum because of the combined effect of the uterine contraction and the maternal voluntary pushing. Furthermore, at this last part, the fetal head is stuck within the PFMs with a permanent and massive strain which is accentuated with the maternal pushing efforts [62].

Even if data are lacking regarding objective, quantitative, *in vivo* measures, it is obvious that the last part of the second stage of labor represents the highest risk situation for the occurrence of perineal trauma. Indeed, the strain applied to the perineum has been estimated by measuring the intrauterine pressure during the pushing phase with values recorded up to 150 mmHg [60]. In addition, finite elements modelling suggested that, during this phases, some PFMs may increase in length up to 300% [1, 2, 67, 68].

It is possible that the pushing (maternal pushing efforts) phase may not be the one being at risk of perineal trauma at childbirth. Indeed, a recent study confirmed that the first part of the second stage of labor (fetal head descent into the pelvic inlet before maternal pushing) may be associated with a risk of perineal trauma [62]. The investigator's hypothesis is that the repetitive strain applied on PFMs during this phase, even if it is much less than that during the pushing phase, could induce repetitive, passive tissue microdamage. PFMs could be weakened by these microdamages, and is therefore more likely to break during the expulsion of the fetal head [62]. This theory is interesting but needs to be supported by additional models and *in vivo* studies.

Finally, a prolonged second stage of labor is also widely reported as a risk factor for perineal damage in the literature mainly because of its strong association with instrumental delivery requirement, fetal macrosomia (fetus with a large head), first delivery (25% of women), all these outcomes being strong risk factors for the occurrence of perineal trauma at childbirth [5, 14].

As mentioned previously, the second stage of labor is considered as prolonged when it lasts for more than 120 minutes. In clinical practice, when the fetal head is still above the mother's ischiatic spines for more than 3 hours, a cesarean section is performed to avoid vaginal birth complications. This intervention is not performed earlier because it has its own morbidity and because we first try to improve the fetal head descent (labor augmentation using oxytocin, maternal position).

2.2.3 – Instrumental delivery

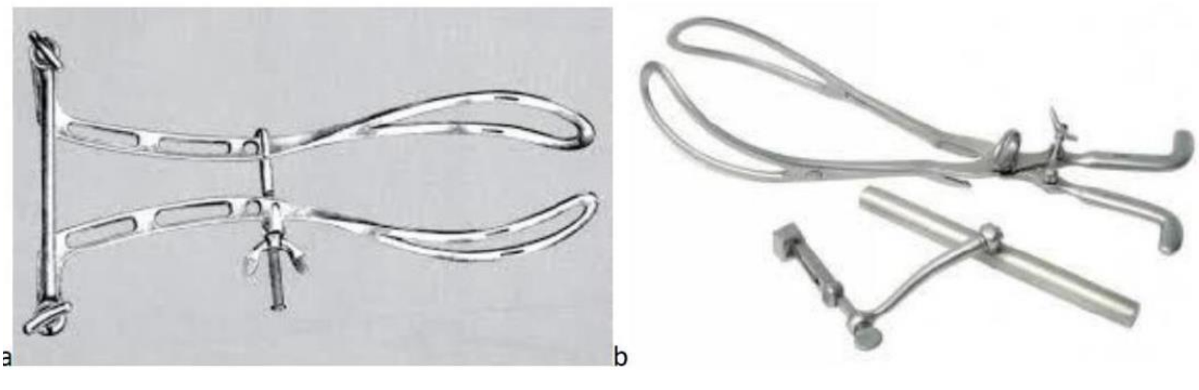
In case of fetal distress and/or in case of insufficient fetal progression during the pushing phase, an instrumental assistance for the delivery could be required. Instrumental delivery is widely reported as the main risk factor for perineal trauma at childbirth. However, the risk of OASIs or the risk of LAM avulsion with an odds ratio (OR) remains higher than 5.0 [5, 14, 53]. Nevertheless, this mode of delivery is frequent and represents 12% of the whole population of deliveries in France per year and 25% when only considering nulliparous women (delivery of the first baby) [11]. The risk of perineal trauma at childbirth could be different according to the type of instrument used for the delivery.

Currently, there are three main types of instruments. The first one is the vacuum, which is placed at the top of the fetal head (Figure 7). This instrument is the most often used in French practices (50% of instrumental deliveries). Its advantage is that it doesn't increase the fetal head diameter and thus the strain applied to PFMs is less. Comparatively with other instruments, the risk of perineal trauma in case of vacuum delivery is low [69]. The risk appears the highest for LAM avulsion, which is reported up to 50% of forceps deliveries in a recent meta-analysis [3]. Latest French guidelines recommend its use at first intention, when an instrumental delivery is required, for preventing perineal trauma at childbirth [14].

a- Kobayashi's vacuum b- Kiwi vacuum c- i-cup vacuum

Figure 7: Examples of obstetrical vacuum

The two other instruments are the forceps (28% of instrumental deliveries; Figure 8) and the spatulas (22% of instrumental deliveries; Figure 9). These instruments consist of two branches applied on each lateral side of the fetal head. This means that, using these types of instruments significantly increases the fetal head diameter. The forceps is a traction instrument which allows to pull the fetus through the pelvic inlet and the perineum. With this instrument the pulling force could exceed 200N [70, 71]. This instrument is considered associated with the highest risk of perineal trauma at childbirth [5, 14, 53]. Spatulas function in a different way. The branches are placed in a same way that we reported for the forceps. The difference is that the obstetrician will not pull the baby using the instrument but will push aside each branch to propel the baby.



a- Suzor's forceps b- Tarnier's forceps

Figure 8: Examples of obstetrical forceps

Figure 9: Thierry's obstetrical spatulas

2.2.4 – Manual perineal protection

Manual perineal protection is a method well-reported for more than 20 years, and its use is widely spread in some countries since a much longer time.

Two opposite politics for manual perineal protection at childbirth were advocated with a "hands on" versus a "hands off" policy. We have data suggesting that in countries with a "hands off" policy, meaning without manual perineal protection, switching to a "hands on policy" led to a massive reduction in the occurrence of OASIs [13, 72-74]. The "hands on" practice is now recommended to prevent perineal trauma at childbirth, especially from the occurrence of OASI [14, 53].

The principle of manual perineal protection is to slow down the progression of the fetal head with one hand and to support the posterior perineum with the other hand (Figure 10). This could avoid an abrupt delivery of the fetal head and decrease the strain exerted on PFMs. One major difficulty is to standardize this technique because a large number of different maneuvers have been reported [12].

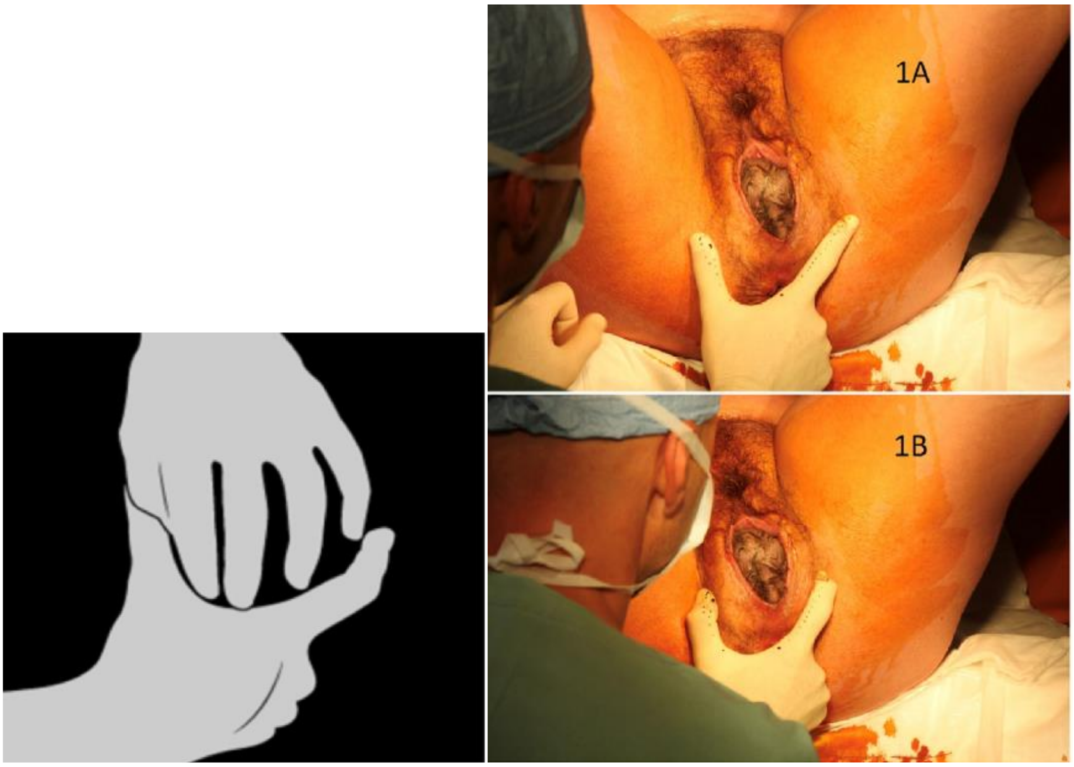


Figure 10: Manual perineal protection, the Viennese Method [75, 76]

3 – Epidemiology of perineal trauma at childbirth

3.1 – Obstetric anal sphincter injuries (OASIs)

A perineal tear is a usual outcome during a vaginal delivery with 50% of perineal tears at delivery reported in a national 2016 French database [11]. Most of these perineal tears only involve the vaginal mucosae, the skin, and some superficial perineal muscles representing 1st and 2nd degrees perineal tears (Table 1) [14, 53]. No negative long-term outcomes are associated with these tears, even if a perineal suture is indicated for perineal repair or there is increased short-term perineal pain. The problem is that for 0.25% to 6% of women, irrespective of the mode of vaginal delivery, a more extensive tear occurs involving the anal sphincter complex and represents the 3rd and 4th degrees perineal tears [4, 53]. These 3rd and 4th degrees perineal tears represent the group of OASIs (Table 1).

Table 1 – Classification of perineal tears [14, 53]

<table>
<tr><th></th><th colspan="2">Degree</th><th>Type of tissue injured</th></tr>
<tr><td rowspan="2"></td><td colspan="2">1st degree</td><td>Vaginal or vulvar epithelium</td></tr>
<tr><td colspan="2">2nd degree</td><td>Perineal muscles (perineal body)</td></tr>
<tr><td rowspan="4">OASIs</td><td rowspan="3">3rd degree</td><td>A</td><td>Less than 50% of the external anal sphincter</td></tr>
<tr><td>B</td><td>More than 50% of the external anal sphincter</td></tr>
<tr><td>C</td><td>External and Internal anal sphincters</td></tr>
<tr><td colspan="2">4th degree</td><td>Anal sphincter complex and anorectal mucosa</td></tr>
</table>

The main risk factors for occurrence of OASI have been already reported earlier in this thesis, which include nulliparity, instrumental vaginal delivery, large birthweight, and occiput posterior presentation [4, 5, 14].

As we reported above, the prevalence of OASIs in the literature is estimated between 0.25% and 6% of all the deliveries, which is meaningful and highlights the difficulty in comparing the results between one team and another and/or from one country to another. Moreover, the data from a European registry report a large range in the prevalence values of OASIs within the European countries [77]. This heterogeneity is likely because of the difficulty in diagnosis, which sometimes requires special expertise. Obstetrical habits, which are still different from one country to another (type of instrument in case of instrumental delivery, manual perineal protection, episiotomy policy), could also explain this variability.

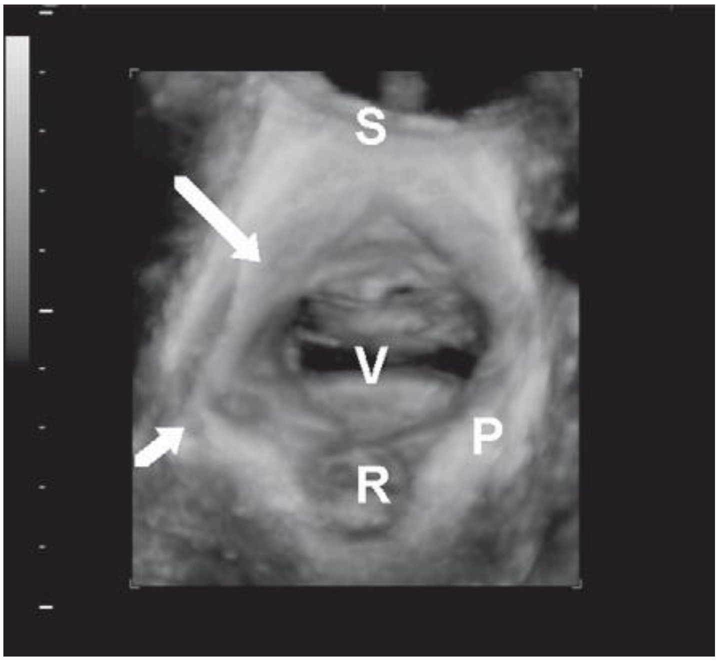
The outcomes of OASIs could be important because of the associated short- and long-term complications. During the first year after delivery, there is a risk of perineal pain and anal incontinence [78, 79]. These symptoms are associated with increased incidence of postnatal maternal depression and/or low quality of mother-child relationship [17]. Further, anal incontinence is a physically and psychosocially debilitating disorder which is associated with depression, especially in young women [17]. The risk of anal incontinence decreases (from an OR at 6.8 to 1.7) in the long term; however, anal incontinence is more persistent in women with OASIs compared to those without OASIs. OASI is also associated with perineal pain, urinary incontinence, and symptoms of genital prolapse with approximately 50% of women being symptomatic at least once a week [15, 16, 54, 78, 80].

3.2 – Levator ani avulsion

As previously mentioned, LAM avulsion is defined as the disinsertion of the LAM from its pubic insertion during a vaginal delivery. Such an injury cannot be seen immediately after a vaginal delivery, and physical examination at this time is not definitive for diagnosis because of the massive distension of PFMs. Therefore, in most cases of LAM avulsion, diagnoses can be confirmed by physical examination several weeks after the delivery, but it requires a special expertise. Perineal ultrasound, which allows for an easy and rapid diagnosis (Figure 11), could be considered as a diagnostic modality, or perhaps the MRI, which has more difficult accessibility but provides high quality diagnosis [3, 81]. Therefore, currently, most of the data about LAM avulsions come from ultrasound studies.

A recent large systematic review, used data from more than 5000 women and reported the prevalence of LAM as 15% in case of spontaneous delivery, 21% in case of vacuum delivery, and 52% in case of forceps delivery [3]. In this study, the investigators reported an increased risk of LAM avulsion in case of spontaneous versus cesarean delivery (OR = 10.69 [5.44-21.00]); in case of forceps versus spontaneous delivery (OR = 6.32 [4.56-8.76]); in case of forceps versus vacuum delivery (OR = 4.09 [2.87-5.84]). Interestingly, they reported that LAM avulsion was unilateral in most cases of spontaneous and vacuum delivery. Conversely, in case of forceps delivery, approximately half of the women had a bilateral avulsion [3].

Some previous as well as recent data suggest that the risk factors for occurrence of LAM avulsion appear similar to the risk factors for the occurrence of OASIs, which include nulliparity, instrumental delivery, especially in case of forceps delivery, large birthweight, and posterior occiput presentation [3, 18, 19] suggesting that these two types of injuries probably share a common pathophysiological process.



S: pubic symphysis V: Vagina R: Rectum P: Puborectal muscle

Figure 11: Ultrasound (3D) view of unilateral levator ani muscle avulsion (right) [82]

As reported in the anatomical description of PFMs previously in this thesis, the LAM is mainly responsible for pelvic organ mobility. Moreover, LAM avulsion, especially in cases of bilateral avulsions, leads to an increase in the levator hiatus area, which is associated with an increased occurrence of pelvic floor disorders. The more frequently occurring disorders include pelvic organ prolapse, the symptom of vaginal bulge, perineal pain, urinary incontinence, and obstructive rectal symptoms [18, 19].

Regarding the massive strain applied on PFMs during childbirth, the ability for PFMs to sustain this strain could vary among women. Therefore, implication of the elastic properties of biomechanical tissues may be meaningful in evaluating the risk of perineal trauma. Several investigators have evaluated changes in biomechanical characteristics of tissues associated with pregnancy and delivery.

4 – Changes in women's intrinsic characteristics during pregnancy

4.1 – Changes in joint laxity during pregnancy

The changes in joint mobility during pregnancy, which were evaluated by different modalities, have been reported inconsistently in the literature. There are two main possibilities for investigating the joint mobility in humans: a general assessment of the joint mobility using a global score, such as the Beighton's score, versus a focused assessment considering a specific joint [21, 83]. The approach using a global score has the advantage of being simple and reliable. Nevertheless, this mode of assessment is weak for investigating the changes over time. Some studies have investigated the changes in general joint mobility during pregnancy, with contradictory results [21, 22, 84, 85].

Changes in the joint mobility during pregnancy were investigated for many years, considering that Abramson *et al*. in 1934 reported an increase in joint mobility in the pubic symphysis using radiography [86]. Some recent studies, used measurement strategies focused on a specific joint and reported an increase in joint mobility during pregnancy for several measures, such as the mobility of the metacarpo-phalangeal joint, abduction of the fourth finger, and anterior drawer test for the knee [21-26, 84]. Therefore, it appears that multiple joints in a woman's body show increased mobility during pregnancy whether these are upper or lower limb joints, up to 180% for some joints [21]. This is often considered as an increase in ligamentous laxity, despite the absence of studies advocating any direct assessments of the joints or ligaments involved in childbirth, which is a major limitation of the existing data. Furthermore, these observations about an increase in joint mobility are in contradiction with a recent report using dynamic B-mode ultrasound to investigate (indirectly) the elastic properties of the patellar tendon during pregnancy, which failed to report any change over time [87].

Despite these limitations, changes in joint mobility, such as a gain in mobility, occur during pregnancy which could be a preparative process to afford the vaginal delivery (especially for pelvic joints). This led to the hypothesis that such an increase in joint mobility could reflect widespread changes in the biomechanical tissues of pregnant women that could involve other tissues such as the PFMs, with a potential impact on the mode of delivery, the risk of perineal trauma at childbirth, and the risk of pelvic floor disorders [23].

4.2 – Changes in spinal curvature during pregnancy

Several changes have been described about women's spinal curve during pregnancy, such as lumbar lordosis and lateral inclination [88-90]. These changes are necessary for maintaining the woman's center of gravity at the center of her support polygon [89]. The mechanisms of these changes probably involve a modification in joint mobility and ligamentous laxity, as reported above [23]. It is likely that these changes can affect obstetrical issues, especially in the mode of delivery by inducing modifications in the pelvic inlet inclination. As explained above, any impact on the mode of delivery will have an impact on the risk of perineal trauma at childbirth (operative vaginal delivery being the most important one). Furthermore, it could be hypothesized that women having the largest changes in the spinal curvature could be those having important changes in the biomechanical characteristic of the soft tissues such as PFMs, which could further be associated with the risk of perineal trauma at childbirth [23].

4.3 - Changes in pelvic organ mobility during pregnancy

As previously mentioned in this thesis, we do have data from studies investigating the changes in the biomechanical characteristics of the upper and lower limbs in women during pregnancy [21, 23]. However, data are lacking about the anatomical sites directly involved in childbirth, especially related to the pelvic floor in women. Although a direct assessment of PFMs and pelvic ligaments performed *in vivo* has not been performed sufficiently, abundant information about pelvic organ mobility during pregnancy is available in the literature. Investigating pelvic organ mobility includes measuring the displacement of some pelvic structures from the rest posture to a strained posture, which could be related to either perineal contraction or a Valsalva maneuver. The Valsalva maneuver involves performing a maximal pushing effort with a closed glottis which increases the intraabdominal pressure significantly, thereby exerting strain on the pelvic floor in women. This pressure is exactly the same as the effort required during the pushing phase of the vaginal delivery in women. Therefore, investigating the pelvic organ mobility may, indirectly, allow for an estimation of the mechanical properties of the pelvic floor in women. Most available data are from clinical studies and/or ultrasound studies. Although pelvic organ mobility can be studied by using an MRI, but this strategy is difficult during pregnancy because of the time taken in performing the analysis, which could be difficult for pregnant women, the limitation for accessing this procedure. Conversely, ultrasound is an easy, safe, and acceptable way to investigate the pelvic organ mobility during pregnancy. In addition, considering that ultrasound is already being widely used in the follow-up of fetus, it would be easy to add measurements dedicated to the pelvic organs of women.

4.3.1 – Clinical considerations

Pelvic organ mobility can be easily assessed clinically using a standardized approach, such as the Pelvic Organ Prolapse Quantification procedure (POP-Q) which has been developed with an international consensus (Figure 12) [91]. It brings information about the position of several fixed points at the anterior and the posterior vaginal wall, the cervix, with reference to the hymen while performing a Valsalva maneuver. Distance between the considered point and the hymen is measured in centimeter (using a ruler) and reported as negative values when above the hymen; as positive values when below the hymen. The defined points are as follows [91]:

- Aa: Located in the midline of the anterior vaginal wall; 3 cm proximal to the external urethral meatus. By definition, the range of position of this point relative to the hymen is from -3 to +3cm.

- Ba: Represents the most distal position of any part of the upper vaginal wall. By definition, Ba is at -3 cm in absence of prolapse and could have positive value according the degree of a potential pelvic organ prolapse.

- C: Represents the most distal edge of the cervix.

- D: Represents the location of the posterior fornix.

- Ap: Is a point located in the midline of the posterior vaginal wall; 3 cm proximal to the hymen with a range of position from -3 to +3cm.

- Bp: Represents the most distal position of any part of the upper posterior vaginal wall. It is at -3cm in absence of genital prolapse and could have positive value according the severity of a potential pelvic organ prolapse.

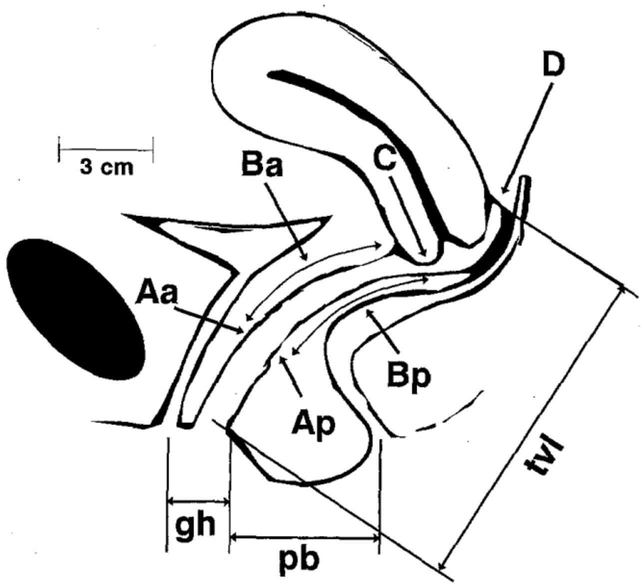


Figure 12: Six points (Aa, Ba, C, D, Ap, Bp), Genital hiatus (gh), Perineal body (pb) and total vaginal length (tvl) used for the POP-Q procedure [91]

Associated with the measure of these 6-point positions, the procedure includes the measure of lengths of three segments [91]:

- **gh (genital hiatus):** Distance from the midline of the external urethral meatus to the posterior midline hymen.

- **pb (perineal body):** Distance from the posterior margin of the genital hiatus to the mid-anal opening

- **tvl (total vaginal length):** Is the greatest depth of the vagina. It is the only measure done at rest.

These measures are used to defined five stages of pelvic organ prolapse as follow [91]:

- **Stage 0:** No prolapse is demonstrated
- **Stage 1:** The criteria for stage 0 are not met, but the most distal portion of the prolapse is more than 1 cm above the hymen
- **Stage 2:** The most distal part of the prolapse is between -1 and +1 cm from the hymen. This stage is usually considered as a clinically significant prolapse.
- **Stage 3:** The most distal portion of the prolapse is >1 cm lower than the hymen (+1cm) but protrudes no further than 2 cm less than the tvl length.
- **Stage 4:** Complete eversion of the total length of lower genital tract.

There is an abundant literature reporting changes in clinical pelvic organ mobility throughout pregnancy [21, 22, 27, 28, 92]. Most of the studies reported that the position of POP-Q points became lower through pregnancy [21, 22, 27, 28]. Only Reimers *et al*. reported a moderate cranial shift of POP-Q points during pregnancy, which the investigators explained because of the specific position of a woman during the measure in their study (sitting 45° upright position) compared to the usual position (supine lithotomy position) [92]. All studies report an increase in the length of the different measured segments through pregnancy, which could be interpreted as a distension of the pelvic floor through pregnancy [21, 22, 27, 28, 92]. Regarding these changes, the prevalence of clinically significant clinical prolapse (stage 2 or more) during pregnancy is relatively high, which affects up to 30% of women in late pregnancy [93]. This increase in mobility and distension appears continuous through pregnancy with a progressive recovery in the postpartum period. This recovery appears faster in case of cesarean section compared to vaginal delivery but without any significant difference between these two groups 12 months after the delivery [92].

4.3.2 – Ultrasound considerations

Many studies have investigated pelvic organ mobility during pregnancy using perineal ultrasound. The most reported technique is the transperineal ultrasound as described by Dietz *et al.* [82, 94].

Some studies report an increase in bladder neck descent during pregnancy beyond a threshold considered as associated with stress urinary incontinence (more than 15-20 mm) [22, 95-98]. This bladder neck descent can be easily measured in 2D transperineal ultrasound by comparing the distance between the pubic symphysis and the bladder neck at rest and then during Valsalva maneuver, the difference between the two measures is reported as the bladder neck descent [99]. This ultrasonographic observation of an increase in bladder neck descent is consistent with clinical observations reporting a low position of the Aa point (bladder neck) through pregnancy and also with the high prevalence of stress urinary incontinence during pregnancy (up to 50% of women) [22, 27, 28, 100]. An increase in bladder neck descent is usually because of a weak or an injured pelvic floor in that specific case of pregnancy. Considering that this observation is performed before the occurrence of any perineal trauma, it is likely that it is associated to a weak pelvic floor. This supports the hypothesis of a change in the biomechanical characteristics of the pelvic floor in women during pregnancy.

An increase in the levator hiatus area is another widely reported observation, considering the measures taken at rest, during Valsalva maneuver or perineal contraction [22, 29, 30, 98, 101, 102]. This observation is consistent with those reporting a low position for most of the POP-Q points during pregnancy and the high prevalence of stage 2 pelvic organ prolapse in late pregnancy.

As previously reported, two phenomena could lead to an increase in levator hiatus area: an anatomical muscle damage (avulsion) or an overdistension of the LAMs. Some studies report that women having the lowest levator hiatus area and or the lowest bladder neck descent in late pregnancy will be those who require an operative delivery (instrumental vaginal delivery or a cesarean section) [101, 103]. This supports the hypothesis of a significant change in the biomechanical properties of the pelvic floor in women and that this change could be associated with the mode of delivery and the risk of perineal trauma.

4.4 – Pathophysiological process

The biological mechanisms involved in these biomechanical changes remain unknown. One recurrent hypothesis is the involvement of the role of relaxin. This hormone is produced by the ovaries, the mammary tissue, and the placenta and has a role in conjunctive tissue remodeling [25]. An association between high maternal serum levels of relaxin and high joint mobility and ligamentous laxity has been reported [32, 85]. Nevertheless, this point remains debated since this association has not been reported in other studies [25, 31]. Another hypothesis is the effect of sexual hormones, especially estradiol, whose expression is important during pregnancy. Nevertheless, the impact of these hormones is unclear since different studies have reported contradictory results (an increase or a decrease in stiffness) for muscle and tendons [104, 105], and one study did not report any association between sexual hormones and joint laxity during pregnancy [31].

Regardless of the potential role of relaxin or estradiol, the main hypothesis consists of a change in collagen modeling with a decrease in the ratio of type 1/type 3 collagen. Collagen is the main component of the muscular extracellular matrix that determines the biomechanical properties of muscles and their ability to sustain a load [34]. This point remains hypothetical because we cannot report data for ex vivo histological analysis of tissues in pregnant women [23].

Finally, these hypotheses are mainly related to joint mobility and ligamentous laxity but not directly to the muscles, in particular the PFMs. Intriguingly, most of the *in vivo* measurements of tissue mechanics in pregnant women were performed at the level of joints and that no information exists at the muscle level [21, 23]. A recent study reported that the stiffness of the patellar tendon does not decrease during pregnancy which suggests the possibility that the biomechanical behavior might be different from one tissue to another [23, 87]. Such biomechanical changes that occur in the PFMs may be a form of physiological preparation of the woman's pelvic floor for childbirth to accommodate the major distension of the perineal muscles during vaginal delivery [23]. Although no data exist about muscle mechanical changes during pregnancy in humans, animal studies provided *ex vivo* evidence of biomechanical changes that are related to the effect of pregnancy.

5 – Animal experimental data suggesting biomechanical behavior of women's pelvic floor during pregnancy and childbirth

To analyze PFMs, the most often considered animal model is the rat model, as the organization of the PFMs in rats is similar to that in humans [34]. An increase in muscle fiber length of the PFMs of rats during pregnancy has been reported, which is explained by an increase in the number of sarcomeres in series. A concomitant increase in passive muscle stiffness has been found [33], which can be explained by a drastic increase in the total collagen content in PFMs [33, 34]. This increase in stiffness can be seen as a physiological mechanism that strengthens the muscular structure during pregnancy and induces an important increase in the muscle fiber length. Considering that tissues with low stiffness have high plasticity or rupture thresholds, representing the limit at which irreversible damage can occur in a structure [106]. The increase in muscle stiffness can be considered a protective process against perineal trauma, especially against muscle rupture. Of interest, these changes in fiber length and muscle stiffness occur only in PFMs (the coccygeus, iliocaudalis, and pubocaudalis muscles) whereas no significant changes occur in the peripheral muscles, such as the *anterior tibialis* muscle. The investigators conclude that these changes are probably because of the increase in the localized mechanical loading applied to the PFMs rather than the hormonal systemic effect [34]. This hypothesis has been confirmed in a recent study from the same research team wherein they compared the mechanical behavior of PFMs in several groups of rats with/without PFMs load and with/without hormonal impregnation (pregnant/non-pregnant) [36]. They reported an increase in normalized muscle fiber length in rats with PFM load whether they were pregnant or not, but without significant change in the sarcomere length. These results suggests that the increase in fiber length was the result of adaptative sarcomerogenesis (and not sarcomere stretch) and highlights the importance of the local environment (the load) more than the hormonal influence [36]. Consistent with previous studies, no changes were observed in peripheral muscles (tibialis anterior). Additionally, the investigators reported an increase in the intramuscular collagen content in PFMs of rats with PFM load; whether they were under hormonal impregnation or not (pregnant or not), confirming the importance of the conjunctive tissue remodeling and the impact of the local mechanical environment more than the systemic hormonal influence [36]. These observation in rats, could be interpreted as the results of an eccentric training in athletes which consists of performing muscular contraction in a stretched muscle. This training technique is expected to increase the ability of the muscle to elongate (without damage) by optimizing collagen synthesis.

There are animal experimental data about the impact of perineal distension during childbirth on these PFMs. Investigators from the same team as previous studies simulated the strain exerted by vaginal delivery by inducing vaginal distension, which replicates fetal crowning, in pregnant and nonpregnant rats [35]. They reported an increase in sarcomere length that was dramatically high in nonpregnant rats. This result indicated that pregnancy-induced adaptations were efficient in limiting the sarcomere hyperelongation which may induce muscle damage [35]. In the recent study evocated above, these researchers exposed PFMs to physiologic and supraphysiologic strains and reported significant sarcomere elongation in groups without PFM load (compared to PFM loading), suggesting that the loading on PFMs during pregnancy should be considered as a preparative process for childbirth to avoid the risk of perineal trauma [36]. In this analysis, the sarcomere elongation was less important in case of physiologic strain in pregnant rats compared to those not under hormonal impregnation (not pregnant), but the difference disappears in case of supraphysiologic strain [36]. This means that hormonal impregnation may have a limited protective effect, but it is not sufficient to prevent perineal trauma in case of massive strain. The largest differences between pregnant and nonpregnant rats were reported for the *pubocaudalis* and *coccygeus* muscles, especially for the entheseal region of the *pubocaudalis* muscle, which became translucent [35]. This observation was reliable in terms of human clinical considerations because this region is the one in which LAM avulsion occurs.

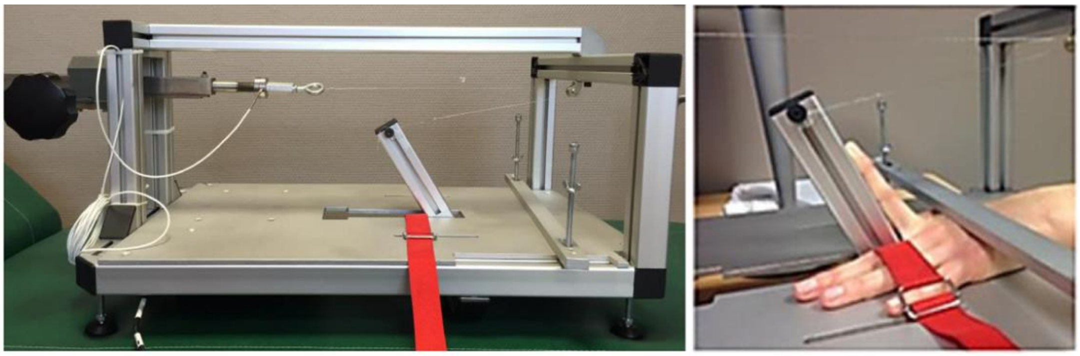
These muscular adaptations contrast those observed with animal data on the elastic properties of the vaginal wall. Indeed, several investigators reported a decrease in stiffness of the vaginal wall during pregnancy, which is consistent with previously described observations in humans [107-109]. They concluded that this decrease in stiffness might be a physiological process that accommodates vaginal distension during childbirth [107-109].

Because this decrease in stiffness is observed for the pelvic floor and some peripheral tissues, it might be related to hormonal systemic changes. In contrast, PFMs may have a specific behavior during pregnancy, and this can be considered as a protective process that avoids muscular rupture during childbirth [23]. Pelvic floor damage may occur when the strain is too important and/or when the biomechanical changes induced by pregnancy are not sufficient to accommodate the strain induced by delivery [23].

6 – Association between women's intrinsic biomechanical characteristics and perineal trauma at childbirth

To date, data on the impact of the intrinsic biomechanical properties of the tissues in a woman and the risk of perineal trauma at childbirth are limited. Meriwether *et al.* investigated whether there is an association between the perineal body stretch during delivery and the risk of OASIs [63]. These investigators reported a 65% increase in perineal body length from the antepartum to the expulsive phase. In this study, the importance of the perineal body stretch was not associated with the occurrence of OASI or any postnatal pelvic floor disorder [63].

We reported a prospective study of 300 women with an assessment of ligamentous laxity between 36 weeks of pregnancy and the onset of labor [37]. Ligamentous laxity was assessed at the second metacarpo-phalangeal joint (MCP laxity) by measuring the passive extension of the nondominant index finger for a 0.26N.m fixed torque using a specific extensometer [37]. Women with high ligamentous laxity were those with the high risk of OASI. An MCP laxity higher than 64° was associated with the occurrence of OASI with 75% sensitivity, 56% specificity, and an area under the curve of 0.65 (Figure 13) [37]. Therefore, the intrinsic biomechanical properties seem to be related to perineal trauma. We hypothesized that women with the greatest ligamentous laxity may be those with the weakest PFMs and, by extension, those with the highest risk of OASI [23, 37]. However, considering that the mechanisms involved in the increase in ligament laxity and the increase in PFMs stiffness are different (see previous section), we currently have no direct evidence to validate this hypothesis. Therefore, it is now crucial to assess the biomechanical behavior of PFMs *in vivo* in pregnant women to determine whether such measurements can help predict perineal trauma at childbirth [23].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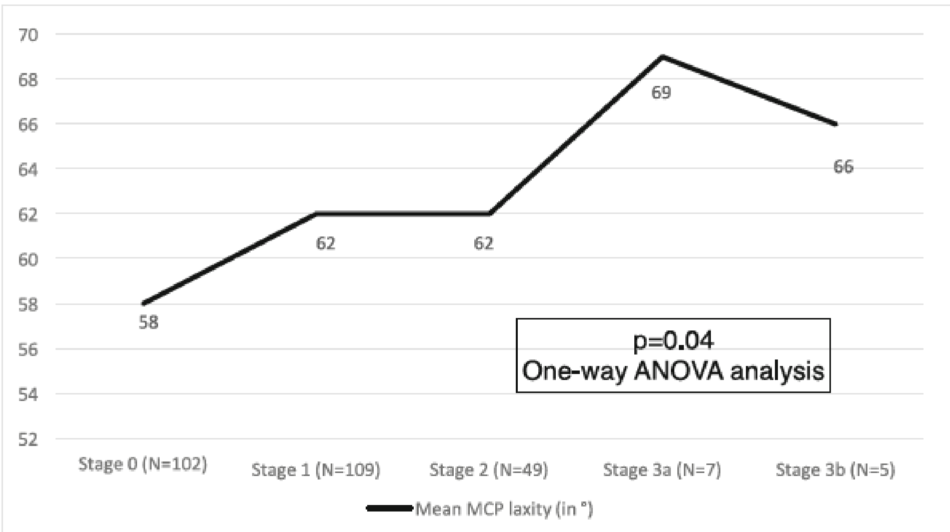

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Figure 13 : Assessment of metacarpo-phalangeal laxity (a) and its distribution according to the severity of perineal tears at childbirth [37]

7 – Innovative methods for investigating the elastic properties of women's pelvic floor

Kruger *et al.* used an elastometer to assess the elastic properties of the LAM in pregnant and non-pregnant women [41, 110]. Their device is similar to a vaginal speculum supplemented with force sensors. This elastometer provides the force/displacement curve with good reproducibility. Using this method, the investigators reported that the stiffness of the LAM is higher in the postpartum period compared to that observed during the prenatal assessments. Although this innovative approach provides relevant information about pelvic floor behavior, it suffers from two main drawbacks. First, the device measures the displacement of the speculum, which is inserted within the vaginal. Thus, it evaluates the elastic properties of both the LAM and the vaginal wall. Considering that the elastic properties of the vaginal wall and PFMs could be very different during pregnancy [23, 33-36, 107-109], this can lead to results that may be difficult to interpret. Second, this remains an intrusive vaginal examination that may be hard to accept for pregnant women.

Morin et al. reported the use of a vaginal dynamometer for reporting PFM function [111]. The device consists of a vaginal speculum which allow an assessment of passive force applied on the speculum at different vaginal aperture. This device has been used in clinical study reporting that continent women demonstrate higher passive force and higher absolute endurance compared to women suffering from stress urinary incontinence. Such an assessment provide a global assessment of PFM function and not a specific assessment of PFM's elastic properties [111]. Furthermore, as for the Kruger *et al.* device it is an intrusive one requiring a vaginal examination.

Egorov *et al*. developed a vaginal tactile imaging device consisting of a vaginal ultrasound probe supplemented with force and temperature sensors [40, 112, 113]. Such a device is expected to provide an assessment of the elastic properties of the pelvic floor. We consider that this technique presents the same limitations as the vaginal elastometer of Kruger *et al*. [23, 41, 110].

Recent technologies of functional ultrasound imaging have been proposed for *in vivo* and noninvasive investigation of the elastic properties of peripheral muscles [38]. Chen *et al*. reported the use of static elastography to assess the elastic properties of the perineal body in nonpregnant women [39]. This was the first study that used elastography for the pelvic floor. Because the static elastography technique provides a qualitative evaluation, it requires the interposition of a custom standoff pad to estimate the elastic properties of the perineal body in comparison to this reference. The investigators reported that the mean compression modulus of the perineal body region was 28.9 kPa. The main strength of this technique is that it allows an *in vivo* assessment with a noninvasive approach. The main limitation is that the measurement is influenced by surrounding tissues, and we do not know which anatomical structure is actually measured (muscles, vaginal wall) [23, 39]. In addition, this technique provides a measurement along the transverse direction of the muscles that does not correspond to the "physiological" stiffness measured along the shortened length, as performed in animal studies [23]. Other research teams suggest similar procedures to provide qualitative assessments of the elastic properties in a woman's pelvic floor, especially for LAMs [114-116].

Shear wave elastography (SWE) is another elastography method that is considered more relevant in the investigation of the elastic properties of peripheral muscles [38]. SWE allows a quantitative, *in vivo* assessment of tissues during a classic ultrasound examination [38, 42]. A remote mechanical perturbation is applied to the tissue using a specific ultrasound sequence to induce the propagation of a shear wave into the tissue of interest using ultrafast acquisition systems; the wave's propagation speed is measured perpendicular to the ultrasound beam (i.e., possibly along the muscle shortening direction). This shear wave speed propagation is linked with the elastic modulus of the tissue: the stiffer is the tissue, the higher the wave's propagation is [38, 42, 117, 118]. The elastic properties of the tissues are reported in terms of Young's modulus, which represents the link between a stress and a strain in an isotropic tissue (similar mechanical properties in all directions). Considering an isotropic solid, the device gives E (Young's modulus) as a measurement with $E = 3\mu = \rho V^2$ with μ representing the shear modulus, ρ the density, and V the shear wave speed. Muscles are stiffer along the fiber direction and thus cannot be considered isotropic. In anisotropic solid, the equation $E = 3\mu$ is no more valid. Therefore, measurements should be divided by a factor 3 to obtain measurement of the shear modulus of a muscle [38, 43, 44, 118]. A previous study has demonstrated that the shear modulus is strongly and linearly related to the Young's modulus, which supports the relevance of shear modulus measurements obtained with a device for the study of muscle biomechanics [38, 43, 118]. Excellent reliability has been reported for SWE assessments of multiple peripheral muscles [45].

MRI also offers the possibility to investigate the elastic properties of several tissues *in vivo* [119]. Nevertheless, we chose not to explore this area in our study because of practical and ethical constraints in performing MRI examinations in pregnant women for research. The other challenge is the accessibility of the device. Moreover, in a clinical approach within the exam should be offered to all pregnant women. It is likely that MRI could offer excellent quality assessments but with too many practical difficulties.

Regarding the necessity to obtain *in vivo* measurements, using a noninvasive and easily accessible method that enables direct and quantitative measurements, and PFMs are easily investigated using a transperineal ultrasound; we made the hypothesis that SWE could be the most effective option [118]. Therefore, we chose to develop the application of this technique to PFM's assessment in this thesis.

8 – For an individual approach of perineal trauma prediction

Different predictive algorithms have been proposed for perineal trauma at childbirth and more specifically for the occurrence of OASIs. Jelovsek *et al.* reported a model for fecal incontinence, and McPherson *et al*. reported a model for OASI, but these models showed poor reliability, with areas under the curve of 0.68 and 0.64, respectively [7, 120]. We consider that these approaches represent too much risk for an incorrect conclusion about the high or low risk of developing the outcome measured. Meister *et al.* reported a more satisfactory predictive model of OASI (area under the curve of 0.83); however, its predictive value has not been validated in another sample, which is a main limitation for its clinical use [6].

All these predictive models are focused on the mode of delivery without any (or very limited) considerations to the biomechanical characteristics of the tissues in women, which might explain the limitations of these predictive tools [23]. A strong evidence exists for large and specific changes in the biomechanical behavior of a woman's pelvic floor during pregnancy in both animals and humans, and this is probably a process that makes childbirth possible. Thus, it could also be considered as a protective mechanism against perineal trauma. Therefore, we hypothesized that taking this biomechanical behavior into account in our risk prediction of perineal trauma at childbirth will probably improve the efficiency of the predictive models, leading to individual risk assessments [23]. In this perspective, we believe that SWE would be a useful tool. All women could undergo several ultrasounds during their pregnancy monitoring, and it is easy to consider performing a short assessment of the viscoelastic properties of PFMs during one of these ultrasound assessments, especially in the third trimester [23]. By including these biomechanical properties of tissues in the risk prediction of perineal trauma at childbirth, we may optimize the efficiency of the existing algorithms with a better identification of high-risk woman. Such an individualized risk assessment can give personalized information to a pregnant woman about her risk of perineal trauma, allowing personalized counselling for the mode of delivery and/or implementation of preventive strategies (e.g., episiotomy, restriction of surgical delivery) [23]. More specifically, the place of protective interventions, such as episiotomy, would be individually discussed. Indeed, there is no benefit of a routine use of episiotomy to prevent perineal trauma and/or pelvic floor dysfunction [121]. A recent biomechanical study using a computational modeling approach reported that a mediolateral episiotomy decreases the stress on PFMs and the force required to deliver successfully [122]. Nevertheless, owing to the morbidity of this intervention (infection, bleeding, pain) and the absence of benefits in the overall population, the answer is to find out how women at high-risk could benefit from mediolateral episiotomy and be correctly identified [23, 121, 123, 124].

Tissue biomechanical behavior consideration, assessed noninvasively using SWE during the last obstetrical ultrasound visit, would allow the identification of women with an intrinsic high-risk of perineal trauma. These women could benefit from personalized information about their risk and the potential preventive strategies that could be offered. Such an antenatal information will probably lead to a better acceptability of these interventions (such as episiotomy) and offer the possibility to collect a real free and informed consent compare to an emergency information during the delivery [23].

This prospect requires, first, to investigate the feasibility of SWE to assess PFM’s elastic properties. It will be necessary to study the reliability of this procedure. Last, a longitudinal study will be required to look for changes in the elastic properties of the PFM in pregnant women and its association with perineal trauma occurrence at childbirth. All these points will be consecutively discussed in this thesis.

9 - Conclusion

Pregnancy is associated with significant changes in biomechanical behavior of the pelvic floor tissues that can be considered as a protective mechanism against perineal trauma at childbirth. Recent functional ultrasound imaging technologies, such as SWE, allow for an *in vivo* assessment of the elastic properties of PFM in women, which may be useful for identifying women with an intrinsic high risk of perineal trauma. We contend that intrinsic tissue biomechanical behavior should be considered in the risk assessment of perineal trauma at childbirth to improve the individualized risk assessment with the goal of providing personalized counseling to women in prenatal courses or during labor and developing preventive strategies [23].

Study 2 – Feasibility of measuring the viscoelastic properties of the levator ani muscle in women using shear wave elastography [49]

With the prospect to consider the elastic properties of PFMs in women for risk prediction of perineal trauma at childbirth, it is necessary to develop tools that allow an *in vivo* assessment of these properties. Our research approach was focused on the technique of SWE, and the first step was to assess the feasibility of this technique to assess PFMs.

1 – Objective

The main endpoint of this study was to evaluate the feasibility of an *in vivo* assessment of the elastic properties of the LAM using SWE technology in a cohort of nonpregnant women. The secondary endpoint was to evaluate objective changes in the elastic properties of the muscles by comparing measurements at rest, when the muscle is in a neutral position, and while performing Valsalva maneuver, when the muscle is in a stretched position.

2 – Material and Methods

This prospective longitudinal study was conducted in the Department of Obstetrics and Gynecology of our university from November 17, 2016, to December 12, 2016.

Eligible participants were volunteer nonpregnant women who had participated in a previous study, which evaluated the association between ligamentous laxity and levator hiatus distension during pregnancy [22]. Exclusion criteria were previous pelvic floor disorders (urinary incontinence, anal incontinence, pelvic organ prolapse) and/or a joint disease.

Only one visit was scheduled for each participant during which we assessed the LAMs using SWE technology. We collected the following anthropometrics data and socio-demographic data: age, body mass index (BMI), and delay since the delivery.

At the time of inclusion, the women underwent an ultrasound assessment of the LAMs using SWE performed using Aixplorer V11 device (SuperSonic Imagine, Aix en Provence, France). The Aixplorer device allows the user to perform both classical two-dimensional B-mode ultrasound acquisition and SWE during the same assessment and using the same equipment. The assessments were performed after voiding and with the woman in lithotomy position at rest, and then at maximal strain during the Valsalva maneuver. We asked the participants to perform two initial Valsalva maneuvers with biofeedback instructions to prevent LAM coactivation from serving as a confounding factor in our analysis [51]. Indeed, performing a Valsalva maneuver require contraction of the diaphragm and abdominal muscle in order to increase abdominal pressure. In physiological conditions, this increase in abdominal pressure is associated with a reflex contraction of PFM for maintaining a normal continence [51]. For voiding or defecation, a relaxation of PFM is required to achieve it. It has been reported that performing PFM ultrasound imaging during Valsalva maneuver is associated with a reflex PFM contraction which is an important confounder. The same study reported that this reflex contraction could be controlled by repeating the Valsalva maneuver with biofeedback [51]. So in our experience, women performed 3 consecutives Valsalva maneuver with biofeedback regarding that they can observe their organs displacement on a recall ultrasound screen.

We first located the LAM, at its pubic insertion, using the classic two-dimensional ultrasound mode with an SL-15-4 linear probe (4-15MHz) of 5 cm in length [50]. This method was previously used to assess LAM avulsions and led to an 87% agreement between the observers [50]. The probe was first placed on the perineum in the sagittal plane. We then applied a 10° inclination to identify the pubic insertion of the LAM (Figure 14). Once the LAM was correctly identified, we performed the SWE assessment.

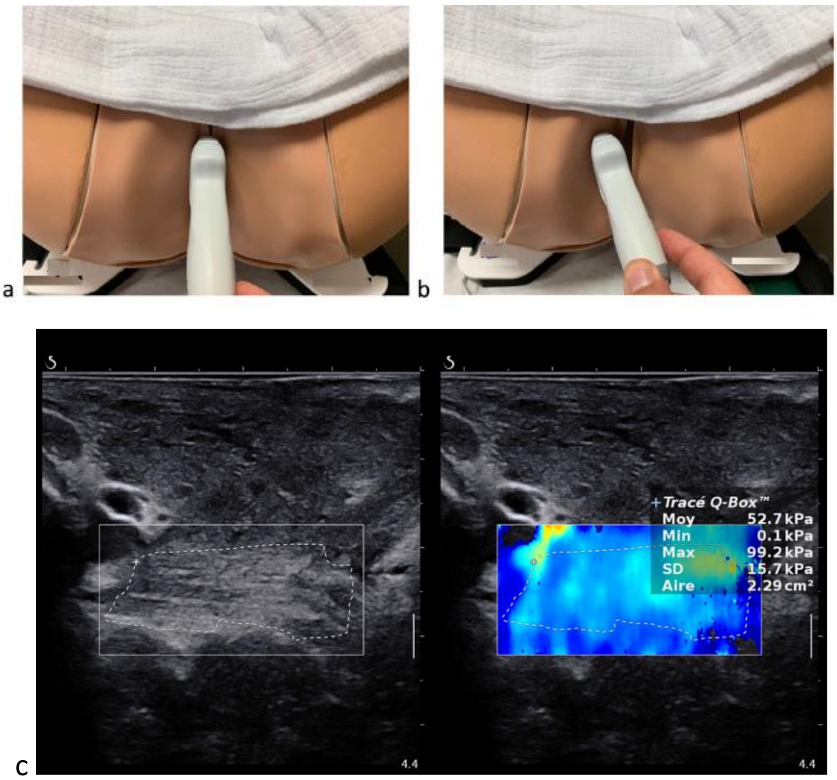
The assessment at rest consisted of a static assessment with one single picture. The limits of the LAM were outlined by hand, and the Young's modulus (in kPa) was obtained within these limits. As reported previously in this thesis, the study of the shear modulus is more relevant than the Young's modulus for muscles [38, 43, 44]. Therefore, we considered the shear modulus for the analysis, which was obtained by dividing the Young's modulus by a factor 3 [38, 43, 44].

For the assessment during Valsalva maneuver, we performed a dynamic acquisition from the rest position to 5s of maximal strain during the Valsalva maneuver. For this dynamic acquisition, we outlined by hand the limits of the LAM in each picture, and the Young's modulus and then the shear modulus were reported for each picture, as described for the assessment at rest. The highest shear modulus obtained during the acquisition was reported as the shear modulus of the LAM during Valsalva maneuver. We performed a dynamic acquisition during the Valsalva maneuver with interval measures during the process to systematically record the highest shear modulus that a static measure, not exactly at the maximal Valsalva, might have missed.

The procedure was performed for both the right and left sides, and the shear modulus was reported at rest and during Valsalva maneuver for the two sides.

We reported the participant characteristics for age, BMI, and delay since the last delivery in terms of the mean and standard deviation (SD), and we reported the number of successfully completed procedures and the number of failed procedures. We then reported the mean and SD for the shear modulus at rest and during Valsalva maneuver for both right and left LAMs to check the feasibility for the two sides.

We assessed the changes in LAM shear modulus from rest to Valsalva maneuver using a Wilcoxon test. We chose this test, a non-parametric one, regarding our sample size which is low with a probably non-normal distribution of measured values. Because the main endpoint was to describe the feasibility of the technique and not its reliability; therefore, a power calculation was not performed. Furthermore, no previous studies would have allowed such a calculation.



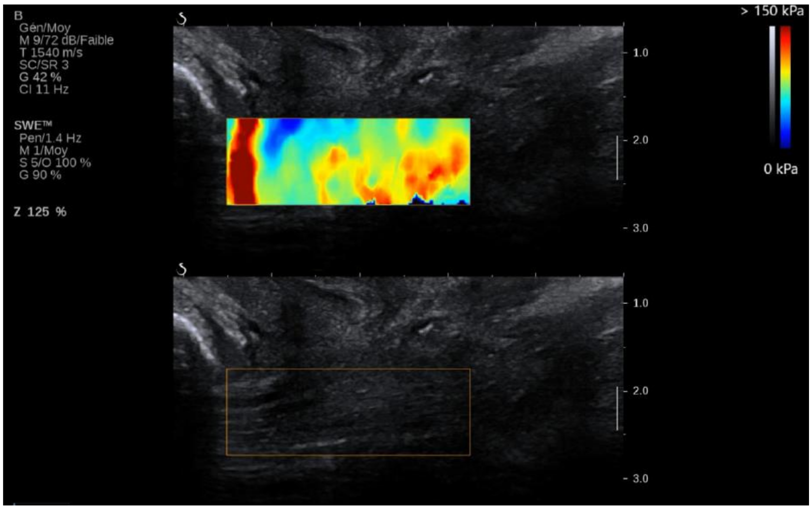


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Figure 14: Levator ani muscle SWE assesment. Probe position (a,b), example of acquisition at rest (c) and at Valsalva maneuver (d) [49, 118].

Levator ani muscle; MSK resolution mode

Analyses were performed using the Stata software (version V14IC; Stata Corporation, College Station, TX, USA). For all analyses, the statistical significance threshold (alpha) used was 5%.

Dr Bertrand GACHON was the coordinating investigator for this study and performed all the assessments.

The ethics committee (protocol no: 2014-A01467-40, *Comité de Protection des Personnes Ouest-III*) and the National Drug Safety Agency (protocol no: 141380B-22, *Agence Nationale de Sécurité du Médicament et des produits de santé)* reviewed and approved the protocol. Written informed consent was obtained from each study participant before inclusion in the study and the realization of any investigations.

3 – Results

A total of 12 parous women were included in this study who had a history of at least one delivery, with 10 exclusively delivered vaginally and 2 delivered with at least one cesarean section. The characteristics of the study population are reported in Table 2.

Table 2: Characteristics of the participants in the feasibility study

	Mean (SD)
Age, in years	31 (2.6)
Body mass index, in kg.m^{-2}	28 (7.4)
Parity	1.9 (0.7)
Delay since the last delivery, in months	14 (2)

SD: standard deviation

All assessments performed at rest were successfully completed. We reported two assessment failures during the Valsalva maneuver, which were related to the women with the highest BMI (37.7 and 42.2 kg.m^{-2}).

The mean shear modulus assessed at rest and during Valsalva maneuver for both the right and left LAM is reported in table 3. The mean shear modulus increased by a factor of more than 2 from rest to while performing the Valsalva maneuver. No significant differences were observed in any measurements between the left and the right side.

Table 3: Elastic properties of the *levator ani muscle* at rest and during Valsalva maneuver, feasibility study

	Mean shear modulus at rest, in kPa (SD)	**Mean shear modulus at Valsalva, in kPa (SD)**	**p***
Right LAM	16.0 (6.9)	35.4 (13.9)	<0.005
Left LAM	17.1 (7.6)	37.6 (13.1)	<0.005

LAM: *levator ani muscle* SD: standard Deviation

*Wilcoxon test

4 – Discussion

4.1 – Main findings

In nonpregnant women, it is possible to assess the elastic properties of the LAMs *in vivo* using SWE at rest and during Valsalva maneuver. The mean shear modulus and thus the stiffness of the LAM increased by a factor of more than 2 from rest to Valsalva maneuver.

4.2 – Strengths and limitations

The first limitation of this study is that only parous women were included, who potentially had existing pelvic floor damage. Thus, the shear modulus that we reported for the LAM may not be representative of the elastic properties of the LAM in nulliparous women because a damaged LAM probably exhibits different biomechanical behavior than an undamaged one [122]. Nevertheless, our analysis was not biased because our main objective was to assess the feasibility of the procedure and not to describe the elastic properties of the LAM.

In addition, when this study was published, no previously published data evaluating the reliability of SWE for this technique in the pelvic floor assessment were available. Nevertheless, considering the easy access to PFMs when using ultrasound, the feasibility of SWE measurement for the LAM in the present study, and the good reliability reported for other muscles, such as abdominal muscles, *gastrocnemius medialis,* and *biceps brachii*, we are confident that a future study will demonstrate good reliability of this method for PFMs [45, 125].

Another limitation of this study was the small number of women included, which is inherent to the pilot feasibility design of the study. Our results must be considered as a proof of concept of the feasibility of the procedure. This feasibility would have to be confirmed and its reproducibility investigated before any application in clinical practice.

4.3 – Interpretation

To our knowledge, this was the first study to report the use of SWE technology and evaluated the elastic properties of the PFMs *in vivo* in parous women. At the time of publication of this study, only one investigator described the assessment of elastic properties of the LAMs *in vivo*. Kruger *et al*. used a vaginal elastometer (vaginal speculum coupled with force sensors) for measuring the elastic properties of the LAMs in pregnant and nonpregnant women. As mentioned previously (see study 1, section 7) this approach is quite interesting but has some major limitations, such as being an intrusive vaginal examination, not providing an accurate measure of the elastic properties of the LAM but the strain applied by the whole pelvic floor on the speculum [41, 110]. Nevertheless, the global technique used by *Kruger et* *al.* remains quite interesting because it provides an assessment of the whole perineum, including the vaginal wall, the LAMs, and the fascia. This is a different approach than ours, as we aimed to specifically investigate the elastic properties of the LAM. The two procedures may be complementary because SWE allows an individual assessment of the PFMs and the device by Kruger *et al.* provides an assessment of the whole pelvic floor; thus, the potential interactions between these different structures can be addressed.

We reported high SD values for the right and left LAM at rest and Valsalva maneuver. We consider that this might be related to the heterogeneity of our population. Indeed, some women had several vaginal delivery, some women had only cesarean section, some had a normal spontaneous delivery and some an operative one.

Chen *et al.* assessed the elastic properties of the perineal body using elastography in nonpregnant women [39]. To our knowledge, this was the first description of the use of elastography to assess the pelvic floor. The investigators reported that the mean compression modulus of the perineal body was 28.9 kPa. As we reported previously (study 1, section 7) this technique has the limitation of requiring the interposition of a standoff pad and thus providing undirect assessment of the elastic properties. Furthermore, it investigates the elastic properties of a large region of interest and not limited to any one specific anatomical structure.

After the publication of this study, new descriptions of the elastic properties of PFMs which were evaluated by using elastography surfaced, especially the experience of Tang *et al.* which reported the SWE assessment of LAM in a population of women aged at mean of 56 years with and without pelvic organ prolapse [126]. They reported a 28 kPa shear modulus for the LAM at rest (versus 17 kPa in ours) and a 57 kPa shear modulus while performing the Valsalva maneuver (versus 36 kPa in ours). However, in they reported slightly increased LAM stiffness in a different population (in terms of mean age and women with pelvic organ prolapse [126]. Li *et al.* reported a comparative analysis of the elastic properties of LAM, using SWE, between continent women and women with stress urinary incontinence [127]. They reported an elastic modulus in continent women of 56 kPa at rest and 82 kPa while performing the Valsalva maneuver versus 48 kPa and 72 kPa in women with stress urinary incontinence. These elastic modulus data should be divided by a factor 3 to obtain the shear modulus which is finally in the same range as that reported by us. Interestingly, the authors reported that the differential in LAM's stiffness from rest to Valsalva maneuver was more obvious in continent women [127].

Silva *et al.* published a work in which the elastic properties of the *pubovisceral* muscle were elegantly calculated using an inverse finite element [128]. They reported the material constant of the *pubovisceral* muscle for continent women that lead to shear modulus values of 78 +/- 44 kPa (using shear modulus = 2*C1 for the neo-Hookean model), 80 +/- 48 kPa (using shear modulus = 2*(C1+C2) for the Mooney-Rivlin model), and 62 +/- 46 kPa (using shear modulus = 2*C1 for the Yeoh model). These values are in the same range, but notably higher than the values reported in our feasibility study (17 +/- 7 kPa). Nevertheless, the number of volunteers in each study was low, and the studies used very different methods; thus, the comparison should be considered carefully. Furthermore, comparing the results of these studies may be difficult because the study populations are quite different (continent and noncontinent women in the study of Silva *et al.* versus recent parous women in our study). The assessments were also done in different positions (dorsal decubitus for MRI acquisition in the study by silva *et al*. versus the lithotomy position in our study). Finally, the technique used in the study of Silva *et al*., inverse finite element, is quite different than our technique, which involves a direct assessment with an instant measure of the shear modulus [128]. This is probably the reason for the difference observed in these two studies.

We reported a 100% success rate using SWE for the assessment at rest, but we reported two failures during the Valsalva maneuver. As previously stated, the failures occurred in the women with the highest BMI. These difficulties were due to the loss of visibility of the LAM during the Valsalva maneuver, as the muscle became too deep to be clearly located using our 15-4 linear probe. In women with a very high BMI, these difficulties are more apparent owing to the thickness of the soft parts of the woman's pelvic floor. To fulfill the objective of assessing elasticity during the Valsalva maneuver in all women, it would be necessary to use different probes that allow deeper assessments.

The results of this study are encouraging but need to be confirmed in a large population, including a reliability assessment. Furthermore, the association between the elastic properties of the pelvic floor in women, as assessed using SWE, and the clinical and ultrasound pelvic floor distension measures should be evaluated. Indeed, if no association between elastic properties and pelvic floor distension exists, it would question the relevance of these measures.

Future studies should investigate the feasibility of assessing other components of the pelvic floor complex, such as ligaments and the vaginal wall. The biomechanical behavior of muscles depends on their intrinsic elastic properties and their attachments. There are reports in the literature that assess peripheral ligaments using SWE [129]. However, the measurements are more challenging for thin and stiff structures, such as tendons and ligaments [38]. Therefore, the feasibility, validity, and reliability of this techniques need to be demonstrated for pelvic floor ligaments and the vaginal wall.

In our experience, the stiffness of the LAM significantly increased from rest to Valsalva maneuver, which means that the stretched LAM is stiffer than it is at rest. This observation is in agreement with the clinical observation made during childbirth; during the period between the onset of pushing and the fetal head delivery, (the period of maximal distension of the perineum) the pelvic floor is stiffer than it is at the beginning of the second stage of labor. The tissues with the least stiffness may easily reach their plasticity threshold, which is the threshold beyond which irreversible damage to the intrinsic material's structure occurs [130]. Plasticity is a material intrinsic characteristic and means that a material remains deformed after being stressed. Elasticity characterizes the ability of a material to recover its initial state after being stressed by an external force [130]. A plastic deformation consists of an irreversible deformation because of permanent changes in the intrinsic structure of a material. Conversely, an elastic deformation constitutes a reversible process caused by an external force, with a return to the initial stage because this force is no longer applied [130]. Thus, it would be helpful to measure the stiffness of the stretched LAM before predicting the risk of pelvic floor trauma at childbirth that is implicated in the occurrence of pelvic floor disorders. To predict pelvic floor trauma, other biomechanical factors can be included in a hypothetical predictive model. One factor is the maximal strength that the tissue can support before rupture. This threshold is impossible to measure in individual patients. One alternative approach would be to perform measurements of muscle volume, which should be related to the maximal strength that it can support. Thus, the combination of both volume and the elastic modulus of PFMs could provide good predictive measures of the risk of damage. These studies may provide information about the intrinsic characteristics of the pelvic floor, especially the rupture threshold. In addition, the potential for any individual material to reach its plasticity or rupture threshold depends on its mechanical characteristics, but also on the stress applied to the material. A predictive model for perineal trauma at childbirth could also include data on the stress applied: fetal head circumference, fetal weight, and operative vaginal delivery. Excessive stress, such as that caused by a large fetal head circumference, could lead to excessive muscular distension beyond the physiological range; if the muscle reaches its plasticity threshold, plastic deformation could occur. The mechanical properties of the ligaments and tendons should be assessed and probably included in such a predictive model because of the ability of muscle to distend is also related to the flexibility of its attachments, which plays the role of a “shock absorber”.

Other studies have reported the use of SWE in pregnant women without any fetal complications [46-48, 131]. It would be interesting to ascertain whether the elastic properties of the PFMs assessed using SWE during pregnancy are predictive of the risk of pelvic floor damage at childbirth and the risk of pelvic floor disorders after childbirth. Every woman undergoes ultrasound during pregnancy, and the possibility of performing an assessment of the elastic properties of the PFMs during the same visit, with the same device, would likely be considered acceptable by most women.

5 - Conclusion

The assessment of the elastic properties of the LAM *in vivo* using SWE is feasible in a cohort of nonpregnant women. This was the first report of such an *in vivo* assessment of the elastic properties of the LAM using a noninvasive technology similar to ultrasound. Before considering its use in our clinical practice, the next step was to assess the reliability of the procedure in addition to the concordance between the elastic properties and clinical distention of the pelvic floor. Future studies will determine whether this technique can provide data to support individual risk prediction of perineal trauma at childbirth and/or pelvic floor disorders and thereby enable us to better individualize treatment decisions (e.g., type of physiotherapy, type of surgery).

Study 3 – Reliability of assessing the viscoelastic properties of the levator ani muscle, biceps brachii, and gastrocnemius medialis using shear wave elastography [52, 118]

1 - Objectives

As reported in the previous study, we described the feasibility of assessing the elastic properties of LAM in women using SWE with a transperineal approach. In the present study, we investigated the interday and intraoperator reliability of SWE for LAM to validate its use in future prospective studies and to compare its reliability with that for the peripheral muscles (*biceps brachii¸ gastrocnemius medialis*), which is reported as excellent [45]. For the LAM, we also investigated the intrasession reliability to check whether the procedure could be simplified by recording only one single measure instead of three consecutive measures [49].

Therefore, the main objective of this study was to assess the intraoperator intersession reliability of ultrasound SWE to measure the elastic properties of the LAM, *biceps brachii,* and *gastrocnemius medialis* in women [118]. The secondary objectives were as follows: (i) to investigate the intrasession reproducibility of the procedure used for the LAM and (ii) to compare intersession reproducibility of the assessment for the LAM when considering the mean of three consecutive measures versus one single measurement.

2 – Material and methods

2.1 – Study settings

This prospective monocentric study was conducted in the Department of Obstetrics and Gynecology of our University from July 2019 to August 2020. In the protocol, the time interval between two visits ranged from 12h to 7 days.

2.2 – Population

Eligible participants were nonpregnant, nulliparous women who visited our gynecology unit. The exclusion criteria were as follows: history of previous delivery (vaginal or cesarean section), personal history of pelvic floor disorders, women with obesity and a BMI higher than 35 $kg.m^{-2}$, women with muscular disease, women requiring admission to a psychiatric unit, women under judicial protection, and those who were unable to understand French language.

2.3 – Data collection

2.3.1 – Participant characteristics

At the first visit, the participant's age, height, and weight were recorded, and their BMIs were calculated.

2.3.2 – Shear wave elastography assessments

The evaluation protocol during the two visits was similar:

- SWE assessment of the right LAM: at rest, during subjective maximal Valsalva maneuver, and during subjective maximal perineal contraction.
- SWE assessment of the right *biceps brachii*: at rest, during a standardized stretch, and during a subjective maximal contraction.
- SWE assessment of the right *gastrocnemius medialis*: at rest, during a standardized stretch, and during a subjective maximal contraction.

All ultrasound measurements were performed using Aixplorer V12 device (SuperSonic Imagine, France) with a SL 18-5 linear probe (5-18 MHz). As detailed below, the muscle location was assessed in B-mode; after which SWE acquisition was performed in a 5-seconds video clip. Shear modulus values were averaged over this period. The clip was obtained to limit the influence of inevitable temporal changes (5%) [45]. Three consecutive measurements for each muscle and under each condition (rest/stretch or Valsalva/contraction) were performed. All measurements during both the visits were performed by a single operator, a senior urogynecologist (BG) with a special interest in pelvic floor imaging. We chose to consistently obtain ultrasound measurements on the right side of the participants based on the convenience of the operator, who was at the right side when the participant was in the supine position, and to standardize the procedure.

2.3.2.1 – Levator ani muscle

For LAM measurements recorded under each condition, the participants laid down in the lithotomy position with an empty bladder. The pubic insertion of the right

LAM was identified using the same procedure reported by Dietz *et al.,* using B-mode ultrasound with a transperineal approach, after which we proceeded to perform the SWE acquisition, as reported in our previous study and in Figure 14 [49, 50, 118]. Before any LAM assessment, the participants performed two initial Valsalva maneuvers with biofeedback, in which visible pelvic floor displacements on the B-mode image were shown to the participant on the screen to prevent LAM coactivation [51].

For assessments at rest, the participants were asked to relax as much as possible. This position represents the condition with the lowest load to estimate the intrinsic resting elastic properties of the LAM.

For assessment during the Valsalva maneuver, the participants were requested to perform a maximal Valsalva maneuver for at least 5s. For this maneuver, the participants had to take a deep breath and push down as much as possible with a closed glottis. This maneuver increases the intraabdominal pressure and induces a cranio-caudal descent of the pelvic organs leading to a distension of the levator hiatus with resultant lengthening of the LAMs. It can be considered as a lengthening of the LAM that should induce an increase in shear modulus [38]. This is in accordance with the childbirth condition because the effort required from the mother is the same and that the same phenomena of LAM lengthening that occurs at childbirth even if the strain magnitude is much higher. This condition is also seen in pelvic floor disorders because the occurrence of pelvic organ prolapse is associated with an overlengthening of the LAMs when intraabdominal pressure increases leading to a prolapse of the pelvic organs.

For assessment during subjective maximal contraction, the participants were asked to contract and tighten the PFMs as much as possible in a similar manner to avoid gas leakage for at least 5s [52, 118]. This procedure is in accordance with the effort performed during physiotherapy procedures which is an important part of pelvic floor disorder management.

2.3.2.2 – Biceps brachii muscle

First, we identified the proximal and distal insertions of the *biceps brachii* using B-mode ultrasound and performed SWE acquisition midway between these insertions for three conditions: at rest, standardized extension, and subjective maximal contraction. We proceeded to an assessment performed at rest with the upper limb having a 90° flexion of the elbow, which was at the same height as the shoulder, with the hand pronated. The forearm rested on a flat support, allowing the *biceps brachii* to be totally free and accessible (Figure 15). We systematically verified the 90° flexion of the elbow using a digital goniometer. For the assessment during extension, the position was the same but with a 180° extension of the elbow (verified with the digital goniometer), and the hand pronated. Finally, for the measurements during contraction, we asked the women to have a subjective maximal contraction of the *biceps brachii* in the rest assessment position. A previous study, using the same procedure, reported that the shear modulus measured in *biceps brachii* muscle in volunteer nonpregnant women is about 3kPa at rest and 19kPa when stretched [45, 132].

a b

Figure 15: Shear wave elastography assessment of the *biceps brachii* muscle at rest (a) and standardized extension (b) [118]

2.3.2.3 – Gastrocnemius medialis

Usually, this measure is performed with the volunteer lying down in ventral decubitus. With the prospect to perform these measures in pregnant women, it is evident that such a position is not ideal because of the risk of compression of the gravid uterus. Therefore, we chose to perform the assessments in women in left lateral decubitus. First, we identified the proximal and distal insertions as well as the lateral borders of the *gastrocnemius medialis* in B-mode ultrasound. We performed the SWE acquisition midway between the lateral borders and midway between the proximal and distal insertions of the muscle under the three conditions: rest; standardized extension, and subjective maximal contraction. For the assessment at rest, the left leg was flexed, the right leg was fully extended (180° verified with a digital goniometer), and the ankle was in neutral position (Figure 16). For the measurement during extension, the participants were in the same position but the right foot supported on a 20° inclined plane for applying a standardized extension of the *gastrocnemius medialis*. Finally, we proceeded to obtain the measurement during contraction with the participants in the same position as that for the assessment at rest but with a voluntary maximal contraction of the *gastrocnemius medialis.*

Figure 16: Shear wave elastography assessment of the *gastrocnemius medialis* at rest (a) and in standardized extension (b,c) [118]

2.4 – Data analysis and statistics

The region of interest was identified and contoured manually using MATLAB scripts (the MathWorks, Inc., 2016). For assessment at rest, standardized extension or while performing Valsalva maneuver, the mean shear modulus for the whole acquisition was considered. For assessments during subjective maximal contraction, the maximal shear modulus for the acquisition was considered. In case of limited region for which the measurement was not possible, the software automatically excluded it from the analysis. As mentioned in the previous study, the Aixplorer device provides a measurement of the Young's modulus that is valid for isotropic tissues. Because muscles are transverse anisotropic tissues, the shear modulus was measured by dividing the Young's modulus by 3 [38, 43, 44].

We first described our participants in terms of age, mean BMI, and the interval between the two assessments. Continuous variables were reported as mean and standard deviations, and categorical variables by numbers and percentages. On the basis of our primary objective, we analyzed the intersession reproducibility for each mode of assessment (at rest, while performing Valsalva maneuver, and contraction) for the LAM, with Intraclass Correlation Coefficient (ICC), the Standard Error of Measurement (SEM) and the Coefficient of Variation (CV) serving as the main judgement criteria. For this analysis, we considered the mean of the three consecutive measurements performed in each session for the analysis. We computed the ICC with the 95% confidence interval for each assessment and calculated the CV [133]. Bland–Altman plots were built according to the methods reported in the original publication [134]. Regarding the ICC value, we considered that the reliability was excellent if equal or higher than 0.90, good if between 0.75 and 0.89, moderate if between 0.50 and 0.74, and poor if lower than 0.50 [133]. We chose the ICC as main judgement criteria regarding its widespread use for investigating the reliability of imaging procedures (especially ultrasound) in clinical studies [82, 133]. In order to perform a more detailed report of the reliability we also reported the CV which could be considered as excellent when lower than 10% and good when lower than 10%. Bland-Altman plots are useful for reporting the distribution of the mean difference according the mean of two measures allowing to check if the procedure is more reliable for low/high values. Such an analysis also report the agreement interval within we can find 95% of the differences between the two techniques.

Regarding our secondary objectives, we used exactly the same methodology to assess the intersession reproducibility for the *biceps brachii* muscle and the *gastrocnemius medialis* muscle.

Last, for the LAM, we investigated the intrasession reproducibility with three consecutive measurements by using the same methods as for the primary objective: ICC, SEM, and CV. ICC values were interpreted as above. We then compared the reproducibility performance when considering the mean of the three measurements or the first of the three consecutive measurements. This analysis was not done for peripheral muscles (*biceps brachii* and *gastrocnemius medialis)* because these assessments are already reported as reliable [45]. Data about the LAM were original.

A priori power calculation was not performed. Considering other studies, which reported the reliability analysis for ultrasound SWE in peripheral muscles; a study population of 20 women appears to be sufficiently effective [45].

Statistical analysis was performed using the STATA software (version V14IC; Stata corporation, College Station, TX, USA). For all analyses, significance level was set at $p<0.05$.

2.5 – Ethical and reglementary considerations

Dr Bertrand GACHON was the coordinating investigator for this study and performed all the assessments.

The study was approved by an ethics committee (*Comité de Protection des Personnes Ile de France 8,* ethical committee for human protection from Ile de France) on the July 16, 2018 and is referenced with the ID RCB: 2018-A011422-53. The study was registered on https://clinicaltrials.gov (NCT03602196) on the July 26, 2018. All methods were performed in accordance with relevant guidelines and regulations. Written informed consent was obtained from all participants before any investigation.

3 - Results

Twenty women were included in this study; their mean age was 23 years (SD = 4 years) with a mean BMI of 22.6 $kg.m^{-2}$ (SD = 3.2 $kg.m^{-2}$). The mean interval between, the two visits was 46.6 hours (SD = 39.6h; range 24-166h). All included women completed the full study protocol.

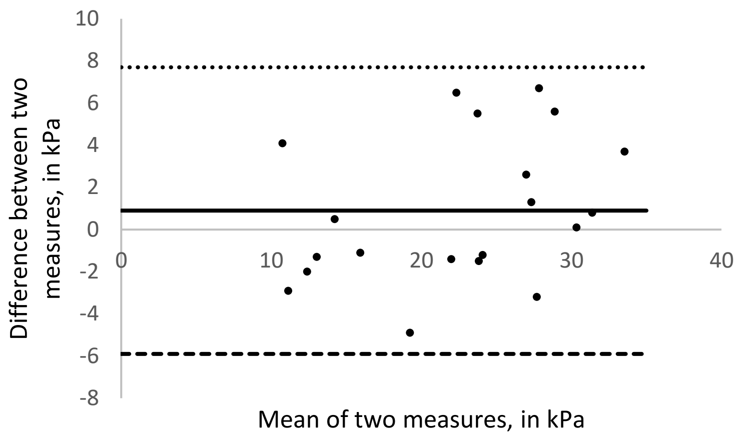
In our main analysis, the ICC was excellent for the LAM in terms of the intersession reproducibility, considering the mean of the three consecutive measures at rest and during Valsalva maneuver (Table 4). Conversely, ICC was poor for measurements performed during subjective maximal contraction. Bland–Altman plots are shown in Figure 147. The results for the intrasession reproducibility for the LAM are reported in Table 5, and they show good reliability at rest and during the Valsalva maneuver, but moderate during subjective maximal contraction. In table 1, we also report the reproducibility performance indicators for both analyses when considering the mean of the three measurements for each visit and when considering the first of the three consecutive measurements. ICC and other reliability indicators were high when the mean of the three measures for the rest and Valsalva maneuvers measurements were considered (Table 4). Reliability during subjective maximal contraction was poor regardless of whether we used the mean or the first measurement alone.

Table 4: Intersession reproducibility performances for the assessment of the LAM's shear modulus [52].

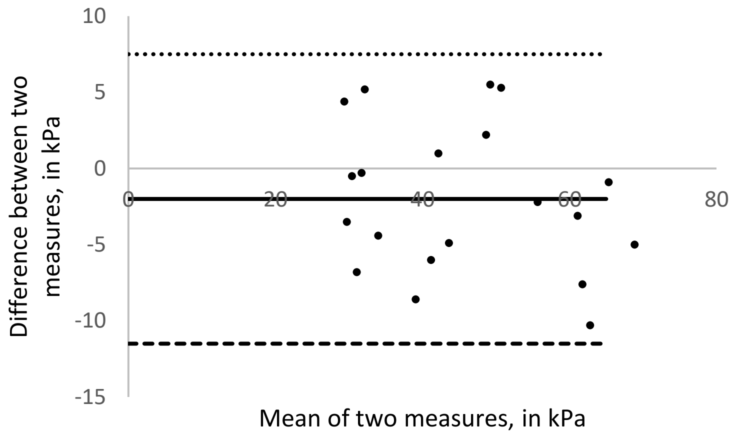
	Mean shear modulus at V1, in kPa (SD)	Mean shear modulus at V2, in kPa (SD)	ICC [95%CI]	CV, in %	SEM, in kPa
Intersession reproducibility performances by considering the mean of the 3 measures at each visit					
Rest	22.8 (8.0)	21.9 (6.8)	0.90 [0.80-0.95]	15.7	3.5
Valsalva	44.5 (13.1)	46.5 (14.2)	0.94 [0.88-0.97]	10.6	4.8
Contraction	59.3 (11.8)	55.1 (15.7)	0.43 [0.07-0.69]	25.1	14.8
Intersession reproducibility performances by considering one single measure at each visit					
Rest	22.2 (8.3)	22.0 (7.0)	0.87 [0.74-0.94]	18.6	4.1
Valsalva	43.2 (13.1)	44.2 (16.1)	0.84 [0.68-0.92]	19.9	8.7
Contraction	60.2 (12.0)	56.2 (16.8)	0.61 [0.31-0.80]	22.9	13.3

Table 5: Intrasession reproducibility performances for the assessment of the right LAM's shear modulus with 3 consecutive measures [52]

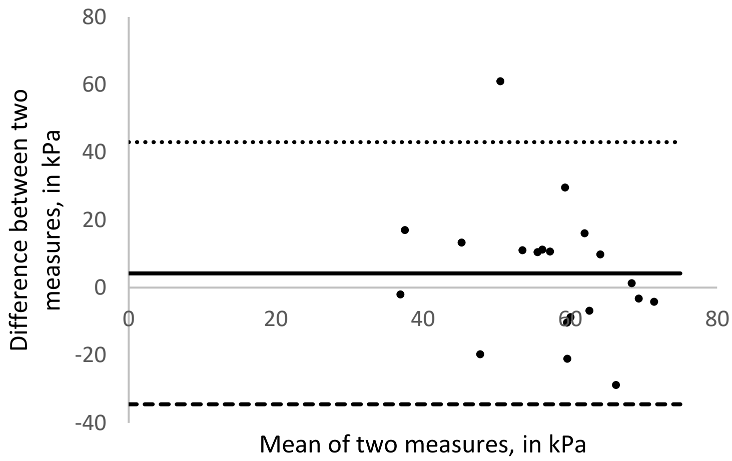
	1st measure mean shear modulus in kPa (SD)	2nd measure mean shear modulus in kPa (SD)	3rd measure men shear modulus in kPa (SD)	ICC [95% CI]	CV, in %	SEM, in kPa
Rest	22.1 (7.6)	22.7 (8.4)	22.2 (7.8)	0.84 [0.75-0.89]	21.1	4.7
Valsalva	43.7 (14.5)	46.9 (13.5)	46.8 (15.6)	0.88 [0.75-0.91]	16.6	7.6
Contraction	58.2 (14.6)	61.4 (14.9)	57.2 (15.5)	0.70 [0.57-0.80]	20.2	11.9



a - Levator ani at rest



b – Levator ani during Valsalva



c – Levator ani during contraction

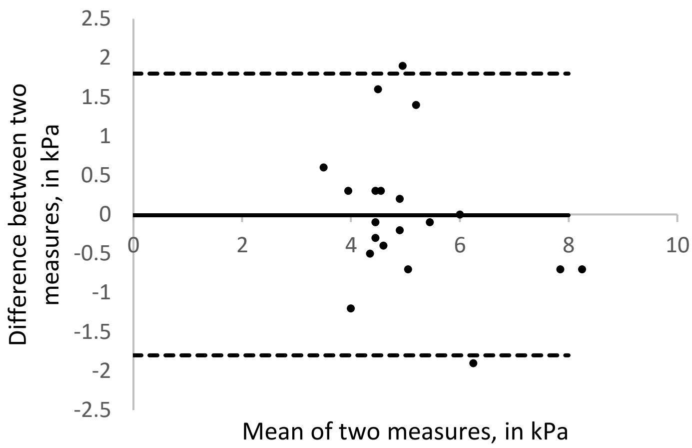
LOA: Limit of Agreement — Lower LOA ······· Upper LOA — Bias

<u>Figure 17: Bland-Altman plots of agreement between VA (first visit) and V2 (second visit) for the mean LAM's shear modulus assessment at each visit and each condition [52]</u>

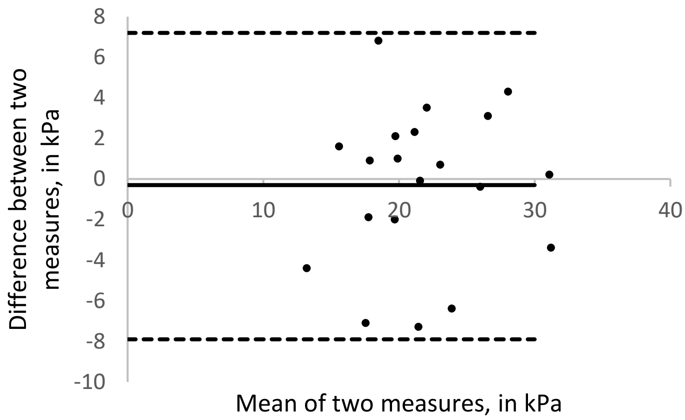
Table 6 : Intersession reproducibility performances for the assessment of the *biceps brachii* and the *gastrocnemius medialis* shear modulus

	Mean shear modulus at V1, in kPa (SD)	**Mean shear modulus at V2, in kPa (SD)**	**ICC [95%CI]**	**CV, in %**	**SEM, in kPa**
Intersession reproducibility performances for the *biceps brachii* muscle					
Rest	5.1 (1.1)	5.1 (1.4)	0.77 [0.56-0.89]	17.6	0.9
Stretch	21.6 (5.4)	22.0 (5.0)	0.75 [0.52-0.87]	17.9	3.9
Contraction	83.4 (28.4)	87.2 (22.3)	0.56 [0.25-0.77]	28.6	24.4
Intersession reproducibility performances for the *gastrocnemius medialis* muscle					
Rest	4.7 (1.2)	5.1 (1.3)	0.49 [0.15-0.73]	24.5	1.2
Stretch	25.4 (11.4)	23.7 (8.3)	0.70 [0.45-0.85]	32.6	8.0
Contraction	82.3 (30.6)	77.9 (32.1)	0.56 [0.24-0.77]	37.8	30.3

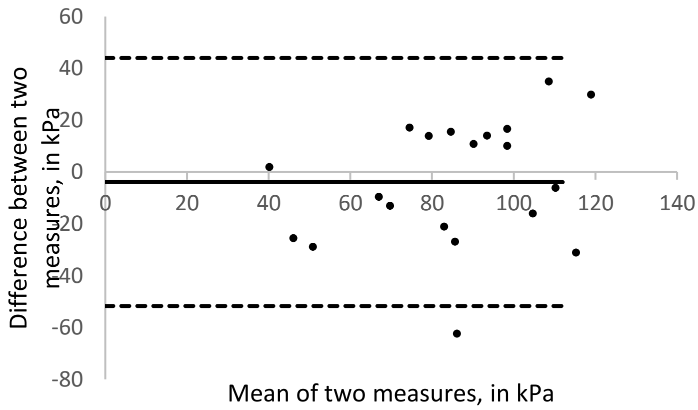
We reported a good reliability for the assessment of the *biceps brachii* at rest and during stretching but the reliability was moderate for the assessment at contraction (Table 6, Figure 18). Regarding the *gastrocnemius medialis,* the reliability was poor for assessment at rest and moderate for assessments at stretch or during contraction (Table 6, Figure 19).



a – Biceps brachii at rest



b – Biceps brachii at stretch

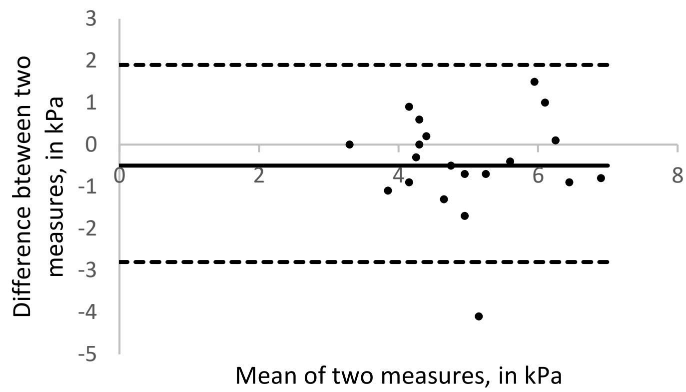


c – Biceps brachii at contraction

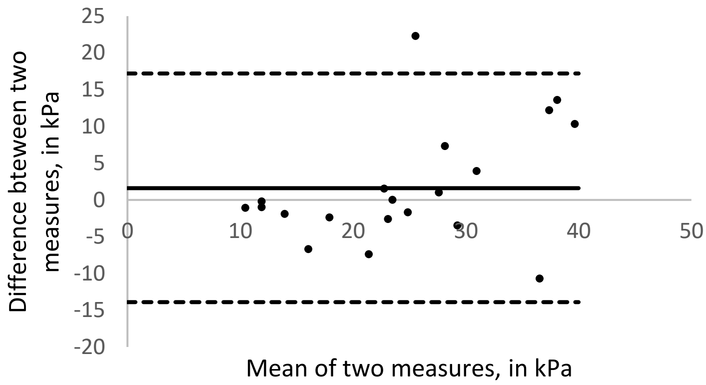
LOA: Limit of Agreement

Lower LOA ······· Upper LOA Bias

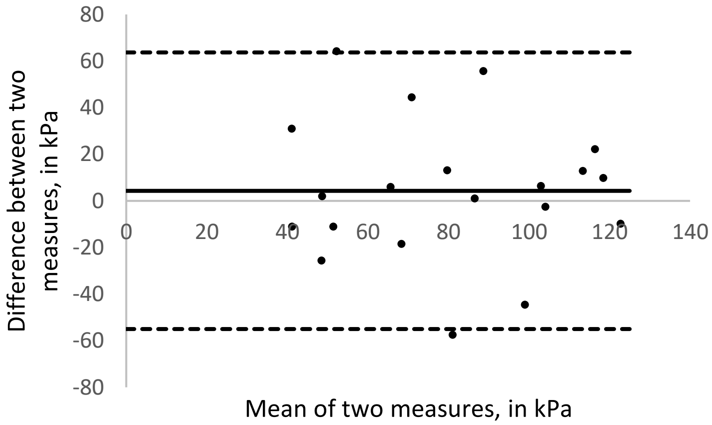
Figure 18: Bland-Altman plots of agreement between VA (first visit) and V2 (second visit) for the mean *biceps brachii* shear modulus assessment at each visit and each condition



a – Gastrocnemius at rest



b – Gastrocnemius at stretch



c – Gastrocnemius s at contraction

LOA: Limit of Agreement — Lower LOA — Upper LOA — Bias

Figure 19: Bland-Altman plots of agreement between VA (first visit) and V2 (second visit) for the mean *gastrocnemius medialis* shear modulus assessment at each visit and each condition

4 – Discussion

4.1 – Main results

The intersession reproducibility of ultrasound SWE for measuring the elastic properties of the LAM were excellent at rest and during Valsalva maneuver but poor during subjective maximal contraction. The reproducibility performance of the mean of three consecutive measurements for each session was higher than that of the first measurement of the three consecutive measurements. Reliability for peripheral muscles was good to moderate for the assessment of the elastic properties of the *biceps brachii* muscle whereas it was moderate to poor for the *gastrocnemius medialis* muscle.

4.2 – Justification of methodological choices

We chose the ultrasound SWE method because it allows noninvasive and quantitative assessment of the the PFMs. We have previously reported the feasibility of measuring LAM elastic properties without difficulties, supporting our choice to focus on this approach in the present study [49]. We systematically investigated the right LAM to ensure operator convenience (since the operator was usually on the right side of the supine participant). This approach appears safe because we only included nulliparous women, thereby avoiding women with LAM avulsion. Furthermore, a previous feasibility pilot study reported no differences in the shear modulus measured on the right versus the left LAM [49]. We considered BMI higher than 35 $kg.m^{-2}$ as an exclusion criterion because measurements for women with very high BMIs could not be performed because of the loss of LAM visibility in B-mode transperineal ultrasound during the Valsalva maneuver.

For the main analysis, we chose to consider the mean of three consecutive measurements performed at each visit instead of a direct single measurement. We hypothesized that reproducibility will probably be better with this approach because it is difficult to standardize a lithotomy position and even more difficult to standardize a Valsalva maneuver.

We chose to perform measurements on peripheral muscles with the prospect of implementing a prospective longitudinal study comparing the biomechanical behavior of PFMs (especially the LAM) versus peripheral muscles during pregnancy. We focused on the *biceps brachii* and the *gastrocnemius medialis* muscle because they are easily accessible and because some studies reported a good reproducibility of such measurements [45]. Nevertheless, these results came from research teams with a specific interest in these muscles, and we thought necessary to investigate the reproducibility in a research team without a great experience of these measures. The other point is that we had to adapt the measurement protocol for the *gastrocnemius medialis* muscle which is usually investigated with the volunteer in ventral decubitus position[45]. Indeed, with the prospect to perform these measures in pregnant women, it is obvious that such a position would not be possible. This is why we chose an installation in left lateral decubitus [118].

4.3 – Strengths and limitations

The main strength of this study is that it provides data about an innovative approach to investigate the elastic properties of PFMs with a non-invasive approach that will be much more acceptable for women than other techniques using vaginal speculums or vaginal ultrasound probes [40, 41, 110, 113, 135, 136].

The primary limitation of this study is that we only reported intraoperator reproducibility data. This was due to the lack of additional available investigator, and because we aimed to use only one investigator in our projects [118]. However, measurements performed by two investigators may show specific interoperator discrepancies, and the interoperator reliability will have to be determined by groups that aim to have more than one investigator in their protocol.

In this study, we made measurements of the mean shear modulus for the largest visible muscle region. Indeed, the viscoelastic properties of a tissue may differ according to the region. This might be true for the muscle which is generally stiffer near to its insertion. Therefore, the good reliability reported in the present study for the LAM suggests that we were able to reproduce the measurements in a similar region, and it is probably a criterion to get reliable measurements. We chose to measure the shear modulus in one single area because, in a clinical view, the part of the LAM accessible with a transperineal approach is considered as the pubic insertion of the LAM (the one affected by obstetric perineal trauma), and it would not have been clinically justified to perform several measures in different areas. To be consistent within our whole study protocol, we chose to have the same approach for peripheral muscles by investigating one single area.

Another limitation is the standardization of the LAM SWE measurement. Indeed, we only required the participants to lie down in the lithotomy position with an empty bladder, without any measurement of the thigh opening. This is particularly true for the subjective maximal contraction condition, wherein the intensity of the contraction was not controlled, because the measurement was highly dependent on the contraction level [137]. Thus, the conditions across measurements may not have been exactly comparable. However, this was a voluntary choice because we aimed to assess the reproducibility in a real-life condition, and considering that we aimed to perform such measurements in a clinical environment with pregnant women.

Lastly, we did not report any clinical examination related data for pelvic organ mobility and therefore were not able to investigate the correlations between SWE considerations and clinical observations for PFMs. Such an analysis is performed in our study 5 in a pregnant women population.

4.4 – Interpretation

We reported excellent reproducibility of the assessments performed at rest and with Valsalva maneuver for LAM related measurements. Only one previous study described such a reproducible analysis of the LAM assessment using a transperineal approach, but that study used an abdominal curved probe. Further, the investigators reported good reproducibility of intraoperator intersession assessments at rest (ICC = 0.86 [0.58-0.95]) and during the Valsalva maneuver (ICC = 0.79 [0.54-0.95]), and did not report measures during contractions [126]. In our results, the reliabilities at rest and during Valsalva maneuver were excellent both when considering the mean of the three consecutive measurements and when considering the first of the three consecutive measurements. This observation would have been the same if we had considered the second or the third of the three measures because the intraoperator intrasession reliability was good among these three measurements. This result is interesting and may have direct applications. On the basis of this result, it appears safe to perform a single measurement for the LAM using the transperineal ultrasound SWE when the objective is to assess the elastic properties of this muscle at a specific and unique time. If the technique is used to investigate changes over time, it is probably safe to perform three measurements and consider their mean for the analysis to increase the sensitivity of the examination.

For the LAM, mean shear modulus values while performing Valsalva maneuver and during contractions were within the same range. This could be surprising because, in skeletal muscles, increases in shear modulus are much greater during contractions compared with passive lengthening [137, 138]. A first explanation could be the contraction of the LAM during the Valsalva maneuver that would have led to an overestimation of the muscle stiffness in this condition. However, this possibility was ruled out because we systematically took care of avoiding any LAM coactivation during Valsalva maneuver based on the biofeedback procedures recommended by Orno *et al.* [51]. In addition, we observed peculiar changes in the muscle characteristics which differed between the tasks while using the B-mode ultrasound, such as an increase in the muscle length and a horizontal orientation of the muscle fibers during Valsalva maneuver, whereas a shortening of the muscle and a downward tilt of its fibers occurred during contractions. This supports the fact that we effectively measured the muscle properties under two different conditions. These results highlight the excessive lengthening of the LAM during a Valsalva maneuver that significantly increase the muscle stiffness in a manner similar to that during contractions. The increase in stiffness is probably much larger during childbirth, which explains the risk of muscle trauma. Lastly, the value of shear modulus of the LAM at contraction should be carefully interpreted considering the poor reliability of this measure, the difficulty to standardize the task, and to be certain that the contraction is maximal.

The comparison with the existing literature on the elastic behavior of LAM remains complex because various methods do not provide values in the same metrics. We cannot compare our results about the LAM viscoelastic properties to biomechanical studies on cadaveric tissues because in these works, researchers aim to identify the level of strain for which the damage occurs and not the intrinsic elastic properties. Our results are not comparable to the studies involving the use of vaginal speculum as an elastometer or vaginal probe because it measures a torque or a force applied on the device, which is recorded by a force sensor and is not a direct quantitative assessment of the elastic properties such as that obtained from using elastography [40, 41, 110, 113]. We can compare our data to other elastography studies. A more direct comparison can be done with the study of Tang *et al*. using SWE, reporting a 28 kPa shear modulus for the LAM at rest (versus 22 in our study) and 57 kPa during Valsalva maneuver (vs 45 in our study). Therefore, Tang *et al*. report a slight increase in LAM stiffness but in a very different population with a mean age of 56 years versus 23 in our study [126]. Finally, our results are comparable to a study by Silva *et al*. that calculated the elastic properties of the pubovisceral muscle using inverse finite element with three models. They reported a shear modulus of 78 ± 44 kPa with the first one, 80 ± 48 kPa with the second one and 62 ± 46 kPa with the last one [128]. Using a comparable approach, Li *et al.* measured the elastic properties of the LAM at rest and while performing Valsalva maneuver using SWE with a comparative analysis between continent women and women with stress urinary incontinence [127]. They chose to report the elastic modulus, which should be divided by a factor 3 to obtain the shear modulus, and finally reported values in the same range as that reported in our study [127]. Silva *et al.* reported an increased stiffness of LAM as compared to that reported in the present study, but values remained in the same range although different methods were used. Taking these comparisons all together, the range of values reported in the present study seems consistent with the literature.

The LAM appears much stiffer than the peripheral muscles. Indeed, we reported an SM of 22 kPa for the LAM at rest, whereas it has been reported to be between 2 and 5 kPa for peripheral muscles of both the upper and lower limbs [45]. This difference may be primarily associated with differences in the intrinsic structure of these muscles, because the LAM mainly consists of type 1 muscular fibers (mainly involved in prolonged effort), whereas peripheral limb muscles mainly comprise type 2 muscle fibers [55, 139]. This could have been controlled by investigating the *soleus* muscle which is mainly composed of type 1 fibers. Another hypothesis could be that measurements in the assessment for LAM were performed near the muscle's pubic insertion, whereas measurements for peripheral muscles were mainly performed at the middle of the muscle from a distance to its insertions [45]. Furthermore, even if measurements were performed without the Valsalva maneuver or subjective maximal contraction and in a lithotomy position, there was always a constraint applied by the abdominal pressure on the PFMs that could never be fully removed under *in vivo* conditions.

Our results showed that SWE is a reliable tool to investigate the elastic properties of PFMs *in vivo*. This offers interesting prospects for research that will aim to improve our knowledge of the pathological process associated with obstetric perineal trauma and/or pelvic floor disorder occurrence.

We reported disappointing results for the reliability of SWE assessment for elastic properties of peripheral muscles. However, we reported a good to moderate reliability for the *biceps brachii* whereas it was reported as excellent in previous studies [45]. One explanation could be that in the present study, all the assessments were performed by a senior urogynecologist with a specific interest in pelvic floor imaging but without any previous experience in the imaging of peripheral muscles. We obtained acceptable results by repeating exactly the same protocol than previously reported, but it is likely that a more experienced observer for the peripheral muscles would have ensured better results. Regarding this point, an observer without a good experience of pelvic floor imaging is more likely to give less satisfactory results, in terms or reliability, than those reported in the present study. This becomes more relevant for the *gastrocnemius medialis* muscle because the main observer did not have any prior experience of such muscle imaging. Furthermore, as we explained it above, we chose to modify the usual protocol (ventral decubitus with the ankle in 90° flexed position, with the foot leaning on the wall) with the prospects to perform these measures in pregnant women. In our study, women laid down in the left lateral decubitus with the ankle's angle controlled using a goniometer. Therefore, the ankle's flexion was controlled but we were not able to control the strict lateral decubitus from one visit to another. It is possible that the flexion of the left leg was different between two visits, and that the left lateral decubitus position could be different from one visit to another with an anterior or posterior inclination. We consider that these limitations regarding the experience of the observer and difficulties to standardize the position could explain the moderate to poor reliability indicators for this muscle in our study whereas they are excellent in other studies [45]. This lack of reliability for peripheral muscles, especially for the *gastrocnemius medialis,* must be taken into account in future studies from our team aiming to investigate changes in elastic properties of both PFM and peripheral muscles during pregnancy [118].

5 – Conclusion

Ultrasound SWE is a reliable tool to investigate the elastic properties of LAM at rest and while performing the Valsalva maneuver; however, this study failed to perform reliable measurements during subjective maximal perineal contractions. This technology might be useful to improve our knowledge of the pathological processes associated with the occurrence of obstetric perineal trauma and/or pelvic floor disorders.

Study 4 – Feasibility, reliability, and acceptability of assessing the viscoelastic properties of the external anal sphincter in term pregnant women by using shear wave elastography

1 – Introduction

We reported that investigating the elastic properties of the LAM in women using SWE is both feasible and reliable [49, 52]. This innovative strategy might be useful in the near future to improve our understanding of the pathophysiology of LAM avulsion and to identify the women at high risk to offer them an individualized management.

As we explained in the first section of this thesis that another type of perineal trauma at childbirth is represented by OASI. We further acknowledge the lack of an efficient strategy that identifies the women at high risk for OASI before the delivery.

With the same mechanism as that for the LAM and the risk of LAM avulsion, the anal sphincter complex is exposed to massive strain during the vaginal delivery. Moreover, the ability of the EAS to lengthen and thus the women's biomechanical intrinsic characteristics could be associated with the risk of OASIs at childbirth.

Additionally, the lack of validated methods for measuring the elastic properties of the EAS in pregnant women quantitatively and *in vivo* is a major problem. We hypothesized that SWE could be used for such an assessment, as we reported for the LAM assessment.

Before using the elastic properties of EAS to improve the predictive strategies for OASI, the reliability of such a measure and the acceptability in women must be determined. The present study was designed to assess the intraoperator intersession reliability, the interoperator intrasession reliability, and the acceptability of SWE applied to the EAS in pregnant women.

2 – Material and methods

2.1 – Study settings

A prospective unicentric study with two planned visits, spaced by a minimal interval of 12 h and a maximum interval of 7 days, was conducted from July 2020 to April 2021, and the inclusion of 40 women was planned.

2.2 – Population

The inclusion criteria were as follows: nulliparous pregnant women at >37 weeks of gestation, women aged >18 years, women with a single fetus with a cephalic presentation, and women with a normal pregnancy. Premature term (before 37 weeks), history of previous delivery (regardless of the mode of delivery), multiple pregnancies, noncephalic presentation, consultation and/or hospitalization within the pathological pregnancy unit, obesity with a prepregnancy BMI higher than 35 kg/m^2, and personal history of pelvic floor disorders were the exclusion criteria. Obesity was considered an exclusion criterion because pilot measurements showed that this assessment was challenging in participants with obesity [49].

2.3 – Data collection

2.3.1 – Participant characteristics

We collected the following data at the first visit: age (in years), height (in cm), weight before pregnancy (in kg), and obstetrical term (in weeks) at the first visit. We calculated the BMI, which was reported in kg/m^2. At the second visit, we collected only one additional characteristic: obstetrical term at the second visit (in weeks). The time interval between the two visits was reported in hours.

2.3.2 – Shear Wave Elastography assessments

During the first visit, women underwent one SWE assessment of the elastic properties of the EAS under 3 conditions: at rest, while performing the Valsalva maneuver, and during perineal contraction. All procedures at the first visit were performed by a single senior urogynecologist (always the same: BG) with a specific interest in pelvic floor imaging. For the second visit, women underwent 2 SWE assessments of the EAS. The first assessment was performed by the same observer as in the first visit. The second assessment was performed by a registrar in urogynecology (always the same: OC) who was blind to the two previous assessments.

The protocol was identical for the three assessments. Women laid supine in a gynecological position with an empty bladder. All assessments were performed using the Aixplorer V12 device (SuperSonic Imagine, France) and an SL 18-5 (5-18 MHz) linear probe wrapped in single-use protection. The probe was placed immediately above the anus in a transverse plane using a transperineal approach (Figure 20) [118]. EAS was identified in two-dimensional ultrasound mode, and thereafter SWE acquisition was performed. For assessment at rest, participants were asked to relax as much as possible. For assessment during the Valsalva maneuver, participants were requested to bear down as much as possible with a closed glottis. This induces an increase in the intraabdominal pressure and strain on the PFMs in the same way (with a lower magnitude) as during childbirth. For assessment during perineal contraction, women were asked to squeeze the perineum as if the woman wanted to avoid flatulence leakage. Three consecutive acquisitions were performed for each condition (at rest, while performing Valsalva maneuver, and at contraction) as a 5s video clip.

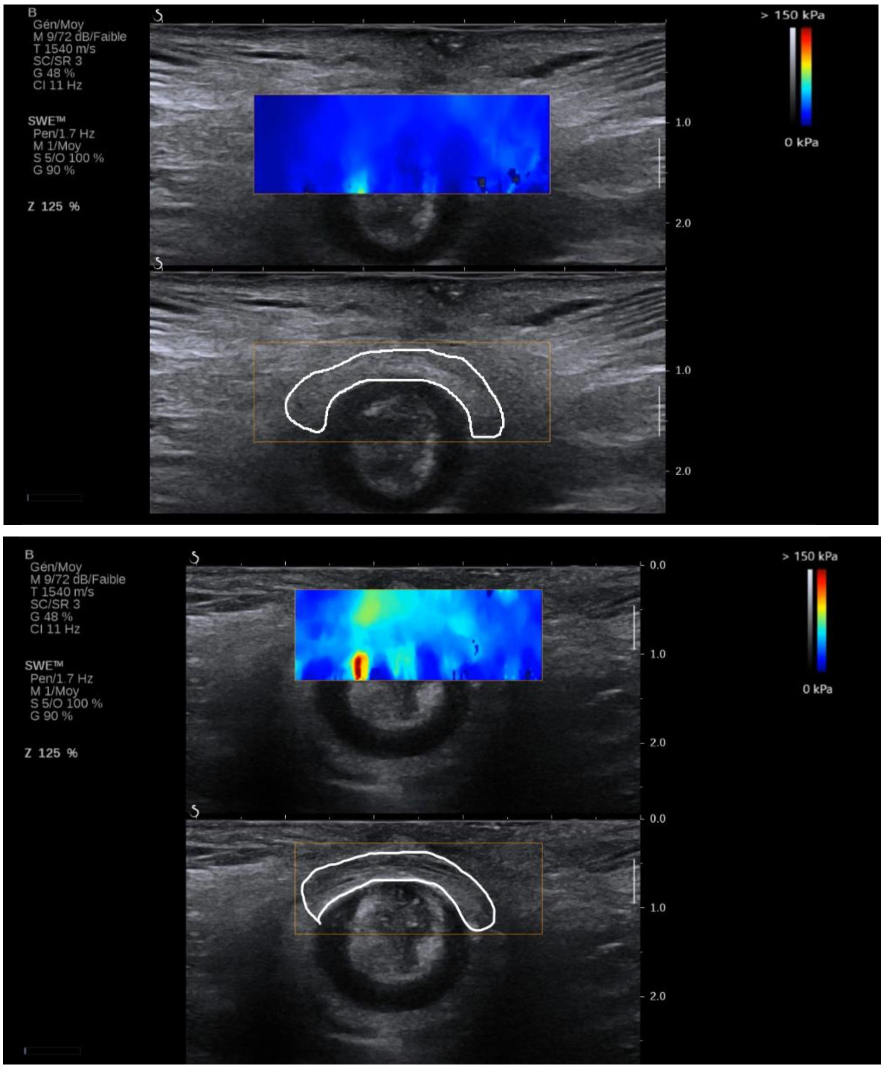
Figure 20: External anal sphincter shear wave elastography assessment: probe position

Figure 21 provides an example of SWE assessment for each of the three conditions. For all assessments, the region of interest was identified and contoured manually (Figure 21) using MATLAB scripts (The MathWorks, Inc., 2016). The Aixplorer device provides a Young's modulus assessment (in kPa) within the region of interest, which is suitable for isotropic tissues. Because muscles are anisotropic tissues, Young's modulus is not suitable, and it is safer to report the shear modulus (in kPa) as reported in previous chapters of this thesis [38, 43, 44].

For assessment at rest and during the Valsalva maneuver, we collected the mean shear modulus for each acquisition, and the mean of the three measures was considered for analyses. For assessment during contraction, we collected the maximal shear modulus for each acquisition, and the mean of the three measures was considered for analyses.

2.3.3 – Feasibility and acceptability

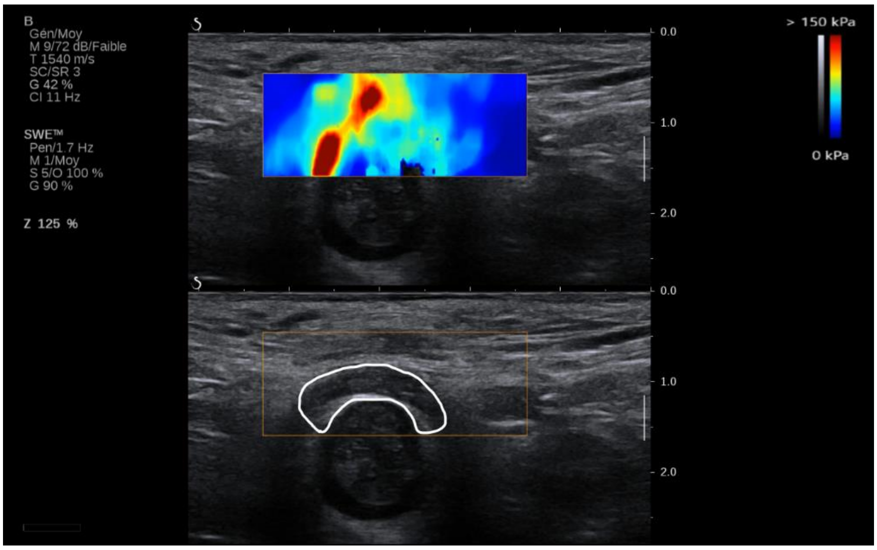
At the end of the second visit, acceptability of the procedure was assessed by asking women “If your practitioner offers you the possibility of this examination in your third trimester to estimate your risk of obstetric anal sphincter injury at childbirth, would you agree to undergo it? Please answer from 0 (certainly not) to 10 (yes, certainly).” Acceptability was investigated by reporting the mean score for the question asked to the participants at the end of the second visit. Acceptability was considered excellent in cases of scores higher than 8/10.



a –

b –

c –



The colored scales indicated the range of shear modulus value. Areas contoured in white lines represent the external anal sphincter muscle (region of interest).

External anal sphincter muscle; MSK resolution mode

<u>Figure 21: Shear wave elastography assessment of the external anal sphincter in term pregnant women at rest (a), during Valsalva maneuver (b) and perineal contraction (c).</u>

2.4 – Data analysis and statistics

Only women who completed the study protocol (both visits) were considered in the analysis. We first reported our population's characteristics and the time interval between the two visits. Continuous variables were reported as means and standard deviation (SD). Categorical variables were reported as numbers and percentages. We first described the elastic properties of the EAS and observed changes from rest to Valsalva and contraction using a Friedmann test for each observer and each session. Regarding the main objective, we investigated the intraoperator and interoperator intrasession reliability by calculating the ICC and its 95% confidence interval, the SEM in kPa, and the CV in % as we calculated in previous studies reported earlier in this thesis[133]. Bland–Altman plots were computed for both intraoperator and interoperator reliability [134]. Reliability was considered excellent in cases of ICCs higher than 0.90, good between 0.76 and 0.90, moderate between 0.50 and 0.75, and poor if less than 0.50 [133]. Methodological justification for choosing these statistical tools is reported in the previous study, in the methods section.

No *a priori* power calculation was performed. After review of other reliability studies, a sample size of 35 women appeared to be sufficiently robust. Assuming that approximately 10% were lost to follow-up (given the inclusion criteria, some women might deliver between the two visits), we planned to recruit 40 women. Statistical analyses were performed using STATA V14 software (Stata Corporation, College Station, TX, USA). For all analyses, statistical significance was set at $p < 0.05$.

2.5 – Ethical and reglementary considerations

Dr Bertrand GACHON was the coordinating investigator for this study and performed all the assessments for the first visit and the first observer measurements for the second visit.

All women received information and gave written informed consent before any investigation. The study was approved by the ethics committee (*Comité de Protection des Personnes Sud Est VI, France;* ID RCB: 2020-A00764-65). The study was registered at https://clinicaltrials.gov before the first inclusion on April 17, 2020 (NCT04350632).

3 – Results

Among the 40 included women, 3 delivered between the two visits and were excluded, leaving 37 women eligible for the analysis. The mean age of the participants was 29 years old (SD=4.9) with a mean BMI of 23.2 kg.m^2 (SD=4.2). The mean term at both the first and second visits was 37 weeks (SD=0.7), with a mean time interval of 42.3 hours (SD=0.7) between the two visits.

Feasibility was excellent: all procedures (100%) were successfully completed.

Regardless of the session and the observer, the mean SM significantly increased from 10.1 kPa at rest to 17.5 kPa during the Valsalva maneuver and 35.8 kPa during perineal contraction ($p<0.005$; Tables 7 and 8).

Results for the intraoperator intersession analysis are reported in Table 7 and Figure 22. We reported excellent reliability for the assessment at rest (ICC= 0.91 [0.84-0.95]; SEM=1.9 kPa; CV=18.8%). Reliability was good for assessments during the Valsalva maneuver (ICC= 0.83 [0.72-0.90]; SEM=4.0 kPa, CV=23.7%) and at contraction (ICC= 0.85 [0.75-0.91], SEM=7.4 kPa, CV= 20.5%).

Results for the interoperator intrasession reliability are reported in Table 8 and Figure 23. Reliability was good at rest (ICC= 0.79 [0.66-0.87], SEM=2.6 kPa; CV=25.5%) and while performing the Valsalva maneuver (ICC= 0.84 [0.73-0.90], SEM=4.4 kPa; CV=23.9%). It was moderate for the assessment during contraction (ICC= 0.70 [0.53-0.82]; SEM=11.0 kPa, CV=30.2%).

Table 7: Intraoperator intersession reproducibility performances for the assessment of the external anal sphincter's shear modulus in term pregnant women.

	Mean shear modulus at V1, in kPa (SD)	Mean shear modulus at V2, in kPa (SD)	ICC [95% CI]	CV, in %	SEM, in kPa
Rest	10.0 (4.4)	10.1 (3.9)	0.91 [0.84-0.95]	18.8	1.9
Valsalva	16.2 (6.6)	17.6 (7.0)	0.83 [0.72-0.90]	23.7	4.0
Contraction	34.6 (11.8)	37.5 (14.0)	0.85 [0.75-0.91]	20.5	7.4

ICC: Intraclass Correlation Coefficient CI: Confidence Interval CV: Coefficient of variation

SEM: standard error of measurement

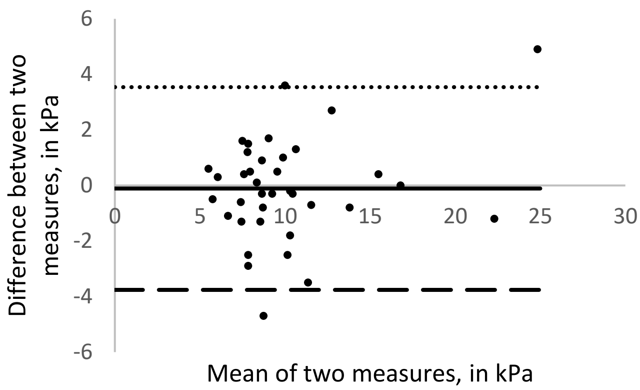
Acceptability was excellent: the mean score for the acceptability question was 9.6/10 (SD=0.5), and no participant was assigned a score lower than 9/10.

Table 8: Interoperator intrasession reproducibility performances for the assessment of the external anal sphincter's shear modulus in term pregnant women.

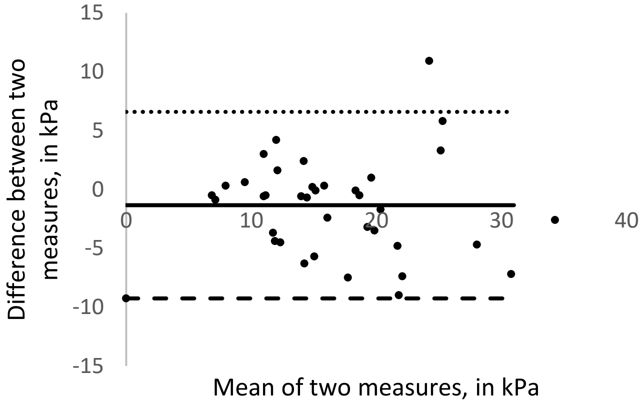
	Mean shear modulus at V1, in kPa (SD)	Mean shear modulus at V2, in kPa (SD)	ICC [95% CI]	CV, in %	SEM, in kPa
Rest	10.1 (3.9)	10.3 (4.0)	0.79 [0.66-0.87]	25.5	2.6
Valsalva	17.6 (7.0)	18.6 (8.0)	0.84 [0.73-0.90]	23.9	4.4
Contraction	37.5 (14.0)	35.4 (13.9)	0.70 [0.53-0.82]	30.2	11.0

ICC: Intraclass Correlation Coefficient CI: Confidence Interval CV: Coefficient of variation

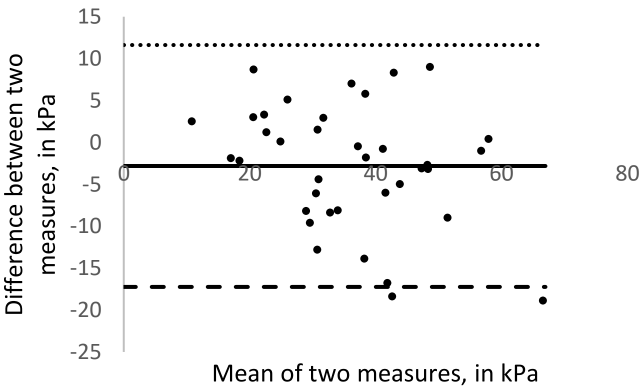
SEM: standard error of measurement



a – Assessment at rest



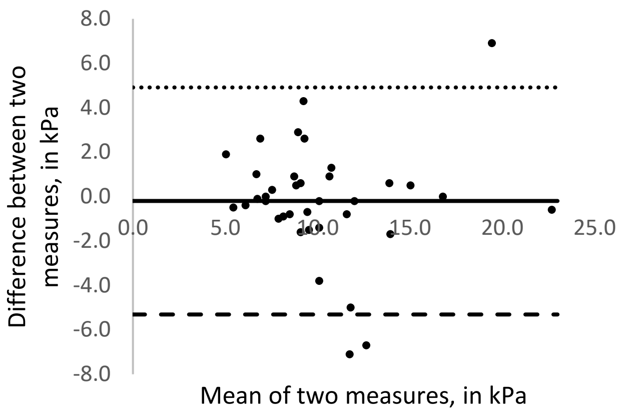
b – Assessment during Valsalva



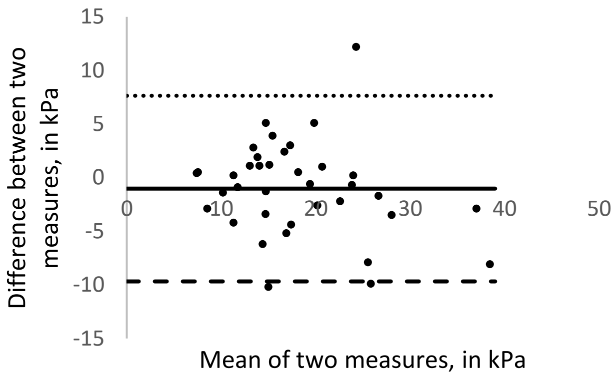
c – Assessment during contraction

LOA: Limit of Agreement

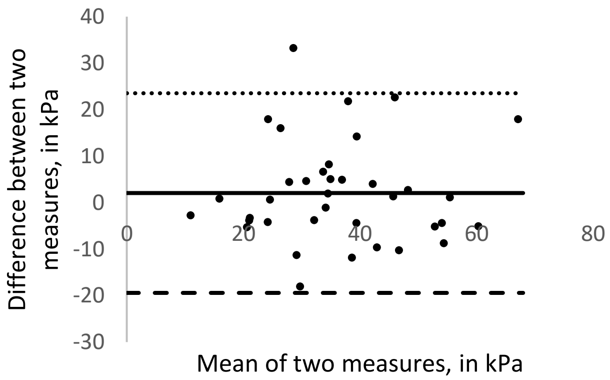
Figure 22: Bland-Altman plots of intraoperator intersession agreement for external anal sphincter assessment at rest, while performing Valsalva maneuver, and during contraction



a – Assessment at rest



b – Assessment during Valsalva



c – Assessment during contraction

LOA: Limit of Agreement

Figure 23: Bland-Altman plots of interoperator intrasession agreement for external anal sphincter assessment at rest, while performing Valsalva maneuver, and during contraction

4 – Discussion

4.1 – Main results

This is the first report of an *in vivo* assessment of the elastic properties of EAS in pregnant women using SWE. The technique appears feasible, highly acceptable to women, and reliable, with good to excellent reliability parameters.

4.2 – Strengths and limitations

The main strength of this study is that it reports the first published data on *in vivo* assessment of the EAS in women. In addition, this study provides the first published data collected in term pregnant women using SWE. The second major strength is the choice of SWE technology instead of other elastography techniques, such as static or quasistatic elastography. SWE allows direct and quantitative assessment of elastic properties, whereas other techniques often involve the use of a standoff pad between the tissue and the ultrasound probe and/or only allow differential assessment [38, 39, 114, 115].

The main limitation of this study is that it reports results from a single center, which is the one where the technique was initially described.

4.3 – Interpretation

In this study, the EAS was stiffer during the perineal contraction than while performing the Valsalva maneuver and at rest. This is consistent with other studies involving peripheral muscles assessed at rest and in stretched positions during contractions [45, 137, 138]. This is also consistent with our previous reports regarding the LAM. We reported excellent and good reliability indicators in this study, similar to those reported for the LAM [52]. This supports the use of SWE as a potentially innovative tool for the assessment of PFMs and its relevance for the field of obstetric-related pelvic floor trauma [23].

Interestingly, the EAS appears less stiff than the LAM, although their histological composition is quite similar [49, 52, 56, 126]. This difference might be associated with a difference in the muscle function. Indeed, the LAM has a major role in maintaining the pelvic organ stability and continence in women. Owing to this function, the LAM is under permanent strain (the intraabdominal pressure is never zero), whereas the EAS is generally involved in active control of anal continence and is mainly strained in cases of urgent situations to avoid anal leakage. Another factor that may contribute to the difference between the EAS and LAM is that the available data for the LAM come from studies involving nonpregnant women, whereas our study is the first to be conducted on pregnant women [49, 52, 126]. It has been reported in human and animal experimentation that pregnancy can affect the elasticity of women's pelvic tissues, and this could partially explain such differences [23, 33, 34].

As previously reported, this is the first description of an assessment of the EAS's elastic properties. Our results must be validated before considering some clinical applications. Such a validation could be obtained by replicating this research protocol in another unit with other investigators looking for comparable results. Another possibility, could be to develop an animal experimentation research protocol with an association of SWE measurements of the EAS and ex vivo elastic properties measurements after sacrifice. Regarding the muscle's volume, it would be necessary to consider bigger animal than rats (monkey, goat).

The possibility of assessing the elastic properties of the EAS among pregnant women offers interesting prospects. First, SWE could be used to report the first human data about changes that occur in the elastic properties of the pelvic floor muscles during pregnancy. This study has been conducted for the assessment of both the LAM and EAS in each trimester of pregnancy (ELASTOPELV; NCT03602196), and the results are reported in the next section (study 5) of this thesis [118]. Second, this technique may improve our knowledge about the biomechanical behavior of pelvic floor muscles during childbirth. Indeed, it will be very interesting to assess several measures at each stage of labor (onset, 5 cm cervical dilatation, complete cervical dilatation, mid-pelvic fetal head station, and crowning). Herein, again, these would be the first human *in vivo* data. Third, SWE measurements on the pelvic floor could be used to improve the prediction of OASI occurrence. Given the low prevalence of OASI, such a research approach would require a large multicentric study to enroll enough women. One difficulty with such a multicentric approach is that we will not be able to control clinical politics of childbirth management in each participating center. The most important heterogeneity is about the diagnosis of OASI which requires some expertise with the risk of undiagnosed injuries at delivery. This could be handle by planning pelvic floor ultrasound few weeks after the delivery in order to look for the existence of an OASI.

The final objective was to improve existing predictive algorithms that remain disappointing for predicting OASI, leading to a more personalized predictive approach that allows individual counseling for pregnant women [6, 7, 23]. This is a topic of major importance considering that women frequently ask for increasing amounts of prenatal information about childbirth, and often have a strong desire to participate in decision-making about their delivery. Thus, the ability to perform individual risk assessment that could include individual women's characteristics in addition to classical delivery characteristics is very compelling. In addition, SWE of the EAS could be used in clinical practice for other applications. One example is the assessment of the EAS in postpartum symptomatic women with anal incontinence. For these women, it would be interesting to perform noninvasive SWE assessment of the EAS to check for sphincter defects or insufficient contraction instead of requiring invasive procedures such as endoanal ultrasound and/or anorectal manometry.

5 – Conclusion

The present study reports the first *in vivo* assessment of the elastic properties of the EAS in term pregnant women. Such assessment using SWE technology appears feasible and highly acceptable to pregnant women. Its reliability is good to excellent regardless of whether an intra- or interobserver approach was considered (except for contraction with an interobserver approach). SWE could be an innovative technique, allowing broad improvement of our knowledge about the biomechanical behavior of pelvic floor muscles during vaginal delivery and individual risk assessment of OASI occurrence for personalized counseling of pregnant women.

Study 5 – Changes in the viscoelastic properties of the pelvic floor and peripheral muscles during pregnancy (ELASTOPELV study) [118]

1 - Introduction

Perineal trauma at childbirth is a frequent outcome which can have a major negative impact on women's health. It is mainly represented by LAM avulsion and OASIs [23]. A recent meta-analysis reported that LAM avulsion occurs in 15% of spontaneous deliveries and 52% of forceps deliveries. Further, LAM is associated with an enlarged levator hiatus area leading to pelvic organ prolapse, sexual dysfunction, perineal pain, and incontinence [3, 18]. The prevalence of OASI is estimated between 0.25% and 6% in the cases of spontaneous deliveries, but it could considerably increase in case of operative vaginal deliveries [14, 53]. The main outcomes associated with OASI are anal incontinence, perineal pain, sexual dysfunction, and postpartum depression [15, 17, 53].

Both LAM avulsion and OASIs share common risk factors, such as nulliparity, operative vaginal delivery, posterior occiput presentation, and high birthweight, and probably a common pathophysiology [3, 5, 14, 53]. Despite these well-known risk factors, to date, attempts for implementing predictive strategies remain disappointing [6, 140]. According to one hypothesis these predictive strategies do not include women's intrinsic characteristics and are focused on the mode of delivery as well as anthropometric data related to the mother and the child [23]. Nevertheless, some reports suggest that intrinsic biomechanical properties of women's tissues could be associated with perineal trauma at childbirth. As an example, a recent study reported that women with the highest joint mobility were those with the highest risk of OASIs [37]. Furthermore, results from animal studies show an increased PFM stiffness during pregnancy, which could be a protective mechanism against perineal trauma at childbirth [33, 34].

We hypothesized that considering the elastic properties of PFMs in women could improve the efficiency of risk prediction for perineal trauma at childbirth. Currently, there is a lack of available data about the changes in the elastic properties of PFMs *in vivo* and their changes during pregnancy. SWE is a recent technology that allows a direct, quantitative, and *in vivo* assessment of the elastic properties of muscles [38]. Its reliability has been recently reported for the LAM and the EAS in women, including during pregnancy, previously in this thesis [52]

The main objective of this study was to describe the elastic properties of the PFMs (LAM and EAS), and their changes during pregnancy using SWE technology. The secondary objectives were: i) to look for specific changes in the PFMs compared to peripheral muscles; ii) to determine whether an association between the elastic properties of PFMs and perineal clinical and B-mode ultrasound measures exists; and iii) to provide explorative data of the association between characteristics of PFMs and the mode of delivery as well as the risk of perineal tear [118].

2 – Material and methods

2.1 – Study settings

A prospective, longitudinal, monocentric study was conducted in the department of Obstetrics and Gynecology of the Poitiers University Hospital, in France. The study's scheme involved three visits during pregnancy: first, between 14 and 18 weeks; second, between 24 and 28 weeks; and the last, between 34 and 38 weeks of pregnancy. For each of the three visits, the protocol followed these steps: clinical perineal assessment, ultrasound B-mode perineal assessment, SWE assessment of the LAM, the EAS, the *biceps brachii* muscle, and the *gastrocnemius medialis* muscle.

2.2 – Population

2.2.1 – Inclusion criteria

The inclusion criteria were: women >18 years, volunteers, nulliparous, with a normal singleton pregnancy, and who were benefited from health insurance.

2.2.2 – Non-inclusion criteria

The non-inclusion criteria were women with a previous vaginal and/or cesarean delivery, women with a personal history of pelvic floor disorders (urinary incontinence, anal incontinence, pelvic organ prolapse), women with a BMI higher than 35 $kg.m^{-2}$, women with chronic muscular diseases, women requiring admission to a psychiatric unit, women under judicial protection, and women unable to understand French language.

2.2.3 – Exclusion procedure

Included women with pathological pregnancy (defined by the necessity of follow-up for consultations which were categorized as pathological pregnancies and/or admission to the pathological pregnancy unit) were excluded.

Women who wished to cease their participation during the study were excluded in the same way. No further data were collected because they expressed their wish, and they were excluded from the analysis.

During this study, the COVID-19 outbreak was declared as a global pandemic. Women having visits planned during the lockdown periods and/or those confirmed with the coronavirus disease were not able to attend the planned visits according to the protocol. Therefore, no data were collected from the cancelled visits, and these women were excluded from the analysis.

2.2.4 – Sample size

In the absence of previous data that would have allowed for a power calculation, this study dealt with exploratory data. Furthermore, the main endpoint was a descriptive one; therefore, *a priori* power calculation did not appear necessary. We initially aimed to obtain the data from at least 50 women in this study. We considered this sample size because the previous studies which reported an increase in levator hiatus area and ligamentous laxity during pregnancy as well as the changes in intrinsic biomechanical characteristics of pregnant women, from 20 to 50 women [22, 25, 31, 118]. We initially estimated that 20% of the women would be excluded during pregnancy for one of the previously reported reasons, leading to an objective of 60 inclusions. Owing to the COVID-19 pandemic, this exclusion criterion was underestimated and the protocol was modified allowing the inclusion of 77 pregnant women.

2.3 – Data collection

2.3.1 – Participant characteristics

At the first visit, after validation of the eligibility criteria, we collected the following data of the participant: height and weight for calculating the BMI (in $kg.m^{-2}$); demographic data and obstetrical history were also collected during the visit, such as age (in years) and gestity.

2.3.2 – Clinical assessment

We performed the POP-Q procedure for clinical assessment of the pelvic organ mobility (Figure 9). The exact procedure is detailed in section 4.3. in this thesis with a measure of the 6 POP-Q point positions (reported in centimeters with negative or positive values) and the length of the 3 POP-Q segments (reported in centimeters). Clinical perineal distension was appreciated as the addition of gh and pb segments.

2.3.3 – B-mode ultrasound assessment

We performed an ultrasound B-mode pelvic floor assessment at each visit of the study. This examination was performed with the woman in the lithotomy position after voiding. We used the Aixplorer (V12, SuperSonic Imagine, France) with an XC6-1 1-6 MHz curved probe. We measured the anteroposterior hiatal diameter as the distance between the anteroinferior extremity of the pubic symphysis and the anorectal junction (in cm). We performed one measure at rest and one during a maximal strain while performing the Valsalva maneuver. For these measures, we used the translabial perineal approach widely described by Dietz *et al*. [82, 141]. We asked women to perform two initial Valsalva maneuvers with biofeedback instructions to prevent levator coactivation from serving as a confounding factor in our analysis [51].
We reported the anteroposterior diameter measure at rest and during Valsalva maneuver. Lastly, we reported the elevator hiatus distension represented by the difference between the measure during Valsalva maneuver and the measure at rest. All these measures were reported in centimeters.

2.3.4 – Shear wave elastography assessment

As previously mentioned, SWE assessments were performed for the LAM, EAS, *biceps brachii* muscle, and *gastrocnemius medialis* muscle. For the LAM, we used exactly the same protocol as reported in the section 2.3.2.1 of the study 3 in this thesis with excellent reliability indicators. For the EAS, we used exactly the same protocol as the one reported in the section 2.3.2 of the study 4 in this thesis (Figure 20 and 21) with again excellent reliability indicators. These measures were added to the protocol after the study's onset explaining why data will not be available for all the included women. For the *biceps brachii* and the *gastrocnemius* *medialis* muscles, we used exactly the same protocol that was reported in the section 2.3.2.2 (Figure 15) and 2.3.2.3 (Figure 16) of the study 3 of this thesis.

For each assessment we collected a 5s video clip. For each muscle we performed 3 measures at rest, 3 measures at stretch (*biceps and gastrocnemius)* or during Valsalva maneuver (LAM and SEA). We calculated the mean shear modulus within each video clip at rest, stretch or during Valsalva. We considered for the analysis the mean shear modulus (in kPa) of the three consecutive measures as reported in our previous studies.

We chose not to perform measurements at contraction because of the moderate/weak reliability reported, high difficulties to standardize the intensity of contraction, and the women's implication in the procedure.

All assessments were performed using an Aixplorer V12 device (SuperSonic Imagine, France) and an SL 18-5 (5-18 MHz) linear probe. The region of interest was identified and contoured manually using MATLAB scripts (The MathWorks, Inc., 2016). The Aixplorer device provides a Young's modulus assessment (in kPa) within the region of interest, which is suitable for isotropic tissues. Because muscles are anisotropic tissues, Young's modulus is not suitable, and it is safe to report the shear modulus (in kPa) as reported in previous chapters of this thesis [43, 44].

2.3.5 – Mode of delivery characteristics

After the delivery, we collected the following data from the volunteer's medical files:

- Mode of delivery: spontaneous/instrumental/cesarean section. We defined a group "operative delivery" that included instrumental and cesarean deliveries.
- Term at delivery (in weeks)
- Birthweight (in grams)
- Perineal tear occurrence according the RCOG-OMS classification [53, 142]. An OASI was considered in case of 3rd or 4th degree perineal tears meaning including at least a partial rupture of the EAS. A perineal tear was considered in case of any injury irrespective of the degree.

2.4 – Data analysis and statistics

2.4.1 – Population's description

We first described the population in terms of characteristics of pregnant women and the type of delivery. Continuous variables were reported as means with SD. Categorical variables were reported as numbers and percentages.

We compared these characteristics between the group of women considered for the analysis and the group of excluded women using a student *t* test for continuous variables and a χ^2 test or a Fischer's exact test for categorical outcomes.

2.4.2 – Changes pelvic organ mobility parameters and in elastic properties of pelvic floor and peripheral muscles through pregnancy

We investigated any changes in the POP-Q, ultrasound B-mode, and SWE measures across the time (the three visits) using a one-way repeated measures ANOVA analysis. Before performing such an analysis, the normality of data was checked using a Shapiro–Wilk test. This first step was necessary to check that we were in the acceptable condition for the one-way ANOVA for repeated measure analysis. This last test was chosen regarding our study design with repeated measures for a same subject. The alternative would have been a Friedman test which is suitable to look for changes across the time but which would not have allowed to control the fact that the measures were obtained in the same subject across the time (repeated measures).

Regarding existing animal data, we expected that the main biomechanical behavior would be an increase in LAM's shear modulus during pregnancy [23, 33, 34]. We also expected that some women would probably have no change or a decrease in their LAM's shear modulus. We reported the number of women having an increase in their LAM's shear modulus at rest with the mean increase (in kPa and in %). In a same way we reported the number of women having no change or a decrease in their LAM's shear modulus at rest with the mean decrease. We focused on measurements at rest because we considered that this one offers the best reflect of the intrinsic elastic properties of the muscle.

We performed the same analysis for the elastic properties of EAS but only in the group of women that underwent this assessment for the three visits (only 37 women).

2.4.3 – Association between elastic properties of the pelvic floor muscles and clinical / ultrasound perineal measurements

First, we investigated the association between clinical perineal distension (addition of segment gh and pb lengths in the POP-Q procedure) and the shear modulus of both the LAM and the EAS at rest and at Valsalva maneuver for each trimester of pregnancy. This analysis was done using a Spearman correlation test, with a report of the R coefficient.

Second, we investigated the association between ultrasound B-mode perineal distension (difference between the anteroposterior levator hiatus diameter at Valsalva and at rest) and the LAM's shear modulus at rest and Valsalva maneuver for each pregnancy trimester using the same method.

2.4.4 – Association between the elastic properties of women's PFM and both, mode of delivery and perineal tears occurrence

Using Student t test, we first compared the mean shear modulus of both LAM and EAS at rest and Valsalva maneuver for the third trimester visit between women having an operative delivery (cesarean or instrumental delivery) and those with a spontaneous delivery.

We performed the same analysis for perineal tears occurrence using the same methods in the subgroup of women having a vaginal delivery.

Then, we investigated the association between an increase or a decrease in LAM's shear modulus at rest through pregnancy and an operative delivery occurrence. We focused this analysis on the LAM's characteristics regarding the association between levator hiatus area and the mode of delivery [101, 103]. This analysis was performed using a χ^2 test reporting OR and 95% confidence interval. Last, using the same methods we investigated the association between an increase or a decrease in EAS's shear modulus at rest through pregnancy and a perineal tear occurrence in the subgroup of women with a vaginal delivery. We focused this analysis on the EAS because this anatomical region is directly involved with perineal tears occurrence whereas the LAM is more specifically involved with LAM avulsion.

2.5 – Statistics

Statistical analysis was performed using STATA V14 software (Stata Corporation, College Station, TX, USA). For all analyses, statistical significance was considered to be $p < 0.05$.

2.6 – Ethical and reglementary considerations

Dr Bertrand GACHON was the coordinating investigator for this study and performed all the assessments.

The study was approved by an ethics committee (*Comité de Protection des Personnes Ile de France 8,* ethical committee for human protection from Ile de France) on the 16/07/2018 and is referenced with the ID RCB: 2018-A011422-53. The study was registered on https://clinicaltrials.gov (NCT03602196) on the26/07/2018. All methods were carried out in accordance with relevant guidelines and regulations. Written and informed consent was obtained from all subjects before any investigation.

3 – Results

3.1- Population's description

Seventy seven pregnant women were included between the April 2, 2019 and the June 24, 2021 among them 30 were excluded, leading to 47 women considered for the analysis (Figure 24). Among the included women population, two did not deliver in our institution and therefore no data were available about the mode of delivery. Regarding that the SWE assessment of the EAS was added to the protocol after the study's onset, full data were available for 37 women.

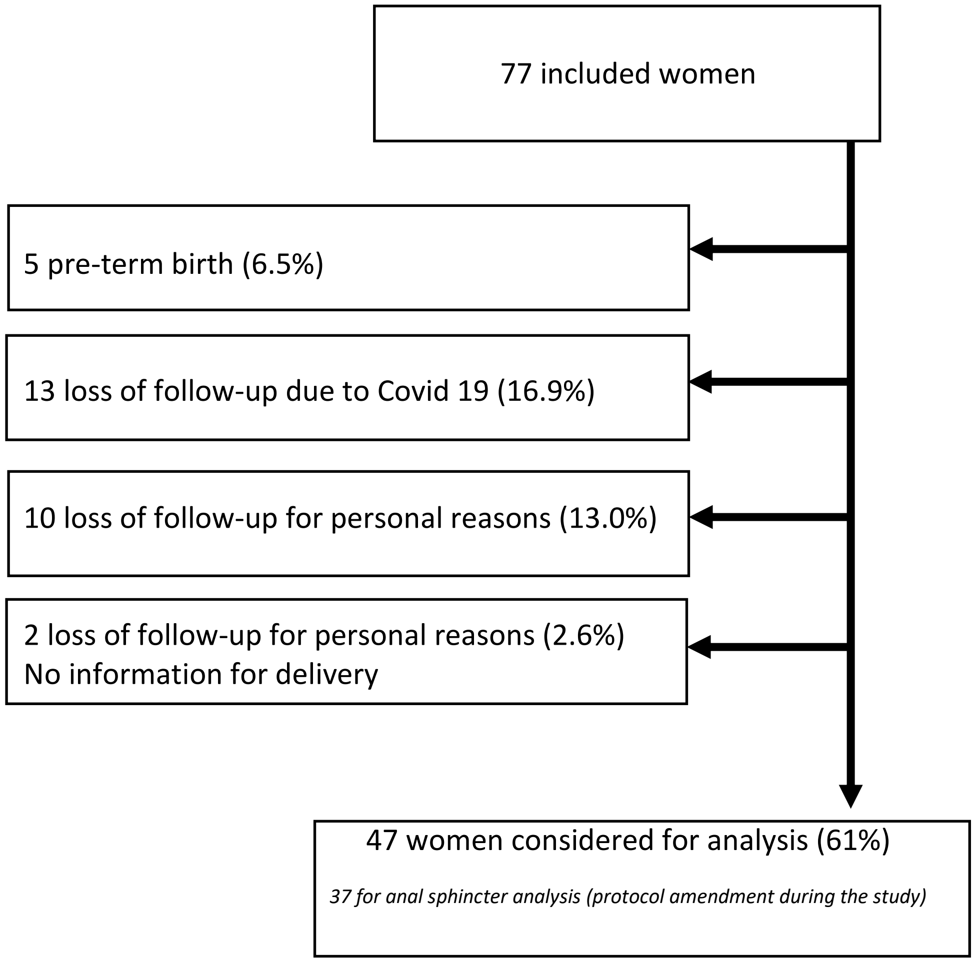


Figure 24: Flow chart of the ELASTOPELV study

Comparison of excluded women and women considered for the analysis is reported in the table 9. There were no differences for any investigated outcomes between these groups.

Among women in the group "operative delivery", 8 undergone an instrumental vaginal delivery and two a cesarean section.

Table 9: Comparison of women and delivery characteristics between include and excluded women

	Women NOT considered for analysis (N=30)	Women considered for analysis (N=47)	p or OR [95%CI]
Mean age (SD), in years	28.5 (0.9)	28.3 (0.6)	0.90
Mean BMI (SD), in Kg.m^{-2}	22.7 (0.5)	22.1 (0.5)	0.4
Operative delivery, n (%)*	11 (39.3)	10 (21.3)	0.42 [0.1-1.3]
Mean term at delivery (SD), in weeks*	38.3 (0.6)	39.4 (0.2)	0.05
Mean birthweight (SD), in g *	3042.5 (129.9)	3258 (61.8)	0.09
Overall perineal tear, n (%)*	21 (75.0)	38 (80.1)	1.41 [0.4-4.9]
Obstetric anal sphincter injury, n (%)*	1 (3.6)	1 (2.1)	0.59 [0.1-47.8]

*data missing for two cases SD: standard deviation

3.2 – Changes in clinical, ultrasound and SWE measured parameters through pregnancy

For clinical measurements, the position of the POP-Q points became lower through pregnancy excepted for the point D (Table 10). The length of all the POP-Q segments increased through pregnancy (Table 10). The overall clinical pelvic organ mobility increased through pregnancy.

Table 10: Changes in POP-Q parameters through pregnancy in the overall population (N=47)

POP-Q measures, in cm	1st trimester mean (SD)	2nd trimester mean (SD)	3rd trimester mean (SD)	p
Ba	-2.6 (0.08)	-2.2 (0.10)	-1.7 (0.11)	<0.0005
Bp	-2.9 (0.04)	-2.8 (0.06)	-2.5 (0.08)	<0.0005
C	-7.2 (0.13)	-7.4 (0.13)	-7.0 (0.14)	0.03
D	-7.6 (0.12)	-7.8 (0.14)	-7.7 (0.16)	0.45
Tvl	8.9 (0.14)	9.7 (0.16)	10.2 (0.20)	<0.0005
Gh	2.8 (0.07)	3.1 (0.06)	3.5 (0.08)	<0.0005
Pb	2.8 (0.07)	3.4 (0.08)	3.8 (0.08)	<0.0005
Clinical distension Gh+Pb	5.6 (0.12)	6.5 (0.12)	7.4 (0.15)	<0.0005

SD: Standard deviation

About ultrasound parameters, we reported an increase in the anteroposterior diameter of the levator hiatus through pregnancy at rest and Valsalva maneuver (Table 11). Nevertheless, the levator hiatus distension did not change across the time.

Table 11: Changes in ultrasound parameters through pregnancy in the overall population (N=47)

	1st trimester mean (SD)	2nd trimester mean (SD)	3rd Trimester mean (SD)	p
Rest, in mm	43.3 (0.7)	47.9 (0.8)	51.6 (0.8)	<0.0005
Valsalva, in mm	48.2 (0.9)	53.0 (0.8)	56.6 (1.1)	<0.0005
Distension (Rest to Valsalva), in mm	4.9 (0.5)	5.1 (0.6)	3.4 (0.8)	0.16

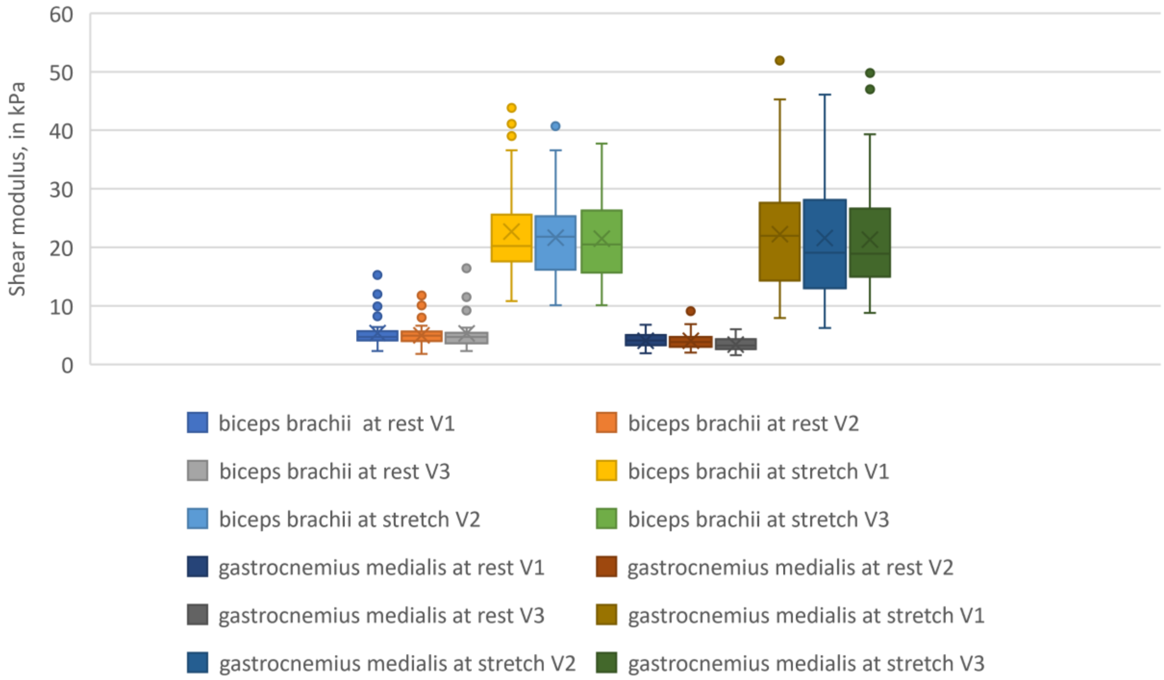
SD: Standard deviation

Regarding the PFM and peripheral muscles, the elastic properties assessed using SWE show no significant changes through pregnancy, except a decrease in *gastrocnemius medialis* shear modulus at rest (Table 12, Figure 25).

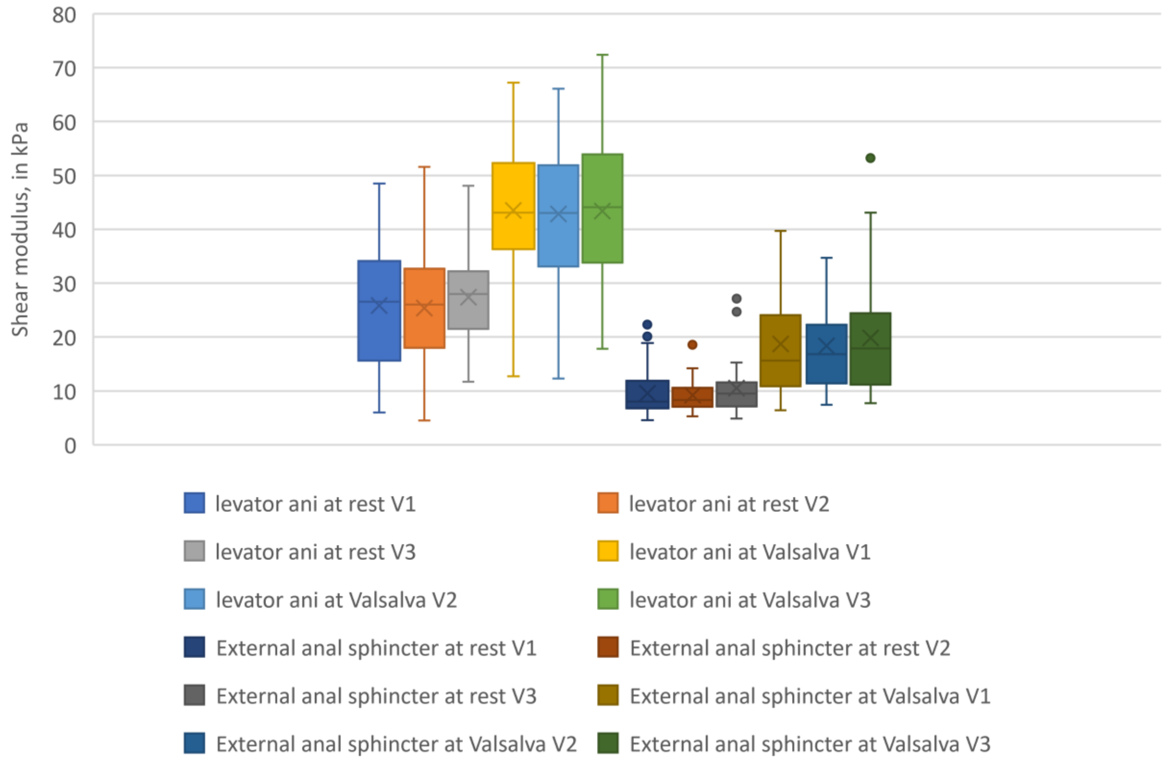
Table 12: Changes in the elastic properties of pelvic floor and peripheral muscles through pregnancy in the overall population

	1st trimester mean SM (SD)	2nd trimester mean SM (SD)	3rd trimester mean SM (SD)	p
***Biceps brachii:* N=47, in kPa**				
Rest, in kPa	5.4 (0.4)	5.0 (0.3)	5.3 (0.4)	0.48
Stretch, in kPa	22.7 (1.1)	21.7 (1.0)	21.5 (1.0)	0.53
***Gastrocnemius medialis*: N=47, in kPa**				
Rest, in kPa	4.1 (0.2)	4.0 (0.2)	3.4 (0.2)	0.004
Stretch, in kPa	22.2 (1.5)	21.6 (1.5)	21.3 (1.4)	0.79
***Levator ani* muscle : N=47, in kPa**				
Rest, in kPa	25.8 (1.7)	25.4 (1.6)	27.4 (1.3)	0.43
Valsalva, in kPa	43.5 (1.8)	42.8 (1.8)	43.4 (2.0)	0.93
External anal sphincter: N=37, in kPa				
Rest, in kPa	9.6 (0.7)	9.4 (0.6)	10.5 (0.6)	0.15
Valsalva, in kPa	18.7 (1.5)	19.2 (1.4)	19.6 (1.4)	0.43

SM: Shear modulus SD: Standard deviation



a



b

<u>Figure 25: Changes in the elastic properties of peripheral (a) and pelvic floor (b) muscles during pregnancy</u>

V1: First visit V2: Second visit V3: Third visit

3.2.2 – Changes in the population of women with an increase in LAM's shear modulus at rest through pregnancy

Among the 47 considered women, 24 women showed can increase in the LAM's shear modulus through pregnancy. The mean increase was 9.9 kPa representing a 76.4% increase. The maximal increase was 29.7 kPa (366.8%) and the minimal one was 2.1 kPa (7.6%). A decrease or no change in the LAM's shear modulus at rest through pregnancy with a mean decrease of 7.7 kPa representing a 22.3% decrease was observed in 23 women. The minimal decrease was 0.8 kPa (4.8%) and the maximal one was 17.9 kPa (53.1%).

Among the 37 women that underwent EAS's SWE assessment at three visits there were 23 women (62.2%) with an increase in EAS's shear modulus during pregnancy. The mean increase was 3.4 kPa representing a 38.2% increase. The maximal increase was 14.8 kPa (120.3%) and the minimal one was 0.2 kPa (2.9%). A decrease or no change in EAS's shear modulus during pregnancy (37.8%) was observed in 14 women. The mean decrease was -3.1 kPa representing a 25.6% decrease. The maximal decrease was -11.7 kPa (52.5%) and the minimal one was -0.1 kPa (-1.6%).

3.3 – Association between clinical, ultrasound and SWE parameters

3.3.1 – Association between clinical parameters and both LAM and EAS elastic properties

We reported a positive and significant correlation (Spearman test) between the elastic properties of the EAS during Valsalva maneuver and clinical perineal distension (Gh+Pb) at each trimester of pregnancy (Table 13). At rest, there was no significant correlation between the elastic properties of the EAS and clinical perineal distension, irrespective of the trimester.

About the LAM's elastic properties, the only significant negative correlation with clinical perineal distension was with the LAM's shear modulus at rest and at 1st trimester of pregnancy (Table 13).

Table 13: Association between clinical perineal distension and PFM's elastic properties

		1st trimester	2nd trimester	3rd trimester
***Levator ani* muscle**				
	Rest	R = -0.3	R = -0.2	R = -0.2
		p = 0.04	p = 0.30	p = 0.19
	Valsalva	R = -0.1	R = -0.1	R = -0.3
		p = 0.53	p = 0.42	p = 0.08
External anal sphincter				
	Rest	R = 0.3	R = 0.2	R = 0.01
		p = 0.06	p = 0.15	p = 0.93
	Valsalva	R = 0.4	R = 0.3	R = 0.28
		p = 0.03	p = 0.02	p = 0.04

R: Spearman's coefficient p: level of significance

3.3.2 – Association between ultrasound levator hiatus distension and LAM's elastic properties

There was no significant association between the elastic properties of the LAM and the ultrasound levator hiatus distension irrespective of the trimester and the condition (measurements taken at rest or during Valsalva maneuver) (Table 14).

Table 14: Association between ultrasound levator hiatus distension and LAM's elastic properties

		1st trimester	2nd trimester	3rd trimester
***Levator ani* muscle**				
	Rest	R = -0.2	R = 0.004	R = -0.02
		p = 0.20	p = 0.98	p = 0.89
	Valsalva	R = -0.2	R = -0.29	R = -0.03
		p = 0.29	p = 0.05	p = 0.82

R: Spearman's coefficient p: level of significance

3.4 – Association between PFM's elastic properties and delivery's characteristics

3.4.1 – Association between PFM's elastic properties in third trimester and delivery's characteristics

We did not report any difference in the mean shear modulus of the LAM at third trimester (at rest or during Valsalva maneuver) and the occurrence of an operative delivery (cesarean section or instrumental vaginal delivery; Table 15) or a perineal tear.

Among women with a vaginal delivery (spontaneous or instrumental), the mean EAS's shear modulus at Valsalva maneuver was higher in those for whom a perineal tear occurred (Table 15). There was no association for measurements at rest on perineal tear occurrence and no association at all for instrumental delivery occurrence.

Table 15: Association between third trimester PFM's elastic properties and characteristics of the delivery

	Operative delivery (Instrumental or cesarean delivery) (N=47)*			**Perineal tears (N=45)****		
	Yes (n=10)	No (n=37)	p	Yes (n=38)	No (n=7)	p
***Levator ani* muscle**						
Rest, mean SM in kPa (SD)	28.4 (3.4)	27.1 (1.4)	0.69	27.1 (1.5)	27.5 (3.5)	0.91
Valsalva, mean SM in kPa (SD)	42.7 (4.6)	43.6 (2.2)	0.86	43.5 (2.1)	41.6 (5.5)	0.73
External anal sphincter						
Rest, mean SM in kPa (SD)	8.7 (0.6)	11.0 (0.8)	0.15	10.3 (0.8)	12.9 (0.9)	0.17
Valsalva, mean SM in kPa (SD)	19.6 (1.6)	19.6 (2.7)	0.99	18.2 (1.3)	27.0 (5.6)	0.02

SM: Shear Modulus SD: Standard Deviation

* Overall population **Only women with a vaginal delivery (spontaneous or instrumental)

3.4.2 – Association between changes in PFM’s elastic properties during pregnancy and delivery’s characteristics

An operative delivery occurred in 21.7% (n=5) of women having an increase in the LAM’s shear modulus at rest through pregnancy versus 20.8% (n=5); OR = 0.9 [0.18-4-89].

All women having a decrease of the EAS’s shear modulus at rest through pregnancy suffered from perineal tear versus 78.3% (n=18) of those with a decrease in EAS’s shear modulus (p = 0.08). Regarding the association between the elastic properties of the EAS during Valsalva maneuver at third trimester and perineal tear occurrence, we repeated the analysis by comparing women with an increase or a decrease of the EAS’s shear modulus during pregnancy to those with a decrease. Ten women with a decrease in EAS’s shear modulus at Valsalva suffered from perineal tears (90.9%) versus 20 (83.3%) in the group with an increase in EAS’s shear modulus at Valsalva (OR = 0.5 [0.01-6.11]).

4 - Discussion

4.1 – Main findings

No significant changes in the elastic properties of PFMs were observed during pregnancy, measured at rest and while performing Valsalva maneuver. The elastic properties of the EAS while performing the Valsalva maneuver were associated with clinical perineal distension during pregnancy, but there was no association between the elastic properties of LAM and both clinical or ultrasound pelvic floor distension.

Women with an intact perineum at delivery had a stiffer EAS at third trimester than those with perineal tears. No association was observed between an increase or decrease in the stiffness of EAS during pregnancy and the occurrence of perineal tears. The stiffness of LAM was not associated with the occurrence of an operative delivery in our population.

4.2 – Strengths and limitations

The main strength of this study is its originality regarding the data representing the first report of an *in vivo* quantitative direct assessment of elasticity of the PFMs, focused specifically on the muscle’s properties during pregnancy. Until now, the existing data were only reported from animal studies, biomechanical modelling or undirect *in vivo* assessment involving the whole perineal assessment [23, 33-36, 110, 143]. It provides a better understanding in the pathophysiology of perineal trauma at childbirth allowing comparisons with existing data.

Another important strength is that we performed an assessment of the PFM's elastic properties with an original approach but with a safe strategy considering that the procedures were reported as feasible, acceptable, and reliable [52].

The major limitation of this study was that a strong association between PFM's elastic properties and consequently the perineal trauma at childbirth (OASI and/or LAM avulsion) was not established. Indeed, regarding the reported prevalence of these injuries, it would have been necessary to include a large number of women to draw significant outcomes. This limitation was expected regarding the study design and the primary objective was to describe the potential changes that could occur in PFM's elastic properties through pregnancy. A study with an analytic approach about the association between these elastic properties and perineal trauma at childbirth occurrence is still necessary and will require a large number of participants. Furthermore, even if we were not able to provide an analysis concerning OASI or LAM avulsion, our results report an association between third trimester elastic properties of the EAS and perineal tear occurrence (irrespective of the severity), which support the prospect of such an upcoming large study. Further, we did not provide postnatal assessment of either the muscle's elastic properties or the perineal trauma assessment, especially pelvic floor ultrasound for LAM avulsion diagnosis. This is related with a methodological choice for optimizing the study's feasibility. Indeed, regarding our previous experiences about prospective studies during pregnancy and postpartum, we expected that a significant number of women would be discouraged in the perspectives of a one-year follow up with at least 5 visits. We chose to prioritize our approach in the antenatal assessment. Wherein, these postnatal assessments will be essential in future research studies, especially postnatal pelvic floor ultrasound assessments for the diagnosis of LAM avulsion.

Another important limitation is that, as reported in study 3, the reliability of assessments for peripheral muscles was moderate; therefore, the results should be carefully interpreted. As reported in the discussion section of study 3, we argue that this lack of reliability could be related to the low experience of the observer in peripheral muscles. Even if we tried to standardize the procedures, our assessment protocol was primarily oriented clinically (installation of pregnant women). A recent paper supports this by reporting a lower reliability of SWE assessment for peripheral muscles using a “clinical feasibility approach” compared to an “optimized, rigid protocol” [144]. Even regarding this limitation, we probably could consider our results as safe because we reported a homogenous behavior between upper and lower limbs (no change), which is consistent with the existing literature from animal studies [33-36, 143].

4.3 – Interpretation

We did not report any change in the elastic properties of the LAM or the EAS during pregnancy. This result is in opposition with our initial hypothesis and with existing literature from animal experimentation reporting an increase in stiffness of PFMs in rats during pregnancy [33-36, 143]. A first explanation could be related to a different biomechanical behavior between women’s PFM and those of rats, meaning a biped human versus a quadruped animal with probably different constraints applied to the PFMs. Moreover, in animal experimentation, the assessment of the elastic properties were performed at a given sarcomere length and *ex vivo*, meaning in the exact same length [33-36, 143]. This was accompanied by an increase in the sarcomere length. Such a protocol is not possible for an *in vivo* assessment, and we were neither able to control the sarcomere length at the time of measurement nor observed a change in sarcomere length through pregnancy. It is possible that the sarcomere length was increased during pregnancy in LAM and EAS. However, in that case, an evaluation at the same sarcomere length would induce SWE measurements at a shorter muscle length. Considering that the shear modulus is increased when the muscle is stretched [138], it would mean that we overestimated the shear modulus at the end of the pregnancy, and therefore our results would suggest a decrease in the intrinsic muscle stiffness. This would provide results contradictory to those of animal studies.

Another interpretation could be that the strain or the stress applied on the LAM during pregnancy is progressively more and more important because of the increasing weight of the gravid uterus. This phenomenon is expected to be associated with changes that occurred in PFM stiffness during pregnancy in rats [33, 34]. If the LAM’s shear modulus did not change while there was an increase in the load applied to this muscle, it could be that its stiffness at the same load decreased. This interpretation remains hypothetical because data are lacking about the increasing load applied to the PFMs during pregnancy, which could be explored in the future using SWE by performing measurements in women in both lithotomy and in upright positions to investigate whether the position induces a change in the muscle's elastic properties. This interpretation combined with the previous one on the sarcomere length suggest strong differences in the LAM behavior during pregnancy between rats and women.

We reported a descent of most of the POP-Q points, an increase of all POP-Q lengths and an increase in the levator hiatus anteroposterior diameter. All these expected changes are in accordance with the literature [21-23, 27-30]. These observations support the validity of our results considering that our population behaves as expected regarding the more frequently reported outcomes in the literature.

There was an association between the EAS's shear modulus and clinical perineal distension (Gh + Pb) at Valsalva maneuver irrespective of the trimester. It was a positive correlation meaning that stiffer the EAS was, the more important was the clinical perineal distension. The interpretation of this result is that women with the most important distension of the perineal body were those with the stiffest EAS and so the more stretched is the EAS, stiffer it is.

This result is supported by the association with the elastic properties in late pregnancy during Valsalva and perineal tears occurrence. It confirms the possibility of an association between the strain magnitude, the intrinsic elastic properties of the muscle, and the perineal trauma occurrence [23]. Herein, we reported that women suffering from perineal tears at childbirth had a lower EAS's shear modulus in late pregnancy than those with an intact perineum. This is in accordance with data from animal experimentation reporting that an increase in PFM's stiffness during pregnancy could be interpreted as a protective process against perineal tears occurrence at childbirth [33, 34]. Indeed, women with a lower perineal distension during pregnancy may not experiment an increase in their EAS's stiffness leading to an increase in perineal trauma risk. This hypothesis supports the importance of the mechanical environment of PFM's during pregnancy and the risk of perineal trauma [33, 34]. This is also in accordance with one of our previous clinical study showing that women with perineal tears at childbirth had more joint mobility in late pregnancy than those with an intact perineum [37]. Our hypothesis following this study was that stiffest tissues may later increase their plasticity or rupture threshold [130]. This plasticity threshold is raised when irreversible damage occurs to the intrinsic structures. For the tissues with lower stiffness, with the maximal capacity of distension, this plasticity threshold may be easily raised, and the tissue is more likely to suffer from irreversible damage [37]. One we reported these results, the main limitation was that it was an interpretation from data obtained from the study of an upper limb joint (second metacarpophalangeal joint) with difficulties to safely extrapolate these results to pelvic floor. The present study with *in vivo* assessments of PFMs supports that the elastic properties of women's PFM could be used to predict perineal trauma at childbirth [23]. With this prospect of improving predictive strategies for perineal trauma, it appears safe to consider the elastic properties of the EAS form perineal tears prediction in the absence of an association between LAM's properties and this outcome. Nevertheless, the LAM's properties could probably be useful to predict the other type of perineal trauma, such as LAM avulsion. This association has not been investigated in the present study because of our low number of participants, but should be investigated through further studies.

We did not report any change in the elastic properties of the *biceps brachii* muscle at rest or under stretched condition through pregnancy. This is in accordance with animal experimental studies evocated above within there was no changes in the elastic properties of peripheral muscles through pregnancy [33, 34]. A recent ultrasound study did not report any change in the elastic properties of the patellar tendon during pregnancy so did our study [87]. Conversely, it is in contradiction with studies reporting an increase in joint mobility in upper limbs through pregnancy [21-23, 26, 31]. This difference may be related to a specific behavior of muscles compared to ligaments, perhaps because of a different intrinsic composition. The other point is, as reported previously on the reliability of *biceps brachii* SWE assessment that was moderate. Therefore, our results should be carefully interpreted.

For the *gastrocnemius medialis*, we expected a change in the elastic properties of this muscle because, even if it was not directly impacted by the load induced by the gravid uterus, pregnancy is associated with a global weight gain, with changes in spinal curvature that could modify the biomechanical behavior of this postural muscle [21, 23]. Furthermore, several studies reported an increased joint mobility in lower limbs during pregnancy, and a recent study reported some changes in the muscular architecture of lower limbs muscles [21, 23, 25, 31, 145]. We finally reported only one significant change as a decrease in the *gastrocnemius medialis* shear modulus at rest during pregnancy. One interpretation could be that the increasing load in the upright position during pregnancy induces a change in the muscle's elastic properties to increase its ability to sustain this load. The measurements being performed in left lateral decubitus, meaning without any strain applied on the muscle, could result in the observation of a decreased muscle shear modulus. This phenomenon might not be observed for the *biceps brachii* muscle because of the absence of muscle load increase because of pregnancy. The phenomenon is not observed for the LAM because even if we performed measures at rest for this muscle, it is impossible to totally suppress the loading applied to the LAM and there is probably permanently a strain applied to the LAM even in decubitus position. It is probably much lower than in upright position but not totally controlled in lithotomy position and probably more and more important through pregnancy. Two elements moderate this interpretation. The first one is that we need data to confirm this hypothesis of an increase in PFM loading, even in lithotomy position, through pregnancy which could probably be done using repeated SWE assessments in both upright and lithotomy position. The second is related to the results of changes in the elastic properties of the *gastrocnemius medialis* at rest which should be very carefully considered because of moderate reliability of this measure in our experience (study 3).

5 – Conclusion

We did not report significant changes in the elastic properties of both peripheral muscles and PFMs. The perineal clinical distension was positively associated with the EAS's elastic properties during the Valsalva maneuver through pregnancy. These elastic properties in late pregnancy were associated with perineal tears occurrence at childbirth with women suffering from perineal tears having less stiffer EAS muscle. These results support the consideration of PFM biomechanical characteristics in perineal trauma at childbirth prediction. Further, studies with a large sample size are required to specifically investigate the association between the elastic properties of PFMs and the risk of OASI and LAM avulsion.

General discussion

Through this thesis we reported that it is feasible, acceptable, and reliable to use SWE for measuring *in vivo* the elastic properties of PFMs in women. Using this technique, we were the first research team to report a quantitative direct assessment of the LAM's elastic properties, then the EAS's elastic properties in women. We also reported the first application of SWE in PFM's assessment in a pregnant women population allowing the description of *in vivo* elastic properties of these PFMs during pregnancy. We believe that these results are of great importance regarding both their clinical and research applications. It is likely that this SWE technology could give us the opportunity to improve our knowledge of the pathophysiology associated with perineal trauma and to improve our level of care and counselling given to women within a predictive, preventive, or therapeutic strategy.

Further studies are required to optimize our clinical and research skills in the field of perineal trauma at childbirth. Indeed, we need to more deeply investigate the association between the impact of pregnancy, labor and delivery on perineal trauma occurring at childbirth.

The first point to investigate is the impact of the local environment on the PFM's elastic pregnancy during pregnancy. Indeed, as previously mentioned, some animal experimentations reported that the increasing load applied by the gravid uterus during pregnancy could induce some changes in the PFM's mechanical behavior [33, 34]. Furthermore, our results suggested that the absence of change in the LAM's shear modulus at rest or Valsalva during pregnancy whereas the load applied to this muscle progressively increase might be interpreted as a gain of muscle's elasticity (i.e., decrease in stiffness for a given load or a given sarcomere length). Nevertheless, these interpretation remains at a hypothetical step regarding that data are lacking for quantifying this increasing loading on PFM's during pregnancy. We believe that this assumption could be investigated thanks to SWE. Indeed, it reasonable to expect implementing a prospective longitudinal study with repeated measures of the LAM's elastic properties at rest and Valsalva maneuver during pregnancy using SWE with the same technique than the one reported in this thesis. The original approach would be to perform these measures in two different positions: in upright position and in lithotomy position. If the gravid uterus effectively increases the load applied on PFM's we should observe an increase in the LAM's shear modulus from lithotomy to upright position and the magnitude of the increase would probably be more important in late pregnancy, when the gravid uterus is the heaviest. We expect that such a procedure would not be associated with major problematics regarding the easy access in women perineum, the use of a translabial approach, which is totally compatible with an upright position (which wouldn't have been the case for an intravaginal approach), and the easy process to visualize the LAM using SWE. To our knowledge, such a research approach has never been reported and these results would be of great importance. Another possibility could be to combine animal experimentation and SWE assessment. It could be original to perform a prospective study on pregnant animals with an investigation of PFM's elastic properties *in vivo* in late pregnancy using SWE. Immediately after this *in vivo* assessment, a sacrifice could be performed allowing an *ex vivo* assessment of the PFM's elastic properties using the same approach than the one reported by Alperin *et al.,* at a given sarcomere length [33, 34]. The main limitation is that it could be difficult to perform in the same animal model as the one used by Alperin *et al*., the rat. Indeed, it is likely that we would be confronted by muscular targets that are too small to perform an ultrasound PFM assessment with SWE. The alternative could be to perform the study on bigger animals, such as goat or squirrel but with the difficulty of the *in vivo* examination acceptability. Furthermore, the usual model for PFM's study is the rat, since the anatomy of pelvic is quite similar to the one in women. Regarding these elements, we consider that this animal experimental approach appears not possible at this moment.

The second point that would require more detailed research is the impact of the labor itself on PFM's elastic properties and on perineal trauma occurrence at childbirth. Indeed, during the different stage of labor, and especially during the second one (from full cervix dilatation to the birth), a massive perineal distension occurs. Some modelling studies suggested that PFM's length could be increased by 300% during this phase (compared to the onset of labor) [1, 2]. A clinical study investigated the changes and clinical perineal distension during the different stages of labor by repeating measures of the Pb segment length [63]. These authors reported a mean antenatal pb length of 3.7cm versus 6.1cm of mean maximal length during second stage of labor, representing approximately a 65% increase [63]. It is likely that such a strain during labor is associated with changes in the elastic properties of PFM's and especially the LAMs which delimitate the levator hiatus within the fetus must progress.

To our knowledge, all the currently available data about PFM's mechanical behavior during childbirth are obtained from modelling studies [61, 68, 146, 147]. There are no experimental data about an *in vivo* characterization of this muscle's behavior. Here again, we believe that SWE and the techniques for PFM's assessment could be used with such a prospect. It could be relevant to perform a prospective study with repeated measures on PFMs (LAM and SEA) using the SWE techniques reported in this thesis at different step of the labor (the onset, full cervix dilatation, different stages of fetal head station, at crowning). Such an approach would allow to report the first human, *in vivo* data about the biomechanical behavior of PFMs during the human delivery. This will not be difficult for the EAS because this muscle would remain easily accessible through the different stages of childbirth using a trans perineal approach. The procedure should be feasible for the LAM during the first stages of labor but might more difficult in the last ones and especially at crowing for whom we can expect a lack of visibility of the LAM's pubic insertion with the most important view of the region of interest occupied by the fetal head. Nevertheless, these difficulties remain hypothetical and pilot data acquisition is required to allow the collection of crucial data for a better understanding of the perineal mechanisms involved in parturition. Such a study will be pivotal to investigate the potential association between perineal distension during childbirth, the PFM's elastic properties and the risk of perineal trauma. The efficiency of this analysis could be improved thanks to an innovative in vivo continuous collection of the strain applied to PFMs during childbirth. Indeed, we actually lead a research protocol consisting in the collection of intra bladder pressure during vaginal delivery (ACCOUPIV study; NCT04544488 https://clinicaltrials.gov). Such an approach allows a continuous recording of the pelvic pressure during childbirth using a pressure sensor connected to a woman's bladder catheter. Thanks to this approach we could be able to measure the strain applied for PFMs and so to provide SWE measurements for maximal distension but also for a given level of strain.

It also necessary to prospect some technological improvements in the elastic properties assessment of PFMs using SWE. The most important one is the possibility to perform a volume SWE acquisition allowing a 3D reconstruction of the woman's pelvic floor. Indeed, we were only able to perform bidimensional SWE acquisitions. This is not depreciable for the EAS assessment but is highly more problematic for the LAM's assessment. Indeed, the LAM is muscle inserted on the pubic bone and who gives distal insertion to the EAS. The right and left LAM's delimitate the levator hiatus plane having a craniocaudal and an anteroposterior inclination. Current SWE technology only allows an assessment of the pubic insertion of the LAM which is easy to investigate. For chance, this region is of interest because it represents the anatomical site of obstetrical LAM avulsion in case of perineal trauma. Nevertheless, it would be of major interest to be able to perform a SWE assessment of the whole levator hiatus. Indeed, this levator hiatus is the major actor of pelvic floor function, stability and its injury highly associated with pelvic floor dysfunction. Furthermore, this levator hiatus size consists in an anatomical hernia within the fetus have to progress during the vaginal delivery. Here again, data available about the biomechanical behavior of the LAMs during childbirth come from modelization study and improvement in SWE technology allowing a dynamic assessment of the whole levator hiatus would be a major contribution.

Last, research studies on the clinical application of SWE for the PFM's assessment are warranted. As we previously mentioned, the prospect is to improve our predictive and preventive strategy for perineal trauma at childbirth, especially OASI and LAM avulsion. To understand the prevalence of these negative outcomes, a large multicentric (if possible international) observational study including at least a SWE of PFM's at late pregnancy (third trimester), a collection of data about the mode of delivery and a postnatal pelvic floor ultrasound assessment (LAM avulsion and OASI), and questionnaires based on symptoms (*Pelvic Floor Distress Inventory 20 Questions)* to identify a sufficient number of both anatomical and clinical outcomes [148] may be considered. Ultrasound assessment of PFM to investigate LAM avulsion is necessary; ideally 3 months after the delivery. During the same examination, an assessment of the anal sphincter complex would also be necessary for undiagnosed OASI at delivery. These ultrasound assessments could be performed using 3D transperineal ultrasound techniques [57, 82, 94, 141]. Moreover, perineal symptoms (urinary incontinence, anal incontinence, vaginal bulge) progress in the following six postpartum months; therefore, we consider that it is necessary to plan a 6-month analysis with a symptomatic assessment (PFDI-20), an ultrasound anatomical pelvic floor assessment (LAM avulsion OASI), and SWE assessment which allow to report the first human data about the progression of women's PFM elastic properties during the postpartum period. Before their active participation, investigators in the different recruiting centers should be aware that it is absolutely necessary to be trained to perform SWE assessment before including women in the research project. Indeed, even if the technique is reliable and that the procedure is quickly accessible for someone trained in pelvic floor ultrasound some specificities associated with SWE are important to consider. The most important one is to perform the assessments by inducing minimal pelvic floor compression with a perineal probe to avoid an artificial increase in the tissue's elastic properties. This reliability consideration is a major one. Before implementing any longitudinal multicentric study it will be necessary to specifically train each investigator and to assess his/her reliability in order to check if his/her reliability indicator are as good as the one reported in this work. This reliability will be assessed on 5 to 10 consecutives women using the same methods as the one used in this thesis (ICC, CV, Bland Altman). The preliminary results from this thesis allow to perform *a priori* power calculation. Indeed, we expect in late pregnancy a mean shear modulus measured at Valsalva for the LAM of 43.4 kPa (SD:2.0) and 19.6 kPa (SD:1.4) for the EAS at Valsalva. We chose to perform this calculation considering values during Valsalva maneuver because of the significant association between EAS's shear modulus during this time and the occurrence of perineal tears. We consider that a 10% difference of shear modulus in LAM while performing Valsalva maneuver between women with LAM avulsion and those without LAM avulsion, and between women with/without OASI would be clinically significant. For an alpha risk of 5% and a power of 90%, it would be necessary to consider 10 women (5 in each group) for the LAM avulsion related analysis and 22 women (11 in each group) for the OASI related analysis. Regarding the expected prevalence of 15% of LAM avulsion and 1% of OASI in a primiparous cohort, it would be necessary to at least include 67 women for the LAM avulsion analysis and 1,100 women for the OASI analysis [3, 11, 14, 53]. In obstetrical studies including a longitudinal follow-up from pregnancy to the postpartum period, a 20% loss of follow-up is reported. By anticipating this loss of follow-up, we consider that it would be necessary to include at least 1,320 women in such a research project. The main objective would be to look for an association between the elastic properties of the LAM and the EAS in women while performing the Valsalva maneuver in late pregnancy with respect to LAM avulsion and OASI occurrence. This analysis would be performed using a χ^2 test reporting OR and 95% confidence interval. A first secondary objective would be to look for an association between PFM's elastic properties in late pregnancy and perineal symptoms (urinary incontinence, anal incontinence, vaginal bulge identified using the PFDI-20 questionnaire) at 6 months postpartum using the same methods.

A last secondary objective would be to observe changes in the elastic properties of PFM from late pregnancy to 6 months postpartum using a one-way ANOVA for repeated measures analysis, such report is a must to support the research conducted in this thesis.

Only such a study would be able to confirm our results suggesting an association between PFMs elastic properties at childbirth. If our hypothesis is supported by such a study, we will be then able to access the final step which should be a clinical trial to determine the benefits of including women's elastic properties in existing predictive algorithms to predict perineal trauma and propose individualized intervention. Judgement's criteria should be both the improvement of the ability to predict the complication, and the impact of a better risk identification. Indeed, such a strategy of improved risk prediction would only be beneficial if we are able to offer women solutions to control this risk and avoid injury. The aim is to identify high risk women for offer them an individualized counselling and preventive strategy based on their intrinsic characteristics. We are currently building European collaborations to implement such a research in the near future. Another clinical issue that should be addressed is probably the interest of PFM's SWE assessment in perineal physiotherapy. Indeed, in the postpartum period a weak pelvic floor is often observed and one objective of the physiotherapy is to recover a more tonic pelvic floor allowing a better support of pelvic organ and so avoid pelvic floor disorders. One criterion for assessing the efficacy of physiotherapy is the subjective assessment of PMF contraction's length and intensity. We hypothesize that this subjective assessment could be improved using a quantitative and reliable assessment thanks to SWE. It is also likely that such an elastic properties assessment could allow to individualize the care strategy according the intrinsic women's characteristics.

General conclusion

SWE is an innovative technology that allows a non-invasive, quantitative, direct and reliable assessment of PFM's elastic properties in women. We described the first report of such an assessment in a non-pregnant then in a pregnant women cohort for the LAM and the EAS. We did not report any significant changes in the elastic properties through pregnancy *in vivo*. This result is not in contradiction with our hypothesis of an optimized risk prediction of perineal trauma at childbirth by considering tissue's biomechanical behavior. Indeed, the elastic properties of the EAS during Valsalva maneuver were associated with the importance of perineal distension during pregnancy. Furthermore, the EAS of women without any perineal injury at childbirth was stiffer in late pregnancy than those suffering from perineal tears. Our results support the consideration of the PFM's biomechanical behavior in our predictive strategy for perineal trauma at childbirth. Further studies are required for a better knowledge of the pathophysiology of perineal trauma and to develop clinical applications of this technology to optimize the risk prediction for perineal trauma at childbirth leading to an individual counseling of pregnant women.

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- Annex 2: Bertrand Gachon, Antoine Nordez, Fabrice Pierre, Laetitia Fradet, Xavier Fritel and David Desseauve. In vivo assessment of the levator ani muscles using shear wave elastography: a feasibility study in women. Int Urogynecol J. 2019;30:1179-86.
- Annex 3: Bertrand Gachon, Xavier Fritel, Fabrice Pierre and Antoine Nordez. In vivo assessment of the elastic properties of women's pelvic floor during pregnancy using shear wave elastography: design and protocol of the ELASTOPELV study. BMC Musculoskelet Disord. 2020;21:305.
- Annex 4: Bertrand Gachon, Xavier Fritel, Fabrice Pierre and Antoine Nordez. Transperineal ultrasound shear wave elastography is a reliable tool for assessment of the elastic properties of the levator ani muscle in women. Scientific Reports. 2021;11:15532.

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GUIDELINES AND POSITION STATEMENTS

Tissue biomechanical behavior should be considered in the risk assessment of perineal trauma at childbirth

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Abstract
Perineal trauma at childbirth is associated with strong negative impacts on a woman's health but remains unpredictable. Pregnancy induces several changes in biomechanical behavior in humans as in animals, namely, an increase in ligamentous laxity and an increase in vaginal distensibility. Pelvic floor muscles in rats are reported to exhibit specific behaviors during pregnancy. Increases in both stiffness and the number of sarcomeres in series are observed and might process that protect against perineal trauma at childbirth. Some data in humans have shown that the risk of perineal trauma is highly linked to the intrinsic characteristics of the tissue, suggesting the potential benefit of incorporating intrinsic biomechanical characteristics in the risk prediction for perineal trauma. Shear wave elastography might be a useful noninvasive tool to investigate the elastic properties of these tissues in pregnant women in vivo, with the goal of implementing these properties as a predictive strategy.

Keywords Obstetric anal sphincter injury · Shear wave elastography · Childbirth · Ligamentous laxity · Perineal trauma · Individualized strategy

Introduction

Perineal trauma is a frequent complication of vaginal delivery, and in the most severe cases, the trauma is complicated by obstetric anal sphincter injury (OASI). Perineal trauma can have major repercussions on a woman's physical health and quality of life, including sexual dysfunction, urinary and/or fecal incontinence, and pelvic organ prolapse [1]. The literature on this topic is abundant and reports several risk factors, including surgical delivery, nulliparity, and fetal macrosomia. Nevertheless, even when these risk factors are considered, perineal trauma at childbirth remains unsatisfactorily predicted, indicating that additional risk factors must be identified [1]. Some recent data suggest that the elastic properties of tissues can be a relevant factor that can be used to improve the risk prediction strategy [2, 3]. The objective of this opinion paper is to provide a synthesis of the published data about the changes in the biomechanical behavior of the tissue and the associated physiology during pregnancy. Recent data show the feasibility of assessing, in vivo and with noninvasive techniques, the elastic properties of a woman's pelvic floor. The expected prospects for perineal trauma prediction and prevention are discussed.

Changes in a woman's joint laxity during pregnancy

In a previous prospective study, we reported an increase in the peripheral ligamentous laxity of women from the first to the third trimester using both a general scoring system (the Beighton score) and a specific assessment on the second metacarpophalangeal joint [4]. Similar increases in ligamentous laxity were reported for other joints by

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several authors [5, 6]. Another example, reported by different teams, is the change in the spinal curvature, which is probably allowed by the changes in ligamentous laxity, to maintain the women's center of gravity at the center of her support polygon [7]. Therefore, it seems that there is a decrease in the stiffness of the tissues during pregnancy that leads to some changes in joint biomechanics to provide physiological accommodation of pregnancy weight gain and the gravid uterus. These biomechanical changes might be considered to indicate the physiological adaptation of a woman to pregnancy-related constraints.

The biological mechanisms involved in these biomechanical changes are hardly known. One recurrent hypothesis involves the role of relaxin. This hormone is produced by the ovaries, the mammary tissue and the placenta and has a role in conjunctive tissue remodeling [5]. There are data reporting an association between higher maternal serum levels of relaxin and higher joint mobility and ligamentous laxity [8, 9]. Nevertheless, this point remains debated since such this association has not been reported in other studies [5, 6]. Another hypothesis is the effect of sexual hormones, especially estradiol, whose expression is important during pregnancy. Nevertheless, the impact of these hormones is unclear since different studies have reported contradictory results (an increase or a decrease in stiffness) for muscle and tendons [10, 11], and one study did not report any association between sex hormones and joint laxity during pregnancy [6]. Regardless of the potential role of relaxin or estradiol, the main hypothesis is a change in collagen modeling with a decrease in the ratio of type 1/type 3 collagen. Collagen is the main component of the muscular extracellular matrix that determines muscle biomechanical properties and its ability to sustain a load [12]. This point remains hypothetical since we cannot report data for ex vivo histological analysis of tissues in pregnant women. Finally, these hypotheses are mainly related to joint mobility and ligamentous laxity but not directly related to muscles or, particularly, pelvic floor muscles. Indeed, it is notable that most of the in vivo measurements of tissue mechanics in pregnant women were performed at the joint level and that no information exists for at the muscle level [13]. A recent study report that the stiffness of the patellar tendon does not decrease during pregnancy which suggest the possibility that the biomechanical behavior might be different from one tissue to another [14]. Such biomechanical changes that may exist in pelvic floor muscles may also be a form of physiological preparation of the woman's pelvic floor for childbirth to accommodate the major distension of perineal muscles during vaginal delivery. While no data exist about muscle mechanical changes during pregnancy in humans, animal experimentation has provided ex vivo evidence of biomechanical changes that are related to the effect of pregnancy.

Animal experimental data about pregnancy-associated changes in the biomechanical behavior of the pelvic floor

To analyze pelvic floor muscles, the most often considered animal model is the rat, as the organization of the pelvic floor muscles in rats is similar to that in humans [12]. There are data reporting an increase in muscular fiber length for the pelvic floor muscles of rats during pregnancy. The increase in muscle fiber length is explained by an increase in the number of sarcomeres in series. A concomitant increase in passive muscle stiffness has been found [15], which can be explained by a drastic increase in the total collagen content in pelvic floor muscles [12, 15]. This increase in stiffness can be seen as a physiological mechanism that strengthens the muscular structure during pregnancy and induces an important increase in muscle fiber length. Considering that tissue with lower stiffness has higher plasticity or rupture thresholds, representing the limit at which irreversible damage can occur in a structure [16], the increase in muscle stiffness can be considered to be a protective process against perineal trauma, especially muscle rupture. Of interest, these changes in fiber length and muscle stiffness occur only in pelvic floor muscles (the coccygeus, iliocaudalis and pubocaudalis muscles), while no significant changes occur in peripheral muscle (i.e., the anterior tibialis muscle). The authors conclude that these changes are probably due more to the local increase in mechanical loading applied to pelvic floor muscles than to a hormonal systemic effect [12].

There are animal experimental data about the impact of perineal distension during childbirth on these pelvic floor muscles. Authors from the same team as previous studies simulated the strain exerted by vaginal delivery by inducing vaginal distension, which replicates fetal crowning, in pregnant and nonpregnant rats [17]. They reported an increase in sarcomere length that was dramatically higher in nonpregnant rats. This result indicated that pregnancy-induced adaptations were efficient in limiting the sarcomere hyperelongation that may induce muscle damage. The largest differences between pregnant and nonpregnant rats were reported for the pubocaudalis and coccygeus muscles, especially for the entheseal region of the pubocaudalis muscle, which became translucent. This observation was reliable in terms of human clinical considerations since this region is the one in which levator avulsion due to childbirth occurs [17]. These observations were also consistent with computer modeling studies that identified the dorsal–caudal portion and its bilateral attachments to the pubis as the most stretched portions of the levator ani during vaginal delivery simulation [18].

These muscular adaptations contrast those observed with animal data on the elastic properties of the vaginal wall. Several authors have reported a decrease in stiffness of the vaginal wall during pregnancy [19–21], which is consistent with previously described observations in humans. These authors concluded that this decrease in stiffness might be a physiological process that accommodates vaginal distension during childbirth [19–21].

Since this decrease in stiffness is observed for the pelvic floor and peripheral tissues, it might be related to hormonal systemic changes. In contrast, pelvic floor muscles may have a specific behavior during pregnancy that is induced more by the mechanical loading applied to pelvic floor muscles than by hormonal mechanisms. The length and stiffness of pelvic floor muscles are increased during pregnancy, and this can be considered a protective process that avoids muscular rupture during childbirth. Pelvic floor damage may occur when the strain is too important and/or when the biomechanical changes induced by pregnancy are not sufficient to accommodate the strain induced by delivery.

Association between biomechanical characteristics and obstetrical pelvic floor trauma

To date, data on the impact of the intrinsic biomechanical properties of a woman and the risk of obstetrical pelvic floor trauma are limited. Meriwether et al. investigated whether there is an association between the perineal body stretch during delivery and the risk of OASI [22]. These authors reported a 65% increase in perineal body length from the antepartum to the expulsive phase, which is consistent with the previously reported data [22]. In this study, the importance of the perineal body stretch was not associated with OASI occurrence or any pelvic floor disorders [22].

Our research team recently published a prospective study of 300 women with an assessment of ligamentous laxity between 36 weeks of pregnancy and the onset of labor [2]. Ligamentous laxity was assessed at the second metacarpophalangeal joint (MCP laxity) by measuring the passive extension of the nondominant index finger for a 0.26-N m fixed torque using a specific extensometer. Women with higher ligamentous laxity were those with a higher risk of OASI. An MCP laxity greater than 64° was associated with OASI, with 75% sensitivity, 56% specificity and an area under the curve of 0.65 [2]. Therefore, the intrinsic biomechanical properties seem to be related to perineal trauma. We hypothesized that women with the greatest ligamentous laxity may be those with the weakest pelvic floor muscles and, by extension, those with the highest risk of OASI [2].

However, considering that the mechanisms involved in the increase in ligament laxity and the increase in pelvic floor muscle stiffness are different (see previous section), we currently have no direct evidence to validate this hypothesis. Therefore, it is now crucial to assess the biomechanical behavior of pelvic floor muscles in vivo in pregnant women to determine whether such measurements can help to predict OASI. The next section is dedicated to the available methods that can be used for this purpose.

How to assess the pelvic floor biomechanical behavior in a woman in vivo

Kruger et al. used an elastometer to assess the elastic properties of the levator ani muscle in pregnant and nonpregnant women [23, 24]. Their device is similar to a vaginal speculum associated with force sensors. This elastometer seems to provide the force/displacement curve with good reproducibility. Using this method, the authors reported that the stiffness of the levator ani muscle is higher in the postpartum assessment than in the prenatal assessment. While this innovative approach provides relevant information about pelvic floor behavior, it suffers from two main drawbacks. First, the device measures the displacement of the speculum, which is inserted within the vagina. Thus, it evaluates the elastic properties of both the levator ani and the vaginal wall. Considering that the changes in the stiffness of the levator ani and vaginal wall are opposite during pregnancy [12, 15, 17, 19, 20], this can lead to results that may be difficult to interpret. Second, this remains an intrusive vaginal examination that may be hard to accept for pregnant women [3, 23, 24].

Recent technologies of functional ultrasound imaging have been proposed for in vivo and noninvasive investigations of the elastic properties of several peripheral muscles [25]. Chen et al. reported the use of static elastography to assess the elastic properties of the perineal body in nonpregnant women [26]. This was the first study that used elastography for the pelvic floor. Because the static elastography technique provides a qualitative evaluation, it requires the interposition of a custom standoff pad to estimate the elastic properties of the perineal body in comparison to this reference. The authors reported that the mean compression modulus of the perineal body region was 28.9 kPa. The main strength of this technique is that it allows an in vivo assessment with a noninvasive approach. The main limitation is that the measurement is not directly focused to the pelvic floor, and we do not know which anatomical structure is measured (muscles, vaginal wall, etc.) [3, 26]. In addition, this technique provides a measurement along the transverse direction of the muscles that does not correspond to the "physiological" stiffness measured along the shortening direction, as performed in animal studies. Other research teams suggest similar procedures to provide qualitative assessments of a woman's pelvic floor elastic properties, especially for levator ani muscles [27–29].

We recently reported the use of shear wave elastography for direct and in vivo measurements of the elastic properties of levator ani muscles in nonpregnant women [3]. The levator ani muscle is identified with classical B-mode ultrasound using a transperineal approach with a linear probe. Once the muscle is identified, the shear wave elastography acquisition is performed to evaluate a shear modulus along the muscle shortening direction. This shear modulus is linked to Young's modulus for muscles [30]. In a feasibility study, we reported that from rest to the Valsalva maneuver, the shear modulus of the levator ani muscle increases by a factor of 2 (16–35 kPa for the shear modulus) [3]. This technique provides a direct and noninvasive elasticity measure of the levator ani muscle. However, this technique has not yet been used for pregnant women, and there are no data about the reproducibility of pelvic floor muscle measurements [3].

Therefore, techniques have been proposed for in vivo investigations of the elastic properties of a woman's pelvic floor. To directly and specifically investigate the properties of the pelvic floor muscles, shear wave elastography seems to be a more appropriate technology, but data on the feasibility of the technique in pregnant women and the reproducibility of the data are needed.

Prediction of perineal trauma

There are different predictive algorithms proposed for perineal trauma at childbirth and, more specifically, for OASI occurrence. Jelovskek et al. reported a model for fecal incontinence, and McPherson et al. reported a model for OASI; these models showed poor reliability, with areas under the curve of 0.68 and 0.64, respectively. This seems to be associated with too high a risk for an incorrect conclusion about the high or low risk of developing the outcome measured [31, 32]. Meister et al. reported a more satisfactory predictive model of OASI (area under the curve of 0.83); however, its use has not been reported in other studies, and its predictive value has not been validated in another sample, which is a main limitation for its clinical use [33].

All these predictive models are focused on the mode of delivery without any considerations of the woman's tissue biomechanical characteristics, which might explain the limitations of these predictive tools. There is strong evidence in animals and humans for large and specific changes in a woman's pelvic floor biomechanical behavior during pregnancy, and this is probably a protective process against perineal trauma. Therefore, we hypothesize that taking this biomechanical behavior into account in our risk prediction of perineal trauma at childbirth will probably improve the efficiency of the predictive models, leading to individualized risk assessments. In this perspective, we believe that shear wave elastography would be a very useful tool. All women will undergo several ultrasounds during their pregnancy monitoring, and it is easy to consider performing a short assessment of the viscoelastic properties of pelvic floor muscles during one of these ultrasound assessments, especially in the third trimester. By including these biomechanical properties in the risk prediction of perineal trauma at childbirth we may optimize the efficiency of the existing algorithms with a better identification of high-risk woman. Such an individualized risk assessments can lead to personalized information for a pregnant woman about her risk of perineal trauma, allowing personalized advice for the mode of delivery and/or implementation of preventive strategies (e.g., episiotomy, restriction of surgical delivery). More specifically, the place of protective interventions such as episiotomy would be individually discussed. Indeed, there are strong data reporting that there is no benefit of a routine use of episiotomy to prevent from perineal trauma or anal/urinary incontinence [34–36]. A recent biomechanical study using a computational modeling approach report that a mediolateral episiotomy decreases the stress on pelvic floor muscles and the force required to deliver successfully [37]. Nevertheless, due to the morbidity of the intervention (infection, bleeding, pain) and the absence of benefits in the overall population, the answer is to find out how high-risk women which could have a benefit of mediolateral episiotomy could be correctly identified [34–36].

Tissue biomechanical behavior consideration, assessed non-invasively using shear wave elastography during the last obstetrical ultrasound visit, would allow to identify women with an intrinsic high-risk of perineal trauma allowing a selective use of episiotomy. These women could benefit a personalized information on mediolateral episiotomy in their specific cases, how the intervention is performed, and what are the required cares after the delivery. Such an antenatal information will probably lead to better acceptability of the intervention and offer the possibility to collect a real free and informed consent compare to an emergency information during the delivery.

Conclusion

Pregnancy is associated with significant changes in pelvic floor biomechanical behavior that can be considered a protective process from perineal trauma during childbirth. Recent functional ultrasound imaging technologies, such as shear wave elastography, allow an in vivo assessment of the elastic properties of a woman's pelvic floor muscle elastic properties and may be useful for identifying women with an intrinsic high risk of perineal trauma. We contend that intrinsic tissue biomechanical behavior should be considered in the risk assessment of perineal trauma at childbirth to improve the individualized risk assessment with the goal of providing personalized counseling to women in prenatal courses or during labor and developing preventive strategies.

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ORIGINAL ARTICLE

In vivo assessment of the levator ani muscles using shear wave elastography: a feasibility study in women

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Abstract

Introduction and hypothesis We hypothesized that shear wave elastography (SWE) technology might be useful for assessing the elastic properties of the pelvic floor in women. Our primary objective was to evaluate the feasibility of assessing the levator ani muscles using SWE in women. Our secondary aim was to investigate the changes in their elastic properties from rest to Valsalva maneuver.

Methods During this prospective feasibility study in nonpregnant female volunteers, we collected data on participant age, body mass index (BMI), parity, and time since the delivery. The levator ani muscles of each participant were assessed using SWE technology at rest and during a Valsalva maneuver by measuring the shear modulus (in kilopascals). We then assessed the changes in the shear modulus at rest and during the Valsalva maneuver using a Wilcoxon test.

Results Twelve parous women participated in this study. The mean time since the last delivery was 14 months, the mean age was 31 years, and mean BMI was 28 kg.m^{-2}. All the assessments performed at rest were successfully completed, but we encountered two failures during the Valsalva maneuver. The mean shear modulus increased by a factor of more than 2 from rest to the Valsalva maneuver for both the right (16.0 vs 35.4 kPa) and left side (17.1 vs 37.6 kPa).

Conclusions An assessment of the elastic properties of the levator ani muscles is feasible for nonpregnant women. The reproducibility of the technique and its application in pregnant women and women with pelvic floor disorders must be investigated.

Keywords Biomechanics · Levator ani muscle · Elastography · Shear wave · Pelvic floor · Women

Introduction

Pelvic floor disorders (PFDs), including pelvic organ prolapse and urinary and anal incontinence, are common conditions that can strongly affect women's health [1, 2]. Despite a lack of data on the pathophysiology of these disorders, vaginal delivery is a well-known risk factor for pelvic floor damage. To date, the role of the intrinsic biomechanical properties of pelvic tissues (pelvic floor elasticity, stiffness, distension) in pelvic floor damage and PFD occurrence remains poorly

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investigated [3–6]. Furthermore, both the preventive and predictive strategies for pelvic floor damage at childbirth and PFD occurrence remain disappointing [7–11].

This potential impact of the intrinsic characteristics of the pelvic floor on PFD occurrence is supported by several publications reporting an association between joint mobility and PFD [5, 12–14]. These studies' results suggest that women with particular intrinsic biomechanical characteristics may have tissues with specific properties, leading to excessive mobility that could be established as excessive joint mobility for a peripheral joint and PFD for the pelvic floor. However, these biomechanical characteristics of the pelvic floor are not considered in either the risk prediction of PFD or the prediction of PFD treatment effectiveness (surgery and or physiotherapy). It is possible that taking these characteristics into account will improve our predictive and preventive strategies and lead to an individualized assessment.

The anatomical structure that is most involved in supporting the pelvic organs includes the levator ani muscles, which can be damaged during childbirth [15]. The levator hiatus is represented by the space between the two levator ani muscles (left and right) and constitutes the widest hernial opening in the human body. Damage to the levator ani muscles (avulsion, over-distension, etc.) may have an impact on the size and distension of the levator hiatus. It has been reported that the size of the levator hiatus is associated with the risk of PFD and especially with pelvic organ prolapse [16]. This observation suggests that taking the biomechanical characteristics of the levator ani muscle into account might be useful for predicting PFD occurrence and treatment effectiveness.

The main risk factor described for PFD occurrence is vaginal delivery, which is hypothesized to induce perineal trauma and, in particular, levator ani muscle trauma. Indeed, an overdistended levator ani muscle and/or a muscle with an avulsion may lead to an oversized levator hiatus, leading to PFD. If we can predict which women are at a high risk of levator ani muscle damage, we may be able to predict which women are at risk of PFD after childbirth. This risk prediction should consider both the effect of the delivery and the effect of the pregnancy itself on the intrinsic characteristics of the pelvic floor.

During pregnancy, there is an increase in both pelvic floor distension and in peripheral ligamentous laxity [5, 17]. The maximal pelvic floor distension occurs during childbirth, when the pelvic floor muscles can be stretched to up to three times their initial length [15]. In a recent study, we reported an association between increased ligamentous laxity and levator hiatus distension in a cohort of pregnant women [6]. In that study, the pregnant women with the greatest peripheral ligamentous laxity (assessed at the second metacarpo-phalangeal joint using a specific extensometer) were those with the greatest levator hiatus distension (assessed using 4D perineal ultrasound) [6].

We hypothesized that the newest functional imaging technologies may be useful for assessing in vivo the biomechanical characteristics of the pelvic floor.

Shear wave elastography (SWE) technology (Supersonic Imagine, Aix en Provence, France) is an innovative technology used to perform quantitative in vivo biomechanical assessments of tissues during an ultrasound examination [18–20]. The procedure consists of applying a mechanical perturbation to induce the propagation of a shear wave into the tissue of interest by using a specific ultrasound probe [18, 19]. The shear wave is an acoustic wave that propagates in the transverse plane into the tissue, where it induces the mechanical perturbation [21]. The device can measure the wave's propagation speed because of its ultrafast ultrasound acquisition. This propagation speed correlates with the stiffness of the tissue: the stiffer the tissue, the faster the wave's propagation speed [18, 19]. This technology has already been used to assess the elastic properties of superficial muscles, especially in the sports domain [18, 19]. It has also been used in pregnant women to assess the elastic properties of the cervix and the myometrium [22, 23]. The shear wave signal is close to the acoustic signal used for conventional ultrasound and is a compression wave that propagates in a longitudinal plane into the tissue [21]. The potential risks and use restrictions for SWE are not different from those for classic ultrasound.

The main endpoint of this study was to evaluate the feasibility of an in vivo assessment of the properties of the levator ani muscle using SWE technology in a cohort of nonpregnant women. The secondary endpoint was to evaluate the capacity of the device to evaluate objective changes in the elastic properties of the muscles by comparing measurements at rest, when the muscle is in a neutral position, and during the Valsalva maneuver, when the muscle is in a stretched position.

Materials and methods

This prospective longitudinal study was conducted at our University Department of Obstetrics and Gynecology from 17 November 2016, to 12 December 2016.

Eligible participants were volunteer nonpregnant women who had participated in a previous study, assessing the association between ligamentous laxity and levator hiatus distension during pregnancy [6]. Exclusion criteria were previous PFD and/or a personal history of joint disease.

There was one clinic visit for each participant during which we assessed the levator ani muscles using SWE technology.

We collected the following anthropometric data and sociodemographic data: age, body mass index (BMI), and time since the last delivery.

At the time of inclusion, the women underwent an ultrasound assessment of the levator ani muscle using SWE performed using an Aixplorer V11 ® device (Supersonic

Imagine, France). The Aixplorer scanner allows the user to perform both classical two-dimensional B-mode ultrasound acquisition and SWE during the same assessment and with the same material. The assessments were performed after voiding with the woman in the lithotomy position at rest, and then at maximal strain during the Valsalva maneuver. We asked participants to perform two initial Valsalva maneuvers with biofeedback instruction to prevent levator co-activation from serving a confounding factor in our analysis [24].

We first located the levator ani muscle, at its pubic insertion, using the classic two-dimensional ultrasound mode with an SL 15–4 linear probe (4–15 MHz) of 5 cm in length [25]. This method was previously used to assess levator ani avulsion and led to an 87% agreement between observers [25]. The probe was first placed in the sagittal plane on the perineum. We then applied an inclination of 10° to identify the pubic insertion of the levator ani [25]. Once the levator ani muscle was correctly identified, we performed the SWE assessment. The assessment at rest, consisted of a static assessment with one picture. The limits of the levator ani muscles were outlined by hand, and the Young modulus (in kilopascals) was obtained within these limits (Figs. 1, 2). The Young modulus characterizes the stiffness of a tissue and is reported to be the relation between a stress and a strain in an isotropic tissue (a tissue whose mechanical properties are similar in all directions). The shear modulus represents the stiffness of a tissue in an anisotropic tissue, such as muscle. However, the calculation for the Young modulus using the device assumes that the tissue is isotropic (a tissue in which mechanical properties are similar in all directions). Because this assumption is not true for the muscle, the Young modulus was divided by a factor of 3 to obtain the shear modulus (in kilopascals) [19, 26]. A previous study showed that the shear modulus is strongly and linearly related to the Young modulus measured using traditional methods of material testing [19, 26]. This observation clearly demonstrates the relevance of shear modulus measurements obtained using ultrasound SWE for the study of muscle biomechanics [19, 26]. For the assessment during the Valsalva maneuver, we performed a dynamic acquisition from the rest position to 5 s of maximal strain during the Valsalva. For this dynamic acquisition, we outlined by hand the limits of the levator ani muscle in each picture, and the Young modulus and then the shear modulus were reported for each picture, as described for the assessment at rest. The highest shear modulus obtained during the acquisition was reported as the shear modulus of the levator ani muscle during the Valsalva maneuver. We performed a dynamic acquisition during the Valsalva maneuver with interval measures during the process to systematically record the highest shear modulus that a static measure, not exactly at the maximal Valsalva, might have missed.

The procedure was performed for both the right and left sides, and the shear modulus was reported at rest and during the Valsalva maneuver for the two sides.

We reported the population characteristics for age, BMI, and time since the last delivery in terms of the mean and standard deviation (SD), and we reported the number of successfully completed procedures and the number of failed

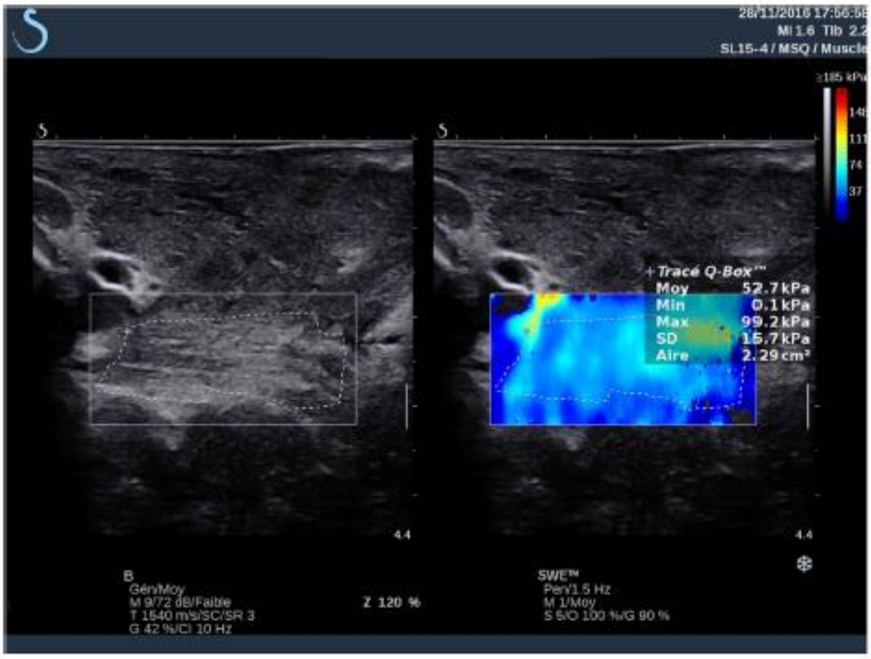

Fig. 1 Levator ani muscle assessment at rest using shear wave elastography technology

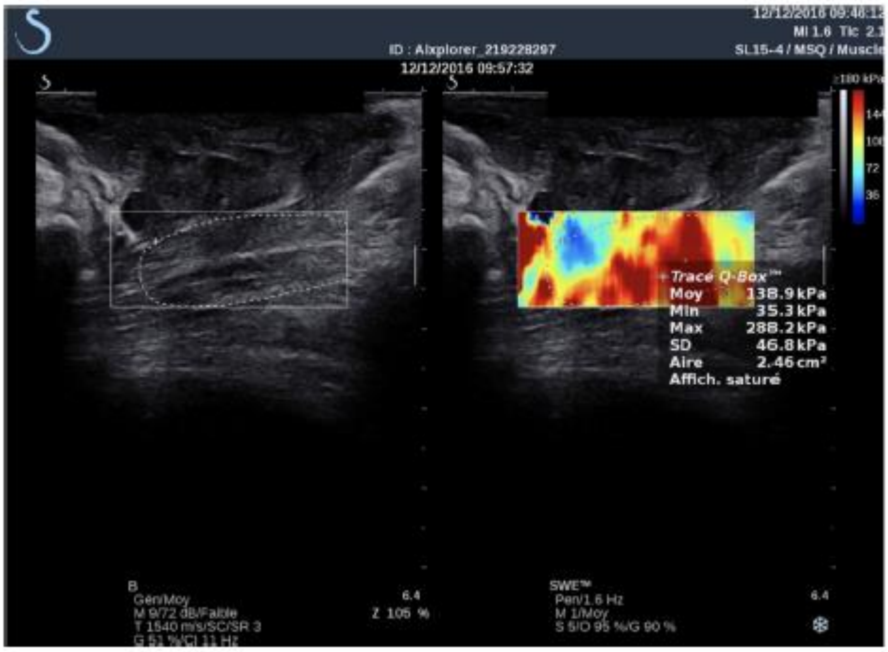

Fig. 2 Levator ani muscle assessment during Valsalva maneuver using shear wave elastography technology

procedures. We then reported the mean and SD for the shear modulus at rest and during the Valsalva maneuver for both right and left levator ani muscles (as means and SD) to check the feasibility of the technique for the two sides.

We assessed changes in levator ani shear modulus from rest to the Valsalva maneuver using a Wilcoxon test.

Because the main endpoint was to describe the feasibility of the technique and not its reliability, a power calculation was not performed. Furthermore, there are no previously published data that would have allowed such a calculation.

For all analyses, the statistical significance threshold (alpha) used was 5%.

Analyses were performed using the Stata software (version V14IC; Stata Corporation, College Station, TX, USA).

Our local ethics committee (protocol no: 2014-A01467–40, Comité de Protection des Personnes Ouest-III) and the National Drug Safety Agency (protocol no: 141380B-22, Agence Nationale de Sécurité du Médicament et des produits de santé) reviewed and approved the protocol. Written, freely given informed consent was obtained from each study participant before inclusion in the study and the realization of any investigations.

Results

A total of 12 women were included in this study. They were all parous women. All 12 had a history of almost one delivery, with 10 vaginally delivered and 2 delivered by cesarean section. The characteristics of the study population are reported in Table 1.

All assessments performed at rest were successfully completed. We reported two assessment failures during the Valsalva maneuver, which corresponded to the two women with the highest BMI (37.7 and 42.2 kg.m^{-2}).

The mean shear modulus assessed at rest and during the Valsalva maneuver for both the right and left levator ani muscles is reported in Table 2. The mean shear modulus increased by a factor of more than 2 from rest to the Valsalva maneuver. There were no significant differences in any measurements between the left and the right sides.

Comment

Main findings

In nonpregnant women, it is possible to assess the elastic properties of the levator ani muscles in vivo using SWE at

Table 1 Characteristics of the participants

	Mean (SD)
Age, in years	31 (2.6)
Body mass index, in kg/m^2	28 (7.4)
Parity	1.9 (0.7)
Delay since the last delivery, in months	14 (2)

SD standard deviation

Table 2 Elastic properties of levator ani muscles at rest and at the Valsalva maneuver

	Mean shear modulus at rest, in kPa (SD)	Mean shear modulus at Valsalva, in kPa (SD)	p*
Right side	16 (6.9)	35.4 (13.9)	< 0.005
Left side	17.1 (7.6)	37.6 (13.1)	< 0.005

* Wilcoxon test

rest and during the Valsalva maneuver. The mean shear modulus and, therefore, the stiffness of the levator ani muscle increased by a factor of more than 2 from rest to the Valsalva maneuver.

Strengths and limitations

The first limitation of this study is that it deals with parous women, who potentially have existing pelvic floor damage. Thus, the shear modulus that we reported for the levator ani muscle may not be representative of the elastic properties of the levator ani muscle in nulliparous women, because a damaged levator ani muscle probably exhibits different biomechanical behavior than an undamaged one [27]. Nevertheless, this limitation did not bias our analysis because our main objective was to assess the feasibility of the procedure and not to describe the elastic properties of the levator ani muscle.

In addition, there are no previously published data concerning the reliability of the SWE technique for this specific use in pelvic floor assessment. Nevertheless, considering the easy access to the pelvic floor when using ultrasound, the feasibility of SWE measurement for this muscle reported in the present study, and the good reliability reported for other muscles, such as the abdominal muscles, gastrocnemius muscle, and biceps brachii, we are confident that a future study will demonstrate the good reliability of this method for pelvic floor muscles [28, 29].

Another limitation of this study is the small number of women included, which is inherent to the pilot feasibility design of the study. Our results must be considered proof of concept of the feasibility of the procedure. This feasibility would have to be confirmed and its reproducibility investigated before any application in clinical practice.

Interpretation

To our knowledge, this is the first study to report the use of SWE technology to assess the elastic properties of the pelvic floor in vivo in women. Only one author has described an in vivo assessment of the elastic properties of the levator ani muscles. Kruger et al. used an elastometer for measuring levator ani muscle stiffness at rest in pregnant and nonpregnant women [30, 31]. The device used in the study consisted of a vaginal speculum coupled with force sensors, which enabled a force/displacement curve to be obtained to calculate the stiffness of the levator ani muscle with good reproducibility [30, 31]. In their work, Kruger et al. reported that the stiffness of the levator ani muscle is more significant in postpartum versus antenatal assessment (436 N/m ± 198 N/m vs 325 N/m ± 14 N/m) [30, 31]. This procedure is interesting, but has several limitations. First, because the device is placed into the vagina and measures the displacement of the speculum, the result may be influenced by the elastic properties of the vaginal wall. Thus, the stiffness that is measured may reflect the global stiffness, including at least the vaginal wall and the levator ani muscle. Second, the authors did not perform the assessment in cases of vaginal infection or when the fetal head was too low. This could highlight some possible difficulties for its wider use in pregnant women [30, 31]. Third, there may be a problem of acceptability for pregnant women to undergo an intrusive vaginal examination. Nevertheless, the global technique used by Kruger et al. remains quite interesting because it provides an assessment of the whole perineum including the vagina, the levator ani muscles, and the fascia. This is a different approach than ours, as we aimed to specifically investigate the elastic properties of the levator ani muscle. The two procedures may be complementary because SWE allows an individual assessment of the pelvic floor tissues and Kruger et al.'s device provides an assessment of the whole pelvic floor; thus, the potential interactions between these different structures can be addressed.

Chen et al. attempted to assess the elastic properties of the perineal body using elastography in nonpregnant women [32]. To our knowledge, this was the first description of the use of elastography to assess the pelvic floor. The author reported that the mean compression modulus of the perineal body was 28.9 kPa [32]. The first limitation of this technique is that it requires the interposition of a custom reference standoff pad made of liquid plastic and plastic softener [32]. The elastic properties of this structure are known, and this technique allows the elastic properties of the target tissue to be measured compared with this reference pad [32]. This type of measure, which uses an interface between the probe and the tissue, may be less efficient than a direct assessment of the tissue without any interference. The use of SWE avoids the use of a standoff pad when performing a direct quantitative assessment of the pelvic floor. This technique remains quite interesting because, as noted for Kruger et al.'s device, it provides a global assessment of the region of interest, including muscles, ligaments, and fascia [32]. This technique may be complementary to our technique, which enables a direct assessment of one structure.

Silva et al. published a work in which the elastic properties of the pubovisceral muscle were elegantly calculated using an inverse finite element [33]. They reported the material constants of the pubovisceral muscle for continent women that lead to shear modulus values of 78 ± 44 kPa (using shear modulus = 2*C1 for the neo-Hookean model), 80 ± 48 kPa (using shear modulus = 2*(C1 + C2) for the Mooney–Rivlin model) and 62 ± 46 KPa (using shear modulus = 2*C1 for the Yeoh model). These values are in the same range, but are notably higher than the values reported in the present study (17 ± 7 kPa). Nevertheless, the number of volunteers in both studies was low, and the studies used very different methods; thus, the comparison should be considered carefully. Furthermore, comparing the results of these studies may be difficult because the study populations are quite different (continent and noncontinent women in the study by Silva et al. versus parous women in our study). The assessments were also done in different positions (dorsal decubitus for MRI acquisition in the study by Silva et al. versus the lithotomy position in our study). Finally, the technique used in the study of Silva et al., inverse finite element, is quite different than our technique, which involves a direct assessment with an instant measure of the shear modulus [33]. This is probably the reason for the differences observed in these two studies.

We reported a 100 % success rate using SWE for the assessment at rest, but we reported two failures of the assessment during the Valsalva maneuver. As previously stated, these failures occurred in the women with the highest BMI. These difficulties were due to the loss of visibility of the levator ani muscle during the Valsalva maneuver, as the muscle became too deep to be clearly located using our 15–4 linear probe. In women with a very high BMI, these difficulties are more apparent owing to the thickness of the soft parts of the pelvic floor. To fulfill the objective of assessing elasticity during the Valsalva maneuver in all women, it would be necessary to use different probes that allow deeper assessments.

The results of this study are encouraging, but need to be confirmed in a larger population and include a reliability assessment. Furthermore, the association between the elastic properties of the pelvic floor in women, as assessed using SWE, and the clinical and ultrasound pelvic floor distension measures should be evaluated. Indeed, if there is no association between elastic properties and pelvic floor distension, it would question the relevance of these measures.

Future studies should investigate the feasibility of assessing other components of the pelvic floor complex, such as ligaments and the vaginal wall. Indeed, the biomechanical behavior on muscles depends on their elastic properties and their attachments (ligaments). There are reports in the literature that assess peripheral ligaments using SWE [21]. However, the measurements are more challenging for thin and stiff structures such as tendons and ligaments [19]. Therefore, the feasibility, validity, and reliability of this technique need to be demonstrated for pelvic floor ligaments and the vaginal wall.

In our experience, the stiffness of the levator ani muscle significantly increased from rest to the Valsalva maneuver, which means that the stretched levator ani muscle is stiffer than it is at rest. This observation is in agreement with clinical observations made during childbirth: during the period between the onset of pushing and fetal head delivery (the period of maximal distension of the perineum) the pelvic floor is stiffer than it is at the beginning of the second stage of labor. It has been reported that the tissues with the least stiffness may easily reach their plasticity threshold, which is the threshold beyond which irreversible damage to the intrinsic material's structure occurs [34]. Plasticity is a material intrinsic characteristic and means that a material remains deformed after being stressed. Elasticity characterizes the ability of a material to recover its initial state after being stressed by an external force [34]. A plastic deformation consists of an irreversible deformation due to permanent changes in the intrinsic structure of a material. Conversely, an elastic deformation constitutes a reversible process caused by an external force, with a return to the initial state once this force is no longer applied [34]. Thus, it would be very helpful to be able to measure the stiffness of the stretched levator ani muscle before attempting to predict the risk of pelvic floor damage, such as levator ani avulsion during childbirth, that is implicated in PFD occurrence. To predict pelvic floor traumas, other biomechanical factors can be included in a hypothetical predictive model. One factor is the maximal strength that the tissue can support before rupture. This threshold is impossible to measure in individual patients. One alternative approach would be to perform measurements of muscle volume, which should be related to the maximal strength that it can support. Thus, the combination of both volume and the elastic modulus of the pelvic floor could provide good predictive measures of the risk of damage. These studies may provide information about the intrinsic characteristics of the pelvic floor and especially its rupture threshold. In addition, the potential for an individual material to reach its plasticity or rupture threshold depends on its mechanical characteristics, but also on the stress applied to the material. A predictive model for pelvic floor trauma could also include data on the stress applied: fetal head circumference, fetal weight, instrumental delivery, etc. Excessive stress, such as that caused by a large fetal head circumference, could lead to excessive muscular distension beyond the physiological range; if the muscle reaches its plasticity threshold, plastic deformation could occur. The mechanical properties of the ligaments and tendons should be assessed and probably included in such a predictive model because the ability of muscle to distend is also related to the flexibility of its attachments, which play the role of a "shock absorber."

Other studies have reported the use of SWE in pregnant women without any fetal complications [22, 23]. It would be interesting to ascertain if the elastic properties of the pelvic floor muscle assessed using SWE during pregnancy are predictive of the risk of pelvic floor damage at childbirth and the risk of PFD after childbirth. Every woman undergoes ultrasound during pregnancy, and the possibility of performing an assessment of the elastic properties of the pelvic floor during the same visit with the same device would likely be considered acceptable by most women.

Conclusion

It is feasible to assess the elastic properties of the levator ani muscle in vivo using SWE in a cohort of nonpregnant women. This is the first report of such an in vivo assessment of the elastic properties of the levator ani muscles using a non-invasive technology similar to ultrasound. The next step is to assess the reliability of the procedure (intra- and inter- observer concordance) in addition to the concordance between the elastic properties and clinical distension of the pelvic floor, before considering its use in our clinical practice. Future studies will determine whether this technique can provide data to support individual risk prediction of PFD and thereby enable us to better individualize treatment decisions (e.g., type of physiotherapy, type of surgery).

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Compliance with ethical standards

Conflicts of interest None.

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STUDY PROTOCOL **Open Access**

In vivo assessment of the elastic properties of women's pelvic floor during pregnancy using shear wave elastography: design and protocol of the ELASTOPELV study

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Abstract

Background: Animal studies have reported an increase in pelvic floor muscle stiffness during pregnancy, which might be a protective process against perineal trauma at delivery. Our main objective is to describe the changes in the elastic properties of the pelvic floor muscles (*levator ani*, external anal sphincter) during human pregnancy using shear wave elastography (SWE) technology. Secondary objectives are as follows: i) to look for specific changes of the pelvic floor muscles compared to peripheral muscles; ii) to determine whether an association between the elastic properties of the *levator ani* and perineal clinical and B-mode ultrasound measures exists; and iii) to provide explorative data about an association between pelvic floor muscle characteristics and the risk of perineal tears.

Methods: Our prospective monocentric study will involve three visits (14–18, 24–28, and 34–38 weeks of pregnancy) and include nulliparous women older than 18 years, with a normal pregnancy and a body mass index (BMI) lower than 35 $kg.m^{-2}$. Each visit will consist of a clinical pelvic floor assessment (using the Pelvic Organ Prolapse Quantification system), an ultrasound perineal measure of the anteroposterior hiatal diameter and SWE assessment of the *levator ani* and the external anal sphincter muscles (at rest, during the Valsalva maneuver and during pelvic floor contraction), and SWE assessment of both the *biceps brachii* and the *gastrocnemius medialis* (at rest, extension and contraction). We will collect data about the mode of delivery and the occurrence of perineal tears. We will investigate changes in continuous variables collected using the Friedman test. We will look for an association between the elastic properties of the *levator ani* muscle and clinical / ultrasound measures using a Spearman test at each trimester. We will investigate the association between the elastic properties of the pelvic floor muscles and perineal tear occurrence using a multivariate analysis with logistic regression.

Discussion: This study will provide original in vivo human data about the biomechanical changes of pregnant women's pelvic floor. The results may lead to an individualized risk assessment of perineal trauma at childbirth.

Trial registration: This study was registered on https://clinicaltrials.gov on July 26, 2018 (NCT03602196).

Keywords: Perineal trauma, Shear wave elastography, Pregnancy, Levator ani muscle, Anal sphincter, Childbirth, Obstetric anal sphincter injury

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Background

Perineal trauma is a frequent complication of childbirth, which may lead to several pelvic floor disorders, such as anal incontinence, urinary incontinence, pelvic organ prolapse and sexual dysfunction [1–4] In the most severe cases, perineal trauma could involve an obstetric anal sphincter injury (OASI) (rupture of the external anal sphincter and, worse still, opening of the rectal mucosae) and/or levator avulsion. OASI, which occurs in nearly 5% of first deliveries, is associated with postnatal anal incontinence and dyspareunia [1]. Levator avulsion, which occurs in nearly 10% of first deliveries, is associated with pelvic organ prolapse and sexual dysfunction [3]. These injuries are associated with trauma of the pelvic floor muscles at vaginal delivery during which these muscles are overstretched, up to three times their initial length [5]. Several risk factors are described in the literature (forceps delivery, fetal macrosomia, etc.) However, the occurrence of these complications remains very difficult to predict [1–4]. It is likely that the risk of pelvic floor trauma can be influenced by intrinsic characteristics of the pelvic floor muscles and their ability to lengthen sufficiently to enable passage of the fetus through the birth canal without being damaged. Identifying women with a high risk of perineal trauma antenatally would enable clinicians to propose individualized counseling and preventive strategies for these women.

Few studies have indicated that some intrinsic biomechanical characteristics of pregnant women could be associated with the risk of perineal trauma [6–8]. In a recent prospective study, we reported an association between peripheral ligamentous laxity (assessed at the metacarpophalangeal joint) and the risk of OASI. In that study, the women with the greatest ligamentous laxity had the greatest risk of OASI [8]. This result supports the hypothesis of an association between a woman's individual biomechanical characteristics and her risk of perineal trauma. However, the main limitation of this study was that it was designed to analyze data about an upper limb joint, which is probably very different from pelvic floor muscle tissues [8].

Data about changes in intrinsic characteristics of women's pelvic floor muscles during pregnancy has mainly originated from experiments on rats [9–11]. Some authors have reported that an increase in muscular fiber length and an increase in pelvic floor muscle stiffness occurs during pregnancy, while no changes were reported for peripheral muscles [9–11]. This could be explained by the increase in mechanical loading (force due to gravity of growing fetus) applied to pelvic floor muscles during pregnancy [10]. This increase in elastic modulus may be a protective process from perineal trauma. On rats, studies reported an increase during pregnancy in both fiber length and stiffness measured at a given sarcomere length [9, 10]. These changes could be interpreted consequently from the increase of loading. The increase in fiber length was thought as a mechanism to limit the fiber strain that can cause injury. The increase in stiffness was thought to be related to extracellular matrix content and would likely reduce the risk of injury due to large strain that occurs during parturition [5, 9]. This is supported by a higher ultimate stress in biological tissues that have higher stiffness [12]. These data about animal experimentation need to be read with caution because there is no data proven that these phenomena occur in a same way in women.

To date, several techniques have been described to assess the in vivo elastic properties of the pelvic floor muscles (vaginal elastometry, tactile imaging, elastography) [13, 14]. One of the most relevant techniques is shear wave elastography (SWE), which allows a direct, quantitative and noninvasive assessment of the muscles [15]. Recently, we reported the feasibility of an in vivo assessment of the elastic properties of the *levator ani* muscle using this technique (100% of procedures allowing a visualization of the levator ani muscle and a measure of elastic properties in women with a lower than 35 $Kg.m^{-2}$ body mass index) [13].

In accordance with animal experimentation, we hypothesize that there are changes in elastic properties of women's pelvic floor through pregnancy and that SWE is relevant to follow these changes [9, 10]. They might be specific to pelvic floor muscles without, or less, significant changes for peripheral muscles such as *biceps brachii* or *gastrocnemius medialis*. Finally, the hypothetical changes in elastic properties of women's pelvic floor may be associated with their intrinsic risk of perineal trauma at childbirth. Indeed, during vaginal delivery, a major strain is applied to pelvic floor muscles which are stretched up to 3 times their initial length [5, 16]. Thus, intrinsic elastic properties of pelvic floor muscles may be associated with their ability to support this strain without being damaged.

Our main objective is to describe the changes in the elastic properties of the pelvic floor muscles (*levator ani*, external anal sphincter) during human pregnancy using shear wave elastography (SWE) technology. The secondary objectives are as follows: i) to look for specific changes of the pelvic floor muscles compared to peripheral muscles; ii) to determine whether an association between the elastic properties of the *levator ani* and perineal clinical and B-mode ultrasound measures exists; and iii) to provide explorative data about an association between pelvic floor muscle characteristics and the risk of perineal tears.

Methods

Design

The ELASTOPELV study will be a prospective, longitudinal, monocentric study. The scheme of the study will involve 3 visits during pregnancy: the first one between 14 and 18 weeks, the second between 24 and 28 weeks and the last between 34 and 38 weeks of pregnancy (Fig. 1). For each of these three visits, the protocol will follow these steps: clinical perineal assessment, ultrasound B-mode perineal assessment, SWE assessment of the *levator ani* muscle, the external anal sphincter, the *biceps brachii* muscle and the *gastrocnemius medialis* muscle (Fig. 1).

Setting

The study will take place in the department of Obstetrics and Gynecology of the Poitiers University Hospital, Poitiers, France.

Population

The inclusion criteria are as follows: women older than 18 years, volunteers, nulliparous, with a normal singleton pregnancy, and who benefit from health insurance.

The exclusion criteria are as follows: women with previous vaginal and/or cesarean delivery, women with a personal history of pelvic floor disorders (urinary incontinence, anal incontinence, pelvic organ prolapse), women with a body mass index (BMI) higher than 35 $kg.m^{-2}$, women with chronic muscular disease, women requiring admission into a psychiatric unit, women under judicial protection, and women unable to understand the French language.

If an included woman has a pregnancy who became pathological (define by the necessity of follow-up into pathological pregnancies consultations and/or admission in pathological pregnancies unit) she will no longer participate to the study and no data will be collected after this event. If a woman wants to stop its participation, no more data will be collected.

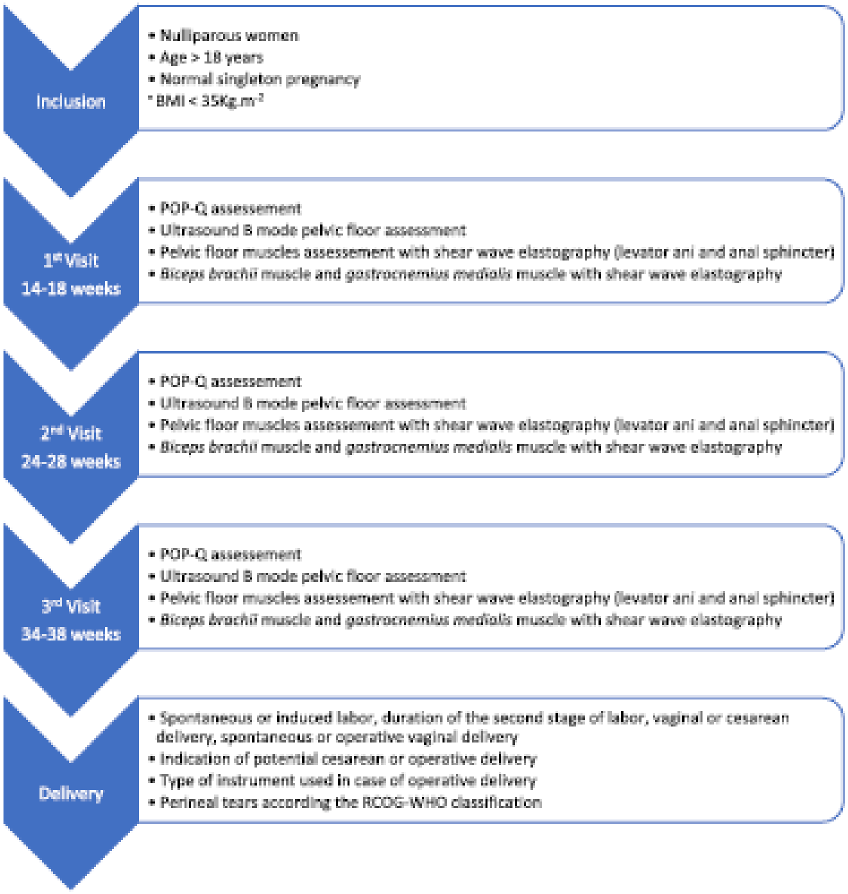

Fig. 1 ELASTOPELV study design, inclusion criteria and data collected

Power calculation

This study deals with exploratory data with an absence of previous data that would allow a power calculation. Furthermore, the main endpoint of the project is descriptive (to describe changes in the elastic properties of the *levator ani* muscle during pregnancy), and, therefore, an a priori power calculation does not appear necessary. We aim to obtain and study the data from at least 50 women. We considered this sample size in part due to previous studies that reported an increase in *levator hiatus* area and ligamentous laxity during pregnancy, as well as changes in the intrinsic biomechanical characteristics of pregnant women, from between 20 to 50 women [17–19]. We estimate that 20% of the women will be excluded during pregnancy because of a complicated pregnancy and/or their own choice, leading to an objective of 60 inclusions.

Recruiting procedure

Women eligible for the ELASTOPELV study will be informed about the study during their clinical consultations and/or ultrasound consultations during a normal pregnancy follow-up by their obstetrician and/or midwife. Eligible women interested in this study will be contacted by the investigator to obtain more information about the study and proceed with the inclusion if they give their free informed consent.

Shear wave elastography principles

The novelty of the ELASTOPELV study is based on the use of SWE to investigate the in vivo elastic properties of the pelvic floor muscles of pregnant women. SWE allows a quantitative in vivo assessment of tissues during a classic ultrasound examination [15, 20]. An Aixplorer® device (Supersonic Imagine, Aix-en-Provence, France) will be used. A remote mechanical perturbation is applied to the tissue using a specific ultrasound sequence to induce the propagation of a shear wave into the tissue of interest. Due to the ultrafast ultrasound acquisition, the wave's propagation speed is measured perpendicular to the ultrasound beam. This shear wave speed propagation is linked with the elastic modulus of the tissue: the stiffer the tissue, the higher the wave's propagation speed is [15, 20, 21]. The elastic properties of the tissue are reported as the Young modulus, which represents the link between a stress and a strain in an isotropic tissue (similar mechanical properties in all directions). Muscles are stiffer along the fiber direction and thus cannot be considered isotropic. Considering an isotropic solid, the Aixplorer device gives E (Young's modulus) as a measurement with, $E = 3\mu = \rho V^2$. with μ the shear modulus, ρ the density, V the shear wave speed.

In anisotropic solid the eq. $E = 3\mu$ is no more valid. So, measurements should be divided by a factor 3 to obtain measurement of the muscle shear modulus [15, 22]. A previous study has demonstrated that the shear modulus is strongly and linearly related to the Young modulus, which supports the relevance of shear modulus measurements obtained with the Aixplorer® device for the study of muscle biomechanics [15, 23].

SWE is based on the hypothesis of a linear elasticity that is commonly assumed in both magnetic resonance elastography and ultrasound SWE. A lot of SWE studies analyzed the effects of loading on changes in muscle elasticity [15]. The effect of nonlinear elasticity should be studied in the future.

Safety

The protocol will be performed with a commercialized ultrasound scanner. This is considered a noninvasive and very safe examination [24]. The technology is widely used to assess the elastic properties of peripheral muscles without any adverse outcomes [15, 25]. Previous studies have reported the use of SWE during pregnancy for both mother and fetal tissue assessment without any adverse outcomes. Therefore, the use of SWE for the assessment of the pelvic floor muscles of pregnant women is safe [26, 27].

Data collection

Women's characteristics

At the first visit, after validation of the inclusion and exclusion criteria, we will collect anthropometric data about the women: height (in cm), weight (in kg) and BMI (in $kg.m^{-2}$). Demographic data and obstetric history will also be collected during the first visit: age (in years), gestity, and verification of the absence of a previous delivery (cesarean or vaginal). The dominant side will be recorded: right-handed or left-handed.

Clinical pelvic floor assessment

We will perform a clinical pelvic floor assessment at each visit. This examination will follow the recommendation of the Pelvic Organ Prolapse Quantification system (POP-Q) [28]. We will perform the procedure with women in the lithotomy position after voiding and maximal strain on the Valsalva maneuver. The position of each point of the POP-Q will be expressed in negative or positive values (in cm), and the length of each segment of the POP-Q (genital hiatus (gh), perineal body (pb), total vaginal length (tvl)) will also be expressed in centimeters [28].

Ultrasound B-mode pelvic floor assessment

We will perform an ultrasound B-mode pelvic floor assessment at each visit of the study. This examination is performed with the woman in the lithotomy position after voiding. We will use an Aixplorer® device with an XC6–1 1–6 MHz abdominal curved probe (V12, Supersonic Imagine, France). We will measure the anteroposterior hiatal diameter (distance between the antero-inferior extremity of the pubic symphysis and the anorectal junction, in cm) at rest, during a maximal strain on the Valsalva maneuver and at maximal perineal contraction. For these measures, we will use the translabial perineal ultrasound approach widely described by Dietz et al. [29, 30]. We will ask women to perform two initial Valsalva maneuvers with biofeedback instruction to prevent levator coactivation from serving as a confounding factor in our analysis [31].

Shear wave elastography assessments

As previously stated, an assessment of the elastic properties of the pelvic floor muscles of pregnant women will be performed at each visit using SWE. These measures will be performed for the *levator ani*, the external anal sphincter, *biceps brachii and gastrocnemius medialis* muscles. Each measurement will be performed on the right side of the woman, as it would be ideal to obtain all the measurements for the same side, preferentially while the women are in left lateral decubitus, which offers the possibility of accessing the right limbs. For each muscle's location, we will investigate the muscle during three conditions: rest, stretch and subjective maximal contraction. We will use an Aixplorer® device (V12, Supersonic Imagine, France) with a linear SL 18–5 probe (5–18 MHz). Every measure will begin by performing a B-mode procedure to locate the muscle. Then, we will proceed with recording a 10-s video clip of the SWE measurements. The region of interest will be outlined by hand and the measure of the shear modulus will be obtained within this region in postprocessing. For assessment at rest and during a stretch, we will consider the mean shear modulus of the video clip, whereas for assessment during contraction, we will consider the maximal shear modulus.

We will perform three measures for each condition (rest, stretch and contraction) and consider the mean of the three measures for analysis. We choose to consider the mean of the 3 acquisitions for each condition to maximize the reliability of the measurement by considering all the measures (the most intense and the weakest contraction, the first measure after installation, etc.)

As previously mentioned, we will measure the Young modulus using the Aixplorer® device, which will be divided by a factor of 3 to obtain the shear modulus, which is more accurate for anisotropic tissues such as muscle [23].

One single investigator will perform all the measurements.

– Specificity for the *levator ani* muscle assessment

For this assessment, we will use the procedure that we described for nonpregnant women in a previous publication [13]. The examination will be performed with the woman in the lithotomy position after voiding. We will first locate the *levator ani* muscle at its pubic insertion during a B-mode ultrasound using the procedure described by Dietz et al. for the diagnosis of levator avulsion, with 87% agreement between observers. We will place the probe in the sagittal plane on the perineum and apply a 10° parasagittal inclination to identify the muscle (Fig. 2) [32]. We will perform assessments during the three considered conditions: rest, stretch and subjective maximal contraction. For the stretch condition, the woman will be asked to perform a maximal Valsalva maneuver. We will prevent levator coactivation in the same way that we described for ultrasound pelvic floor assessment [31]. With this procedure, a previous study reported that the shear modulus measured in *levator ani* muscle in non pregnant women is about 16 kPa at rest and 35 kPa during Valsalva maneuver [13].

– Specificity for external anal sphincter assessment

The woman's position will be the same as for the levator ani muscle. We will place the probe on the perineum immediately above the anus in the axial plane (Fig. 3). We will first locate the external muscle using a B-mode ultrasound and then proceed to the SWE assessments in the middle of the anterior zenith of the sphincter ring for the three conditions: rest, maximal Valsalva maneuver and subjective maximal perineal contraction [33].

– Specificity for biceps brachii muscle assessment

First, we will identify the proximal and distal insertion of the biceps brachii using B-mode ultrasound and perform SWE acquisition midway between these insertions for three conditions: rest, standardized extension, and subjective maximal contraction. We will perform an assessment at rest performed with the upper limb having a 90° flexion of the elbow, which will be at the same height as the shoulder, with the hand in the pronation position. The forearm will rest on a flat support, allowing the *biceps brachii* to be totally free and accessible (Fig. 4A). We will systematically verify the 90° flexion of the elbow using a digital goniometer. For the assessment during extension, the position will be the same but with a 180° extension of the elbow (verified with the digital

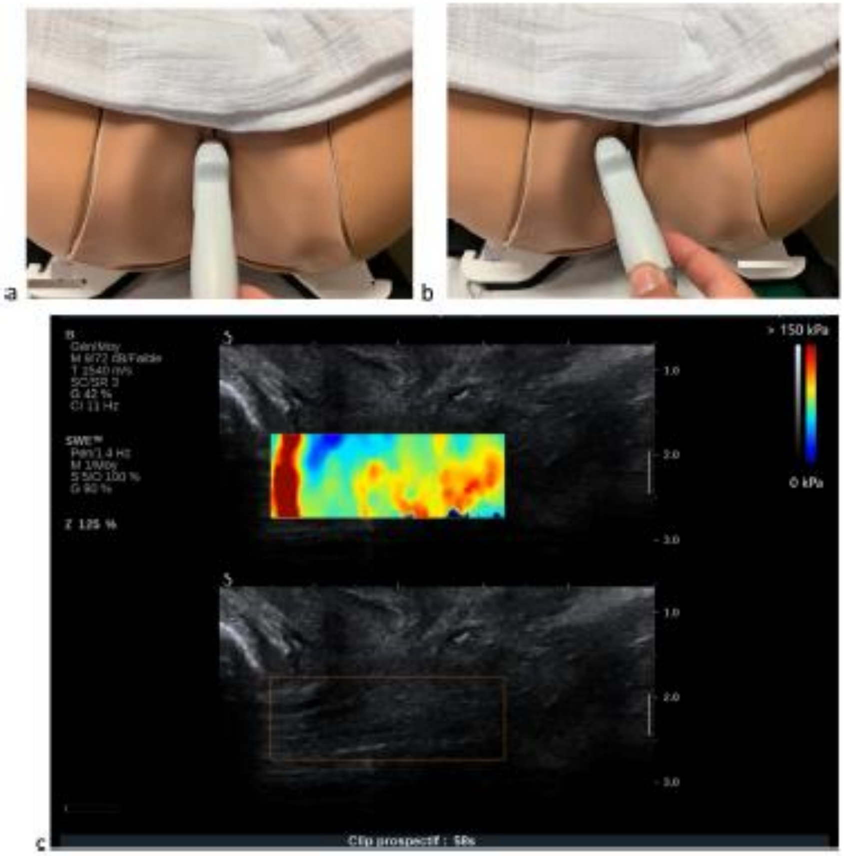

Fig. 2 Levator ani muscle SWE assessment: probe position and example of acquisition

goniometer) and the hand in the pronation position (Fig. 4b). Finally, for the measurements during contraction, we will ask the woman to have a subjective maximal contraction of her *biceps brachii* in the rest assessment position. With this procedure, a previous study reported that the shear modulus measured in *biceps brachii* muscle in non pregnant volunteer is about 3 kPa at rest and 19 kPa when stretched [25, 34].

- Specificity for gastrocnemius medialis muscle assessment

Usually, this measure is performed with the volunteer lying down in ventral decubitus. Because of the evident risks of compression of the gravid uterus, such a position is not ideal for pregnant women, and so the assessments will be performed while the woman is in left lateral decubitus. First, we will identify the proximal and distal insertions as well as the lateral borders of the *gastrocnemius medialis* in B-mode ultrasound. We will perform the SWE acquisition midway between the lateral borders and midway between the proximal and distal insertions of the muscle for the three conditions: rest, standardized extension, and subjective maximal contraction. For the assessment at rest, the left leg will be flexed, the right leg will be fully extended (180°, verified with the digital goniometer) and the ankle will be in a neutral position (Fig. 5a). For the measurement during extension, the woman will be in the same position but with the right foot supported on a 20° inclined plane to apply a standardized extension of the *gastrocnemius medialis* (Fig. 5b). Finally, we will proceed to obtain the measurement during contraction with the woman in the same position as for the assessment at rest but with a voluntary maximal contraction of the *gastrocnemius medialis*. With this procedure, a previous study reported that the shear modulus measured in *gastrocnemius medialis* muscle in non pregnant volunteer is about 3.1 kPa at rest [25].

- *Data related to mode of delivery*

After childbirth, we will obtain the following data from the subjects' medical files:

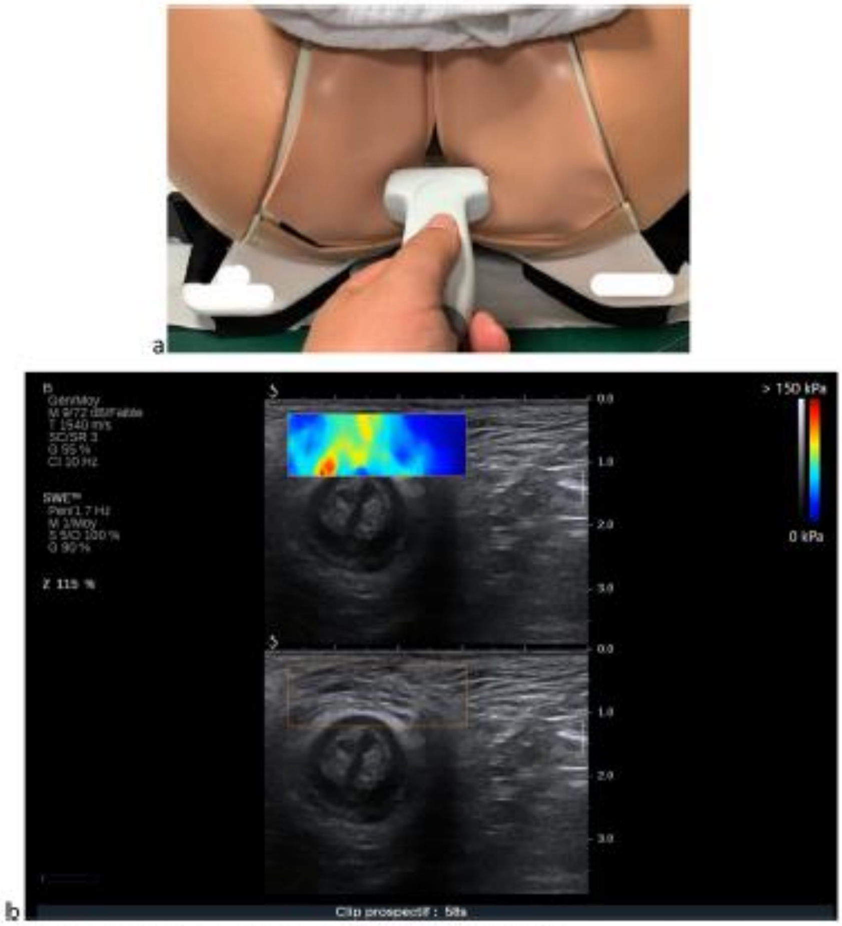

Fig. 3 External anal sphincter SWE assessment: probe position and example of acquisition

- spontaneous or induced labor
- epidural analgesia
- duration of the second stage of labor (time between full cervical dilatation and the birth, in minutes)
- duration of the expulsive phase (time between the onset of pushing and the birth, in minutes)
- mode of delivery (spontaneous vaginal delivery, operative vaginal delivery, cesarean delivery)
- indication for potential cesarean delivery (fetal distress, other)
- type of instrument used for potential operative delivery (vacuum, forceps, spatulas)
- indication for potential operative delivery (fetal distress, other)
- episiotomy use
- potential perineal tears classified according to the Royal College of Obstetricians and Gynaecologists (RCOG) guidelines [35, 36].

Analysis

Judgment criteria

The primary judgment criteria will be the evolution of the shear modulus of the pelvic floor muscle (*levator ani* and external anal sphincter) across the pregnancy assessed at rest, during the Valsalva, and during a contraction.

Secondary judgment criteria will be:

- the association between POP-Q measurements (especially gh and pb) and the elastic properties (shear modulus) of the levator ani muscle at each visit;
- the association between perineal B-mode ultrasound measurements and the elastic properties (shear modulus) of the levator ani muscle at each visit;
- the changes in shear modulus of the *biceps brachii* and *gastrocnemius medialis* muscles during pregnancy compared to the pelvic floor muscles;

Fig. 4 SWE acquisitions of the *biceps brachii* muscle at rest and standardized extension

Fig. 5 SWE acquisitions of the *gastrocnemius medialis* muscle at rest and standardized extension

- the association among the shear modulus of the pelvic floor muscles (*levator ani* and external anal sphincter) at the last visit, the mode of delivery (spontaneous vaginal delivery, operative vaginal delivery, cesarean delivery) and the potential occurrence of a perineal tear (RCOG-WHO classification, French guidelines) [35, 36].

Plan of analysis

We will describe the anthropometric and sociodemographic characteristics of the included women. Age, BMI and term of pregnancy will be reported as the mean and standard deviation (SD). For all the other analysis, we will only consider data about women who completed the three planned visits. For each trimester, we will only collect continuous variables, which will be reported as the means and SDs. Changes in these variables during pregnancy will be investigated using a Friedman test. Obstetric data will be reported as the means and SD for continuous variables and as percentages and frequencies for categorical variables.

Regarding the study endpoints, we will first report the main outcome of this study, which consists of the changes in the pelvic floor muscle's shear modulus across the pregnancy. Then, we will report the changes in all other measured pelvic floor-related parameters (POP-Q measurements, ultrasound B-mode measurements). We will look for an association between the shear modulus of the levator ani muscle and clinical (POP-Q measurements) and ultrasound B-mode assessments at each trimester using a Spearman correlation coefficient calculation. Second, we will report the changes in the shear modulus of the *biceps brachii* and *gastrocnemius medialis* muscles to look for changes between the different investigated locations. Third, we will look for an association between the shear modulus of the pelvic floor muscles (*levator ani* and external anal sphincter) and both the mode of delivery (vaginal or cesarean delivery) and perineal tear occurrence using univariate analysis. Variables with a level of significance greater than $p < 0.15$ in univariate analysis will be included in the multivariate analysis using a logistic regression. We will perform statistical analysis with Stata software (version V14IC; Stata Corporation, College Station, TX, USA). For all analyses, significance will be considered for $p < 0.05$, and we will calculate odds ratios (ORs) with 95% confidence intervals when appropriate.

Study duration

We have planned for an 18-month period of inclusion, which led to a total study duration (from the inclusion of the first women to the end of the follow-up of the last women) of 24 months.

Ethical and reglementary considerations

Every volunteer will receive oral and written information about the study and must give her free and informed written consent before any investigation. The study was approved by an ethical committee (*Comité de Protection des Personnes Ile de France VIII*) and is referenced with the ID RCB: 2018-A01422–53. The study is also registered on https://clinicaltrials.gov (NCT03602196).

Availability of data and materials

Supporting data could be accessed on request to Poitiers University Hospital, Department of gynecology and Obstetrics, France.

Discussion

Short summary of the study

It is difficult to predict the outcome of severe perineal trauma (OASI and/or levator avulsion) at childbirth, as there is a strong potential of an alteration of the woman's health. One hypothesis to optimize the efficiency of risk prediction is to consider the intrinsic biomechanical characteristics of women's pelvic floors. Such an approach may allow an individualized risk assessment personalized information for each woman. Our prospective, monocentric, longitudinal study will include 60 nulliparous pregnant women. Three visits are planned in this protocol (one per trimester of pregnancy) and will include clinical (POP-Q) and ultrasound assessment of the pelvic floor, SWE assessment of the pelvic floor muscles (*levator ani*, external anal sphincter) and the *biceps brachii* and *gastrocnemius medialis* muscles. Finally, data about the mode of delivery (cesarean section or vaginal delivery) and the occurrence of perineal tears will be collected. The main endpoint will be to describe the changes in the elastic properties of the pelvic floor muscles across pregnancy. The secondary endpoints will be to look for an association between SWE measurements of the *levator ani* muscle and clinical and ultrasound perineal assessments, to compare muscular changes during pregnancy among the pelvic floor muscles and the *biceps brachii and gastrocnemius medialis* muscles and to look for an association among the elastic properties of the pelvic floor muscles, the mode of delivery and the occurrence of perineal tears.

Justification of methodological choices

Choice of shear wave elastography technology to investigate pelvic floor muscles

Few other techniques have been proposed for investigating the elastic properties of pelvic floor muscles. Kruger et al. reported the use of a vaginal elastometer to investigate the elastic properties of the *levator ani* muscle in both pregnant and nonpregnant women [14, 37]. This device consists of a vaginal speculum with several force sensors, allowing the acquisition of a force/displacement curve. Such a device is quite interesting, but because it measures the properties of both the vaginal wall and the *levator ani* muscle, the measurements of the elastic properties of the *levator ani muscle* might be biased. Furthermore, we think that the vaginal intrusion could be associated with a lower participation rate since it involves the intromission of a medical device by an investigator. Egorov et al. developed a vaginal tactile imaging device consisting of a vaginal ultrasound probe supplemented with force and temperature sensors [38, 39] . Such a device is expected to provide an assessment of the pelvic floor elastic properties. We consider that this technique presents the same limitations as the vaginal elastometer of Kruger et al. [14, 37]. Static elastography is another ultrasound-related functional imaging technology that can be used to assess women's pelvic floors with a non-invasive approach [40–43]. However, this technique has major limitations in providing non direct and qualitative assessments of the pelvic floor.

The choice of the transperineal approach to assess pelvic floor muscles is supported by an important literature reporting that such an approach is efficient (in terms of acceptability and reliability) to investigate pelvic floor muscles [29, 30, 32, 33]. In 2018, our research team published a feasibility study on the use of SWE to investigate the elastic properties of the *levator ani muscle* in non-pregnant women with this transperineal approach [13]. In this paper, we report that we were able to individualize the *levator ani* muscle and to measure a shear modulus in 100% of women with a lower than 35Kg.m^{-2} BMI which allows to report the feasibility of the procedure. We consider that the fact that we investigate only the right *levator ani* muscle do not induce any bias considering that in this previous study, we reported that there are no differences between the elastic properties of the right and left *levator ani* muscles, assessed using SWE [13]. There is no published technique for investigating in vivo the elastic properties of the external anal sphincter. Considering the easy access to the external anal sphincter using ultrasound with a transperineal approach and the efficiency of SWE in other muscle applications, we consider that this choice is relevant [33]. In the future, this examination could be easily performed in the ultrasound follow-up of pregnant women. We do not have data about the reproducibility of pelvic floor muscles assessment using SWE. Nevertheless, considering the easy access to these muscles with a transperineal approach and the fact that SWE is a reliable tool for assessing peripheral muscles we expected a good reproducibility of the technique.

Choice of investigating biceps brachii and gastrocnemius medialis muscles

We expect to study muscles with different characteristics. Considering that the *biceps brachii* is not exposed to any increases in mechanical loading related to pregnancy, we expect to find a different pattern compared to pelvic floor muscles. The difference might be less pronounced for the *gastrocnemius medialis* since this muscle is exposed to an increase of loading due to the increase in weight that occurs during pregnancy. We also chose these two peripheral muscles because they are superficial, large and easily accessible muscles. Furthermore, we have data reporting that SWE is reliable to investigate these muscles with high performance reliability indicators [25].

Justification of inclusion and exclusion criteria

We choose to include only nulliparous women in this study. This choice is easily understandable by the willingness to avoid bias related to any previous obstetrical perineal trauma. The elastic properties of the pelvic floor muscles that we will report in this study will be solely related to the intrinsic characteristics of the woman and the changes induced by the pregnancy. We will also exclude women with a BMI above 35 kg.m^{-2}. This is due to the results of a feasibility study that reported difficulties in SWE assessments of the *levator ani* muscle during the Valsalva maneuver for women with high BMIs [13]. These difficulties were due to a loss of visibility of the *levator ani* during the maneuver using a superficial linear probe; the muscle became too deep to be clearly located.

Expected results

Concordance with animal experimentations

As we mentioned in the background section, human data about the evolution of pelvic floor muscles during pregnancy are lacking. It has been reported in animal experiments that during pregnancy, the stiffness of the pelvic floor muscle increases due to a drastic increase in total collagen content [9–11]. As we mentioned it in the background section, these animal experimentation related data must be interpreted carefully considering that there is no work with a confirmation that these phenomena occur in a same way in pregnant women.

We expect to report a similar increase in the stiffness of the pelvic floor muscles during pregnancy in our study, which will support the data from animal experiments. Such an increase in stiffness might be a protective process from perineal trauma given that tissue with the lowest stiffness easily raises their plasticity threshold to a level beyond which irreversible damage occurs in the tissue [12].

One potential confounding factor for the interpretation is that we do not have data about the use of perineal stretching device such as Epi-No® during pregnancy. There is no data about the impact of such a practice on pelvic floor muscle elastic properties. Some works reports an increase in perineal extensibility, but it reports maximal vaginal compliance to the Epi-No® without data about a direct assessment of pelvic floor muscles stiffness [44, 45]. Furthermore, its use is not recommended in French guidelines [36]. This considered, we think that the risk of bias in our cohort is weak.

Concordance between SWE measurements and clinical / ultrasound measurements

We expect to report an association between the elastic properties of the *levator ani* muscle and the clinical and ultrasound assessments of pelvic floor distension. For the clinical assessment, we will investigate all POP-Q measurements but with a special interest in the gh and pb measurements that are performed during the Valsalva maneuver and that reflect pelvic floor distension that occurs during the maneuver. A similar association will be investigated for the ultrasound assessment (distance between the pubic symphysis and the anorectal angle). If we can report such a correlation between the elastic properties of the *levator ani* investigated using SWE and clinical / ultrasound pelvic floor distension, it will support the efficiency and the applicability of SWE for functional pelvic floor muscle assessments. We choose to focus on the *levator ani* muscle for this analysis given the well-reported association between levator hiatus and pelvic organ mobility [46].

Preliminary data about the hypothetical association between elastic properties of women's pelvic floors and obstetric perineal trauma

Finally, we will look for a potential association between the elastic properties of the pelvic floor muscles and the occurrence of perineal tears as well as the mode of delivery. Due to the expected number of women, it will not be possible to conclude about such an association. The objective is to provide preliminary data about the distribution of pelvic floor muscles elastic properties according the stage of perineal tear. We expect that these preliminary data would allow the future implementation of a larger multicentric prospective study investigating the interest of including the elastic properties of the pelvic floor muscles in our risk prediction of perineal trauma. This is required to offer each pregnant woman personalized information and an individualized preventive strategy. One prospect might be a selective use of episiotomy in high-risk women considering that this intervention, in a biomechanical study, reduce the stress on the muscles and the force required to delivery successfully [47]. Our data might be helpful in providing a justification for implementing this type of study and offering the possibility of performing a power calculation.

Abbreviations

BMI: Body Mass Index; Gh: Genital hiatus; OASI: Obstetric Anal Sphincter Injury; Pb: Perineal body; POP-Q: Pelvic Organ Prolapse Quantification; RCOG: Royal College of Obstetricians and Gynaecologists; SD: Standard Deviation; SWE: Shear Wave Elastography; Tvl: Total vaginal length; WHO: World Health Organization

Acknowledgments

None.

Authors' contributions

All authors have read and approved the manuscript. BG - Main text writing, main investigator, study design, methods elaboration. XF – Review of each version of the manuscript, methods elaboration (clinical aspects). FP - Review of each version of the manuscript, methods elaboration (clinical aspects). AN – Review of each version of the manuscript, methods elaboration (biomechanical aspects).

Funding

There is no funding for this study.

Availability of data and materials

Not applicable. This is a study protocol.

Ethics approval and consent to participate

Every volunteer receives an oral and written information about the study and give her free and informed written consent before any investigations. The study is approved by an ethical committee (*Comité de Protection des Personnes - Ile de France VII: Hopital Ambroise Paré – 9 avenue Charles de Gaulle 92100 Boulogne-Billancourt, France*) and referenced with the ID RCB: 2018-A01422–53. The study is also registered on https://clinicaltrials.gov (NCT03602196).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Transperineal ultrasound shear-wave elastography is a reliable tool for assessment of the elastic properties of the levator ani muscle in women

Bertrand Gachon[1,2,3]✉, Xavier Fritel[1,3], Fabrice Pierre[1] & Antoine Nordez[2,4]

Our main objective was to assess the intraoperator intersession reproducibility of transperineal ultrasound Shear Wave Elastography (SWE) to measure the levator ani muscle (LAM) elastic properties. Secondary objective was to compare reproducibility when considering the mean of three consecutives measurements versus one. In this prospective study involving non-pregnant nulliparous women, two visits were planned, with a measurement of the shear modulus (SM) on the right LAM at rest, during Valsalva maneuver and maximal contraction. Assessments were done with a transperineal approach, using an AIXPLORER device with a linear SL 18–5 (5-18 MHz) probe. For each condition, 3 consecutive measures were performed at each visit. The mean of the three measures, then the first one, were considered for the reproducibility by calculating intraclass correlation coefficient (ICC), and coefficient of variation (CV). Twenty women were included. Reproducibility was excellent when considering the mean of the 3 measures at rest (ICC = 0.90; CV = 15.7%) and Valsalva maneuver (ICC = 0.94; CV = 10.6%), or the first of the three measures at rest (ICC = 0.87; CV = 18.6%) and Valsalva maneuver (ICC = 0.84; CV = 19.9%). Reproducibility was fair for measurement during contraction. Transperineal ultrasound SWE is a reliable tool to investigate LAM elastic properties at rest and during Valsalva maneuver.

Abbreviations

BMI	Body Mass Index
CV	Coefficient of variation
ICC	Intraclass correlation coefficient
LAM	Levator ani muscle
PFD	Pelvic floor disorders
SEM	Standard error of measurement
SM	Shear modulus
SWE	Shear wave elastography

Pelvic floor disorders (PFDs) are frequently occurring conditions, and up to 20% of women experience PFDs during their lifetime[1]. Although the pathophysiology of PFDs is complex, some of these disorders may be explained by vaginal delivery, which can induce pelvic floor damage involving the levator ani muscle (LAM) and/or anal sphincter in up to 15% of women[2,3]. This is supported by data reporting that LAM avulsion is associated with an increased levator hiatus area, which is a primary risk factor of pelvic organ prolapse[3,4]. However, the individual pathophysiology of pelvic floor damage itself remains poorly understood, and safe strategies for identifying high-risk women based on their intrinsic characteristics are desperately required[2].

Studies using animal models and/or ex vivo human tissue analysis have suggested that since the elastic properties of pelvic floor muscles are the first to change during pregnancy, these elastic properties may be associated

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with PFD[2,5,6]. The current main limitation is that information about the human mechanical behavior of pelvic floor muscles in vivo is lacking. Indeed, until recently, ex vivo and destructive biomechanical analysis was required to characterize muscle's mechanical behavior. Therefore, while the literature suggests an association between pelvic floor muscles (and especially the main one, the LAM) and obstetrical pelvic floor damage and /or pelvic floor disorders, this hypothesis remains to be tested in vivo[7,8]. Thus, one of our current challenges is to identify innovative techniques for the assessment of pelvic floor elastic properties to better understand the potential role of these properties in the occurrence of obstetric pelvic floor damage and/or PFD. Furthermore, this technique must be reliable to be implemented in both research designs and clinical practices. The viscoelastic properties of pelvic floor muscles could be measured using a speculum combined with sensors, i.e., a vaginal probe able to measure the force exerted by the pelvic floor muscles on it[9,10]. However, they require an intravaginal intrusive examination, and it remains an indirect measurement on pelvic floor muscles elastic properties (based on the force exerted on the probe/speculum)[2,11].

Our study focused on a direct quantitative assessment of muscle elasticity using ultrasound shear-wave elastography (SWE). In this technique, a mechanical perturbation was generated using ultrasound to induce the propagation of a shear wave along the main axis of the ultrasound probe[12–14]. The speed of the wave's propagation was correlated to the shear modulus and the stiffness of the tissue, since the stiffer the tissue, faster was the wave's propagation[12–14]. This technique seems relevant compared to static or quasistatic elastography because allows a direct quantitative measurement of muscles elastic properties (without the interposition of a standoff pad). In addition, the measurement can be made along the muscle fiber direction, while static elastography measures the transverse behavior (i.e., hardness) that is probably less physiologically relevant[14–17]. Furthermore, SWE is feasible with a superficial linear probe offering the possibility of a transperineal approach for investigating the LAM using the technique of Dietz et al. without any intravaginal examination[18].

In a previous report we described the feasibility of assessing the elastic properties of LAM in women, using SWE with a transperineal approach. In the present study, we investigate inter-day and intra-operator reliability for LAM to validate its use in future prospective studies. We also investigate the intrasession reliability to check if the procedure could be simplified by doing only one single measure instead of 3 consecutives measures[11]. So, the main objective of the present study was to assess the intraoperator intersession reproducibility of ultrasound SWE to measure the LAM elastic properties in women[12]. The secondary objectives were as follows: (i) to investigate the intrasession reproducibility and (ii) to compare intersession reproducibility when considering the mean of three consecutive measurement versus one single measurement.

Material and methods

Study settings. This prospective monocentric study was conducted in our University Department of Obstetrics and Gynecology from July 2019 to August 2020. In the protocol, the time interval between two visits ranged from 12 h to 7 days.

Population. Eligible participants were non-pregnant, nulliparous women attending a visit to our Gynecology unit. The exclusion criteria were as follows: history of previous delivery (vaginal or cesarean section), personal history of PFD, obese women with a body mass index (BMI) higher than 35 kg·m^{-2}, women with muscular disease, women requiring admission to a psychiatric unit, women under judicial protection, and those who were unable to understand French language.

Data collection. *Participant characteristics.* At the first visit, the participants' age, height, and weight were collected, and their BMI was calculated.

Shear wave elastography assessments. The evaluation protocol during the two visits was similar: ultrasound SWE assessment of the LAM at rest, during subjective maximal Valsalva maneuver, and during subjective maximal perineal contraction. For subjective maximal Valsalva maneuver, it was required from the women to take a deep breath and to push down as much as possible with a closed glottis. This will highly increase the intraabdominal pression and so will induce a cranio-caudal descent of pelvic organs leading to a distension of the levator hiatus with a lengthening of the LAMs. It can be considered as a lengthening of the LAM that should induce an increase in shear modulus[14]. This is in accordance with the childbirth condition because the effort required from the mother is the same and that the same phenomena of LAM lengthening that occurs at childbirth even if the strain magnitude is much higher. This is also in accordance with pelvic floor disorders because the occurrence of pelvic organ prolapse is associated to an overlengthening of the LAMs when intraabdominal pressure increases leading to a prolapse of pelvic organs through it. For the subjective maximal contraction, it was required from the women to contract and tighten her perineum as much as she can. It is also in accordance with the effort performed during physiotherapy procedures. This is an important part of pelvic floor disorders management. Finally, the rest position represents the condition with the lowest load to estimate the intrinsic resting elastic properties of the LAM. For each condition (rest, Valsalva maneuver, and maximal contraction), three consecutive measurements were performed. All measurements during both visits were performed by a single operator, a senior urogynecologist (BG, the first author) with a special interest in pelvic floor imaging. We chose to consistently obtain ultrasound measurements on the right side of the participants based on the convenience of the operator, who was at the right when the participant was in the supine position, and to standardize the procedure.

The principles of ultrasound SWE and the procedure for measuring muscle elastic properties have been widely described and illustrated in previous publications, and specific aspects pertaining to the LAM are described below[11–14].

For measurements in each condition, the participants lay down in the lithotomy position with an empty bladder. The pubic insertion of the right LAM was identified in B-mode ultrasound with a transperineal approach using the procedure reported by Dietz et al., after which we proceeded to perform the SWE acquisition[11,12,18]. Before any LAM assessment, the participants performed 2 initial Valsalva maneuvers with biofeedback, in which visible pelvic floor displacements on the B-mode image were shown to the participant on the screen, to prevent LAM coactivation[19]. For assessments at rest, the participant was asked to relax as much as possible. For assessment during the Valsalva maneuver, the participant was requested to perform a maximal Valsalva maneuver for at least 5 s. For assessment during subjective maximal contraction, the participant was asked to contract her perineum as if she wanted to avoid gas leakage for at least 5 s. Figure 1 shows the LAM assessment at rest (a) and in the subjective maximal Valsalva maneuver (b).

Ultrasound measurements were performed using an AIXPLORER device (V12, SUPERSONIC IMAGINE, France) with a linear SL 18–5 probe (5–18 MHz). As reported above, the muscle location was assessed in B-mode, after which we performed SWE acquisition in a 5-s video clip. Shear modulus (SM) values were averaged over this period. The clip was obtained to limit the influence of inevitable temporal changes (5%)[20]. To provide an idea of the temporal changes during measurements, a video clip of an ultrasound SWE assessment of the LAM during the Valsalva maneuver is provided as online supplementary material (Supplementary material 1).

Data analysis and statistics. The region of interest was identified and contoured manually using Matlab scripts (The Mathworks, Inc., 2016). For assessments at rest and during the Valsalva maneuver, the mean SM for the whole acquisition was considered. For assessments during subjective maximal perineal contraction, the maximal SM for the acquisition was considered. In case of limited region within the measurement is not possible, the software automatically excludes it for the analysis. As mentioned in a previous publication, the AIXPLORER device provides a measurement of the Young's modulus that is valid for isotropic tissues. Since muscles are transverse isotropic tissues, the SM was measured by dividing the Young's modulus by 3[11,12,14,21,22]. In a material, the measure shear wave speed (Vs) along a given direction could be converted to a shear modulus (mu) along this direction thanks to this equation: $mu = rho\ Vs^2$. Considering an incompressible isotropic material, mu (or Vs) could be converted to the Young's modulus (E): $E = 3mu = 3\ rho\ Vs^2$. In a muscle, since it is not an isotropic material, E is not equal to 3 mu anymore. However, the relationship $mu = rho\ Vs^2$ remains valid. The AIXPLORER directly provides $E = 3\ rho\ Vs^2$ (wrong equation for muscles). Therefore, for muscles, it is recommended to divide the values by 3 to assess the shear modulus rather than the Young's modulus. Dividing the Young's modulus values by 3 is equivalent to directly consider $mu = rho\ Vs^2$[14,21,22].

We first described our population in terms of age, mean BMI, and the interval between the two assessments. Continuous variables were reported as mean and standard deviation, and categorical variables by numbers and percentages. On the basis of our primary objective, we analyzed the intersession reproducibility for each mode of assessment (rest, Valsalva, contraction), with the intraclass correlation coefficient (ICC), the standard error of measurement (SEM), and the coefficient of variation (CV) serving as the main judgment criteria. For this analysis, we considered the mean of the three consecutive measurements performed in each session for the analysis. We computed the ICC with 95% confidence intervals for each assessment and calculated the CV[23]. Bland–Altman plots were built according to the methods reported in the original publication[24,25]. Regarding the ICC value we therefore considered that the reliability was excellent if 0.90 or higher, good if between 0.75 and 0.89, moderate if between 0.50 and 0.74 and poor if lower than 0.50[23].

To address our secondary objectives, we investigated the intrasession reproducibility within three consecutive measurements by using the same methods as for the primary objective: ICC, SEM, and CV. ICC values were interpreted as reported above. We then compared the reproducibility performance when considering the mean of the three measurements or the first of the three consecutive measurements. A priori power calculation was not performed. Considering other studies reporting reliability analysis for ultrasound SWE in peripheral muscles, a study population of 20 women appears to be sufficiently effective[20].

Statistical analysis was performed using the STATA software (version V14IC; Stata Corporation, College Station, TX, USA). For all analyses, significance was considered for $p < 0.05$.

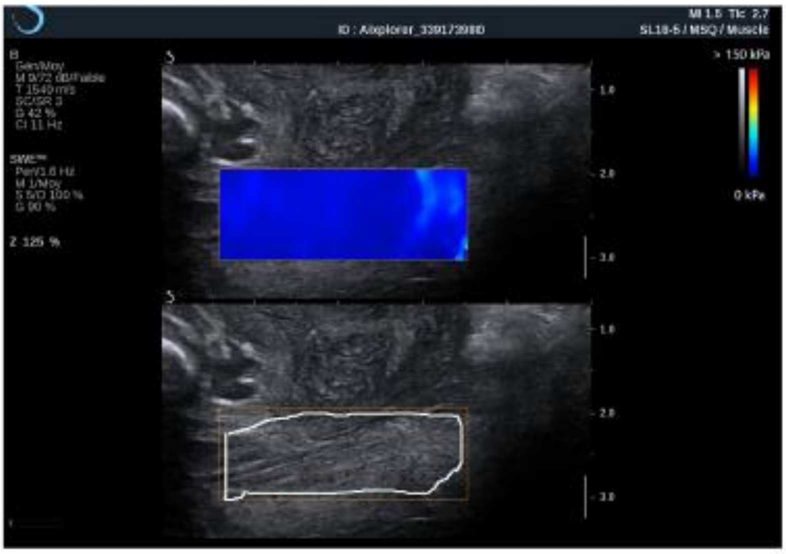
Ethical and reglementary considerations. The study was approved by an ethics committee *(Comité de Protection des Personnes Ile de France 8,* ethical committee for human protection from Ile de France) on the 16/07/2018 and is referenced with the ID RCB: 2018-A01422-53. The study was registered on https://clinicaltrials.gov (NCT03602196) on the 26/07/2018. All methods were carried out in accordance with relevant guidelines and regulations. Written and informed consent was obtained from all subjects before any investigation.

Results

Twenty women were included in this study. Their mean age was 23 years (SD = 4 years) with a mean BMI of 22.6 $kg \cdot m^{-2}$ (SD = 3.2 $kg \cdot m^{-2}$). The mean interval between the two visits was 46.6 h (SD = 39.6 h; range, 24–166 h). All included women completed the study protocol.

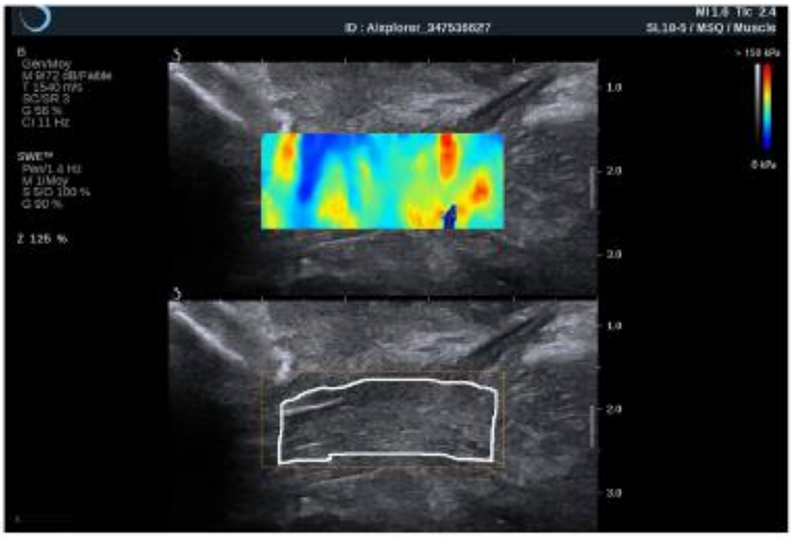
In our main analysis, the ICC was excellent for the intersession reproducibility, considering the mean of the three measures at rest and during the Valsalva maneuver (Table 1). Conversely, ICC was poor for measurements performed during subjective maximal contraction (Table 1). Bland–Altman plots are shown in Fig. 2. The results for intrasession reproducibility are reported in Table 2, and they show good reliability at rest and during the Valsalva maneuver, but moderate during subjective maximal contraction.

In Table 1, we report the reproducibility performance indicators for both analyses when considering the mean of the three measurements for each visit and when considering the first of the three consecutive measurements. ICC and other reliability indicators were higher when considering the mean of the three measurements for the



a – Levator ani muscle assessment at rest (the colored scale indicates Shear modulus values)

zones contoured with withe line represent the levator ani muscle (region of interest)



b - Levator ani muscle assessment at Valsalva maneuver (the colored scale indicates Shear modulus values)

zones contoured with withe line represent the levator ani muscle (region of interest).

Figure 1. Assessment of the right *levator ani* muscle at rest (**a**) and Valsalva maneuver (**b**).

rest and Valsalva maneuver measurements (Table 1). Reliability during subjective maximal contraction was poor, regardless of whether we used the mean or the first measurement alone.

	Mean shear modulus at V1, in kPa (SD)	Mean shear modulus at V2, in kPa (SD)	ICC [95% CI]	CV, in %	SEM, in kPa
Intersession reproducibility performances by considering the mean of the 3 measures at each visit					
Rest	22.8 (8.0)	21.9 (6.8)	0.90 [0.80–0.95]	15.7	3.5
Valsalva	44.5 (13.1)	46.5 (14.2)	0.94 [0.88–0.97]	10.6	4.8
Contraction	59.3 (11.8)	55.1 (15.7)	0.43 [0.07–0.69]	25.1	14.8
Intersession reproducibility performances by considering one single measure at each visit					
Rest	22.2 (8.3)	22.0 (7.0)	0.87 [0.74–0.94]	18.6	4.1
Valsalva	43.2 (13.1)	44.2 (16.1)	0.84 [0.68–0.92]	19.9	8.7
Contraction	60.2 (12.0)	56.2 (16.8)	0.61 [0.31–0.80]	22.9	13.3

Table 1. Intersession reproducibility performances for the assessment of the right levator ani muscle's shear modulus. ICC: Intraclass correlation coefficient, CI: confidence interval, CV: coefficient of variation, SEM: standard error of measurement, V1: first visit, V2: second visit.

Discussion

Main results. The intersession reproducibility of ultrasound SWE for measuring the elastic properties of the LAM was excellent at rest and during the Valsalva maneuver but poor during subjective maximal contraction. The reproducibility performance of the mean of three consecutive measurements for each session was higher than that of the first of the three consecutive measurements.

Justification of methodological choices. We chose the ultrasound SWE method since it is allowing non-invasive and quantitative assessment of the pelvic floor muscles. We have previously reported the feasibility of measuring LAM elastic properties without difficulties, supporting our choice to focus on this approach in the present study[12]. We systematically investigated the right LAM to ensure operator convenience (since the operator was usually on the right side of the supine participant). This approach appears safe since we only included nulliparous women, thereby avoiding women with levator avulsion. Furthermore, a previously feasibility pilot study reported no differences in the SM measured on the right versus left LAM[12]. We considered BMI higher than 35 kg·m^{-2} as an exclusion criterion because measurements for women with very high BMIs could not be performed due to loss of LAM visibility in B-mode ultrasound during the Valsalva maneuver[12]. Finally, for the main analysis, we chose to consider the mean of three consecutives measurements performed at each visit instead of a direct single measurement. We hypothesized that reproducibility will probably be better with this approach since it is difficult to standardize a lithotomy position and even more difficult to standardize a Valsalva maneuver.

Strengths and Limitations. The main strength of this study is that it provides data about an innovative approach to investigate the elastic properties of pelvic floor muscles with a non-invasive approach that will be much more acceptable for women than other techniques using vaginal speculums or vaginal ultrasound probes[9,26–28].

The primary limitation of this study is that we only reported intra-operator reproducibility data. This was due to the lack of an additional available experimenter, and because we aimed to use only one experimenter in our projects[11]. However, measurements performed by two experimenters may show specific interoperator discrepancies, and the interoperator reliability will have to be determined by groups that aim to have more than one experimenter in their protocol.

In this study, we made measurement of the mean shear modulus for the largest visible muscle region. Indeed, the viscoelastic properties of a tissues may differ according to the region. This might be true for the muscle which is generally stiffer near to its insertion. Therefore, the good reliability reported in the present study suggest that we were able to reproduce the measurements in a similar region and it is probably a criterion to get reliable measurements. We chose to measure the shear modulus in one single area because, in a clinical view, the part of the LAM accessible with such a transperineal approach is considered as the pubic insertion of the LAM (the one affected by obstetric perineal trauma) and it would not have been clinically justified to perform several measures in different areas.

Another limitation is the standardization of the LAM SWE measurement. Indeed, we only required the participants to lie down in the lithotomy position with an empty bladder without any measurement of thigh opening. This is particularly true for the subjective maximal contraction condition, where the intensity of the contraction was not controlled, since the measurement was highly dependent on the contraction level[29]. Thus, the conditions across measurements may not have been exactly comparable. However, this was a voluntary choice because we aimed to assess the reproducibility in *real-life* conditions since we aimed to perform such measurements in a clinical environment with pregnant women.

Lastly, we did not report any clinical examinations related data and so were not able to investigate the correlation between elastography considerations and clinical observations. Such an analysis is ongoing in a prospective study in pregnant women (ELASTOPELV)[11].

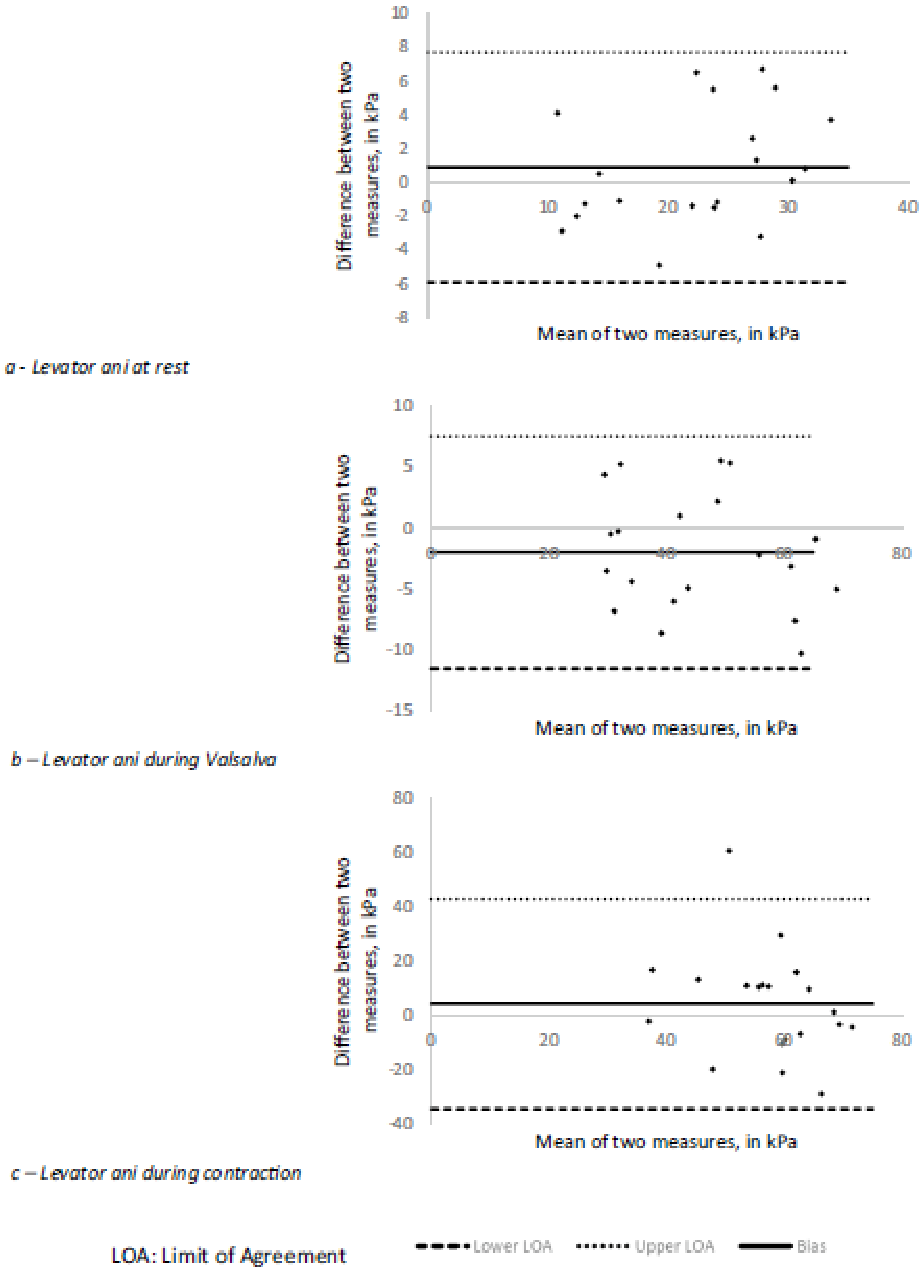


Figure 2. Bland–Altman plots of agreement between V1 (first visit) and V2 (second visit) for the mean levator ani muscle's shear modulus assessment at each visit and each condition.

Interpretation. We reported excellent reproducibility for assessments performed at rest and with the Valsalva maneuver. Only one previous report has described such a reproducibility analysis of LAM assessment using a transperineal approach, but that study used an abdominal curved probe. In that study, the authors reported good reproducibility of intra-operator intersession assessments at rest (ICC = 0.86 [0.58–0.95]) and during the Valsalva maneuver (ICC = 0.79 [0.54–0.91]), and they did not report measures during contraction[30].

In our results, the reliabilities at rest and during the Valsalva maneuver were excellent both when considering the mean of the three consecutive measurements and when considering only the first of the three consecutive

	1st measure mean shear modulus, in kPa (SD)	2nd measure mean shear modulus, in kPa (SD)	3rd measure mean shear modulus, in kPa (SD)	ICC [95% CI]	CV, in %	SEM, in kPa
Rest	22.1 (7.6)	22.7 (8.4)	22.2 (7.8)	0.84 [0.75–0.89]	21.1	4.7
Valsalva	43.7 (14.5)	46.9 (13.5)	46.8 (15.6)	0.88 [0.75–0.91]	16.6	7.6
Contraction	58.2 (14.6)	61.4 (14.9)	57.2 (15.5)	0.70 [0.57–0.80]	20.2	11.9

Table 2. Intrasession reproducibility performances for the assessment of the right levator ani muscle's shear modulus with 3 consecutive measures. ICC: Intraclass correlation coefficient, CI: confidence interval, CV: coefficient of variation, SEM: standard error of measurement.

measurements. This observation would have been the same if we had considered the second or third of the three measurements since the intra-operator intra-session reproducibility was good among these three measurements. This result is interesting and may have direct applications. On the basis of this result, it appears safe to perform a single measurement of the LAM using transperineal ultrasound SWE when the objective is to assess the elastic properties of this muscle at a specific and unique time. If the technique is used to investigate changes across time, it is probably safer to perform three measurements and to consider their mean for the analysis to increase the sensitivity of the examination.

Mean shear modulus for assessment during Valsalva maneuver and contraction were within the same range of values. This could be surprising because, in skeletal muscles, increases in shear modulus are much bigger during contractions[29] compared to passive lengthening[31]. A first explanation would have been a contraction of the LAM during the Valsalva maneuver that would have led to overestimate the stiffness of the muscle in this condition. This was probably not the case because we systematically took care of avoiding any LAM coactivation during Valsalva maneuver thanks to bio feedback procedures as recommended by Orno et al.[19]. In addition, we observed using B-mode ultrasound a very different behavior between tasks: an increase in muscle length and an horizontalization of its fibers during Valsalva maneuver, while a shortening of the muscle and a downward tilt of its fibers occurred during contractions. This supports the fact that we effectively measure the muscle properties in two different conditions. These results highlight the large lengthening of the LAM during a Valsalva maneuver that significantly increase its stiffness in a similar manner than during contractions. The increase in stiffness is probably much larger during childbirth explaining the risk of muscle trauma. Lastly, these interpretations about the value of the shear modulus of the LAM at contraction should be carefully considered regarding the poor reliability of such a measure, the difficulty to standardize the task and to be sure that the contraction is maximal.

The comparison with the literature on LAM elastic behavior remains complex because various methods do not provide values in the same metrics. We cannot compare our results about the LAM viscoelastic properties to biomechanical studies on cadaveric tissues because in these works, researchers aim to identify the level of strain for which damage occurs and not the intrinsic elastic properties. Our results are not comparable to study involving the use of vaginal speculum as an elastometer or vaginal probe because it measures a torque or a force applied on the device and recorded by force sensor, which is not a direct quantitative assessment of elastic properties as using elastography[9,10]. We can compare our data to other elastography studies. A more direct comparison can be done with the study of Tang et al. using SWE, reporting a 28 kPa shear modulus for the LAM at rest (versus 22 in our study) and 57 kPa during Valsalva maneuver (vs 45 in our study). Therefore, Tang et al. report a little stiffer LAM but in a very different population with a mean age of 56 years versus 23 in our study[30]. Finally, as done in our previous study[12], we can compare our results to a study of Silva et al. that calculated the elastic properties of the pubovisceral muscle using inverse finite element with three models. They reported a shear modulus of 78 ± 44 kPa with the first one, 80 + /- 48 kPa with the second one and 62 + /- 46 kPa with the last one[32]. Silva et al.'s reported a stiffer LAM muscle than in the present study, but values remained in the same range and very different methods were used. Taken these comparisons all together, the range of values reported in the present study seems consistent with the literature.

The LAM appears to be much stiffer than the peripheral muscles. Indeed, we reported an SM of 22 kPa for the LAM at rest, whereas it has been reported to be between 2 and 5 kPa for peripheral muscles of both the upper and lower limbs[20]. This difference may be primarily associated with differences in the intrinsic structure of these muscles, since the LAM mainly consists of type 1 muscular fibers (mainly involved in prolonged effort), whereas peripheral limb muscles mainly consist of type 2 muscle fibers[33]. Another hypothesis could be that measurements in the LAM were performed near the muscle's pubic insertion, whereas measurements for peripheral muscles were mainly performed in the middle of the muscle with distance to its insertions[20]. Furthermore, even if measurements were performed without the Valsalva maneuver or subjective maximal contraction and in a lithotomy position, there is always a constraint applied by abdominal pressure into pelvic floor muscles that could never be fully removed in in vivo conditions.

Our results showed that SWE is a reliable tool to investigate the elastic properties of pelvic floor muscles in vivo. This offers interesting prospects for research that will aim to improve our knowledge of the pathological processes associated with obstetric perineal trauma and/or PFD occurrence. A prospective study is ongoing in a pregnant women cohort that investigates changes in the elastic properties of pelvic floor muscles and peripheral muscles during pregnancy by using the same protocol as that described in this paper[11].

Conclusion

Ultrasound SWE is a reliable tool to investigate LAM elastic properties at rest and during the Valsalva maneuver, but the present study failed to perform reliable measurements during perineal subjective maximal contraction. This technology might be useful to improve our knowledge of the pathological processes associated with obstetric perineal trauma and/or pelvic floor disorders.

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Author contributions

Each author of this work meets the criteria for authorship: - BG contribute to the study design, performed the analysis, and wrote each version of the main text of this manuscript. He wrote the main text of the revised version of the manuscript according revivers comments. - XF and FP, contribute to statistical analysis, review each version of the manuscript. They contributed equally to the revision of the manuscript according reviewer comments. - AN contribute to the study design and data analysis, draft the work, and review each version of the manuscript. He contributed equally to XF and FP to the revision of the manuscript according reviewers comments.

Competing interests

The authors declare no competing interests.

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DOCTORAT BRETAGNE LOIRE / EDUCATION LANGAGES, INTERACTIONS COGNITION, CLINIQUE

UNIVERSITÉ DE NANTES

Titre : Caractérisation in vivo des propriétés viscoélastiques du plancher pelvien de la femme au cours de la grossesse

Mots clés : Levator ani ; sphincter anal externe ; accouchement ; prédiction ; élastographie

Résumé : Les déchirures périnéales graves survenant lors d'un accouchement sont des complications qui impactent de manière négative la santé des femmes (douleur, incontinence, sexualité). Nous émettons l'hypothèse que la prise en compte des propriétés élastiques des muscles du plancher pelvien de la femme pourrait optimiser les stratégies de prédiction existantes. La problématique est qu'il n'existait aucune technique permettant de mesurer ces propriétés in vivo, de manière quantitative et non invasive. Nous avons utilisé la technique d'élastographie par onde de cisaillement permettant de mesurer *in vivo* les propriétés élastiques d'un muscle et l'avons appliqué, pour la première fois, à l'étude des muscles du plancher pelvien. Nous avons ainsi pu démontrer qu'il était possible de mesurer les propriétés élastiques du muscle *levator ani* et sphincter anal externe chez la femme et ceci de manière reproductible. Nous avons ensuite utilisé cette technique dans une étude longitudinale évaluant les modifications des propriétés élastiques des muscles du plancher pelvien de la femme au cours de la grossesse. Cette étude a mis en évidence qu'il n'y avait pas de variation significatives des propriétés élastiques des muscles du plancher pelvien au cours de cette période. Les femmes chez qui survenait une déchirure périnéale à l'accouchement avaient un muscle sphincter anal externe moins rigide, en fin de grossesse, que celle avec un périnée intact. Ces résultats confirment notre hypothèse initiale et supportent la mise en place de travaux de recherches futurs et de plus grande ampleur dans cette thématique.

Title : In vivo definition of women's pelvic floor muscles viscoelastic properties through pregnancy

Keywords : Levator ani ; external anal sphincter; childbirth; prediction; elastography

Abstract : Obstetric perineal tears occurring at childbirth are negative outcomes that strongly impact women's health (pain, incontinence, sexuality). We hypothesized that considering the intrinsic elastic properties of women's pelvic floor muscles would optimize the efficiency of existing predictive strategies. However, there was no validated method allowing an *in vivo*, quantitative and non-invasive assessment of these elastic properties. We considered the technology of shear wave elastography allowing an *in vivo* assessment of a muscle's elastic properties and applied it, for the first time, to the study of pelvic floor muscles. Therefore, we reported that it is feasible to measure the elastic properties of the *levator ani* muscle and the external anal sphincter muscle and that these assessments were reliable. Then, we used this technology into a longitudinal study investigating any change in the elastic properties of women's pelvic floor muscles through pregnancy. We failed to report any significant changes in these muscles elastic properties during pregnancy. We reported that women suffering from any perineal tear at childbirth had a less stiff external anal sphincter during late pregnancy than those having an intact perineum at childbirth. This result is in accordance with our initial hypothesis and support the implementation of upcoming larger studies in this thematic.