Assessment of Differential Expression of Progesterone Receptors A & B in Endometriosis Undergoing IVF Treatment: A Personalized Approach for Better IVF Success

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ABSTRACT

Objective: Estrogen and Progesterone hormones play a pivotal role in the pathogenesis of Endometriosis. In the present study, relative quantification of PR-A and PR-B expression in eutopic endometrial tissue and endometriotic implants was done. By examining their expression profiles, we aim to gain deeper insights into the molecular mechanisms that can influence IVF success or failure in endometriotic patients. Our study also observed certain ART outcomes in both endometriosis and control groups, to evaluate the IVF success rate.

Methods: This study was conducted at MHRT, a tertiary care center, and referral centers from other hospitals. The research encompassed an examination of 125 cases of infertility spanning from January 2019 to January 2023. Finally, biopsy samples were collected from 60 patients with endometriosis during laparoscopic procedures, while control biopsy samples were gathered from 18 patients who exhibited no signs of endometriosis lesions during the same laparoscopic procedures. The biopsy samples were then sent for Progesterone assay and later evaluated for ART outcomes.

Results: It was observed from the results of our study that there was a significant downregulation of PR-A in stage III, and IV compared to Stage I and II. However, there was no significant change in PR-B expression in grades I and II, III and IV. Our study also observed certain ART outcomes in both endometriosis and control groups, revealing that women with endometriosis have lower chances of achieving pregnancy than women with other causes of infertility (95% CI, 22/60 vs. 12/18, p-value = 0.024).

Conclusion: Our study results shed light on the complex role of progesterone isoforms in endometriosis. Progesterone receptor-A significant downregulation in severe cases challenges existing paradigms and opens new avenues for research.

Keywords: Endometriosis, IVF, Progesterone receptor-A, Progesterone receptor-B.

1. Introduction

1.1. Background

Implantation is a well-organized procedure in which the blastocyst securely adheres to the endometrium and infiltrates the epithelium, ultimately giving rise to the formation of the placenta. This intricate process hinges on the seamless coordination of the fertilized egg’s development into a blastocyst and the specific transformation of the endometrial tissue, driven by molecular and cellular modifications. These changes are intricately regulated by a variety of agents exhibiting endocrine, paracrine, or autocrine activities [1], [2]. This critical phase, often referred to as the ‘window of implantation,’ necessitates a complex molecular conversation between the secretory activities of the endometrial tissue and those of the blastocyst.
Progesterone and estrogen are the steroid hormones that play an important role in the regulation of the implantation window. Progesterone induces changes in signaling pathways that lead to the establishment of a receptive endometrium [3]. Estrogen receptors (ERα and β) and progesterone receptors (PR) A and -B are expressed in the epithelium and stroma of the human endometrium. The majority of estrogen’s biological effects are orchestrated by the estrogen receptor (ER). ER achieves this by engaging with site-specific DNA and collaborating with various co-regulatory proteins. However, during the process of implantation, the signaling pathways involving estrogen receptor (ER) and progesterone receptor (PR) operate differently. In this context, ER and PR signaling relies on paracrine and autocrine factors facilitated by growth factors and cytokines [4]. Progesterone exerts its influence by activating the canonical progesterone receptors (PRs), which function in a genomic manner. These PRs play a pivotal role in regulating the transcriptional responses of genes associated with implantation [4]. Progesterone stimulates an elevation in the gene expression of integrin αβ3 within epithelial cells [5]. Integrins represent a family of transmembrane-binding glycoproteins composed of two distinct protein subunits, namely α and β. They serve as receptors for various components, including extracellular matrix molecules, glycoproteins, and neighboring cells. The accumulation of integrins at adhesion sites results in the formation of a network of cytoskeleton proteins. Endometrial receptors, including Estrogen (E2) and Progesterone (P4), play a pivotal role in hormonal genomic actions and the expression of specific biomarkers associated with its receptive differentiation. Both of these receptors achieve their peak expression in the glandular epithelium and stromal tissue during the late proliferative and early secretory phases of the menstrual cycle. However, after day 19, there is a sudden disappearance of E2 and P4 receptors from the glandular tissue, likely influenced by the actions of progesterone, while these receptors continue to persist in the stromal region. Various techniques have been used to study the endometrium gene expression of mapping to study receptive endometrium.

Progesterone and progestins have long been used for the treatment of endometriosis to relieve pain, mainly by inducing pseudo-pregnancy, thus suppressing ovarian estrogen biosynthesis, which, in turn, suppresses growth and inflammation in endometriosis [1]. Regrettably, the alleviation of pain seems to provide only temporary relief [2]. In addition, about 9% of women with endometriosis simply do not respond to progestin therapy due to unknown reasons [6]. Certainly, a widespread inclination toward relative progesterone resistance in the eutopic and ectopic endometrial tissues of women diagnosed with endometriosis has been extensively documented [3], [4].

The physiological effects of progesterone (P) are mediated by two isoforms of progesterone receptors (PRs): PR-A and PR-B. Both PR-A and PR-B arise from a single gene and function as transcriptional regulators of progesterone-responsive genes [4]. The two isoforms can be distinguished by the presence of an extra NH2-terminal segment of approximately 165 amino acids in PR-B. This particular segment encompasses a transactivation function unique to PR-B, and it is essential for specifying target genes that can be activated by PR-B, but not by PR-A [7], [8]. PR-B functions as a strong activator of transcription of several PR-dependent promoters while ligand-bound PR-A can repress the transcriptional activity of PR-B and other steroid receptors [9], [10]. As a result, it can be concluded that PR-A and PR-B are functionally distinct agents in mediating the actions of progesterone both in vivo and in vitro [11]. Among individuals diagnosed with endometriosis, studies have indicated that both isoforms of progesterone receptors are present in eutopic endometrial tissue, albeit with irregular expression patterns. However, within endometriotic implants, only PR-A is expressed, and PR-B is not detected [12]. The presence of repressive PR-A and the apparent reduction in stimulatory PR-B expression could reasonably account for the observed progesterone resistance in endometriosis [4], [12], [13].

Despite the existence of a plethora of studies on the extracellular matrix molecules and glycoprotein, there are very few studies on the Progesterone receptor at the endometrium during the implantation window (days 0–5) in humans. A significant contributing factor to this knowledge gap is the necessity for participants in these studies to undergo an endometrial biopsy. Evaluating the variance in the expression of PR-A and PR-B in endometriosis patients undergoing IVF can provide valuable insights into the influence of progesterone on endometrial receptivity and, consequently, the success or failure rates of IVF. This study centers on the assessment of differential Progesterone A and B expression in endometriosis patients undergoing IVF treatment, in comparison to a control group with eutopic endometrium.

2. Materials and Methods

2.1. Study Approval

The study was approved by the Institutional Review Board (IRB) of the Department of Obstetrics and Gynecology, MHRT Hospital & Research Centre, Hyderabad, Telangana, India. Informed written consent from all participants involved in the study was collected prior to the sample collection.

2.2. Inclusion and Exclusion Criteria for Study Participants

The study’s inclusion criteria comprised the following conditions: participants had to be between 33 and 37 years of age at the time of the surgical procedure with duration of marriage of 10 to 15 years, serum anti-mullerian hormone range of 1.5–2.1 ng/mL, individuals with symptoms of pelvic pain, amenorrhea, dyspareunia, dysmenorrhea, intermenstrual discomfort, considering IVF for infertility. The exclusion criteria comprise individuals who have infertility due to factors such as tubal blockage, male-related issues, diminished ovarian reserve, uterine problems, or other unexplained causes. Additionally, it includes individuals with endometriosis who are not seeking IVF for infertility treatment.
2.3. Study Design

We conducted a prospective hospital-based study on infertile patients enrolled for ART between January 2019 to January 2023 at MHRT and other referral centers from other hospitals. The research encompassed an examination of 125 cases of infertility spanning from January 2019 to January 2023. These cases were meticulously scrutinized to identify the underlying causes of infertility like tubal factor, male factor, uterine factor, Diminished ovarian reserve, Endometriosis, and other causes. All the cases had a laparoscopy done as a part of pre-ART workup due to various problems like pelvic pain, amenorrhea, dyspareunia, dysmenorrhea, intermenstrual discomfort, and other reasons, in order to achieve a precise diagnosis and proper staging of endometriosis. They have also undergone hysteroscopy and endometrial biopsy.

The study participants were divided into two groups according to the study protocol: (i) Group A comprised 60 Patients with endometriosis with stage I, stage II with chocolate cyst, stage III, and stage IV, confirmed with laparoscopy (ii) Group B comprised of 18 infertile patients who served as control group, showing no signs of endometriosis, the cause of infertility was due to other factors like tubal factor, male factor, uterine factor and other causes. All the endometriosis patients were diagnosed and classified in different grades clinically after visual confirmation of the presence of endometriosis lesions through laparoscopy and histopathological investigations. The Severity of endometriosis was determined according to the guidelines of the American Society of Reproductive Medicine Revised System. These patients have undergone their endometrial tissue and endometrial suppression analysis for the progesterone receptor studies.

All the sixty patients with Endometriosis underwent IVF and ART outcomes were determined for these patients.

The primary outcomes were PR-A and PR-B expression in the groups and live birth rate after IVF-ET. The secondary outcomes were the number of oocytes retrieved, implantation rate early pregnancy loss rate, and clinical pregnancy rate.

2.4. Sample Collection

Biopsy samples were collected from all 60 female endometriosis patients during laparoscopic procedures and control biopsy samples were obtained from 18 patients having no indications of endometriosis lesions during laparoscopy. All the biopsy samples were collected in 1× phosphate buffer saline (PBS) solution for RNA extraction.

2.5. RNA Extraction

Total RNA was extracted from tissue biopsy using TRIzol reagent which was converted to double-stranded complementary DNA (cDNA) using oligonucleotide primers (oligodT) and reverse transcriptase enzyme. The reliability of cDNA was known through agarose gel electrophoresis, stored at –20 °C for further analysis. The levels of mRNA expression were determined using real-time quantitative PCR using the following primer sequence:

- **MPRα**: 5′-CTGAAGTTTGCCTGACACCA-3′
  5′-AATAGAAGGCAGGTTTCCTGA-3′
- **MPRβ**: 5′-CAGCGAAGGCCACCAAAAATCT-3′
  5′-CAATCCCAAGCCACCCCAT3′
- **GAPDH**: 5′-GAAATCCCATACCCACCATCCTCA-3′
  5′-CAAATGAGCCCCAGCTTC-3′

2.6. Hormonal Therapy

Both the study and control groups did not undergo any hormonal therapy for a duration of three months before the endometrial biopsy. Hormonal treatment was only initiated when they enrolled in the in vitro fertilization (IVF) program. This study also evaluates the outcomes of assisted reproductive technology (ART) in both the endometriosis and control groups after they received the following treatment: Group-A patients received 2 doses of LHRH agonist before IVF, and blastocyst were cultured and frozen. The average oocyte retrieval was between 5–6 oocytes that have been suppressed with LHRH in the interval of 6 months. Group-B patients received 2 doses of Dienogest 2 mg for 3 months before the start of IVF and ovum pickup was done and the numbers of oocytes retrieved were between 4–6 and blastocysts were cultured.
TABLE I: Baseline Characteristics of the Groups Under Study [14]

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Test group (n = 60)</th>
<th>Control group (n = 18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.2 ± 3.8</td>
<td>35.2 ± 3.8</td>
<td>NA</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.5 ± 2.0</td>
<td>24.0 ± 1.0</td>
<td>0.158 &lt; 0.0000001 a</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 ± 1.0</td>
<td>169 ± 1.5</td>
<td>&gt;0.99 a</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.6 ± 8.0</td>
<td>63.5 ± 11.0</td>
<td>0.45 a</td>
</tr>
<tr>
<td>Smoking habits (n%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not once smoked</td>
<td>28 (70)</td>
<td>5 (50)</td>
<td></td>
</tr>
<tr>
<td>Present smoker</td>
<td>7 (17.5)</td>
<td>3 (30)</td>
<td></td>
</tr>
<tr>
<td>Previous smoker</td>
<td>5 (12.5)</td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td>Duration of infertility in years</td>
<td>12 ± 2.5</td>
<td>11 ± 3.1</td>
<td>0.22 a</td>
</tr>
<tr>
<td>Previous live birth rate (n%)</td>
<td>11/60 (18.33)</td>
<td>5/18 (27.77)</td>
<td></td>
</tr>
<tr>
<td>Partners sperm count (x10⁶ per ml)</td>
<td>70.2 ± 2.5</td>
<td>75.2 ± 0.5</td>
<td>&lt;0.0000001 a</td>
</tr>
<tr>
<td>Patients ovarian reserve AMH (ng/mL)</td>
<td>1.8 ± 1.0</td>
<td>1.8 ± 1.0</td>
<td>NA</td>
</tr>
</tbody>
</table>

Note: AMH: Anti-Mullerian hormone. Data is presented as mean ± S.D or n (%). a. student's t-test.

3. RESULTS

3.1. Study Population

The study flow chart is represented in Fig. 1. Overall, 125 infertile patient's cases were assessed to identify the cause of infertility and then 60 patients were considered in Group-A with different stages of endometriosis, and 18 patients were considered in Group-B with other causes of infertility like tubal factor, diminished ovarian reserve, Uterine factor, and others. These women were matched by age serum AMH level or duration of marriage to avoid bias in the study. These groups underwent laparoscopy, hysteroscopy, and endometrial biopsy as a part of the pre-ART work-up, and then samples were sent for progesterone assessment to the laboratory.

3.2. Baseline Characteristics

The baseline characteristics are presented in Table I. The age and serum AMH levels were the same for both groups. The BMI of Group B was slightly higher than that of Group A. Previous live birth rate (%) and partner sperm count (x10⁶ per ml) were higher for Group B than Group A. The question of poor previous live birth rate in Endometriosis patients is a matter of concern and is assessed by Progesterone receptor A and B expression analysis in this study.

In our Real-time quantitative PCR analysis of progesterone isoforms, Figs. 2–4 represents image of RTPCR for PR-A and B, their associated amplification, melt peak, and melt curve. Figs. 5A and 5B indicates Real-time
Fig. 5. (A) Real-time qPCR analysis of Progesterone A using Tukey’s multiple comparison test; (B) Real-time qPCR analysis of Progesterone B using Tukey’s multiple comparison test.

Table II: ART Outcomes after ET for the Study Group and Control Group [14]

<table>
<thead>
<tr>
<th></th>
<th>Study group with endometriosis on ART (n = 60)</th>
<th>Control group without endometriosis on ART group (n = 18)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count of retrieved oocytes</td>
<td>5.23 ± 0.175</td>
<td>4.94 ± 0.36</td>
<td>0.0037a</td>
</tr>
<tr>
<td>Average number of embryos cultured</td>
<td>1.5 ± 0.5</td>
<td>1.3 ± 0.3</td>
<td>0.11a</td>
</tr>
<tr>
<td>Average number of embryos transferred</td>
<td>1.0 ± 0.2</td>
<td>0.99 ± 1.2</td>
<td>0.95a</td>
</tr>
<tr>
<td>Rate of successful embryo implantation</td>
<td>0.42 ± 0.13</td>
<td>0.5 ± 0.21</td>
<td>0.053a</td>
</tr>
<tr>
<td>Rate of clinical pregnancies achieved</td>
<td>22/60 (36.66)</td>
<td>12/18 (66.66)</td>
<td>0.024b</td>
</tr>
<tr>
<td>Rate of live births</td>
<td>15/60 (25)</td>
<td>8/18 (44.44)</td>
<td>0.11b</td>
</tr>
<tr>
<td>Early pregnancy loss rate</td>
<td>7/60 (11.66)</td>
<td>4/18 (22.22)</td>
<td>0.25b</td>
</tr>
</tbody>
</table>

Note: ART: Assisted reproductive technology. ET: Embryo transfer. Data is presented as mean ± S.D or n (%). A student’s t-test. b Pearson’s χ² test.

qPCR analysis of Progesterone A and B using Tukey’s multiple comparison test. We did not observe any significant differences between patients with different stages of endometriosis and the control group for Progesterone receptor B. However, a significant downregulation of Progesterone receptor A was evident in cases of higher-grade endometriosis (Stage III and Stage IV), in contrast to lower-grade cases (Stage I and II).

We also determined ART outcomes after Embryonic transfer for the study group and control group as shown in Table II. We found that there is a significant difference found between the groups in terms of clinical pregnancy rate (22/60 vs. 12/18, p-value = 0.024) but no notable difference was found between the groups in terms of live birth rate (15/60 vs. 8/18, p-value = 0.11) following the first embryonic transfer, as presented in Table II. This suggests that women with endometriosis have lower chances of achieving pregnancy than women with other causes of infertility.

4. Discussion

The role of progesterone in the pathophysiology of endometriosis has long been a subject of interest. Traditionally, progesterone has been considered a protective factor, exerting inhibitory effects on endometrial tissue proliferation and inflammation. However, emerging research suggests that the relationship between progesterone and endometriosis may be more intricate than previously thought.

The maintenance of a balanced uterine environment is intricately linked to the signaling processes of progesterone (P4) and estrogen (E2) [15]. The coordinated interaction and regulation of P4 and E2 pathways are essential for proper uterine function and fertility. When these pathways become disrupted, conditions such as endometriosis and infertility often result.

Progesterone resistance is a term used to describe the unresponsiveness of endometrial tissue to progesterone, resulting in the dysregulation of progesterone signaling and the deregulation of a subset of progesterone-dependent genes in the eutopic secretory endometrium in endometriosis patients [16]. Progesterone resistance is a hallmark of endometriosis and is thought to contribute to the pathogenesis and progression of the condition. The dysregulation of progesterone-dependent genes in the eutopic endometrium is a key feature of progesterone resistance in endometriosis [3]. This resistance may also affect endometrial receptivity and implantation, leading to infertility in some women with endometriosis. One of the most significant mechanisms of progesterone resistance is PGR deficiency [17]–[19].

Progesterone, a steroid hormone, works by attaching to and activating two types of progesterone receptors found in target tissues, known as PR-A and PR-B. These receptors are functionally different and are derived from a single gene via estrogen-regulated promoters. Ligands activate PRs, which act as transcription factors [16]. Cell transfection studies have revealed that PR-A and PR-B have variable abilities to activate promoters sensitive to progesterin hormones [18]: PR-B appears to be a more effective gene transcription regulator than PR-A. PR-B is essential for cellular proliferation in response to progesterone. Conversely, PR-A assumes the role of a primary inhibitor for PR-B and the functions of various steroid receptors, such
as estrogen, androgen, glucocorticoid, and mineralocorticoid receptors. PR-A functions as a dominant repressor of PR-B and the activities of other steroid receptors like estrogen, androgen, glucocorticoid, and mineralocorticoid receptors. The relative expression rates of their respective mRNAs, which reflect the balance between PR-A and PR-B, are crucial in determining the precise cellular response to progesterone. This ratio can vary depending on the species and the cellular environment. While PR-B has gotten less attention in studies, PR-A has historically been connected to the endometrium anti-inflammatory and anti-proliferative effects of progesterone [20].

Several studies have been published, have evaluated the expression of PGR in ectopic endometriosis lesions and eutopic endometrium in endometriosis-affected women. The majority of them demonstrated lower PGR levels in ectopic lesion tissue than in eutopic endometrium and was unable to distinguish between PR-A and PR-B [21]–[24]. Research that distinguished between PR-A and PR-B isoforms frequently found decreased levels of PR-B in both endometriosis lesions and the normal uterine lining, while the results regarding PR-A were inconclusive [24]. In conclusion, in most forms of ectopic lesions, PR-A and PR-B expression are typically lower and PR-B insufficiency is considerably more obvious.

The primary PGR isoform expressed within lesions is PR-A [25], which is thought to result in an elevated PRA:PRB ratio and decreased progesterone activities. However, Misao et al. discovered that in some cases of ovarian endometriosis, PR-B mRNA was expressed at a higher level than in eutopic endometrium with a higher PRB:PRA ratio [21]. These findings highlight the necessity of PR-A/PR-B ratio in endometrial function and imply an imbalance of PGR isoforms in the pathophysiology of endometriosis, although more research is needed to understand the precise variation of PGR expression in endometriotic lesions or eutopic endometrium.

Based on the PR-A and PR-B expression profiles, treatment strategies can also be personalized. Adjustments to treatment plans can be made as necessary, based on changes in PR-A and PR-B expression and the patient’s clinical response. By tailoring treatment plans to the individual characteristics of each patient, including the unique molecular profile of their endometriotic lesions, healthcare providers can optimize therapeutic outcomes and enhance the quality of life for women living with endometriosis. As our understanding of the molecular underpinnings of endometriosis continues to grow, personalized medicine will play an increasingly crucial role in the quest to alleviate the burden of this disease [15].

In the present study, the relative quantification of PR-A and PR-B were assessed in different grades of endometriosis to investigate potential variations in their expression levels and elucidate their possible roles in the pathogenesis of this condition. Both the study and control groups did not undergo any hormonal therapy for a duration of three months before the endometrial biopsy. Hormonal treatment was only initiated when they enrolled in the in vitro fertilization (IVF) program. This study also assesses the ART outcomes in endometriosis and control groups after receiving the following treatment:

Group A patients received 2 doses of LHRH agonist before IVF, froze for all, and average oocyte retrieval was between 5–6 oocytes then were suppressed with LHRH in the interval of 6 months. Group B patients before IVF received 2 doses of Dienogest 2 mg for 3 months before the start of IVF ovum pickup was done and the numbers of oocytes retrieved were between 4–6 and blastocysts were cultured and frozen for all. FET was done with HRT treatment.

The low success rate of Assisted Reproductive Technology (ART) in patients with endometriosis is indeed a significant concern. Few studies have explored the use of progesterone in predicting ART outcomes in such patients [26]. To address this gap, we assessed the differential expression of PR-A and PR-B in endometriotic implants compared to a control group.

It was observed from the results of our study that there was a significant downregulation of PR-A in stages III, and IV compared to Stages I and II and there was no significant change in expression levels of PR-B in grades I and II, III and IV. However, the level of expression of Progesterone receptor B was higher than Progesterone receptor A in Stage III and IV Endometriosis suggesting that Progesterone receptor B may play a more substantial role in the context of endometriosis. In contrast to this, expression levels of Progesterone receptor B did not show significance between different grades of endometriosis and control. This divergence in isoform expression warrants further investigation into the functional differences and unique roles of progesterone A and B in the disease. Our study also observed certain ART outcomes in both endometriosis and control groups, revealing that women with endometriosis have lower chances of achieving pregnancy than women with other causes of infertility (95% CI, 22/60 vs. 12/18, p-value = 0.024). PR-A downregulation in higher stages of endometriosis may be linked to ART failure in patients with endometriosis.

5. Conclusion

In conclusion, our study’s results shed light on the complex role of progesterone isoforms in endometriosis. Progesterone A’s significant downregulation in severe cases challenges existing paradigms and opens new avenues for research into the molecular mechanisms, disease progression, and potential therapeutic targets in endometriosis. Understanding the roles of these progesterone isoforms may ultimately lead to more effective management strategies for this debilitating condition. Further investigations are warranted to confirm and expand upon these findings, ultimately improving the care and outcomes for individuals affected by endometriosis.

5.1. Limitations

It is crucial to acknowledge the limitations of our study, including the relatively small sample size and potential confounding factors. To validate our findings and provide a more comprehensive understanding of progesterone isoforms in endometriosis, larger and more diverse cohorts should be investigated. Furthermore, studies should delve
deeper into the clinical implications of progesterone isoform expression, such as assessing patient outcomes and treatment responses based on isoform profiles.

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**AUTHORS’ CONTRIBUTIONS**

RR, SSA, and AAK contributed equally to this work; RR, SSA, and AAK designed the study; RR, AAK conducted the laboratory work; SSA, AAK wrote the manuscript. SSA performed the statistical analysis. All authors reviewed and approved the final manuscript.

**CONFLICT OF INTEREST**

Authors declare that they do not have any conflict of interest.

**REFERENCES**


