Stimulation protocols for Severe Endometriosis- is there any difference?

Protocolos de estimulação para Endometriose Grave - há alguma diferença?

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ABSTRACT
Endometriosis, being an estrogen-dependent condition, can lead to subfertility in women. Those facing difficulty achieving pregnancy goals may need assistance from assisted reproductive technology (ART). Due to the challenges posed by endometriosis in natural conception, assisted reproductive technologies like IVF and ICSI. Objective of the study: This study aimed to compare ART outcomes in women with endometriosis following the three distinct groups of protocols undergoing various treatment modalities, namely the progestin-primed ovarian Stimulation protocol, GnRH agonist long protocol, and Combined Oral Contraceptives (OCPs). This investigation aims to enhance our understanding of how different stimulation protocols may influence ART outcomes.

Methodology: This is a cross-sectional study between March 2019 to March 2023. The study collected data from 126 infertile women diagnosed with endometriosis, who were undergoing their first intracytoplasmic sperm injection (ICSI) cycle at MHRT Hospital and Research Center, Hyderabad, Telangana India. Patients in each group were classified into three Protocols ie-GnRH agonist Long Protocol, Progestins primed Ovarian Stimulation, and Combined Oral Contraceptives. The outcomes of the ICSI program were evaluated.

Result: The findings from our study suggest that women with endometriosis undergoing ICSI experience improved reproductive outcomes when subjected to prolonged downregulation with GnRH agonist long protocol, Progestin primed Ovarian Stimulation and Combined Oral Contraceptives before initiating ovarian stimulation. Notably, the clinical pregnancy rate is notably higher at GnRH agonist long Protocol 57.5% compared to the alternative protocols: PPOS at 34.2 % and Combined Oral Contraceptives at 40.54 %. The groups subjected to extended pituitary downregulation with GnRH agonists long protocol demonstrated superior clinical pregnancy and live birth rates compared to those following the PPOS and Combined Oral Contraceptives.

Conclusion: This study demonstrates that GnRH agonists' long protocol demonstrated superior clinical pregnancy and live birth rates compared to those following the PPOS and Combined Oral Contraceptives. Better clinical outcomes were observed in the GnRH agonist long protocol group when compared to the PPOS and Combined Oral Contraceptive group.

Keywords: stimulation, GnRH agonist, progestin primed ovarian stimulation, combined oral contraceptives.
saber, o protocolo de estimulação ovárica preparado com progestina, o protocolo longo do agonista GnRH e os contraceptivos orais combinados (PCOs). Esta investigação visa melhorar a nossa compreensão de como diferentes protocolos de estimulação podem influenciar os resultados da ART Metodologia: Este é um estudo transversal entre março de 2019 a março de 2023. O estudo coletou dados de 126 mulheres inférteis diagnosticadas com endometriose, que estavam passando por seu primeiro ciclo de injeção de esperma intracitoplasmática (ICSI) no Hospital e Centro de Pesquisa MHRT, em Hyderabad, Telangana, na Índia. Os pacientes em cada grupo foram classificados em três Protocolos ie-GnRH agonista Long Protocol, Progestins estimulação ovárica primed, e contraceptivos orais combinados. Os resultados do programa ICSI foram avaliados Resultado: Os resultados do nosso estudo sugerem que mulheres com endometriose que estão passando por ICSI experimentaram melhores resultados reprodutivos quando submetidas a downregulation prolongado com GnRH agonista longo protocolo, Progestin estimulação ovárica primed e contraceptivos orais combinados antes de iniciar a estimulação ovárica. Notavelmente, a taxa de gravidez clínica é notavelmente maior no protocolo longo agonista GnRH 57,5% em comparação com os protocolos alternativos: PPOS em 34,2% e contraceptivos orais combinados em 40,54%. Os grupos submetidos a desregulação pituitária prolongada com agonistas GnRH longo protocolo demonstrou gravidez clínica superior e taxas de natalidade ao vivo em comparação com aqueles após o PPOS e contraceptivos orais combinados. Nossa abordagem segmentada de FIV, incluindo ciclos de FET, revelou uma maior taxa de natalidade viva no protocolo agonista GnRH em comparação com os PPOS e contraceptivos orais combinados. Conclusão: Este estudo demonstra que o longo protocolo dos agonistas da GnRH demonstrou uma gravidez clínica superior e taxas de nascimentos vivos em comparação com os que se seguiram aos PPOS e contraceptivos orais combinados. Melhores resultados clínicos foram observados no grupo de protocolo longo agonista GnRH quando comparado ao grupo PPOS e contraceptivo oral combinado.


1 INTRODUCTION

Endometriosis is a prevalent gynecological condition occurring in women of reproductive age, characterized by the existence of active endometrial tissue (including glands and stroma) in areas beyond the confines of the uterus. Primary symptoms associated with endometriosis include lower abdominal pain, dysmenorrhea (painful menstruation), sexual discomfort, and difficulties with fertility(Gruber TM, et al., 2021) Endometriosis-related infertility is believed to be multifaceted, affecting fertility by directly disrupting the typical structure of the fallopian tubes and ovaries. Indirectly, it can impact fertility through inflammatory responses and oxidative stress, leading to deterioration in the quality of oocytes.Indeed, male factors, including sperm quality, also significantly influence women's fertility( AmalineiCet al., 2018.). As assisted reproductive technology has advanced, In Vitro Fertilization with Embryo Transfer (IVF-
ET) has increasingly emerged as a crucial treatment for individuals experiencing infertility due to endometriosis. Pituitary down-regulation stands as a pivotal stage within the IVF-ET procedure. The Gonadotropin-releasing hormone agonist (GnRH-a) operates by competitively interacting with the pituitary gland, impeding the release of GnRH(Mahapatra DK et al., 2015). This action inhibits the secretion of associated hormones in the ovary, thereby achieving pituitary down-regulation. Furthermore, GnRH-a efficiently averts premature luteinization of follicles, enhancing the synchronization of follicular growth and development.(de Ziegler Det al., 2013.) Ongoing research focuses on exploring various down-regulation protocols to facilitate In Vitro Fertilization with Embryo Transfer (IVF-ET) for assisting pregnancy in individuals affected by endometriosis-related infertility. Both National and international studies have investigated these protocols to enhance success rates. However, clinical outcomes from these endeavors remain contentious and continue to spark debate within the medical community. (Cao, X. et al., 2020)

Endometriosis is an estrogen-dependent condition characterized by the presence of endometrial-like tissues outside the uterine cavity. It can result in subfertility among women, in such cases, the utilization of Assisted Reproductive Technology (ART) becomes necessary to facilitate and achieve pregnancy (Broi et al., 2019) and impacts approximately 10% of women of reproductive age, with a higher prevalence of 20%–50% observed among infertile women(Johnson NP et al., 2017). Women with endometriosis commonly experience a reduced pregnancy rate, prompting the frequent use of assisted reproductive techniques to enhance the likelihood of conception. In vitro fertilization (IVF) stands out as a crucial strategy for achieving pregnancy, particularly for infertile women who do not respond well to surgical treatment. It is noteworthy that even mild cases of endometriosis can negatively impact fertility by influencing oocyte development, embryogenesis, and implantation. (Signorile PG et al., 2010, Harb HM et al., 2013)

Intra cytoplasmic sperm injection (ICSI) is recommended as the primary treatment choice for women with stage III to IV endometriosis or those experiencing associated impaired tubal function or male factor infertility, advanced female age, diminishing ovarian reserves, or when repeated attempts (3-4) at intrauterine insemination (IUI) fail to result in conception(Johnson NP et al., 2013). Controversies related to the ICSI process include the choice of protocol and the potential risks associated with ovarian stimulation. Ovarian stimulation for assisted reproductive technology (ART) does not appear to increase the risk of recurrence or exacerbate pain symptoms, and it has minimal impact
on existing endometriomas. Notably, ICSI does not seem to elevate the risk of endometriosis recurrence. (Benaglia l et al., 2009)

For individuals with mild to moderate-stage endometriosis, there's a potential increase in the likelihood of pregnancy by incorporating intrauterine insemination with ovarian stimulation post-surgical treatment. Conversely, in cases of severe endometriosis, a multidisciplinary approach is crucial, considering the patient's specific characteristics, to determine the prioritization of treatment modalities. This may involve a comprehensive evaluation of surgical treatment and assisted reproduction methods, such as in vitro fertilization. Infertile women are significantly more likely to have moderate to severe endometriosis than previously fertile women (D’Hooghe TM et al., 2003).

1.1 WHY PRE-ART TREATMENT IN ENDOMETRIOSIS?

Ovarian stimulation in Assisted Reproductive Technology (ART) comprises three key components: promoting the growth of multiple follicles, suppressing the pituitary to prevent a luteinizing hormone (LH) surge and premature ovulation before oocyte retrieval, and triggering the final maturation of oocytes. (Jirghe PR et al., 2022) Endometriotic lesions in the pelvis create an unfavorable microenvironment for oocyte fertilization and the early development of embryos. In IVF programs, ovarian endometriosis can impact ovarian reserve and responsiveness to ovarian stimulation. This, in turn, leads to reduced fertilization and pregnancy rates among patients with endometriosis compared to a control group. There are elevated levels of proinflammatory cytokines and oxidative damage to ovarian follicles, resulting in compromised oocyte quality, the development of embryos ranging from average to poor quality, or possibly no embryo formation at all. These effects appear to be irrespective of the stage of endometriosis. ART is an appropriate treatment for patients who have advanced endometriosis with reduced Ovarian Reserve or if the tubal function is compromised. ART is the only viable method in cases of endometriosis–induced severe tubal adhesion-related disease or the presence of large endometriomas.

The choice of stimulation protocols for individuals with severe endometriosis undergoing assisted reproductive technology (ART) treatments, such as ICSI are PPOS, GnRH agonist long protocol, and GnRH agonist long protocol.

Ovarian stimulation was successfully carried out through the implementation of one of two distinct protocols, either the GnRH-agonist long protocol, PPOS, or the OCP protocol. The choice between these protocols was made based on careful patient
counseling and the preferences of the attending clinician. Aim of Ovarian Stimulation appropriate no of oocytes, No ovarian stimulation, Endometrium receptivity should not be affected, and Endometrioma shouldn’t be worsened (Somigliana E, et al., 2023.). Stimulation protocols in endometriosis are different for endometriosis compared to non-endometriosis patients. Risk for endometriosis to increase in severity as endometriosis thrives on estrogen and by ovarian stimulation we are increasing estrogen in the body, Endometrioma may increase in size depending on the IVF cycle, fewer oocytes, and higher cancellation rate (Chantalat E, et al., 2020)

1.2 PROGESTIN-PRIMED OVARIAN STIMULATION PROTOCOL

PPOS involves the use of progestins (e.g., Combined Oral Contraceptives) before ovarian stimulation to create a more controlled and synchronized follicular development. This protocol is designed to suppress premature luteinizing hormone (LH) surges. (Huang J, et al., 2019)

In the PPOS (Progestin-Primed Ovarian Stimulation) protocol, Controlled Ovarian Stimulation (COS) commenced on days 2 to 4 of the menstrual cycle. Medroxyprogesterone Acetate (MPA) (10 mg/day, Zhejiang Xianju Pharmaceutical Co., China) or Duphaston (20 mg/day; Abbott Biologicals B.V., Netherlands) was administered alongside gonadotropins from the initiation of COS and continued until the trigger day. Once a minimum of three follicles reached a diameter of ≥18mm, follicular maturation was induced using Decapeptyl (0.1 mg; Ferixing Pharmaceuticals Ltd., SaintPrex, Switzerland) and human chorionic gonadotropin (hCG) (1000 IU; Lizhu Pharmaceutical Trading Co., China) (17). Oocyte aspiration was performed 35.5 to 36.5 hours after the trigger

1.3 GNRH AGONIST PROTOCOL (DEPOT)

GnRH agonists, such as leuprolide, are used to induce a reversible downregulation of the pituitary gland, leading to a temporary suppression of gonadotropins. This suppression aims to prevent premature ovulation and optimize follicular development during ovarian stimulation. (van Loenen AC, et al., 2002.)

Downregulation with GnRH agonist 3-6 months before ART increases the odds of clinical pregnancy by more than fourfold.

In the GnRH agonist long protocol, all patients underwent pituitary down-regulation during the mid-luteal phase (Day 21) of the preceding menstrual cycle. The
GnRH analog was initiated during the mid-luteal phase to induce downregulation and was consistently administered until the day of human chorionic gonadotropin (hCG) administration in the treatment protocol (Ruan M et al., 2021, Ying et al., 2019, Kumar P et al., 2014). It is achieved through the administration of a 0.1 mg triptorelin (decapeptyl, Ferring Pharmaceuticals, Netherlands) injection. Once desensitization was confirmed by plasma E2 levels of ≤ 50 pg/mL, the absence of ovarian follicles, and an endometrial thickness of ≤ 6 mm, ovarian stimulation was initiated by administering recombinant FSH (Gonal-F, Merck Serono, Switzerland) or purified HMG (Menopur, Ferring, Switzerland) injections on Day 2 or 3 of the menstrual period. Final oocyte maturation and release were facilitated by discontinuing GnRH and administering an injection of 5000-10000 international units (IU) of hCG upon the detection of at least three follicles of 17 mm in size.

1.4 COMBINED ORAL CONTRACEPTIVES (OCPS)

OCPs are sometimes used in the management of endometriosis before initiating an IVF cycle. OCPs can provide suppression of the menstrual cycle, helping to control endometriosis symptoms and creating a more predictable and controlled environment for subsequent fertility treatments. (Weisberg E et al., 2015)

OCPs may delay the start of IVF treatment, and the suppression may not be as profound as with GnRH agonists.

1.5 ROLE OF SEGMENTED IVF IN SEVERE FORMS OF ENDOMETRIOSIS

When planning Assisted Reproductive Technology (ART), a segmented approach with an agonist trigger, freeze-all strategy, and embryo transfer scheduled after six cycles with suppressive therapy in between is often considered advantageous. (Zaat T et al., 2021)

Hence, our study aims to compare three distinct groups of patients undergoing various treatment modalities, namely the Progestin Primed Ovarian Stimulation protocol, GnRH agonist long protocol, and Combined Oral Contraceptives. The objective is to evaluate assisted reproductive technology (ART) outcomes, including clinical pregnancy rate, biochemical pregnancy, implantation rate, and other relevant factors. This investigation aims to enhance our understanding of how different stimulation protocols may influence ART outcomes.
2 METHODS

2.1 STUDY SUBJECTS AND DESIGN

This is a cross-sectional study conducted between March 2019 to March 2023. The study collected data from 126 infertile women diagnosed with endometriosis, who were undergoing their first intracytoplasmic sperm injection (ICSI) cycle at MHRT Hospital and Research Center, Hyderabad, Telangana India. Informed consent was obtained from all participants, allowing access to their clinical and laboratory information related to their medical history for Clinical Research.

The diagnosis of endometriosis was established and confirmed through either laparoscopy or pathological examination, following the criteria set forth by the revised American Fertility Sterility (rAFS) classification (Zeng et al., 2014). To be eligible to participate in this study, patients had to meet the following inclusion criteria: (I) be a female aged ≤40 years; (II) have a baseline FSH <10 IU/L, (III) infertility associated with endometriosis, (IV) a body mass index (BMI) of less than 27 kg/m², (V) stage III/IV endometriosis, (VI) the absence of polycystic ovarian syndrome (PCOS), and the absence of other endocrine diseases. The diagnosis of endometriosis was done either by surgical confirmation (laparoscopy) or an indication of likelihood through findings from transvaginal sonography (TVS) or magnetic resonance imaging (MRI). This involves identifying the presence of uni- or bilateral ovarian endometrioma and deep endometriosis. To ensure consistency in the study samples, only fresh embryo transfers were selected from the patients recruited for this research. This study was approved by the ethics board of Medical Health and Research Institute, Hyderabad, and informed consent was taken from all the patients.

The study included women diagnosed with a severe form of endometriosis who underwent intracytoplasmic sperm injection (ICSI) with ovarian stimulation. The study included 42 patients in each group. The long GnRH-agonist protocol was compared to the Combined Oral Contraceptives and PPOS protocol in these patient populations for ART outcomes.

2.2 REGIMEN FOR OVARIAN STIMULATION

Patients underwent transvaginal ultrasound and a baseline hormone test before commencing ovarian stimulation. Those meeting the criteria of all follicles having a diameter <10 mm, FSH <14mIU/ml, and an endometrial thickness <6mm initiated Controlled Ovarian Stimulation (COS). The starting gonadotrophin dose was determined.
based on maternal age, body mass index (BMI), and ovarian reserve. Recombinant/urinary FSH (Gonal-F, Merck Serono, Italy, Urofolitropin for injection, Lizhu Pharmaceutical, China) alone or in combination with hMG (Menotropins, Lizhu Pharmaceutical, China) at total doses of 150~300IU/day were employed for ovarian stimulation. Follicular growth was tracked through transvaginal ultrasound and hormone measurements, with gonadotrophin doses adjusted accordingly.

2.3 IVF/ICSI TREATMENT PROTOCOLS

The Subjects were categorized into three groups depending on the treatment strategy utilized: either the GnRH agonist long protocol, PPOS and OCPs.

2.4 GNRH AGONIST LONG PROTOCOL (DEPOT)

All 42 women will receive three Zoladex 3.65 mg injections subcutaneously (SC). The injections are given either as a single dose or divided into two doses, 6 weeks apart. After the Zoladex injections, controlled ovarian stimulation (COS) will be initiated. The dosage of FSH will be individually determined for each woman. GnRH antagonist will be given if needed during the ovarian stimulation phase. Ovum pickup (egg retrieval) will be performed. Four months after the protocol initiation, women are scheduled for an appointment at the IVF department. Women are scheduled for an appointment precisely 28 days after the last Zoladex injection for further assessment. Full suppression will be done during this appointment. Ovarian stimulation is commenced with subcutaneously injected gonadotrophins (FSH). The protocol concludes with frozen embryo transfer (FET).

2.5 PPOS PROTOCOL

In the PPOS Protocol, all 42 women had COS initiation occurred on days 2 to 4 of the menstrual cycle. Medroxyprogesterone Acetate (MPA) (10 mg/day, Zhejiang Xianju Pharmaceutical Co., China) or Duphaston (20 mg/day; Abbott Biologicals B.V., Netherlands) was administered alongside gonadotrophin from the beginning of COS and continued until the trigger day. Four months before COS after the protocol initiation, women are scheduled for an appointment at the IVF department. Women are scheduled for an appointment precisely 28 days after the last Medroxyprogesterone Acetate injection for further assessment. GnRH antagonists will be given if needed during the ovarian stimulation phase. Full suppression will be done during this appointment. Once
a minimum of three follicles attained a diameter of ≥18mm, follicular maturation, and human chorionic gonadotropin (hCG) (1000 IU; Lizhu Pharmaceutical Trading Co., China) (17). Oocyte aspiration took place 35.5 to 36.5 hours after triggering. The protocol concludes with frozen embryo transfer (FET).

2.6 COMBINED ORAL CONTRACEPTIVES

Women in the Combined Oral Contraceptives group, all 42 women will be given before COS will be given and followed by Combined Oral Contraceptives (50 COCs) continuously for three consecutive months (i.e., 3 × 28 days). Preferably, ethinylestradiol/levonorgestrel 30/150 μg will be prescribed. However, other one-phase sub-50 OCs will be accepted if patients are already using specific types. Upon completion of the three-month oral contraceptive treatment, women are instructed to contact the IVF department on the first day of bleeding. On the second day of withdrawal bleeding, women will visit the IVF center and will be initiated to suppress the luteinizing hormone (LH) peak. Ovarian stimulation with subcutaneously injected gonadotrophins (follicle-stimulating hormone (FSH)) will commence one day later, with the dosage tailored to each individual.

2.7 STATISTICS

A comparison between the three groups treated with different stimulation protocols was conducted. Using different tests like One-way ANOVA for continuous variables and Chi-square test for categorical variables prestimulation cycle characteristics of the patients were assessed using Kruskal Willis H-test for continuous variables and Pearson’s chi-square test or Fisher’s exact test for categorical variables. All statistical tests conducted in the analysis were two-tailed, and a significance level of p < 0.05 was established to determine statistical significance. The confidence interval was set at 95%. All the statistical tests were performed using SPSS 23.0 softwares.
Figure 1—Details of the flowchart illustrating participant progression and outlining the primary reproductive outcomes of the enrolled patients. PPOS, progestin-primed ovarian stimulation; GnRHa, gonadotropin-releasing hormone agonist; Combined Oral Contraceptives, ET, embryo transfer.

3 RESULTS

3.1 BASELINE CHARACTERISTICS

From March 2019 to March 2023 a total of 126 women who underwent surgical diagnosis and were confirmed to have moderate or severe endometriosis patients were enrolled for IVF/ICSI. Among them, 42 patients were subjected to the PPOS protocol, 42 were subjected to the GnRH agonist protocol and 42 were subjected to the Combined Oral Contraceptives in the follicular phase. Among the cycles included were 42 in each group of a total of 126 women who underwent the three protocols PPOS, GnRH agonists, and COCP respectively. The baseline characteristics of the patients are shown in Table 1.
ASRM Stage

<table>
<thead>
<tr>
<th>Stage</th>
<th>PPOS</th>
<th>GnRH agonist long protocol</th>
<th>Combined Oral Contraceptives</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>14(33.3%)</td>
<td>18(42.8%)</td>
<td>16(38%)</td>
<td>0.65</td>
</tr>
<tr>
<td>IV</td>
<td>28(66.7)</td>
<td>24(57.2%)</td>
<td>26(62%)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Previous failed Cycles

<table>
<thead>
<tr>
<th>Cycles</th>
<th>PPOS</th>
<th>GnRH agonist long protocol</th>
<th>Combined Oral Contraceptives</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13(31%)</td>
<td>16(38%)</td>
<td>18(43%)</td>
<td>0.49</td>
</tr>
<tr>
<td>1</td>
<td>16(38)</td>
<td>11(26%)</td>
<td>11(26%)</td>
<td>0.24</td>
</tr>
<tr>
<td>&gt;2</td>
<td>13(31)</td>
<td>15(35)</td>
<td>13(31%)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Gravidity

<table>
<thead>
<tr>
<th>Gravidity</th>
<th>PPOS</th>
<th>GnRH agonist long protocol</th>
<th>Combined Oral Contraceptives</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>26(62%)</td>
<td>22(52.4%)</td>
<td>18(43%)</td>
<td>0.02*</td>
</tr>
<tr>
<td>1</td>
<td>16(38%)</td>
<td>20(47.6%)</td>
<td>24(57%)</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Source: (Lan, J et al., 2021)

One-way ANOVA for continuous variables and Chi-square test for categorical variables.

a. Comparison between PPOS and GnRHa, P < 0.05. b Comparison between PPOS and OCP’s, P < 0.05

The stimulation cycle characteristics of the patients within the three groups are shown in Table 2. ART outcomes of patients after first embryonic transfer within three groups are shown in Table 3.

Table 2 depicts the Outline the pre-stimulation cycle characteristics of the patients within the three groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PPOS</th>
<th>GnRH agonist long protocol</th>
<th>Combined Oral Contraceptives</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Cycles</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>--</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>10±2</td>
<td>11±2</td>
<td>11±2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total of gonadotropins dose</td>
<td>(2662.5-3300.0)</td>
<td>(2700.0-3440.6)</td>
<td>(2400.0-3075.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cancellation rate</td>
<td>8(16.6%)</td>
<td>6(11.9%)</td>
<td>8(14.2%)</td>
<td>0.85</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>(4(2-6))</td>
<td>(5(3-6))</td>
<td>(4(3-5))</td>
<td>0.66</td>
</tr>
<tr>
<td>No of mature Oocytes retrieved</td>
<td>(2(1-2))</td>
<td>(4(3-5))</td>
<td>(3(3-5))</td>
<td>0.911</td>
</tr>
<tr>
<td>No. of Viable Embryos</td>
<td>(2(1-2))</td>
<td>(4(2-4))</td>
<td>(3(2-3))</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Source: (Kalafat, E., et al., 2022)

Categorical variables were expressed as counts and percentages (%), while continuous data were represented as mean ± standard deviation (SD). a. Group comparisons for Continuous variables were conducted by using Kruskal Willis H-test. b. Group comparisons for categorical variables were conducted using Pearson’s chi-square test or Fisher’s exact test as deemed suitable.

Table 3 illustrates Reproductive outcomes of patients’ first embryo transfer cycles originating from the three COS protocols.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PPOS (n=38)</th>
<th>GnRH agonist long protocol (n=40)</th>
<th>Oral Contraceptives (n=37)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ET cycles</td>
<td>38</td>
<td>40</td>
<td>37</td>
<td>--</td>
</tr>
<tr>
<td>Fresh ET Cycles</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>--</td>
</tr>
<tr>
<td>FET cycles</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>--</td>
</tr>
<tr>
<td>Thickness of endometrium (mm)</td>
<td>10.0 (9.0-11.0)</td>
<td>11.0 (9.0-12.0)</td>
<td>10.0 (9.0-11.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>Embryo Class</td>
<td>Cleavage embryo</td>
<td>16(42.10%)</td>
<td>29(72.5%)</td>
<td>20(54.4)</td>
</tr>
<tr>
<td>Blastosyst</td>
<td>22(57.8%)</td>
<td>11(27.5%)</td>
<td>17(45.9%)</td>
<td>0.006</td>
</tr>
<tr>
<td>No. of embryos transferred (n, %)</td>
<td>1</td>
<td>25(65.78%)</td>
<td>15(37.5%)</td>
<td>16(43.2%)</td>
</tr>
<tr>
<td>Implantation rate (n, %)</td>
<td>2</td>
<td>12(31.5%)</td>
<td>22(55%)</td>
<td>21(56.7%)</td>
</tr>
<tr>
<td>Pregnancy Complications</td>
<td>Biochemical pregnancy(n, %)</td>
<td>7(18.42%)</td>
<td>16(40%)</td>
<td>11(29.7%)</td>
</tr>
<tr>
<td>Clinical Pregnancy(n, %)</td>
<td>13(34.2%)</td>
<td>23(57.5%)</td>
<td>15(40.54%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Early miscarriage, (n, %)</td>
<td>5(13.15%)</td>
<td>0</td>
<td>1(2.7%)</td>
<td>--</td>
</tr>
</tbody>
</table>
Ongoing and late miscarriage (n, %) | 3 (7.89%) | 5 (12.5%) | 6 (16.2%) | >0.1

Ectopic pregnancy (n, %) | 2 (5.26%) | 1 (2.5%) | 0 | --

Ongoing Pregnancy | 5 (13.1%) | 18 (45%) * | 8 (21.62%) | 0.002

Live Birth (n, %) | 4 (10.52%) * | 16 (40%) * | 7 (18.9%) | 0.001

Induced abortion | 0 | 1 (2.5%) | 0 | --

Still birth | 1 (2.63%) | 1 (2.5%) | 1 (2.7%) | 0.98

Source: (Gullo, G., 2023)

Categorical variables were expressed as counts and percentages (%), while continuous data were represented as mean ± standard deviation (SD). Group comparisons for categorical variables were conducted using Pearson’s chi-square test or Fisher’s exact test as deemed suitable. For continuous variables, ANOVA with the post-hoc Bonferroni test was employed. PPOS, progestin-primed ovarian stimulation; GnRHa, gonadotrophin-releasing hormone agonist; ET, embryo transfer; FET, Frozen/thawed embryo transfer;

a. Comparison between PPOS and GnRHa, P < 0.05. b Comparison between PPOS and OCP’s, P < 0.05

Figure 2: Pregnancy outcomes of the three different groups PPOS, progestin-primed ovarian stimulation; GnRHa, gonadotropin-releasing hormone agonist; Combined Oral Contraceptives

Figure 2 Illustrates pregnancy outcomes among the three different groups, further comparisons revealed significantly higher clinical pregnancy rates in the GnRH agonist long protocol (57.5%) in comparison to PPOS (34.2%) and Combined Oral Contraceptives (40.54%). There was a significant difference found between GnRH agonist long protocol and PPOS group with a p value of 0.03. Further, a statistically significant difference in Biochemical pregnancy was found between GnRH agonist long protocol and PPOS group with a p value of 0.043. Biochemical pregnancy rate was higher (40%) in GnRH agonist, followed by 18.42% in PPOS, and 29.7% in Combined Oral Contraceptives protocol respectively. There is a statistical significant difference in Live birth rate in GnRH agonists (40%), PPOS (10.52%), with the p value of 0.001. Better
Clinical outcomes were observed in the GnRH agonist long protocol group when compared to the PPOS and Combined Oral Contraceptive group.

Figure 3 Illustrates comparisons of Implantation rate and clinical pregnancy in Progestin Primed Ovarian Stimulation, GnRH Agonist and Combined Oral Contraceptives

The results of our study indicate that the reproductive outcomes for women with endometriosis undergoing ICSI are enhanced when a prolonged downregulation with GnRH agonist is administered before commencing ovarian stimulation and the clinical pregnancy rate is higher 23(57.5%) when compared to the other two protocols PPOS 13(34.2%), Combined Oral Contraceptives 15 (40.54%) depicted in Figure 2. Due to the pituitary downregulation achieved with GnRH agonist (GnRH-a), the extended treatment groups exhibited superior clinical pregnancy rates and live birth rates compared to both the Progestin Primed Ovarian Stimulation (PPOS) and Combined Oral Contraceptives. This conclusion stems from our experience with segmented in vitro fertilization-embryo transfer (IVF-ET) cycles, revealing a notably higher live birth rate in the GnRH agonist protocol when compared to the PPOS and Combined Oral Contraceptives protocols depicted in Figure 3.

Implementing a freeze-all policy in IVF can enhance outcomes, leading to significantly higher rates of implantation, clinical pregnancy, and ongoing pregnancy. This improvement may stem from mitigating the negative impact of exogenous gonadotropins on the endometrium during the assisted reproductive technology (ART) cycle, a phenomenon prevented by the adoption of the freeze-all policy.
4 DISCUSSION

Nearly 15% of individuals experiencing infertility and necessitating assisted reproductive treatment are affected by endometriosis. (Santulli P et al., 2016). The presence of endometriotic lesions in the pelvic region is thought to establish an unfavorable microenvironment for the fertilization of oocytes and the initial stages of embryo development within the fallopian tubes in vivo. (Vassilopoulou L et al., 2018) Moreover, severe endometriosis can potentially impact ovarian reserve and responsiveness to ovarian stimulation in the context of in vitro fertilization (IVF) programs (Zeng C et al., 2022). Consequently, these effects contribute to reduced fertilization and pregnancy rates among individuals with severe endometriosis undergoing in vitro fertilization (IVF) in comparison to other patient groups. This phenomenon is likely associated with elevated levels of proinflammatory cytokines and oxidative damage to ovarian follicles, ultimately leading to compromised oocyte quality irrespective of the stage of endometriosis. (Sanchez AM et al., 2017)

In severe Endometriosis patients, there is the Changes in the pelvic anatomy, the extent of the inflammatory response within the pelvic cavity, and the degree of imbalance in the local microenvironment of the endometrium

In comparison with the three protocols used PPOS is a relatively newer protocol, and its long-term effectiveness is still being studied. Individual responses can vary. OCPs may delay the start of IVF treatment, and the suppression may not be as profound as with GnRH agonists.

GnRH agonists play a crucial role in inhibiting the growth and activity of endometrial tissue by lowering estrogen levels. (Maggi R et al., 2016). The downregulation induced by these agonists not only curtails the inflammatory response but also enhances the pelvic microenvironment. By temporarily "resting" the ovaries and regulating hormonal fluctuations, GnRH agonists contribute to symptom relief, particularly alleviating pain. Moreover, this suppression has been associated with improved prospects for successful embryo implantation and higher pregnancy rates in comparison to individuals not undergoing such suppression during intracytoplasmic sperm injection (ICSI). Patients with advanced endometriosis (stages III/IV) generally experience poorer reproductive outcomes in in vitro fertilization (IVF), although the precise pathogenic mechanisms remain unclear (Luca A et al., 2016, Moreira, M. L et al., 2022.) Endometriosis is linked to a decrease in the number of retrieved oocytes and high-quality embryos, as well as lower implantation rates (IR) and pregnancy rates (PR),
potentially attributable to compromised endometrial receptivity. However, live birth rates (LBR) appear to be approximately comparable to those observed in cases of infertility stemming from other causes (Surrey ES 2013, Daniilidis A et al., 2018, Hamdan M et al., 2015)

High rates of pregnancy after long-term down-regulation of women with severe endometriosis reported by a study group Marcus SF et al., 1994. Numerous studies have explored the reproductive outcomes of patients with endometriosis undergoing ICSI with or without treatment using GnRH agonists. In these studies, GnRH agonist administration typically commenced on day 21 of the menstrual cycle, and gonadotropin stimulation commenced once sufficient pituitary suppression was achieved. Interestingly, there appears to be a tendency for a higher pregnancy rate per cycle in ICSI cycles involving GnRH agonist downregulation compared to cycles without downregulation (Zikopoulos K et al., 2004).

A study reported by Benaglia l et al., 2013 reported after a 3–6 month period of gonadotrophin stimulation following the completion of an Intracytoplasmic Sperm Injection (ICSI) cycle, there was no significant change in overall endometriosis symptom scores. Out of the patients, 11% reported worsening symptoms, while 77% reported improvement. Additionally, the size of Endometriomas remained stable.

D’Hooghe et al. 2003 reported that among patients with stage III/IV endometriosis undergoing gonadotropin stimulation, the utilization of higher gonadotropin doses, resulting in elevated mean circulating estradiol levels, was associated with lower cumulative disease recurrence in Intracytoplasmic Sperm Injection (ICSI) cycles compared to intrauterine insemination (IUI) cycles.

Studies have indicated a higher clinical pregnancy rate per transfer in 21 patients with various stages of endometriosis who underwent ovarian stimulation after receiving GnRH agonist for 60 or more days, compared to 11 patients with endometriosis treated with a standard midluteal GnRH agonist downregulation protocol (67% vs. 27%). (Georgiou EX et al., 2019, Santana, D. C., et al., 2022)

Considering the widely acknowledged link between estrogen stimulation and the sustenance and advancement of endometriosis, one might inquire whether the significantly heightened levels of estradiol induced by gonadotropin stimulation could potentially worsen the underlying condition. The available data addressing this concern are limited but promising. In a particular study, it was observed that 3–6 months post the conclusion of an IVF cycle, the overall symptom scores related to endometriosis remained
unchanged. Approximately 11% of patients reported deterioration, while 77% reported an improvement. (Surrey, E. S. 2015).

The GnRH agonists (GnRH-a) has demonstrated an enhancement in the clinical pregnancy rate among infertile patients, particularly in those with stages III–IV endometriosis. In conclusion, the administration of a GnRH agonist for 3 months preceding Intra cytoplasmic sperm injection and embryo transfer (ICSI-ET) in infertile patients with endometriosis did not have a detrimental impact on the ovarian response to exogenous gonadotropins. (Zareii A et al., 2021) The reproductive outcomes for patients with endometriosis seem to be improved when downregulation with GnRH agonists is employed before ovarian stimulation for ICSI in comparison to PPOS and Combined Oral Contraceptives.

In a prospective randomized multicenter trial, (Surrey et al. 2013) investigated the use of GnRH agonist (GnRHa) prior to the initiation of gonadotropin stimulation for assisted reproductive technologies in 41 patients with surgically confirmed endometriosis. Among them, 25 received a three-month course of GnRHa before ovarian stimulation and in vitro fertilization (IVF), while 26 underwent standard ovarian stimulation before IVF. Despite having a higher percentage of patients with more advanced disease, the group treated with a prolonged course of GnRHa showed a trend towards higher implantation rates (42.7% versus 30.4%) and significantly higher clinical pregnancy rates (80% versus 53.9%, < 0.05) compared to the control group.

GnRH-agonist -long protocol significantly improved the clinical pregnancy rate among endometriosis patients at stages III–IV compared to PPOS and OCPs

5 CONCLUSION

Due to the advancing nature of endometriosis, infertile women often undergo ovarian stimulation as part of Assisted Reproduction treatment, whether or not they have undergone surgical intervention. There is compelling evidence indicating that the extended administration of GnRH agonist (GnRHa) may enhance cycle outcomes in at least a subset of patients with endometriosis. Our study concludes that the reproductive outcomes for women with endometriosis undergoing intracytoplasmic sperm injection (ICSI) show improvement when employing prolonged downregulation with the GnRH agonist long protocol before initiating ovarian stimulation, potentially resulting in an enhanced likelihood of achieving pregnancy. The GnRH agonist long protocol has demonstrated the potential to enhance the clinical pregnancy rate, implantation
Fertilization rate, and number of retrieved oocytes. in infertile patients, particularly those dealing with Severe stages (III–IV) endometriosis. The utilization of downregulation with GnRH agonists before ovarian stimulation for in vitro fertilization (IVF) appears to positively influence the reproductive outcomes of patients with endometriosis. This protocol is associated with improved success rates in achieving clinical pregnancies, emphasizing its significance in optimizing fertility treatment outcomes, especially for individuals with advanced stages of endometriosis. Initiating pre-IVF suppressive treatment in women with endometriosis has been shown to enhance the outcomes of in vitro fertilization (IVF). Thus, Individualized treatment plans may involve a combination of medical and surgical approaches based on the severity and location of endometriosis.
REFERENCES


