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Faculdade de Medicina

Programa de Pós-Graduação Medicina Molecular

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TRANSLOCATOR PROTEIN (TSPO) EXPRESSION AND LOCALIZATION IN HUMAN ENDOMETRIUM AND ENDOMETRIOSIS

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MAÍRA CASALECHI BADIN TELLES

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Tese apresentada ao Programa de Pós-Graduação em Medicina Molecular da Universidade Federal de Minas Gerais como requisito para obtenção do título de Doutor.

Orientador: Prof. Dr. Fernando Marcos dos Reis
Co-orientador: Prof. Dr. Antônio Marcos Coldibelli Francisco

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ATA DE DEFESA DE TESE

Às 09:00 horas do dia vinte e oito de fevereiro de dois mil vinte e quatro, videoconferência por meio da plataforma Zoom, realizou-se a sessão pública para a defesa da Tese de **MAÍRA CASALECHI BADIN TELLES**, número de registro 2019706398, graduada no curso de BIOMEDICINA, como requisito parcial para a obtenção do grau de Doutor em MEDICINA MOLECULAR. A presidência da sessão coube ao professor Fernando Marcos dos Reis, Orientador. Inicialmente, o presidente fez a apresentação da Comissão Examinadora assim constituída: Fernando Marcos dos Reis - Orientador (UFMG), Antônio Marcos Coldibelli Francisco - Coorientador (UNIVAS, Paola Viganò (Policlinico de Milano), Júlio César Rosa e Silva (FMRP-USP), Flávia Ribeiro de Oliveira (UFMG) e Ana Luiza Lunardi Rocha Baroni (UFMG). Em seguida, a candidata fez a apresentação do trabalho que constitui sua Tese de Doutorado, intitulada: **TRANSLOCATOR PROTEIN (TSPO) EXPRESSION AND LOCALIZATION IN HUMAN ENDOMETRIUM AND ENDOMETRIOSIS**. Seguiu-se a arguição pelos examinadores e logo após, a Comissão reuniu-se, sem a presença da candidata e do público e decidiu considerar aprovada a Tese de Doutorado. O resultado final foi comunicado publicamente à candidata pelo presidente da Comissão. Nada mais havendo a tratar, o presidente encerrou a sessão e lavrou a presente ata que, depois de lida, se aprovada, será assinada pela Comissão Examinadora.

Belo Horizonte, 28 de fevereiro de 2024.

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RESUMO

As limitações dos métodos de imagem atuais para detectar pequenas lesões endometrióticas ou lesões superficiais estimulam a busca por novos alvos moleculares. TSPO é uma proteína de 18KDa localizada na membrana externa mitocondrial , que pode ser rastreada por tomografia por emissão de pósitrons (PET) usando ligantes específicos. TSPO está localizada principalmente em neurônios e locais inflamados fora do cérebro. Nossa hipótese é que ela também possa ser expressa no endométrio humano e em tecidos endometrioticos, sendo um alvo para imagens moleculares da endometriose. Este estudo transversal prospectivo incluiu 28 mulheres com endometriose e 11 controles - pacientes sem endometriose. Lesões endometrióticas (n=59) e peritônio normal (n=13) de pacientes com endometriose foram obtidas durante laparoscopia, enquanto amostras de endométrio eutópico de pacientes com endometriose (n=28) e de mulheres controle (n=11) foram coletadas no sala de cirurgia usando um dispositivo flexível. A expressão do mRNA da TSPO foi avaliada por PCR quantitativa em tempo real com transcrição reversa, enquanto a expressão da proteína foi avaliada por imuno-histoquímica com um anticorpo monoclonal anti-TSPO humano. A expressão de mRNA de TSPO foi detectada de forma invariável em todos os tipos de tecidos avaliados; entretanto, descobriu-se que a proteína TSPO é mais abundante no epitélio glandular do que no estroma, tanto no endométrio quanto nas lesões endometrióticas. Curiosamente, as terapias hormonais não alteraram a expressão da TSPO, e a sua presença foi principalmente negativa nos tecidos adjacentes aos implantes endometrióticos. Como prova de conceito, o padrão de expressão proteica do TSPO no tecido endometriótico e ao longo das áreas adjacentes sugere que a imagem molecular baseada no TSPO pode ser usada para detecção não invasiva de endometriose.

Palavras-chave: TSPO; endometriose; endometriose; biomarcadores.

ABSTRACT

The limitations of current imaging methods to detect small or superficial endometriotic lesions prompt the search for new molecular targets. TSPO is an 18KDa protein located in the outer mitochondrial membrane, which can be traced by positron emission tomography (PET) using specific ligands. TSPO is located mostly in neurons and inflammatory sites outside the brain. We hypothesized that it might also be expressed in the human endometrium and endometrial-like tissue, being a target for molecular imaging of endometriosis. This prospective cross-sectional study included 28 women with endometriosis and 11 endometriosis-free controls. Endometriotic lesions (n=59) and normal peritoneum (n=13) from endometriosis patients were obtained during laparoscopy, while samples of eutopic endometrium from patients with endometriosis (n=28) and from control women (n=11) were collected in the operating room using a flexible device. TSPO mRNA expression was evaluated by quantitative reverse-transcription real-time PCR while protein expression was evaluated by immunohistochemistry with a monoclonal antibody anti-human TSPO. TSPO mRNA expression was detected in an invariable fashion in all tissue types evaluated; however, TSPO protein was found to be more abundant in the glandular epithelium than in the stroma, both in the endometrium and in the endometriotic lesions. Interestingly, hormone therapies did not alter the expression of TSPO, and its presence was mostly negative in tissues adjacent to endometriotic implants. As a proof of concept, the protein expression pattern of TSPO in endometriotic tissue and along the adjacent areas suggests that TSPO-based molecular imaging might be used for noninvasive endometriosis detection.

Keywords: TSPO; endometriosis; endometrium; biomarkers.

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(Dunn’s test). SUP: superficial peritoneal endometriosis; OMA: ovarian endometrioma; DE: deep endometriosis; NP: normal peritoneum.

Figure 7: TSPO gene mRNA and protein expression in the control endometrium (A-C), eutopic endometrium from patients with endometriosis (D-F) and endometriotic lesions (G-I). The participants were subdivided into those without any hormone use in the month before surgery (None) or in use of combined oral contraceptives (COC) or isolated progestins. The bars represent group medians (mRNA) or means (protein) and only mRNA levels in endometriotic lesions (G) differed significantly between subgroups ($p<0.05$, Kruskal-Wallis ANOVA).

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LIST OF ABBREVIATIONS AND ACRONYMS

ASRM: American Society of Reproductive Medicine

BMI: body mass index

CAPES: Coordenação de Aperfeiçoamento de Pessoal do Nível Superior

cDNA: complementary deoxyribonucleic acid

CNPq: Conselho Nacional de Desenvolvimento Científico e Tecnológico

COC: combined oral contraceptives

Ct: Threshold cycle

DE: deep endometriosis

FAPEMIG: Fundação de Amparo à Pesquisa de Minas Gerais

GnRH: Gonadotropin-releasing hormone

INCT-HSM: Instituto Nacional de Ciência e Tecnologia em Hormônios e Saúde da Mulher

IRB: Institutional Review Board

MRI: magnetic resonance imaging

mRNA: messenger ribonucleic acid

OMA: ovarian endometrioma

PET: positron emission tomography

RT-qPCR: quantitative reverse-transcription real-time polymerase chain reaction

SRI: Society for Reproductive Investigation

StAR: steroid acute regulatory protein

SUP: superficial endometriosis

TSPO: translocator protein

UFMG: Universidade Federal de Minas Gerais

UNIVÁS: Universidade do Vale do Sapucaí

SUMMARY

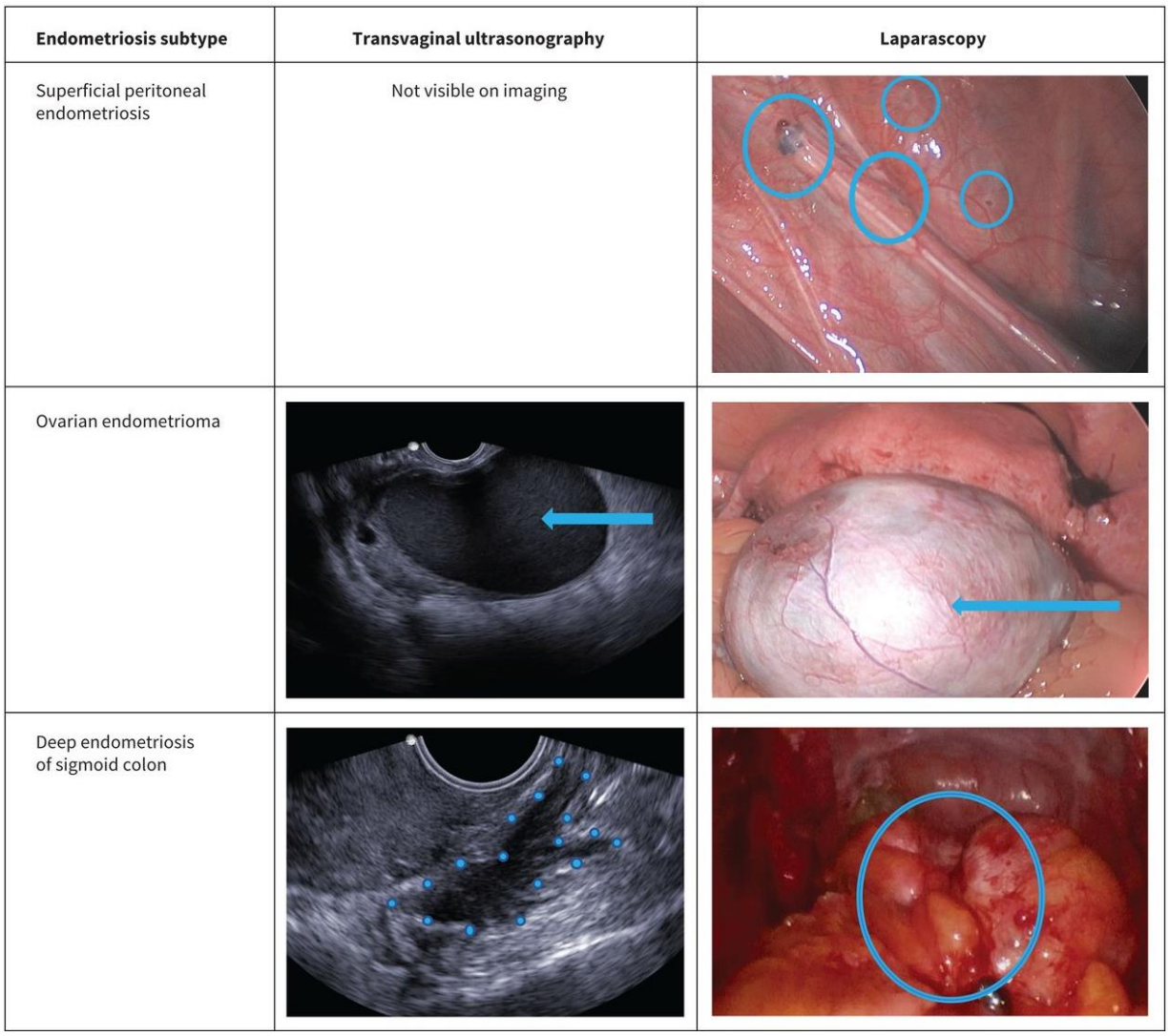
1. INTRODUCTION

Endometriosis is a chronic inflammatory gynecological disorder defined as the presence of endometrial-like tissue outside the uterus that affects up to 10% of women of reproductive age worldwide (Taylor et al., 2021). Its clinical appearance comprises three distinct phenotypes: 1. superficial endometriosis (SUP); which is characterized by thin layer lesions in the peritoneum; 2. ovarian endometrioma (OMA); characterized by cystic lesions in the ovaries, which are filled by a dark fluid; and 3. deep endometriosis (DE), defined by the presence of lesions with more than 5 mm depth into the tissue (Taylor et al., 2021). These appearances might be isolated or combined, and several pelvic organs and tissues might be compromised, including ovaries, intestines, peritoneum, and bladder (Hsu et al., 2010). From the histological point of view, endometriosis is considered the presence of ectopic endometrial glands or stroma, often with haemosiderin outside the uterine cavity (Chapron et al., 2019). Despite being often characterized as a pelvic disease, evidence suggests that it is a multifactorial disorder, which has several effects throughout the body (Taylor et al., 2021). Among them, the most common symptom is infertility and chronic pelvic pain, including dysmenorrhea, non-menstrual pain, dyspareunia, dyschezia, and dysuria, but endometriosis also might cause migraine, fatigue, pelvic fibroids and irritable bowel syndrome (Becker et al., 2022).

Considering endometriosis nonspecific symptoms, as well as its heterogenic manifestations, its diagnosis is challenging, usually taking seven to nine years after its first signs, deeply impacting women' quality of life, and, consequently, being an economic burden: only in the USA of over US$22 billion (Nnoaham et al., 2019; Soliman et al., 2017; Staal et al., 2016; Taylor et al., 2021). Up to now, endometriosis has no specific biochemical marker that could be

used for a clinical diagnosis. Studies have found potential biomarkers, but most of them have been discarded at the research stage, and very few have been translated into clinical practice, with poor accuracy. So far, most diagnosis confirmations comprise laparoscopic surgery with biopsy and histopathological examination of the lesions (Becker et al., 2022). However, surgical diagnosis is not a perfect gold standard, since diagnostic laparoscopy might be inaccurate and can miss the disease (Taylor et al., 2021). Transvaginal ultrasound and magnetic resonance imaging (MRI) performed by trained specialists show good accuracy in detecting OMA and DE (Becker et al., 2022), but SUP lesions develop shallowly on the surface of the peritoneum or ovaries, making their imaging far more difficult (Borghese et al., 2015) (Figure 1). Also, the absence of visible lesions or negative histology do not exclude endometriosis, since occult endometriosis has been in random peritoneal biopsy specimens (Balasch et al., 1996; Kazanegra et al., 2008; Albee, Sinervo and Fisher, 2008; Stegmann et al., 2008). Therefore, reliance on surgery for endometriosis diagnosis only delays the initiation of treatment for this complex disease (Taylor et al., 2018). Therefore, the detection of molecular targets that could be used with imaging techniques aiming to a faster, more precise, and non-invasive diagnosis of endometriosis in general and SUP in particular is urgently needed (Coutinho et al., 2019). The translocator protein (TSPO), also known as peripheral benzodiazepine receptor, (Braestrup & Squires, 1977) is a potential tool for a more specific and sensitive noninvasive imaging of endometriotic lesions. It is an 18 kDa protein with five transmembrane domains, mainly localized in the outer mitochondrial membrane of the neurons and is involved in membrane biogenesis, bioenergetics, cell proliferation, apoptosis, and immunomodulation (Liu et al., 2022; Morin et al., 2016; Taketani et al., 1994). Together with the steroid acute regulatory protein (StAR), TSPO has an important role in the first, rate-limiting step of steroid hormone biosynthesis, as both proteins mobilize cholesterol to the inner mitochondrial

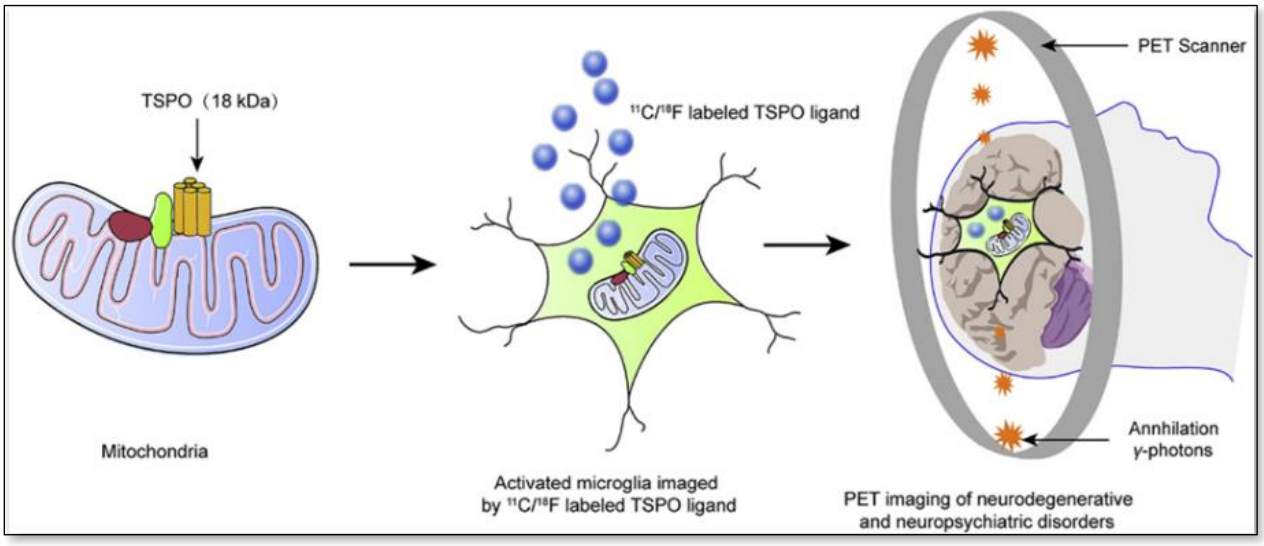
Figure 1. Imaging and laparoscopic appearance of endometriosis subtypes. - Allaire C. et al., 2023. PMID: 36918177 *(modified)*



membrane to be converted into pregnenolone (Stocco et al., 2017). TSPO can be modulated by inflammation (M. K. Chen & Guilarte, 2008; Werry et al., 2019) to the point that it is considered an inflammation biomarker and a natural defense (Rupprecht et al., 2010), as it is effective in brain protection from neurodegeneration, neuroinflammation, and in neuropathic pain (Girard et al., 2008; Lee et al., 2016; Wei et al., 2013). More than that, TSPO expression has been detected in sites outside the nervous system such as the kidney, heart, and liver (Batarseh & Papadopoulos,

2010; Giatzakis & Papadopoulos, 2004; Papadopoulos et al., 2006). Moreover, TSPO has specific ligands – such as the C-11 labeled R-enantiomer of 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide ((R)-[11C]PK11195) and the F-18 labeled N,N-diethyl-2-[4-(2-[18F]fluoroethoxy)phenyl]-5,7-dimethyl-pyrazolo[1,5-a]pyrimidine-3-acetamide ([18F]DPA-714) – that, once conjugated to radioisotopes, allow the non-invasive imaging of TSPO-rich tissues using positron emission tomography (PET) (Figure 2).

Figure 2: TSPO localization in the outer mitochondrial membrane in a neuro cell, which can be used as a target of radiolabeled molecules to PET imaging-based diagnosis. Zhang et al., 2021. PMID: 33643818



Little is known about TSPO expression and function in the female reproductive system. It has been shown that TSPO is present in the rat endometrium and is regulated by steroid hormones (Morohaku et al., 2013). Therefore, we hypothesized that this molecule might also be present in the human endometrium and in endometriosis. Considering that, this study aimed to evaluate TSPO expression in human endometrial tissue and endometriotic lesions. Furthermore, we evaluated TSPO localization in areas adjacent to endometriotic lesions as well as in normal

peritoneum to find out whether this biomarker is circumscribed to endometriosis and thus could be potentially used to identify endometriotic lesions through PET scan.

2. MATERIALS AND METHODS

2.1 Study design and participants

This prospective cross-sectional study included 28 women with endometriosis and 11 patients without endometriosis as controls. All participants were enrolled from March 2017 to September 2018 at the Hospital das Clínicas Samuel Libânio, Pouso Alegre, Brazil. The study design, protocol, and informed consent form were approved by the local Institutional Review Board (IRB; registration number 60378816.1.0000.5149), and all participants freely signed the informed consent upon enrollment.

The main clinical characteristics of the endometriosis and control groups are summarized in Table 1. Briefly, women with endometriosis had been referred for laparoscopic surgery due to pelvic pain of moderate to severe intensity that did not respond satisfactorily to medical treatment, and/or infertility. The control group was composed by women undergoing laparoscopy and/or hysteroscopy for benign gynecological conditions other than endometriosis.

2.2 Sample collection

Samples of endometriotic lesions (n=59) and normal peritoneum (n=13) were obtained during laparoscopy, while samples of eutopic endometrium from patients with endometriosis (n=28) and from control women (n=11) were collected in the operating room using a flexible device (Pipelle®, Laboratoire CCD, Paris, France) (Attachment 1). One portion of each endometrial tissue sample was fixed in HistoChoice® Tissue Fixative (CAT: H2904; Sigma-Aldrich, St. Louis, Missouri, United States) and was paraffin-embedded to allow protein localization by immunohistochemistry. Another portion was stored in 1 mL of RNA*later* (CAT:

AM7021; ThermoFisher, Waltham, Massachusetts, United States) for 24 hours at room temperature, then drained and stored at -80º C for future gene expression analysis.

2.3 RNA extraction, complementary DNA synthesis, and semi-quantitative PCR

Expression of the messenger ribonucleic acid (mRNA) encoding TSPO was evaluated in all samples by quantitative reverse-transcription real-time polymerase chain reaction (RT-qPCR). For processing, endometriotic tissue stored in RNA*later* was removed from the freezer at -80 °C and was washed twice in cold PBS. All endometriotic lesions were manually dissected to minimize adjacent-tissue contamination, followed by disruption and homogenization. Total RNA was isolated using the TRIzol® protocol, unmodified. Total RNA was quantified by light absorbance at 260 nm (NanoDrop - Thermo Fisher Scientific, Wilmington, Delaware, USA), and 1 μg of total RNA was pretreatment with DNase I, Amplification Grade for 15 minutes (CAT: 18068015; Invitrogen, Carlsbad, CA, USA) to remove undesired genomic DNA contamination. First-strand complementary deoxyribonucleic acid (cDNA) was synthesized from 750 ng of DNase I-treated total RNA using Superscript IV first-strand synthesis system (CAT: 18091050; Invitrogen, Carlsbad, CA, USA). Real-time PCR was carried out as described previously (Dela Cruz et al., 2022) in an ABI-Prism 7500 Sequence Detection System using the fluorescent dye Power SYBR Green Master Mix Kit (Invitrogen Life Technologies, Carlsbad, CA, USA). The PCR parameters were: [stage 1] a cycle of 95 °C / 10min; [stage 2] 40 cycles of 95 °C / 15 seconds, 60 °C / 15 seconds and 72 °C / 20 seconds; [Stage 3] 95 °C / 15 seconds, 54 °C / 15 seconds and 95 °C / 15 seconds. The gene encoding the ribosomal protein S26 was used as the internal control. The synthesized primer sequences used for PCR amplification were: TSPO forward 5’CCTACCCCTTGCAAAGAAGC 3’; TSPO reverse 5’TCGGGCACCAAAGAAGATGG 3’; S26 forward 5’CCAAAGGGAGGCTGGTGAAT 3’; S26 reverse 5’

GGTGCCTGCGATATTTGTTAGG 3'. Primers were designed to span two sequential exons and thus anneal only to cDNA. The specificity of PCR products was confirmed by single peak dissociation curves. Threshold cycle (Ct) values were normalized to S26 (ΔCt), and each sample value was reported as 1/ΔCt.

2.4 Immunohistochemistry

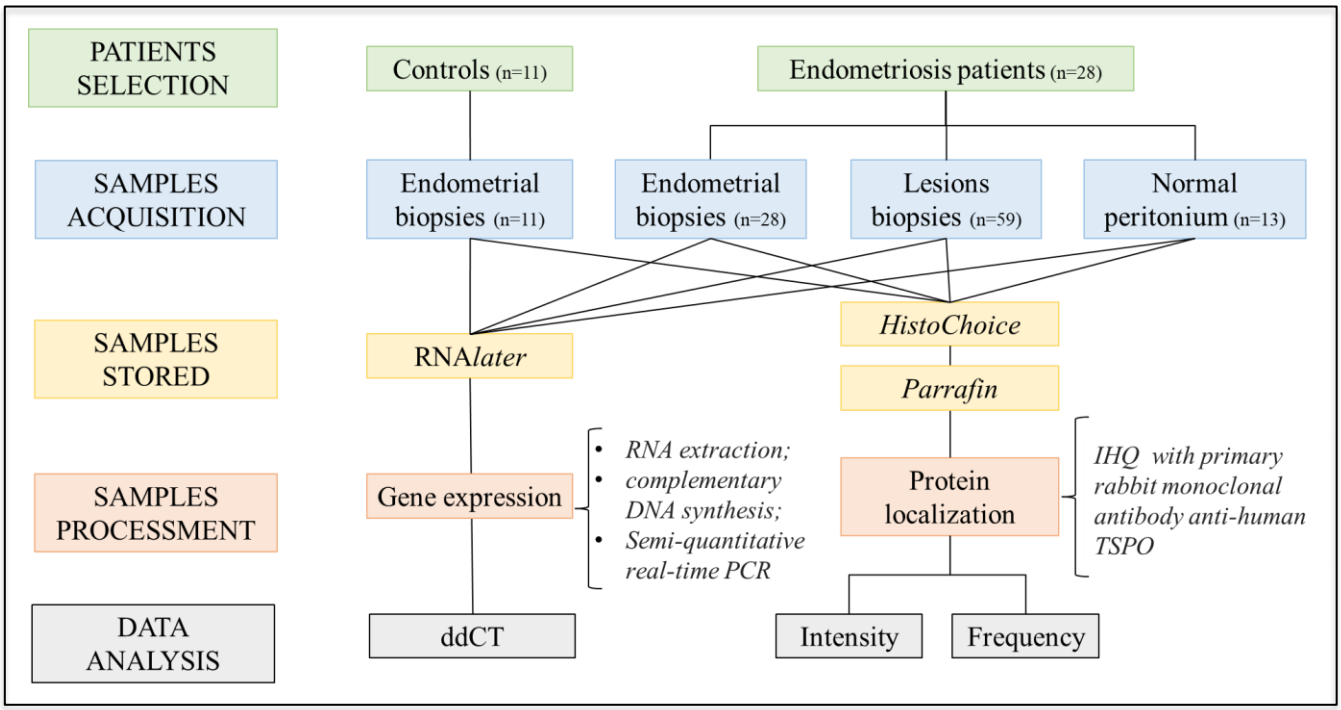
All immunohistochemistry steps were performed using the Novolink™ non-biotin polymer detection system kit (Novocastra®, Newcastle Upon Tyne, UK). The paraffin-embedded samples sectioned at 0.4 µm of thickness were deparaffinized with xylene and hydrated with graded ethanol and PBS. The slides were microwaved in an EDTA buffer for 5 minutes for optimal exposure of the epitopes of interest. The endogenous peroxidase activity was blocked with peroxidase block for 5 minutes. The sections were incubated for 5 minutes to reduce background staining with the protein block solution. The sections were then incubated overnight at 4ºC with primary rabbit monoclonal antibody anti-human TSPO (D1N7Z RabbitmAb #70358 – Cell Signaling – Massachusetts /USA) diluted 1:50. After incubation, each slide was incubated 30 minutes with the post-primary block reagent, and then the reactant polymer was applied for another 30 minutes incubation. The staining was developed by diaminobenzidine and counterstained with hematoxylin. Negative control reactions consisted of omitting the primary antibody step from the protocol. High-resolution images of the stained sections were acquired through a Panoramic Digital Slide Scanner (3DHistech, Budapest, Hungary) and analyzed in full using CaseViewer 2.4 software. Representative areas were chosen to illustrate the findings. The intensity of the immunostaining was graded on a 0 to 3 arbitrary unit scale, and the percentage of cells with positive staining was graded as 0 (absent), 1 (1% to 25%), 2 (26% to 75%), or 3 (76% to 100%).

An individual index for each sample was obtained by summing its immunostaining intensity and the percentage scores (Couto et al., 2018).

2.5 Statistical analysis

The results were analyzed by the D'Agostino-Pearson test to determine normal data distributions. Unless otherwise stated, continuous variables were summarized as mean ± standard error and categorical variables were expressed as frequency (percentage). Differences between groups were assessed using the Kruskal-Wallis analysis of variance followed by Dunn's test, whereas the immunostaining index was compared between epithelial and stromal compartments using the Wilcoxon's paired rank test. All analyses were performed using GraphPad Prism 6.

Figure 2. Overview of study methodology.



3. RESULTS

Considering the two study groups, there was no difference in age, BMI, cycle regularity, or use of hormonal treatments in the month before the endometrial biopsy (Table 1). Considering the women affected by endometriosis, they were predominantly stage III/IV (75%), accordingly to rASRM classification (Practice Committee of the ASRM, 2012), with a higher prevalence of infertility, dysmenorrhea and dyspareunia than the control group (Table 1).

Table 1. Clinical characteristics of the study groups.

	Endometriosis (n = 28)	Control (n = 11)	P value
Age (years)	37.6 ± 6.0	36.1 ± 8.6	0.539
BMI (Kg/m^2)	26.1 ± 3.7	24.8 ± 3.1	0.312
Dysmenorrhea (VAS)	7.2 ± 3.1	1.2 ± 3.0	0.000
Dyspareunia (VAS)	3.3 ± 3.9	0.7 ± 2.4	0.020
Acyclic pelvic pain (VAS)	2.0 ± 3.2	0.6 ± 2.1	0.134
Regular menstrual cycles	7 (25%)	5 (46%)	0.262
Infertility	19 (68%)	3 (27%)	0.033
Adenomyosis (ultrasound)	7 (25%)	0 (0%)	0.159
Family history of endometriosis	3 (11%)	2 (18%)	0.609
Hormonal treatments in the last month			
Combined oral contraceptive	9 (32%)	1 (9%)	0.228
Progestin	14 (50%)	4 (36%)	0.497
GnRH agonist	0 (0%)	0 (0%)	1.000
rASRM Stage of endometriosis			
I	2 (7%)	–	
II	5 (18%)	–	
III	10 (36%)	–	
IV	11 (39%)	–	

TSPO mRNA expression was detected in all tissue types evaluated, with no significant quantitative difference between eutopic endometrium of controls and eutopic endometrium from endometriosis patients, or between SUP, OMA or DE lesions (Figure 4).

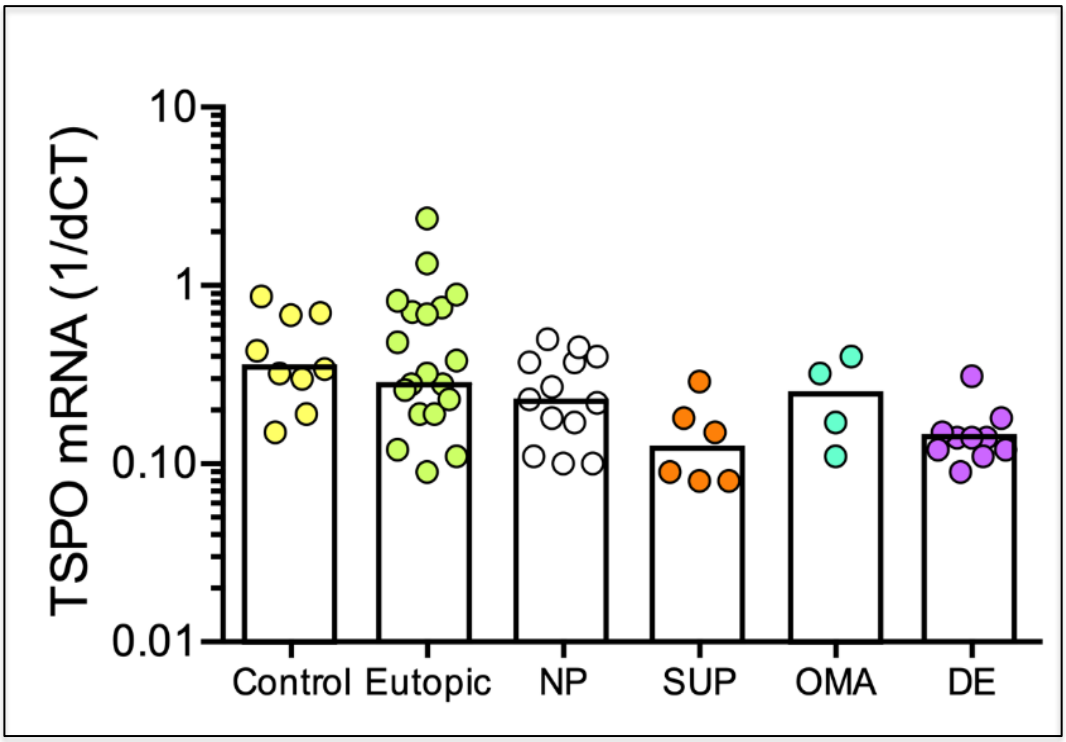
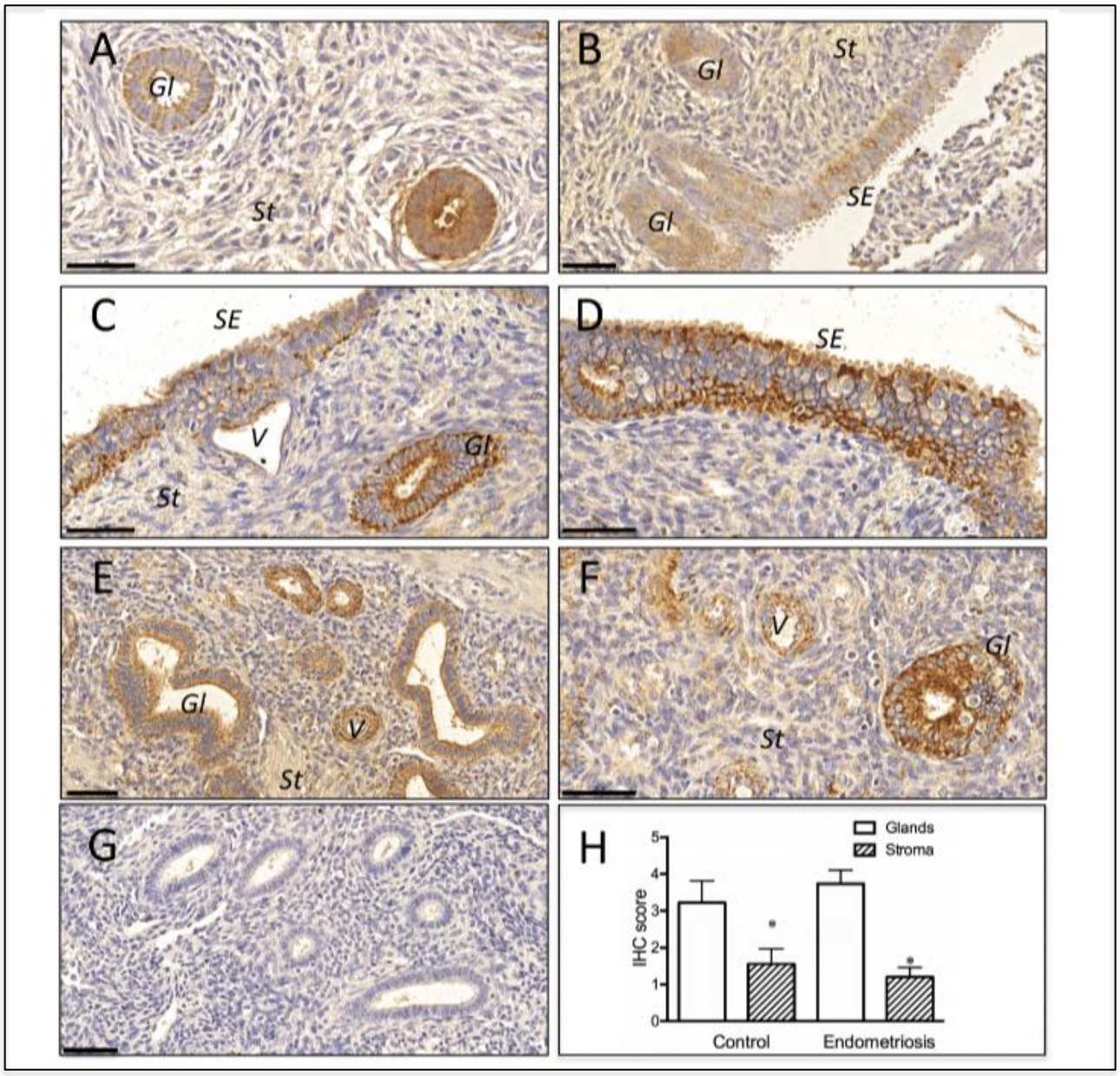


Figure 4: TSPO mRNA expression in endometrium and in endometriotic lesions. The bars represent the group medians, and group comparisons were made with the Kruskal-Wallis test. ENDO: eutopic endometrium from the endometriosis group; NP: normal peritoneum; SUP: superficial peritoneal endometriosis; OMA: ovarian endometrioma; DE: deep endometriosis.

TSPO protein was localized in endometrium from both control women and from endometriosis patients (Figure 5). In controls, the immunostaining index for TSPO expression was higher in the glandular epithelium (3.2 ± 0.6) than in the stroma (1.6 ± 0.4, $p<0.05$, Figure 5H). The same pattern was found in the eutopic endometrium of women with endometriosis, where TSPO immunostaining prevailed in the glandular epithelium (3.7 ± 0.4) over the stroma (1.2 ± 0.3, $p<0.001$). Positive controls had the expected staining pattern (not shown), and negative controls had no staining at all (Figure 5G), demonstrating the reliability of the results.

Figure 5: TSPO localization in endometrium of controls (A-B) and in women with endometriosis (C-F). G = negative control. SE: surface epithelium. Gl: glandular epithelium; St: stroma; V: blood vessel. Scale bar = 50 µm. The immunohistochemistry score (mean ± standard error) is shown in (H). *p<0.05 vs. Glands (Wilcoxon's paired rank test).



Endometriotic lesions of all types evaluated expressed the TSPO protein (Figure 6), with the TSPO immunostaining index being higher in the endometriotic glands (4.5 ± 0.2) than in the stroma (2.0 ± 0.2). Importantly, TSPO was almost undetectable in the non-affected areas adjacent to the endometriotic lesions (0.8 ± 0.2) or in the normal peritoneum (1.5 ± 0.4, p<0.001, Figure

6I). TSPO immunostaining did not differ significantly between SUP, OMA, and DE lesions, either in the glands (Figure 6J) or in the stroma (Figure 6K).

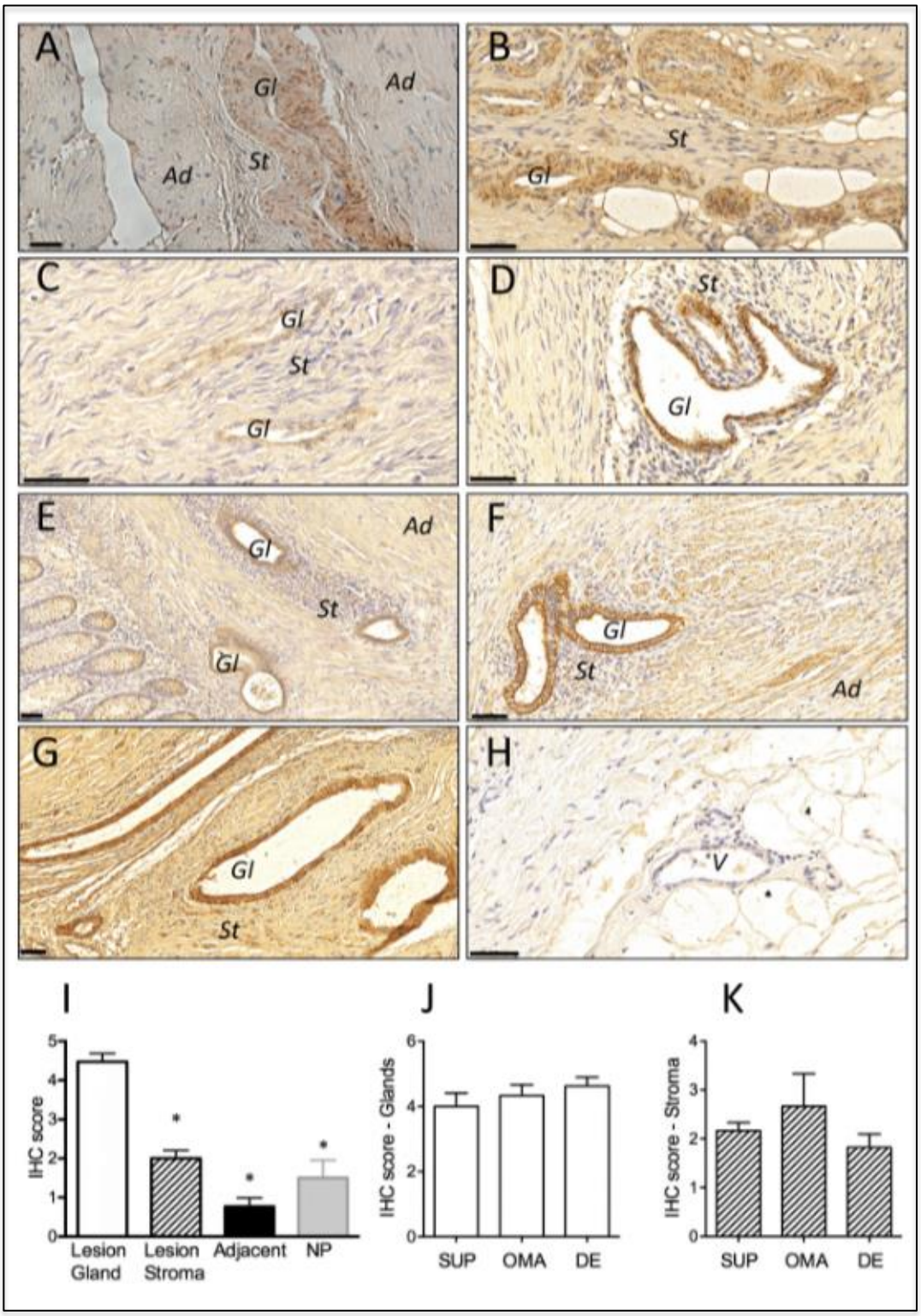


Figure 6: TSPO localization in endometriotic lesions (A-G) and disease-free peritoneum (H). The images are representative of superficial peritoneal endometriosis (A-B), ovarian endometrioma (C), and deep endometriosis of the uterosacral ligament (D), intestine (E-F), and bladder (G). Gl: glandular epithelium; St: stroma; V: blood vessel; Ad: adjacent area. Scale bar = 50 µm. The immunohistochemistry score (mean ± standard error) is shown in (I-K). *p<0.05 vs. Lesion-Gland (Dunn's test). SUP: superficial peritoneal endometriosis; OMA: ovarian endometrioma; DE: deep endometriosis; NP: normal peritoneum.

We also evaluated whether the use of combined oral contraceptives (COC) or isolated progestins could interfere with the expression levels of TSPO in eutopic endometrium or in endometriotic lesions (Figure 7). There was no difference related to hormone therapy use in the expression of TSPO in the endometrium (Figure 7A-F). The use of progestin was associated with decreased TSPO mRNA levels in endometriotic lesions ($p<0.05$, Kruskal-Wallis test, Figure 7G) but, considering protein expression, no difference was found between women using COC or progestin in comparison with women without any hormone therapy, either in the glands or at the stroma of the endometriotic tissue (Figure 7H-I).

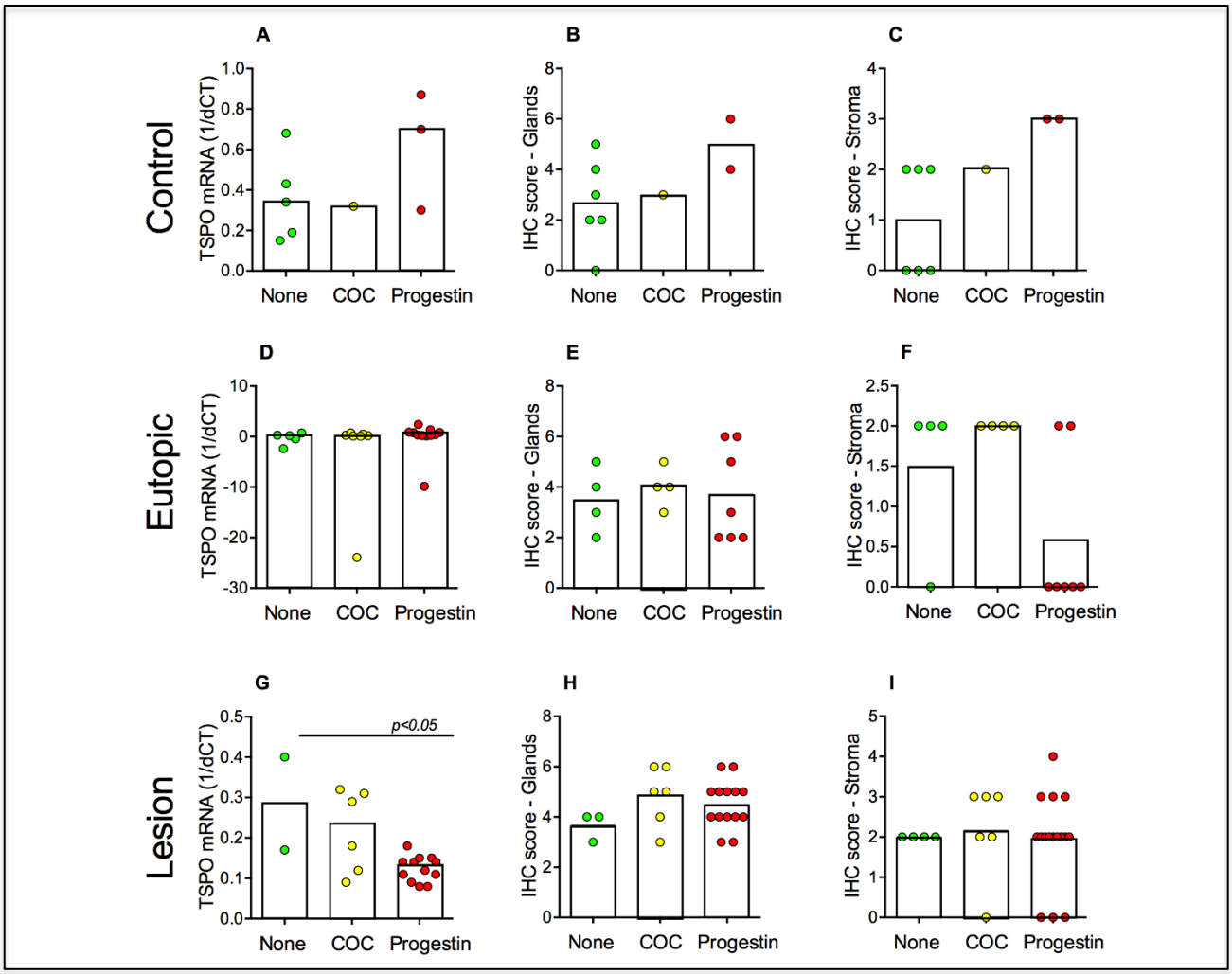


Figure 7: TSPO gene mRNA and protein expression in the control endometrium (A-C), eutopic endometrium from patients with endometriosis (D-F) and endometriotic lesions (G-I). The participants were subdivided into those without any hormone use in the month before surgery (None) or in use of combined oral contraceptives (COC) or isolated progestins. The bars represent group medians (mRNA) or means (protein) and only mRNA levels in endometriotic lesions (G) differed significantly between subgroups ($p<0.05$, Kruskal-Wallis ANOVA).

4. DISCUSSION

Noninvasive diagnosis of endometriosis currently relies on ultrasound and MRI, which should be performed by specialists familiar with its imaging protocols and pitfalls. Although ultrasound has a good sensitivity in diagnosing DIE and OMA, detecting SUP lesions remains a challenge, which contributes to delayed diagnosis and treatment and to the progressive worsening of women's quality of life (Casalechi et al., 2021). In this scenario, a target for PET-ligands allowing the diagnosis and follow-up of endometriotic (especially SUP) lesions would likely improve the treatment timeliness and the quality of life of women suffering with endometriosis.

Previous studies have shown the presence of TPSO in the rat endometrium (Morohaku et al., 2013). On these bases, we hypothesized that TPSO could also be present in human endometrium and, possibly, in endometriotic lesions. In fact, our data showed for the first time the expression of TSPO in the endometrium of women with and without endometriosis and also in endometriotic lesions, having a considerable contrast with the peritoneum (Figure 5). Since TSPO has specific ligands already standardized to be used for PET/Scan imaging (Filippi et al., 2023), our results suggest that TPSO qualifies to be further investigated as a molecular target for endometriosis imaging based on PET/Scan.

Another critical point to be considered is that, in animal models, hormone treatments seem to change TSPO expression patterns (Morohaku et al., 2013). This could challenge the employment of TSPO as a molecular target for endometriosis diagnosis, since treatments would have to be suspended in order to have a clearer picture of the lesions. As a matter of fact, we detected lower TSPO mRNA levels in the endometriotic lesions of women on progesterone treatment compared to those without hormonal therapies. However, considering the results obtained from protein expression, no difference could be observed in women using progesterone.

This finding makes TSPO an even more interesting target for a non-invasive diagnosis of endometriosis, as the test could be performed at any time of the menstrual cycle and especially during hormonal endometriosis treatment. This is an ideal situation, considering that stopping the treatment causes pain and decreases women's quality of life (Szyplowska et al., 2023).

PET-Scan with TSPO ligands has been clinically used to evaluate neurological conditions such as depression (Eggerstorfer et al., 2022), Alzheimer's disease (Rauchmann et al., 2022), and hypothalamic inflammation (Butler et al., 2022). Generally speaking, PET/scan is a reliable technology for the initial workup of several tumor-based diseases and for follow-up settings, providing a better staging and evaluation of the response to the treatment. A great advantage that makes TSPO a potential target for non-invasive diagnosis is that there are already different radioligands for TSPO available for clinical imaging, some of which allow an image detection of lesions as small as 4 mm. Specifically, (R)-[11C]PK11195 is a widely used radioligand for TSPO in clinical imaging nowadays (Ching et al., 2012), and second-generation ligands like [18F] DPA-714 have been developed (James et al., 2008).

The standardization of PET tests, including kinetics analysis and modeling of preclinical data, is challenged by several quantification-related caveats (Z. Chen et al., 2021). Because the clinical trials evaluating TSPO as a diagnostic target were conducted for neuroinflammatory disorders there is no information on how its ligands would work on pelvic evaluations, in terms of sensitivity and specificity. Also, the affinity of TSPO to ligands can be reduced in the presence of a single nucleotide polymorphism (rs6971) in *TSPO* gene exon 4, which might lead to false negative results (Ching et al., 2012). In this sense, evaluating the normal female pelvic imaging with a TSPO tracer is needed before its validation to detect pelvic endometriosis.

It is expected that the development of endometriosis biomarkers could improve diagnosis as well as follow-up after lesion resection and/or medical treatment. A strength of the present study is the analysis of different types of lesions and clinical scenarios, showing that TSPO expression is constant among various endometriotic phenotypes and during hormone therapies. However, we are aware of some limitations of the study, mainly considering that samples were obtained from patients undergoing laparoscopy surgery, and, therefore, our results should not be extrapolated to women with early or asymptomatic disease not requiring surgical intervention. Considering that TSPO is linked to inflammation, it remains to be established whether lesions from the initial stages of endometriosis would present the same expression of TSPO as seen in lesions from women undergoing surgery. Finally, we did not evaluate TSPO localization in other pelvic tissues that express the protein in the mouse, such as fallopian tubes and ovarian cortex (Morohaku et al., 2013). Nevertheless, only further studies with PET/Scan imaging will demonstrate whether the TSPO density in other pelvic organs is low enough to contrast with that of endometrium and endometriosis, allowing the specific detection of endometriotic foci.

In summary, TSPO is present in the human endometrium as well as in several types of endometriotic lesions, being more abundant in the glandular epithelium than in the stroma. Hormone therapies did not alter the expression of TSPO, and its presence was mostly negative in tissues adjacent to endometriotic implants. However, further studies are still needed before the application of TSPO as a diagnostic target. These include performing PET with different TSPO ligands to establish whether the *in vivo* TSPO labeling allows the accurate localization of endometriotic lesions in contrast with the anatomic background and to find out which ligand would perform better in endometriosis detection using TSPO-based molecular imaging.

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ATTACHMENTS

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Projeto: CAAE – 60378816.1.0000.5149

Interessado(a): Prof. Fernando Marcos dos Reis
Departamento de Ginecologia e Obstetrícia
Faculdade de Medicina- UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 26 de outubro de 2016, o projeto de pesquisa intitulado **" Proteína translocadora / receptor benzodiazepínico periférico no endométrio e na endometriose"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto através da Plataforma Brasil.

Profa. Dra. Vivian Resende
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep:31270-901 – BH-MG
Telefax: (031) 3409-4592 - e-mail: coep@prpq.ufmg.br

Attachment 1: Institutional Review Board Approval - UFMG

Attachment 2: Institutional Review Board Approval - UNIVAS

FACULDADE DE CIÊNCIAS MÉDICAS DR.JOSÉ ANTÔNIO GARCIA COUTINHO -

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Proteína translocadora / receptor benzodiazepínico periférico no endométrio e na endometriose UNIVÁS

Pesquisador: Antônio Marcos Coldibelli Francisco

Área Temática: Reprodução Humana (pesquisas que se ocupam com o funcionamento do aparelho reprodutor, procriação e fatores que afetam a saúde reprodutiva de humanos, sendo que nessas pesquisas serão considerados "participantes da pesquisa" todos os que forem afetados pelos procedimentos delas):
(Reprodução Humana que não necessita de análise ética por parte da CONEP;);

Versão: 2

CAAE: 62465416.3.0000.5102

Instituição Proponente: FUNDACAO DE ENSINO SUPERIOR DO VALE DO SAPUCAI

Patrocinador Principal: MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO

DADOS DO PARECER

Número do Parecer: 1.922.375

Apresentação do Projeto:

Endometriose é uma doença ginecológica estrógeno-dependente, caracterizada pela presença de tecido endometrial fora da cavidade uterina, associada a dor pélvica e infertilidade1,2,3. O tempo decorrido entre o início dos sintomas e o diagnóstico da doença é longo, em torno de 6-9 anos4, e o diagnostico é feito por laparoscopia, seguida de confirmação histológica do tecido ectópico endometrial (glândulas endometriais e estroma). Apesar de intensa investigação, o processo patogênico que leva ao desenvolvimento e à manutenção da doença é ainda pouco conhecido5. Ademais, por apresentar uma variedade de sintomas inespecíficos, a endometriose é frequentemente confundida com outras doenças ou tem seus sintomas ignorados4. Portanto, novos estudos são necessários no intuito de entender o mecanismo fisiopatológico da endometriose e propor um biomarcador que torne o diagnóstico mais rápido, não invasivo e preciso. No entanto, as
tentativas foram frustradas até o momento e os biomarcadores testados até hoje têm baixa acurácia e pouca ou nenhuma utilidade clínica3. A proteína translocadora (TSPO) é uma proteína

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Attachment 2: Institutional Review Board Approval – UNIVAS *(continuing)*

de 18kDa localizada predominantemente no exterior da membrana mitocondrial. Originalmente descrita como receptor benzodiazepínico periférico, a TSPO foi descoberta a partir da constatação de que os benzodiazepínicos também ocupavam sítios de ligação específicos fora do sistema nervoso central, além de se ligarem aos sítios centrais, como o receptor GABAA6. A expressão de TSPO é muito baixa no sistema nervoso central, mas eleva-se na presença de inflamação, particularmente nas células da glia6-9. Há evidência do envolvimento da TSPO em diversos processos fisiológicos, como proliferação celular, apoptose, eritropoiese, fluxo de cálcio e biossíntese dos hormônios esteróides9-16. Apesar da TSPO ter sido detectada em diferentes tecidos, ela se mostrou altamente expressa em células produtoras de hormônios esteroides, como glândula adrenal, testículos e ovários6. De outra parte, estudos in vivo têm demonstrado que os hormônios esteroides são
capazes de regular a expressão de TSPO17,18,19,7. A presença de TSPO foi detectada no útero de ratas e mostrou-se suscetível à regulação pelos níveis endógenos de hormônios sexuais20,21. Entretanto, não temos conhecimento de nenhum estudo que tenha avaliado a TSPO no endométrio humano ou em doenças ginecológicas. Uma vez que a TSPO é um importante regulador de eventos celulares como apoptose, proliferação e metabolismo, e que sua expressão é regulada por esteroides sexuais7, a sua expressão/regulação pode ter um papel chave no desenvolvimento de várias doenças ginecológicas, como a endometriose.
Além disso, se a expressão de TSPO for mais acentuada em implantes endometrióticos do que no peritônio, essa molécula poderá representar uma inovação no diagnóstico por imagem de lesões endometrióticas, inclusive as superficiais e de pequenas dimensões, uma vez que existem ligantes específicos da TSPO, como PBR111 e PK11195, que podem ser conjugados a radioisótopos e ser usados para o mapeamento não invasivo dos tecidos ricos em TSPO22.

Objetivo da Pesquisa:

Verificar se existem diferenças de expressão e regulação da TSPO entre o endométrio humano normal e aquele de mulheres com endometriose,bem como no peritônio e em lesões endometrióticas superficiais, profundas, e endometrioma de ovário; correlacionar essas alterações com marcadores inflamatórios e com a intensidade dos sintomas

Avaliação dos Riscos e Benefícios:

Riscos:

No grupo controle: A coleta de amostra do endométrio pode causar desconforto e cólica momentânea. A amostra será colhida em material inteiramente estéril e descartável, que não

Attachment 2: Institutional Review Board Approval – UNIVAS *(continuing)*

FACULDADE DE CIÊNCIAS MÉDICAS DR.JOSÉ ANTÔNIO GARCIA COUTINHO -

Continuação do Parecer: 1.922.375

oferece risco para a sua saúde.

No grupo estudo: A coleta de amostra do endométrio pode aumentar em até 10 minutos o seu tempo de anestesia, mas não influencia a segurança da cirurgia. A amostra será colhida em material inteiramente estéril e descartável, que não oferece risco para a sua saúde.

Benefícios:

Embora não traga benefício imediato para você, este estudo irá contribuir para o conhecimento mais detalhado da endometriose. Esse conhecimento poderá ajudar no desenvolvimento de novos métodos para diagnosticar e tratar a doença.

Comentários e Considerações sobre a Pesquisa:

Pesquisa bem fundamentada com relevância social e científica

Considerações sobre os Termos de apresentação obrigatória:

Folha de rosto readequada conforme solicitação

Foram acrescentados as declaraçoes de infraestrutura dos dois hospitais selecionados para a realização da pesquisa

Recomendações:

Nenhuma

Conclusões ou Pendências e Lista de Inadequações:

Nenhuma

Considerações Finais a critério do CEP:

Apos o término da pesquisa apresentar relatório ao CEP.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Recurso do Parecer	recurso.pdf	19/12/2016 18:52:21		Aceito
Recurso Anexado pelo Pesquisador	Recurso.docx	19/12/2016 18:52:15	Antônio Marcos Coldibelli Francisco	Aceito
Outros	termo1.pdf	19/12/2016 18:51:49	Antônio Marcos Coldibelli Francisco	Aceito
Outros	termo.pdf	19/12/2016 18:51:33	Antônio Marcos Coldibelli Francisco	Aceito

Endereço: Avenida Prefeito Tuany Toledo, 470
Bairro: Campus Fátima I **CEP:** 37.550-000
UF: MG **Município:** POUSO ALEGRE
Telefone: (35)3449-9270 **E-mail:** pesquisa@univas.edu.br

Attachment 2: Institutional Review Board Approval – UNIVAS *(continuing)*

FACULDADE DE CIÊNCIAS MÉDICAS DR.JOSÉ ANTÔNIO GARCIA COUTINHO -

Continuação do Parecer: 1.922.375

Folha de Rosto	folha_de_rosto.pdf	19/12/2016 18:49:24	Antônio Marcos Coldibelli Francisco	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Univas.doc	19/12/2016 18:48:18	Antônio Marcos Coldibelli Francisco	Aceito
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_829008.pdf	28/11/2016 16:23:03		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Univas_grupo_endometriose.doc	20/11/2016 10:34:25	Antônio Marcos Coldibelli Francisco	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Univas_grupo_controle_e_ACO.doc	20/11/2016 10:34:07	Antônio Marcos Coldibelli Francisco	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

POUSO ALEGRE, 15 de Fevereiro de 2017

Assinado por:
Rosa Maria do Nascimento
(Coordenador)

Endereço: Avenida Prefeito Tuany Toledo, 470
Bairro: Campus Fátima I **CEP:** 37.550-000
UF: MG **Município:** POUSO ALEGRE
Telefone: (35)3449-9270 **E-mail:** pesquisa@univas.edu.br

Attachment 3: Patients included in the study and their clinical characteristics. *BMI: Body mass index; COC: Combined oral contraceptive; GnRH: Gonadotropin-releasing hormone; VAS: Visual Analogue Scale; ASRM: American Society of Reproductive Medicine; SUP: superficial endometriosis; OMA: ovarian endometriosis; DE: deep endometriosis.*

Patients	Sample	Age (years)	Number of previous gestations	Parity	Family history	BMI (kg/m2)	Regular cycles	COC last month	Progestin last month	GnRH analog last month	Dysmenorrhea (VAS)	Dyspareunia (VAS)	Non-menstrual pain (VAS)	Infertility	Adenomyosis (US)	ASRM score	ASRM stage	SUP	OMA	DE
Control 01	Endometrium	43	3	2	0	27.34	yes	no	no	no	0	0	0	no	no	0	0	no	no	no
Control 02	Endometrium	42	5	4	0	26.22	yes	no	no	no	0	0	0	no	no	0	0	no	no	no
Control 03	Endometrium	30	0	0	0	22.04	yes	no	no	no	0	0	0	no	no	0	0	no	no	no
Control 04	Endometrium	43	1	0	0	24.46	yes	no	no	no	0	0	0	yes	no	0	0	no	no	no
Control 05	Endometrium	42	2	2	0	32.04	no	no	yes	no	0	0	0	no	no	0	0	no	no	no
Control 06	Endometrium	38	3	3	0	24.86	no	no	yes	no	0	0	0	no	no	0	0	no	no	no
Control 07	Endometrium	22	0	0	1	25.15	no	no	yes	no	10	8	0	yes	no	0	0	no	no	no
Control 09	Endometrium	44	1	0	0	23.73	yes	no	no	no	0	0	0	yes	no	0	0	no	no	no
Control 10	Endometrium	20	0	0	1	21.99	no	no	yes	no	0	0	0	no	no	0	0	no	no	no
Control 11	Endometrium	38	1	1	0	20.52	no	yes	no	no	0	0	7	no	no	0	0	no	no	no
Control 12	Endometrium	34	2	1	0	24.98	no	no	no	no	3	0	0	no	no	0	0	no	no	no
Endometriosis 02	Endometrium	36	1	1	0	35.70	no	yes	no	no	10	10	6	yes	yes	40	IV	yes	no	yes
Endometriosis 02	Lesion GUT	36	1	1	0	35.70	no	yes	no	no	10	10	6	yes	yes	40	IV	yes	no	yes
Endometriosis 02	Lesion surgical site	36	1	1	0	35.70	no	yes	no	no	10	10	6	yes	yes	40	IV	yes	no	yes
Endometriosis 02	Lesion peritoneum	36	1	1	0	35.70	no	yes	no	no	10	10	6	yes	yes	40	IV	yes	no	yes
Endometriosis 02	Normal peritonium	36	1	1	0	35.70	no	yes	no	no	10	10	6	yes	yes	40	IV	yes	no	yes
Endometriosis 03	Endometrium	47	1	1	0	21.09	no	no	yes	no	0	8	0	no	no	10	II	yes	yes	yes
Endometriosis 03	Lesion USL	47	1	1	0	21.09	no	no	yes	no	0	8	0	no	no	10	II	yes	yes	yes
Endometriosis 03	Normal peritonium	47	1	1	0	21.09	no	no	yes	no	0	8	0	no	no	10	II	yes	yes	yes
Endometriosis 04	Endometrium	46	2	2	0	30.80	yes	no	yes	no	10	8	0	no	yes	60	IV	yes	yes	yes
Endometriosis 04	Lesion GUT	46	2	2	0	30.80	yes	no	yes	no	10	8	0	no	yes	60	IV	yes	yes	yes
Endometriosis 04	Lesion ovary	46	2	2	0	30.80	yes	no	yes	no	10	8	0	no	yes	60	IV	yes	yes	yes
Endometriosis 04	Lesion peritoneum	46	2	2	0	30.80	yes	no	yes	no	10	8	0	no	yes	60	IV	yes	yes	yes
Endometriosis 04	Normal peritonium	46	2	2	0	30.80	yes	no	yes	no	10	8	0	no	yes	60	IV	yes	yes	yes
Endometriosis 05	Endometrium	45	2	2	0	23.60	yes	yes	no	no	0	0	6	no	no	4	I	yes	no	yes
Endometriosis 05	Lesion surgical site	45	2	2	0	23.60	yes	yes	no	no	0	0	6	no	no	4	I	yes	no	yes
Endometriosis 05	Lesion belly button	45	2	2	0	23.60	yes	yes	no	no	0	0	6	no	no	4	I	yes	no	yes
Endometriosis 05	Normal peritonium	45	2	2	0	23.60	yes	yes	no	no	0	0	6	no	no	4	I	yes	no	yes
Endometriosis 06	Endometrium	42	1	1	0	33.70	yes	no	no	no	8	0	0	yes	yes	16	III	no	yes	no
Endometriosis 06	Lesion ovary	42	1	1	0	33.70	yes	no	no	no	8	0	0	yes	yes	16	III	no	yes	no
Endometriosis 06	Normal peritonium	42	1	1	0	33.70	yes	no	no	no	8	0	0	yes	yes	16	III	no	yes	no
Endometriosis 07	Endometrium	44	1	1	0	24.70	no	yes	no	no	5	0	0	no	yes	50	IV	yes	yes	yes
Endometriosis 07	Lesion ovary	44	1	1	0	24.70	no	yes	no	no	5	0	0	no	yes	50	IV	yes	yes	yes
Endometriosis 08	Endometrium	36	0	0	0	26.70	no	no	yes	no	10	7	0	yes	yes	80	IV	yes	yes	yes
Endometriosis 08	Lesion GUT	36	0	0	0	26.70	no	no	yes	no	10	7	0	yes	yes	80	IV	yes	yes	yes
Endometriosis 08	Lesion GUT	36	0	0	0	26.70	no	no	yes	no	10	7	0	yes	yes	80	IV	yes	yes	yes
Endometriosis 08	Lesion GUT	36	0	0	0	26.70	no	no	yes	no	10	7	0	yes	yes	80	IV	yes	yes	yes
Endometriosis 08	Lesion ovary	36	0	0	0	26.70	no	no	yes	no	10	7	0	yes	yes	80	IV	yes	yes	yes
Endometriosis 08	Lesion surgical site	36	0	0	0	26.70	no	no	yes	no	10	7	0	yes	yes	80	IV	yes	yes	yes
Endometriosis 08	Normal peritonium	36	0	0	0	26.70	no	no	yes	no	10	7	0	yes	yes	80	IV	yes	yes	yes

Attachment 3: Patients included in the study and their clinical characteristics *(continuing). BMI: Body mass index; COC: Combined oral contraceptive; GnRH: Gonadotropin-releasing hormone; VAS: Visual Analogue Scale; ASRM: American Society of Reproductive Medicine; SUP: superficial endometriosis; OMA: ovarian endometriosis; DE: deep endometriosis.*

Patients	Sample	Age (years)	Number of previous gestations	Parity	Family history	BMI (kg/m2)	Regular cycles	COC last month	Progestin last month	GnRH analog last month	Dysmenorrhea (VAS)	Dyspareunia (VAS)	Non-menstrual pain (VAS)	Infertility	Adenomyosis (US)	ASRM score	ASRM stage	SUP	OMA	DE
Endometriosis 09	Endometrium	45	1	1	0	30.30	no	no	yes	no	8	0	9	yes	yes	62	IV	yes	yes	yes
Endometriosis 09	Lesion GUT	45	1	1	0	30.30	no	no	yes	no	8	0	9	yes	yes	62	IV	yes	yes	yes
Endometriosis 09	Lesion ovary	45	1	1	0	30.30	no	no	yes	no	8	0	9	yes	yes	62	IV	yes	yes	yes
Endometriosis 09	Normal peritonium	45	1	1	0	30.30	no	no	yes	no	8	0	9	yes	yes	62	IV	yes	yes	yes
Endometriosis 10	Endometrium	-	0	0	1	27.59	no	yes	no	no	10	0	0	yes	no	5	I	yes	no	no
Endometriosis 10	Lesion peritoneum	-	0	0	1	27.59	no	yes	no	no	10	0	0	yes	no	5	I	yes	no	no
Endometriosis 10	Normal peritonium	-	0	0	1	27.59	no	yes	no	no	10	0	0	yes	no	5	I	yes	no	no
Endometriosis 11	Endometrium	28	0	0	0	31.18	no	no	yes	no	8	0	5	yes	no	78	IV	yes	yes	no
Endometriosis 11	Lesion USL	28	0	0	0	31.18	no	no	yes	no	8	0	5	yes	no	78	IV	yes	yes	no
Endometriosis 11	Lesion ovary	28	0	0	0	31.18	no	no	yes	no	8	0	5	yes	no	78	IV	yes	yes	no
Endometriosis 12	Endometrium	39	0	0	0	21.64	no	no	no	no	9	8	8	yes	no	52	IV	yes	yes	no
Endometriosis 12	Lesion ovary	39	0	0	0	21.64	no	no	no	no	9	8	8	yes	no	52	IV	yes	yes	no
Endometriosis 12	Lesion peritoneum	39	0	0	0	21.64	no	no	no	no	9	8	8	yes	no	52	IV	yes	yes	no
Endometriosis 13	Endometrium	36	1	0	1	24.09	no	no	yes	no	10	0	0	yes	no	10	II	no	no	yes
Endometriosis 13	Lesion retrocervical	36	1	0	1	24.09	no	no	yes	no	10	0	0	yes	no	10	II	no	no	yes
Endometriosis 13	Normal peritonium	36	1	0	1	24.09	no	no	yes	no	10	0	0	yes	no	10	II	no	no	yes
Endometriosis 14	Endometrium	37	1	0	0	24.00	no	yes	no	no	3	0	0	yes	no	80	IV	no	yes	no
Endometriosis 14	Lesion ovary	37	1	0	0	24.00	no	yes	no	no	3	0	0	yes	no	80	IV	no	yes	no
Endometriosis 14	Normal peritonium	37	1	0	0	24.00	no	yes	no	no	3	0	0	yes	no	80	IV	no	yes	no
Endometriosis 15	Endometrium	25	0	0	0	26.91	no	yes	no	no	10	6	0	yes	no	30	III	yes	no	no
Endometriosis 15	Lesion peritoneum	25	0	0	0	26.91	no	yes	no	no	10	6	0	yes	no	30	III	yes	no	no
Endometriosis 15	Normal peritonium	25	0	0	0	26.91	no	yes	no	no	10	6	0	yes	no	30	III	yes	no	no
Endometriosis 16	Endometrium	38	0	0	0	27.55	no	no	yes	no	8	8	0	yes	no	88	IV	yes	yes	yes
Endometriosis 16	Lesion GUT	38	0	0	0	27.55	no	no	yes	no	8	8	0	yes	no	88	IV	yes	yes	yes
Endometriosis 16	Lesion ovary	38	0	0	0	27.55	no	no	yes	no	8	8	0	yes	no	88	IV	yes	yes	yes
Endometriosis 16	Normal peritonium	38	0	0	0	27.55	no	no	yes	no	8	8	0	yes	no	88	IV	yes	yes	yes
Endometriosis 17	Endometrium	38	0	0	0	27.55	no	no	yes	no	8	8	0	yes	no	88	IV	yes	yes	yes
Endometriosis 17	Lesion GUT	40	1	1	0	29.71	no	yes	no	no	5	5	0	no	no	20	III	no	no	yes
Endometriosis 17	Normal peritonium	40	1	1	0	29.71	no	yes	no	no	5	5	0	no	no	20	III	no	no	yes
Endometriosis 18	Endometrium	28	0	0	0	27.34	no	no	yes	no	8	8	0	yes	no	40	IV	yes	yes	yes
Endometriosis 18	Lesion GUT	28	0	0	0	27.34	no	no	yes	no	8	8	0	yes	no	40	IV	yes	yes	yes
Endometriosis 18	Lesion ovary	28	0	0	0	27.34	no	no	yes	no	8	8	0	yes	no	40	IV	yes	yes	yes
Endometriosis 18	Lesion peritoneum	28	0	0	0	27.34	no	no	yes	no	8	8	0	yes	no	40	IV	yes	yes	yes
Endometriosis 18	Normal peritonium	28	0	0	0	27.34	no	no	yes	no	8	8	0	yes	no	40	IV	yes	yes	yes
Endometriosis 19	Endometrium	47	2	2	0	23.83	no	no	no	no	0	7	1	no	yes	20	III	no	yes	no
Endometriosis 19	Lesion ovary	47	2	2	0	23.83	no	no	no	no	0	7	1	no	yes	20	III	no	yes	no
Endometriosis 19	Normal peritonium	47	2	2	0	23.83	no	no	no	no	0	7	1	no	yes	20	III	no	yes	no
Endometriosis 20	Endometrium	39	1	1	0	21.30	yes	yes	no	no	8	7	8	yes	no	30	IV	no	yes	yes
Endometriosis 20	Lesion GUT	39	1	1	0	21.30	yes	yes	no	no	8	7	8	yes	no	30	IV	no	yes	yes
Endometriosis 20	Lesion ovary	39	1	1	0	21.30	yes	yes	no	no	8	7	8	yes	no	30	IV	no	yes	yes
Endometriosis 20	Lesion surgical site	39	1	1	0	21.30	yes	yes	no	no	8	7	8	yes	no	30	IV	no	yes	yes
Endometriosis 20	Normal peritonium	39	1	1	0	21.30	yes	yes	no	no	8	7	8	yes	no	30	IV	no	yes	yes

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Patients	Sample	Age (years)	Number of previous gestations	Parity	Family history	BMI (kg/m2)	Regular cycles	COC last month	Progestin last month	GnRH analog last month	Dysmenorrhea (VAS)	Dyspareunia (VAS)	Non-menstrual pain (VAS)	Infertility	Adenomyosis (US)	ASRM score	ASRM stage	SUP	OMA	DE
Endometriosis 21	Endometrium	33	1	1	0	23.14	no	no	yes	no	8	0	5	no	no	40	III	yes	yes	no
Endometriosis 21	Lesion ovary	33	1	1	0	23.14	no	no	yes	no	8	0	5	no	no	40	III	yes	yes	no
Endometriosis 21	Lesion peritoneum	33	1	1	0	23.14	no	no	yes	no	8	0	5	no	no	40	III	yes	yes	no
Endometriosis 21	Normal peritonium	33	1	1	0	23.14	no	no	yes	no	8	0	5	no	no	40	III	yes	yes	no
Endometriosis 22	Endometrium	39	2	2	0	27.92	no	yes	no	no	4	0	0	no	no	60	IV	no	yes	no
Endometriosis 22	Lesion ovary	39	2	2	0	27.92	no	yes	no	no	4	0	0	no	no	60	IV	no	yes	no
Endometriosis 22	Normal peritonium	39	2	2	0	27.92	no	yes	no	no	4	0	0	no	no	60	IV	no	yes	no
Endometriosis 23	Endometrium	37	2	2	0	25.21	no	no	yes	no	8	0	8	no	no	72	IV	no	yes	yes
Endometriosis 23	Lesion USL	37	2	2	0	25.21	no	no	yes	no	8	0	8	no	no	72	IV	no	yes	yes
Endometriosis 23	Lesion ovary	37	2	2	0	25.21	no	no	yes	no	8	0	8	no	no	72	IV	no	yes	yes
Endometriosis 23	Lesion retrocervical	37	2	2	0	25.21	no	no	yes	no	8	0	8	no	no	72	IV	no	yes	yes
Endometriosis 23	Lesion ureter	37	2	2	0	25.21	no	no	yes	no	8	0	8	no	no	72	IV	no	yes	yes
Endometriosis 24	Endometrium	30	0	0	0	20.76	yes	no	yes	no	6	0	0	yes	no	25	III	yes	yes	yes
Endometriosis 24	Lesion USL	30	0	0	0	20.76	yes	no	yes	no	6	0	0	yes	no	25	III	yes	yes	yes
Endometriosis 24	Lesion ovary	30	0	0	0	20.76	yes	no	yes	no	6	0	0	yes	no	25	III	yes	yes	yes
Endometriosis 24	Lesion peritoneum	30	0	0	0	20.76	yes	no	yes	no	6	0	0	yes	no	25	III	yes	yes	yes
Endometriosis 24	Normal peritonium	30	0	0	0	20.76	yes	no	yes	no	6	0	0	yes	no	25	III	yes	yes	yes
Endometriosis 25	Endometrium	34	0	0	0	25.71	yes	no	no	no	10	0	0	yes	no	10	II	no	no	yes
Endometriosis 25	Lesion USL	34	0	0	0	25.71	yes	no	no	no	10	0	0	yes	no	10	II	no	no	yes
Endometriosis 25	Lesion peritoneum	34	0	0	0	25.71	yes	no	no	no	10	0	0	yes	no	10	II	no	no	yes
Endometriosis 25	Lesion retrocervical	34	0	0	0	25.71	yes	no	no	no	10	0	0	yes	no	10	II	no	no	yes
Endometriosis 25	Normal peritonium	34	0	0	0	25.71	yes	no	no	no	10	0	0	yes	no	10	II	no	no	yes
Endometriosis 26	Endometrium	39	0	0	1	27.34	yes	no	no	no	9	0	0	yes	no	24	III	no	no	yes
Endometriosis 26	Lesion USL	39	0	0	1	27.34	yes	no	no	no	9	0	0	yes	no	24	III	no	no	yes
Endometriosis 26	Normal peritonium	39	0	0	1	27.34	yes	no	no	no	9	0	0	yes	no	24	III	no	no	yes
Endometriosis 27	Endometrium	34	0	0	0	24.22	no	no	yes	no	10	0	0	yes	no	12	IV	no	no	yes
Endometriosis 27	Lesion bladder	34	0	0	0	24.22	no	no	yes	no	10	0	0	yes	no	12	IV	no	no	yes
Endometriosis 27	Lesion GUT	34	0	0	0	24.22	no	no	yes	no	10	0	0	yes	no	12	IV	no	no	yes
Endometriosis 27	Lesion USL	34	0	0	0	24.22	no	no	yes	no	10	0	0	yes	no	12	IV	no	no	yes
Endometriosis 27	Normal peritonium	34	0	0	0	24.22	no	no	yes	no	10	0	0	yes	no	12	IV	no	no	yes
Endometriosis 28	Endometrium	34	2	2	0	22.06	no	no	yes	no	6	7	0	no	no	10	II	yes	no	yes
Endometriosis 28	Lesion bladder	34	2	2	0	22.06	no	no	yes	no	6	7	0	no	no	10	II	yes	no	yes
Endometriosis 28	Lesion peritoneum	34	2	2	0	22.06	no	no	yes	no	6	7	0	no	no	10	II	yes	no	yes
Endometriosis 28	Normal peritonium	34	2	2	0	22.06	no	no	yes	no	6	7	0	no	no	10	II	yes	no	yes
Endometriosis 29	Endometrium	-	1	1	0	26.35	no	yes	no	no	7	0	0	yes	no	30	III	yes	yes	yes
Endometriosis 29	Lesion USL	-	1	1	0	26.35	no	yes	no	no	7	0	0	yes	no	30	III	yes	yes	yes
Endometriosis 29	Lesion ovary	-	1	1	0	26.35	no	yes	no	no	7	0	0	yes	no	30	III	yes	yes	yes
Endometriosis 29	Lesion peritoneum	-	1	1	0	26.35	no	yes	no	no	7	0	0	yes	no	30	III	yes	yes	yes
Endometriosis 29	Lesion vagina	-	1	1	0	26.35	no	yes	no	no	7	0	0	yes	no	30	III	yes	yes	yes
Endometriosis 29	Normal peritonium	-	1	1	0	26.35	no	yes	no	no	7	0	0	yes	no	30	III	yes	yes	yes

Attachment 4: Manuscript acceptance at Cell Biology International.

Gmail Fernando Reis <reis.ufmg@gmail.com>

Your revised manuscript CBIN.20230824.R1 successfully submitted

1 mensagem

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12-Feb-2024

Dear Prof. Reis:

Your revised manuscript entitled "Translocator protein (TSPO) expression and localization in human endometrium and endometriosis: a potential target for a noninvasive diagnosis?" by Reis, Fernando; Casalechi, Maíra; Cruz, Cynthia Dela; Assis, Wiviane A.; Vieira-Lopes, Millene; F, Felipe Eduardo Lopes; Francisco, Antônio M.C., has been successfully submitted online and is presently being given full consideration for publication in Cell Biology International.

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Attachment 5: Paper published at Cell Biology International.

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RESEARCH ARTICLE

Cell Biology International WILEY

Translocator protein expression and localization in human endometrium and endometriosis: A potential target for a noninvasive diagnosis?

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Abstract

The limitations of current imaging methods to detect small or superficial endometriotic lesions prompt the search for new molecular targets. TSPO is an 18 KDa protein located in the outer mitochondrial membrane, which can be traced by positron emission tomography (PET) using specific ligands. TSPO is located mostly in neurons and inflammatory sites outside the brain. We hypothesized that it might also be expressed in the human endometrium and endometrial-like tissue, being a target for molecular imaging of endometriosis. This prospective cross-sectional study included 28 women with endometriosis and 11 endometriosis-free controls. Endometriotic lesions ($n = 49$) and normal peritoneum ($n = 13$) from endometriosis patients were obtained during laparoscopy, while samples of eutopic endometrium from patients with endometriosis ($n = 28$) and from control women ($n = 11$) were collected in the operating room using a flexible device. TSPO mRNA expression was evaluated by quantitative reverse-transcription real-time PCR while protein expression was evaluated by immunohistochemistry with a monoclonal antibody antihuman TSPO. TSPO mRNA expression was detected in an invariable fashion in all tissue types evaluated; however, TSPO protein was found to be more abundant in the glandular epithelium than in the stroma, both in the endometrium and in the endometriotic lesions. Interestingly, hormone therapies did not alter the expression of TSPO, and its presence was mostly negative in tissues adjacent to endometriotic implants. As a proof of concept, the protein expression pattern of TSPO in endometriotic tissue and along the adjacent areas suggests that TSPO-based molecular imaging might be used for noninvasive endometriosis detection.

KEYWORDS
biomarkers, endometriosis, endometrium, TSPO

