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Influence of progestin use on the IL-10 level in peritoneal endometriosis

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***Abstract*---**Purpose: How can progestin influence the anti-inflammatory cytokine IL-10 level in the peritoneum with endometriosis? Methods: A cross-sectional study with a laboratory analysis carried out in tertiary hospitals in southern Brazil with clinical-demographic data compilation and sample collection of peritoneal lavage and a peritoneum biopsy from 40 patients — 22 with endometriosis (11 with progestin and 11 without it) and 18 in the control group — was conducted. For the sample analysis, the ELISA was performed, and a comparison of the average IL-10 concentrations by ANOVA followed by Bonferroni was carried out. Results: There was a higher concentration of IL-10 among patients with endometriosis compared to the control group ($p \leq 0.05$), and statistical significance was observed with a reduction of IL-10 in the peritoneal lavage of

patients with endometriosis in the use of progestin (8.1 ± 7.4 pg/ml) compared to those with endometriosis without using progestin (17.9 ± 10.5 pg/ml). Considering the disease stage, a higher level of IL-10 ($p \leq 0.05$) was observed both in the initial stages (12.1 ± 10.5 pg/ml) and in advanced stages (15.4 ± 9.7 pg/ml) compared to the control (1.7 ± 1.8 pg/ml), which was significantly higher for advanced endometriosis. Conclusion: This study reinforces the hypotheses of a higher IL-10 concentration in the peritoneum with endometriosis, confirming its increase with disease progression. Nevertheless, it suggests progestin's real influence as a control promoter over the runaway inflammatory process in the peritoneum affected by endometriosis.

***Keywords*---**endometriosis, peritoneum, cytokines, IL-10, progestin.

Introduction

Endometriosis is a benign chronic disease, which results from abnormal implantation of the endometrial tissue outside the uterine cavity, wherein the peritoneum is the leading site. It affects about 6.1 to 10.0% of women of reproductive age, but its prevalence is possibly underestimated due to unspecific or absent symptoms [1-3]. The definitive diagnosis requires laparoscopic confirmation, which allows for its classification in evolution stages according to criteria defined by the American Society for Reproductive Medicine (ASRM) [4-7].

The literature describes pelvic pain (86.2%), dyspareunia (29.5%), and infertility (11.6%) as the most important clinical factors of endometriosis [2,8-10]. The pain intensity is possibly not related to the lesion's extent but rather due to the unrestrained inflammatory process in the peritoneal environment [11-14]. When determining its etiology, the most accepted theory is based on retrograde menstruation. Although it is a prevalent phenomenon (76.0 to 90.0%), only 10.0% of women experience eliminating this refluxed material and its consequent implantation and evolution to a chronic inflammatory process [15-17].

When analyzing the disease's inflammatory profile in the peritoneum, there is a loss of homeostasis because there is an increase in pro-inflammatory and anti-inflammatory cytokines. The progesterone resistance characteristic of endometriosis can be attributed to this lack of control [17-20]. Several studies have been conducted to identify the cytokines involved in the disease, and Interleukin-10 (IL-10) stands out as one of the central anti-inflammatory cytokines associated with the maintenance and growth of the ectopic endometrium [10,21]. It is believed that a possible increase in the level of IL-10 both in the serum and in the peritoneal lavage corroborates with an immune system imbalance and impairs the peritoneal cell capacity to perform the apoptosis of endometriotic lesions, thus favoring the progression of lesions [12,22-23]; however, IL-10 concentrations in patients with endometriosis described in the literature are divergent and are not well-established [20,22].

Endometriosis's clinical and social importance can be illustrated by damaging these patients' quality of life [16]. Because it is characterized as neuropathic pain, its symptoms damage mental health, leading to depression and anxiety [24]. The first choice for pain control and improvement in life quality is progestin use, whether associated or not with estrogen [7,25]. Progesterone is believed to be effective in restoring immune system homeostasis and performing the apoptosis of ectopic endometrial cells [7,26-27]. It is the only natural progestogen produced by the *corpus luteum* after ovulation, the placenta during pregnancy, and the adrenals and the nervous system. Synthetic progestogens attempt to simulate progesterone's effect and are called progestins. Progestins are derived from progesterone itself and testosterone. Small changes in the original molecules can induce considerable differences in the activity of each progestin [28].
Given the high prevalence of endometriosis and the damage caused to these women, the importance of clarifying the phenomena involved is highlighted as well as the need for further studies to investigate inflammatory behavior in heterogeneous groups and under hormonal influence [17,29-31]. Thus, in this study, progestin's influence on the levels of IL-10 in the peritoneum of women with endometriosis was analyzed.

Materials and methods

Study populations

A cross-sectional study with an experimental design was conducted to test the hypothesis, and human tissue samples were analyzed, followed by a laboratory evaluation. The samples were obtained from a peritoneal lavage and a peritoneum biopsy from patients who underwent laparoscopic treatment for peritoneal endometriosis and patients without the disease who underwent a tubal ligation. The collection took place in public and private tertiary hospitals in southern Brazil from August 2019 to January 2020.

Interviews were carried out, and peritoneum biopsy and peritoneal lavage samples were collected from 40 patients who underwent laparoscopic surgery. Of these, 22 correspond to patients with endometriosis (11 without using hormonal medication and 11 using progestin) and 18 without the disease (11 without using hormonal medication and seven using progestin). The predicted sample was 16 patients per group based on the study by Malutan et al. (2015) in which it was assumed that parameters for a two-tailed test, a significance level of 5%, a test power of 80%, a standard deviation of 1.14, and average difference were considered relevant to be detected equal to 0.8 pg/ml of IL-10 [12].

Women in menacme aged ≥18 years were included and agreed to participate in this study by signing the Free and Informed Consent Form (ICF). The exclusion criterion was using any class of anti-inflammatory drugs within seven days before the surgical procedure.

After consenting with the ICF signature during the preoperative period, the eligible patients answered a questionnaire that evaluated clinical and demographic criteria. During the surgical procedure, for all participants, immediately after entering the peritoneal cavity, 5.0mL of 0.9% saline was

injected into the Douglas cul-de-sac and aspirated to 1.5mL for storage in identified plastic tubes. After inspecting the peritoneal cavity in patients with visualized endometriosis, endometrial implants were classified into stages according to the revised ASRM5, and biopsies were performed on the peritoneal endometriotic lesion. In the control group, the biopsy was derived from a healthy peritoneum in the posterior cul-de-sac (Figure 1). The samples were sent to the Experimental Neuroscience Laboratory (LANEX), located at the University of South of Santa Catarina (UNISUL), for conservation in an Ultrafreezer at -80˚C until the time of analysis. The technique adopted for the collection was standardized and was followed according to a predetermined script.

Levels of IL-10 in human samples

To determine the levels of IL-10, specimens of peritoneum and samples of peritoneal lavage stored at -80°C were unfrozen, and a volume of 100 µL was run. According to the manufacturer's instructions, the analyses of the concentrations of this marker were performed by ELISA and Kits Duo Set (R&D Systems). The values obtained were estimated by interpolating data with a standard curve using a colorimetric assay measured at 450 nm (correction with a wavelength of 540 nm) with a spectrophotometer. The values obtained are expressed in picograms per milliliter (pg/ml).

Study's variables

For the study's variables, the dependent ones are the IL-10 concentrations in the peritoneal lavage and peritoneal tissue (in pg/ml or pg/mg of protein). The independent ones are age (years), skin color (white or non-white), parity (number of pregnancies), phase of the menstrual cycle (proliferative/secretory), the stage of endometriosis (minimal/mild and moderate/severe), and the use of hormonal medication (yes/no). Pain (pelvic pain, dysmenorrhea, deep dyspareunia, dysuria, and dyschezia) was evaluated in average scores of values obtained from the Visual Analogue Scale (VAS), whose maximum value is 10, being 0 = no pain and 10 = maximum pain; this scale is validated and widely used in Brazil.

Statistical analyses

The data obtained were input into a database using SPSS software version 18.0. In the descriptive analysis, the variables were expressed as frequencies, means, and medians. The variables were categorized in the bivariate analysis, and the chi-square or Fischer's exact tests were applied. GraphPad Prism© software version 6.0 (GraphPad Software, San Diego, CA, USA) was also used. The independent variables were presented descriptively using frequency distribution tables. The dependent variables were plotted for means and standard deviation (SD). The p-value was considered statistically significant when $p < 0.05$, and there was a 95% confidence interval (95% CI).

The data from the IL-10 means between groups were first subjected to the Shapiro-Wilk normality test, and to identify the normal distribution, they were analyzed by ANOVA followed by Bonferroni.

Compliance with Ethical Standards

Regarding the ethical aspects of the research, it was approved by the Research Ethics Committee (CEP) of UNISUL under CAAE 16719219.9.0000.5369. All participants were assigned the ICF. The researchers declare that there is no conflict of interest.

Results

Demographic and clinical characteristics

Peritoneal samples were collected from 40 patients, 22 with endometriosis and 18 in the control group (without endometriosis). Among women with endometriosis, 11 were using progestins [Dienogest (36.3%), combined oral contraceptives (COC) (27.3%), intramuscular Medroxyprogesterone Acetate (DMPA) (18.2%), and Levonorgestrel intrauterine system (LN-IUD) (18.2%)], and 11 did not use any progestin. The main objective of using progestin for 72.7% of these women was to control pain. Among patients without endometriosis, seven used progesterone for contraception [COC (42.9%) and LN-IUD (57.1%)], and 11 did not use any hormonal medication (Figure 2). According to the ASRM criteria, considering the 22 patients with endometriosis, 16 (72.7%) had a minimal or mild classification and six (27.3%) moderate or severe.

When assessing demographic and clinical characteristics, mean age of 37.7 ± 7.55 years was identified for the patients under study. The nulliparity was higher between the group with endometriosis, and there was no difference in the history of abortion between the two groups. The occurrence of dysmenorrhea and deep dyspareunia was quantified according to VAS. Dysmenorrhea was more intense in patients with endometriosis who did not use progestin, with an average score of 6.65 ± 3.03 points, and was also higher in the control group without progestin use, with an average score of 3.13 ± 3.46 points; however, there was no statistical significance in the difference in means ($p = 0.775$). The same finding was observed concerning deep dyspareunia, for which the highest mean values of the scale were observed in women with endometriosis who did not use progestin (4.27 ± 3.32 points). In comparison, the control group had a lower mean value between women utilizing progestin (0.50 ± 1.41 points), but there was no statistical difference (Table 1).

When considering the variables according to the presence of endometriosis, three characteristics were statistically significant: a history of nulliparity indicated a greater chance of occurring in women who have endometriosis with an odds ratio (OR) of 57 ($p < 0.001$); dysmenorrhea (VAS ≥ 6) is more related to endometriosis (OR 5.08; $p = 0.023$); in the same way as deep dyspareunia (VAS ≥ 6), which is more common in women with the disease (OR 14.17; $p = 0.017$). (Table 2) There was no statistical significance when analyzing these categorized variables among patients who used progestin or did not (data not shown).

Influence of the use of progestin on the levels of IL-10

Considering the laboratory findings for the analysis of the influence of the use of progestin on the levels of the anti-inflammatory cytokine IL-10 in the peritoneal lavage of patients with endometriosis compared to the group without endometriosis, there was a statistically significant increase in IL-10 both in women who used progestin (with endometriosis 8.1 ± 7.4 pg/ml; without endometriosis: 1.2 ± 1.2 pg/ml ;) and in those who did not use it (with endometriosis: 17, 9 ± 10.5 pg/ml; without endometriosis: 1.9 ± 2.2 pg/ml).

When comparing women belonging to the group with endometriosis, a reduction in these cytokine levels were observed in women who used progestin (8.1 ± 7.4 pg/ml) compared to those who did not use it (17.9 ± 10.5 pg/ml) (Figure 3a); however, when this evaluation was performed using samples of peritoneum biopsies, no statistically significant differences were observed in the levels of this cytokine between women with or without endometriosis regardless of the use of progestin (Figure 3b).

Influence of the degree of endometriosis on IL-10 concentrations

The analysis of the influence of the degree of endometriosis on IL-10 concentrations showed a statistically significant difference in both the peritoneal lavage and the peritoneum biopsy. In the study of the peritoneal fluid, a higher concentration of IL-10 was identified in the group with endometriosis, either in the minimal/mild stage (12.1 ± 10.5 pg/ml) or the moderate/severe stage (15.4 ± 9.7 pg/ml), when compared to the group without endometriosis (1.7 ± 1.8 pg/ml) ($p \leq 0.05$); however, there was a significant difference in the mean values of this cytokine between the two staging groups (minimal/mild and moderate/severe) (Figure 4a). Regarding the samples of peritoneum biopsies, a statistically significant increase was observed only among the most advanced stage of the disease group (moderate/severe: 4.5 ± 4.4 pg/ml) for the group without endometriosis (-0.04 ± 0.5 pg/ml) (Figure 4b).

Analysis of the influence of the menstrual cycle on the level of IL-10

Finally, regarding the analysis of the influence of the menstrual cycle (proliferative and secretory phase) on the level of IL-10, in the peritoneal lavage, there was no statistically significant difference between women in the group with endometriosis (proliferative phase: 12.8 ± 8.6 pg/ml; secretory phase: 15.5 ± 11.9 pg/ml) and the group without endometriosis (proliferative phase: 0.8 ± 0.3 pg/ml; secretory phase: 2.4 ± 2.2 pg/ml), and no difference was observed between the phases of the menstrual cycle within the groups. Besides, the peritoneal biopsy samples had no statistically significant difference between the group with endometriosis (proliferative phase: 1.2 ± 2.7 pg/ml and secretory phase: 0.7 ± 1.4 pg/ml) and the group without the disease (proliferative phase: 1.2 ± 2.1 pg/ml and secretory phase: 0.3 ± 0.6 pg/ml). There was no difference observed regarding the phases of the cycle between the groups.

Discussion

This study is believed to be the first to describe progestin's possible action as a reducer of IL-10 associated with endometriosis advancement (Figure 5). Considering the women's demographic and clinical characteristics included in this study, a profile like that described in the literature was obtained, such as age, the number of children, and painful symptoms [20,32]. Statistically significant differences were found for nulliparity between groups with and without endometriosis: those with the disease were less likely to have children. The history of abortion described in the study population did not show any difference between patients with and without endometriosis. Statistical relevance was also obtained for the presence of dysmenorrhea and deep dyspareunia, which were more intense among women with endometriosis. According to previous studies, these obstetric and gynecologic characteristics are frequently highlighted as the primary clinical factors observed among patients with endometriosis [10,32].

There was an influence of progestin use on the anti-inflammatory profile of peritoneal endometriosis, which was suggested by the reduced concentration of IL-10 in the peritoneal lavage of these patients. The only study that indirectly suggests that progestin reduces inflammation in the peritoneum with endometriosis is that of Margatho et al. (2020) [33]; however, the present study indicated differences concerning their research for some inclusion criteria because the participants included were women with deep endometriosis, and the contraceptive method evaluated was an etonogestrel subdermal implant (ENG) or LN-IUD. They performed blood measurements of the CD23 biomarker at six and 24 months after ENG or LN-IUD insertion. The users of these devices showed a reduction in the dosed values compared to non-users, which reinforces the present study's findings because CD23 is a marker that possibly reflects the inflammatory response associated with endometriotic implants [33].

The use of hormonal therapy for pain control of the disease is widely used in clinical practice and has positive results established in the literature [23,27,34-35]. Such use was ratified in this study population as most women used progestin to control pain; furthermore, recent studies have expanded the use of hormonal methods, emphasizing progestin could reduce endometriotic lesions and the volume of endometriomas [32,34]. Barra et al. (2020) observed a significant regression in the volume of lesions caused by rectosigmoid endometriosis [32]. Vignali et al. (2020) followed success in reducing the volume of endometriomas: 76.1% in 12 months ($p < 0.001$) [34]. Both used a transvaginal ultrasound for quantification. To provide these benefits of progestin to women with endometriosis, the possible action of its use to reduce the intense inflammatory process observed in the peritoneal lavage of these patients is highlighted.

Considering the concentration of IL-10—also in the peritoneal lavage—between samples with and without endometriosis, significantly higher levels of this interleukin were observed in patients with endometriosis, importantly among those who did not use hormonal medication. Recent studies using similar methods and comparing women with and without endometriosis also showed a higher concentration of IL-10 in the peritoneal lavage of the group with endometriosis [20,23,36]. Volpato et al. (2018) and Jiang et al. (2019) highlighted

the importance of further investigations, given the relevance of this cytokine in endometriosis's pathogenesis even without obtaining statistically significant results regarding IL-10 [22,31].

In a case-control study with 24 women with endometriosis and 32 belonging to the control group without endometriosis, Bellelis et al. (2019) presented results similar to those of the present study with an emphasis on the increased levels of IL-10 in the peritoneal fluid of patients with endometriosis ($p = 0.025$) and without a sufficient detection of IL-10 in the endometrial biopsy samples of the same group compared to the control [20]. Concerning the discrepancies between the results obtained by these authors in connection with the present study, they could be due to the difference in the severity of endometriosis. Bellelis et al. (2019) only studied deep endometriosis and not superficial peritoneal endometriosis. These authors suggest that the increased anti-inflammatory response in the peritoneum corresponds to an attempt by the immune system to control the intense inflammatory process resulting from endometriotic lesions. This hypothesis was also suggested by Nanda et al. (2020), who used blood samples for analysis [36].

Another differential aspect of the present study is the predominance of women with endometriosis in early stages, given the frequent report of samples' predominance in advanced settings and the interest in identifying the runaway inflammatory process behavior in specimens minimal and mild stages [20-21]. The association between the stage of endometriosis and the concentration of IL-10 is uncertain in the literature [10,21,23]. The concentration of IL-10 in the peritoneal lavage was significantly increased in all stages of endometriosis compared to the control group. It was higher for more advanced stages, but there was no statistical significance when comparing advanced stages to early stages. A similar result was presented by Sikora et al. (2018), who found higher levels of IL-10 in peritoneal fluid samples in stages III and IV compared with stages I and II [35]. Furthermore, it positively contributes to clarifying the existing gap regarding the peritoneum's inflammatory profile behavior to a minimal and mild endometriosis degree. Likewise, it reinforces the hypothesis of a higher concentration of IL-10 in moderate and severe stages [20-21,23].

Some studies suggest that IL-10 has the potential for the development, maintenance, and increase of endometriosis foci because the loss of the refluxed phagocytosis capacity endometrium is attributed to this cytokine [10,21]. Suen et al. (2019) found that samples of endometrial tissue in stages III and IV showed an increase in cells responsible for IL-10 secretion, such as macrophages and Th17 cells, signaling more migration inflammatory cells and stimulating angiogenesis. These mechanisms cause the highest secretion of IL-10 due to the increase in the number of cells responsible for its secretion [21].

Thus, considering the results and the studies cited, it is also suggested that there is an association of IL-10 with the possible uncontrolled inflammation and consequent maintenance of the ectopic endometrium, leading to the cascade of events responsible for chronic inflammation and the loss of immune system homeostasis. Thus, progestin can contribute to stopping this inflammatory

cascade and can consequently prevent the lesions from evolving to advanced stages and can prevent irreversible distortions in the pelvic anatomy.

Limitations

The hormone-dependent characteristic of endometriosis is known, and there have been discussions involving the phase of the menstrual cycle and the inflammatory profile in the peritoneum; however, no significant values were obtained associated with the phase of the menstrual cycle and the concentration of IL-10 [11,20]. This result may be due to the low number of samples in the analyzed groups and the samples' heterogeneity in a proliferative and secretory phase. Still, it indeed serves as a basis for sample calculations.

Conclusion

Although the disease's mechanism remains uncertain, this study contributes to confirming the evidence of a higher IL-10 concentration in the peritoneum of women with endometriosis and its possible influence on the maintenance and progression of the ectopic endometrium in the same way as the identification of a higher concentration of IL-10 in the more advanced stages of the disease. It also suggests progestin's possible action in attenuating the concentration of IL-10 in the peritoneum affected by endometriotic lesions.

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Declarations

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethics approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by thr Research Ethics Committee (CEP) of UNISUL under CAAE 16719219.9.0000.5369

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability
Not applicable

Authors' contributions
All authors whose names appear on this article made substantial contributions to the conception and interpretation of data;

PMP Fermino Project development, data collection, data analysis, manuscript writing
LK Volpato Project development, manuscript writing
AP Piovezan Project development, data analysis, manuscript editing
VV Horewicz data collection, data analysis
DF Martins data collection
RD Nunes Project development, data collection, data analysis, manuscript editing

All authors drafted the work or revised it critically for important intellectual content; approved the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by thr Research Ethics Committee (CEP) of UNISUL under CAAE 16719219.9.0000.5369

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Consent for publication
Not applicable

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Tables and Figures

Table 1 Description of the patients' demographic and clinical characteristics under study and their respective groups: patients with endometriosis (using or not using progestins) and patients without endometriosis (using or not using progestins). Brazil, 2020

Variables	Total	Endometriosis		Control	
		No Progestin	With Progestin	No Progestin	With Progestin
	n (%) average ± SD	n (%) average ± SD	n (%) average ± SD	n (%) average ± SD	n (%) average ± SD
Age(years) (n=40)	37.7 ± 7.55	36.2 ± 5.07	35.4 ± 8.05	44.4 ± 8.34	34.5 ± 4.81
Color (n=40)					
White	36 (90.0)	11 (100.0)	10 (90.9)	8 (77.7)	7 (100.0)
Not white	4 (10.0)	0 (0.0)	1 (9.1)	3 (27.3)	0 (0.0)
Parity (n=40)					
No	18 (45.0)	9 (82.0)	8 (73.0)	1 (9.1)	0 (0.0)
Yes	22 (55.0)	2 (8.0)	3 (27.0)	10 (90.9)	7 (100.0)
Miscarriage (n=40)					
No	34 (85.0)	10 (90.9)	8 (72.7)	9 (81.8)	7 (100.0)
Yes	6 (15.0)	1 (9.1)	3 (27.3)	2 (18.2)	0 (0.0)
Dysmenorrhea (VAS) (n=40)	4.9 ± 3.56	6.65 ± 3.03	5.88 ± 2.91	3.13 ± 3.46	1.25 ± 2.31
Deep dyspareunia (VAS) (n=40)	3.06 ± 3.33	4.27 ± 3.32	3.94 ± 3.71	1.4 ± 2.23	0.50 ± 1.41
Infertility (n=40)					
No	34 (85.0)	6 (54.5)	10 (90.9)	11 (100.0)	7 (100.0)
Yes	6 (15.0)	5 (45.5)	1 (9.1)	0 (0.0)	0 (0.0)
Endometriosis stage (n=22)					
Minimal/mild	16 (72.7)	8 (72.7)	8 (72.7)	-	-
Moderate/severe	6 (27.3)	3 (27.3)	3 (27.3)	-	-

Abbreviation: SD - standard deviation; VAS - Visual Analogue Scale to evaluate pain, whose maximum value is 10, 0 = no pain, and 10 = maximum pain.
Legend: Endometriosis - women underwent laparoscopic treatment for peritoneal endometriosis; Control - healthy women, submitted to laparoscopic tubal ligation. No Progestin – women who did not use progestin; With progestin – women who did use progestin.; Color - skin color (white or no-white); Parity - number of pregnancies; Miscarriage - number of miscarriages; Dysmenorrhea (VAS) - menstrual pain evaluated in average scores of values obtained from VAS; Deep dyspareunia (VAS) - intercourse pain evaluated in average scores of values obtained from VAS; Infertility – women rush for infertility (yes or no). Endometriosis stage: the staging of endometriosis (minimal/mild or moderate/severe) according to the American Society for Reproductive Medicine.

Table 2 Categorized analysis of demographic and clinical characteristics among patients with and without endometriosis

Variables	Endometriosis			p-value	OR (IC95%)
	Yes n (%)	No n (%)	Total n (%)		
Age (years) (n=40)					
18-35	13 (65.0)	7 (35.0)	20 (50.0)	0.207	1
≥ 36	9 (45.0)	11 (55.0)	20 (50.0)		0.44 (0.12,1.57)
Color (n=40)					
Not white	1 (25.0)	3 (75.0)	4 (10.0)		1
White	21 (58.3)	15 (41.7)	36 (90.0)	0.116	4.20 (0.40, 44.40)
Nulliparity (n=40)					
No	5 (22.7)	17 (77.3)	22 (55.0)		1
Yes	17 (94.4)	1 (5.6)	18 (45.0)	<0.001	57.00 (6.09, 548.31)
Miscarriage (n=40)					
No	18 (52.9)	16 (47.1)	34 (85.0)		1
Yes	4 (66.7)	2 (33.3)	6 (15.0)	0.536	1.78 (0.29, 11.04)
Phase of the menstrual cycle (n=22)					
Secretory	6 (50.0)	6 (50.0)	12 (54.5)		1
Proliferative	5 (50.0)	5 (50.0)	10 (45.5)	0.500	1.00 (0.19, 5.36)
Pelvic Pain (VAS) n=40					
0-5	19 (54.3)	16 (45.7)	35 (87.5)	0.810	1
6-10	3 (60.0)	2 (40.0)	5 (12.5)		1.26 (0.19,8.52)
Dysmenorrhea (VAS) n=40					
0-5	9 (39.1)	14 (60.9)	23 (57.5)	0.023	1
6-10	13 (76.5)	4 (23.5)	17 (42.5)		5.06 (1.25,20.48)
Deep dyspareunia (VAS) n=40					
0-5	12 (41.4)	17 (58.6)	29 (72.5)	0.017	1
6-10	10 (90.9)	1 (9.1)	11 (27.5)		14.17 (1.59,125.88)

Dysuria (VAS) n=40					
0-5	20 (52.6)	18 (47.4)	38 (95.0)	0.340	1
6-10	2(100.0)	0 (0.0)	2 (5.0)		4.51 (0.20,1000.24)
Dyschezia (VAS) n=40					
0-5	18 (50.0)	18 (50.0)	36 (90.0)	0.150	1
6-10	4 (100.0)	0 (0.0)	4 (10.0)		9.00 (0.45,179.33)

Abbreviation: OR - Odds Ratio; VAS - Visual Analogue Scale to evaluate pain, whose maximum value is 10, 0 = no pain, and 10 = maximum pain.
Legend: Endometriosis – women with endometriosis (yes or no); Color - skin color (white or non-white); Nuliparity – women with no child (yes or no); Miscarriage – women whit historic of miscarriages (yes or no); Pelvic Pain (VAS) – pelvic pain evaluated in scores of values obtained from VAS; Dysmenorrhea (VAS) - menstrual pain evaluated in scores of values obtained from VAS; Deep dyspareunia (VAS) - intercourse pain evaluated in scores of values obtained from VAS; Dysuria (VAS) – miccional pain evaluated in scores of values obtained from VAS; Dyschezia (VAS) – defecation pain evaluated in scores of values obtained from VAS.

Results found in the present study for endometriotic women:	Supported by:
↑ Nulliparity, dysmenorrhea and deep dyspareunia	References 10, 31
↑ IL-10 in the peritoneal lavage	References 20, 23, 35
↑ IL-10 in the peritoneal lavage in early stages	**NOVELTY**
↓ of pain by progestins	References 23, 27, 33, 34
↓ of IL-10 in peritoneal lavage by progestins	**NOVELTY**

Fig 1 Chart showing the macroscopic characteristics of collected specimens according to ASRM criteria from the different patient groups
Legend: Control- healthy women submitted to laparoscopic tubal ligation. Endometriosis- women who underwent laparoscopic treatment for peritoneal endometriosis.
Abbreviation: ASRM - American Society for Reproductive Medicine.

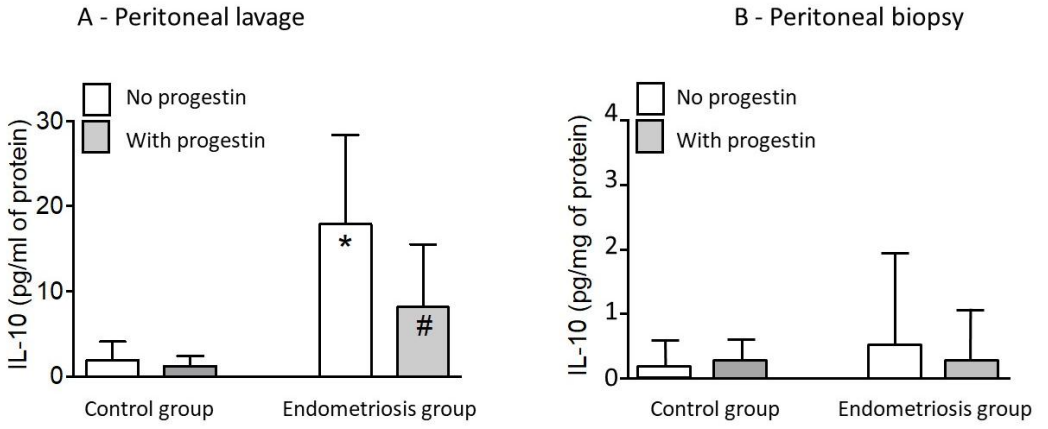

Fig 2 Influence of the use of progestin on IL-10 levels in women with or without endometriosis. Results are presented as mean ± SD. Two-way ANOVA followed by Bonferroni. P <0.05. Brazil, 2020
Legend: A - peritoneal lavage; B - peritoneum biopsy; IL-10 – Interleukin 10; (*): the statistically significant difference of the control group/ endometriosis group without progestin; (#): statistically significant difference of the endometriosis group with/without progestin.
Control group - healthy women submitted to laparoscopic tubal ligation;
Endometriosis group - women who underwent laparoscopic treatment for

peritoneal endometriosis; No Progestin – women who did not use progestin; With progestin – women who did use progestin.

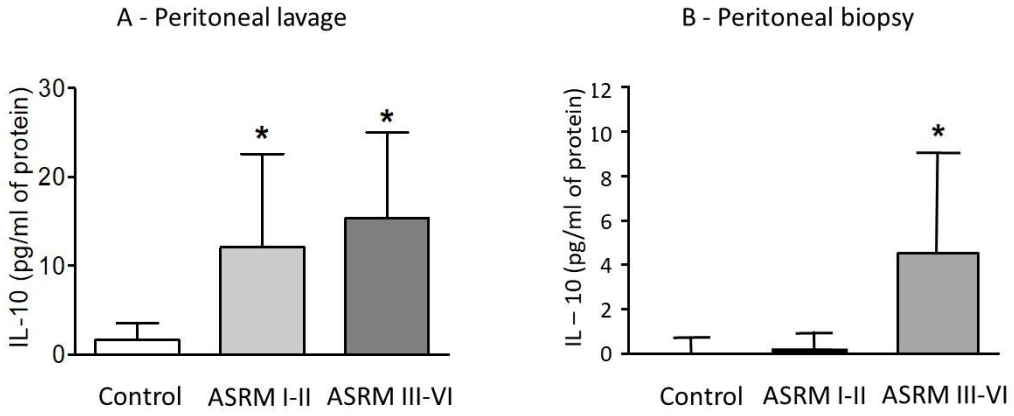

Fig 3 Influence of the endometriosis stage on IL-10 levels in women with or without endometriosis. Results are presented as mean ± SD. Two-way ANOVA followed by Bonferroni. P <0.05. Brazil, 2020
Legend: a - peritoneal lavage; b - peritoneum biopsy; IL-10 – Interleukin 10; ASRM – American Society for Reproductive Medicine; (*): statistically significant difference concerning the control group. P <0.05.
Control group - healthy women submitted to laparoscopic tubal ligation; Endometriosis group - women who underwent laparoscopic treatment for peritoneal endometriosis; ASRM I-II - the staging of endometriosis minimal/mild according to the American Society for Reproductive Medicine (ASRM). ASRM II-IV - the stage of endometriosis moderate/severe according to ASRM.

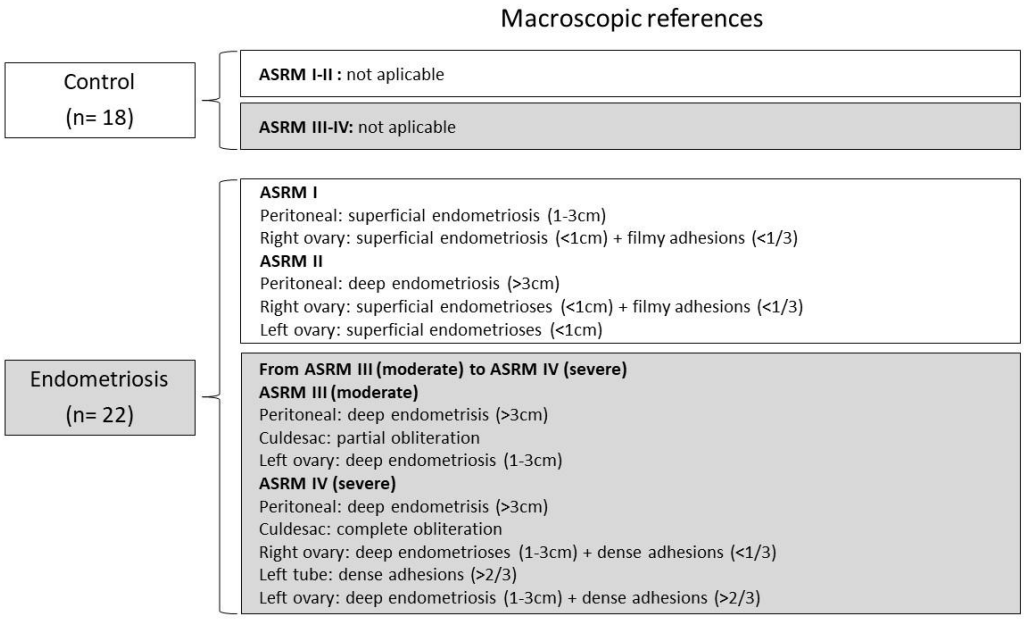

Fig 4 Summary figure with the novelty of the study