# Comparative analysis of IVF-ICSI outcomes between advanced and early stage of endometriosis stimulated with HMG

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## ABSTRACT

**Introduction: Endometriosis is a challenging disease to treat, and patients may eventually need in vitro fertilisation with Intracytoplasmic sperm injection (IVF/ICSI) to conceive after other modalities failed. There are inconsistent outcomes of IVF performance in patients with endometriosis especially with highly purified human menotropin gonadotrophin (hMG). This study was commenced to determine whether the use of hMG affects the IVF outcome in different stage of endometriosis.**

**Materials and Methods: This is an observational study. Eighty-seven women who had endometriosis confirmed surgically and underwent IVF/ICSI treatment, stimulated with hMG alone were included. Based on the revised American Society for Reproductive Medicine (rASRM), the participants were classified as early endometriosis (I/II) (n=39) or advanced endometriosis (III/IV) (n=35). The main outcome measures used were clinical pregnancy rate.**

**Results: Women with advanced endometriosis had a lower oocyte yield, less good quality day-3 embryos and lower clinical pregnancy rate compared with the mild endometriosis. However, higher fertilisation rate were recorded in advanced stage endometriosis compared to milder disease.**

**Conclusions: The rASRM classification of endometriosis is valuable in predicting IVF outcome as advanced endometriosis performs poorly compared to a milder disease. Highly purified hMG could be an alternative as an ovarian stimulation in endometriosis.**

## INTRODUCTION

Endometriosis is a disease characterised by presence of endometrial glands or stroma in sites other than the uterine cavity. It is considered as one of the challenging gynaecologic diseases in reproductive medicine and it affects 2-10% of women in general population.[1] However, the prevalence of endometriosis in women who seek fertility treatment in much higher at 20.50%.[2]

Various reasons have been attributed to cause reduced spontaneous conception, including altered pelvic anatomy due to formation of fibrosis and adhesion, ovulatory dysfunction following chronic ovarian inflammation,[3] dysregulation of folliculogenesis because of elevated radical oxidative stress and impairment in cytokine, growth factor, and interleukin homeostasis as well as flawed implantation due to alteration in endometrial receptivity.[4] All these conditions may adversely affect the reproductive system of women.

To date, endometriosis is commonly staged using the revised American Society Reproductive Medicine (rASRM) classification, in which stage I and II are considered as early stage or mild endometriosis, and stage III and IV considered as advanced.[5] In women with endometriosis, the European Society of Human Reproduction and Embryology recommends *in vitro* fertilisation and embryo transfer (IVF-ET) as a viable fertility treatment.[6]

However, the outcomes of IVF-ET are not the same in women with endometriosis compared to other patients due to different causes of infertility. In one meta-analysis by Rossi et al., advanced endometriosis had a lower live birth rates following IVF compared with mild disease.[7] In contrast, another meta-analysis in a larger endometriosis population revealed a weak association between successful pregnancy outcomes following IVF according to the stage of endometriosis.[8]

The results of different studies differ, and this could be due to the type of gonadotropin stimulation used. Current evidence is still inconclusive with regards to the types of gonadotropin stimulation and a successful IVF outcome in women with endometriosis. One meta-analysis proved that highly purified human menotropin gonadotropin (hMG) has been shown to achieve similar ovarian stimulation compared with recombinant FSH.[9] But the evidence to support the effect of hMG for different stages of endometriosis is limited.

The present study was undertaken to investigate the outcomes of IVF in ovarian stimulation with human menopausal gonadotropin in patients in different stages of endometriosis in order to form the basis of providing the best advice for patient.

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## MATERIALS AND METHODS

*Study participants*

This retrospective observational cohort study was carried out at tertiary teaching hospital in Kuala Lumpur, Malaysia. The study was approved by local research and ethics commission board committee with ethics approval reference code project: FF-2020-375. The data of the participants from January 2016 to June 2020 was retrieved from the Universiti Kebangsaan Malaysia Medical Centre record unit.

The inclusion criteria were women who were surgically diagnosed with endometriosis and have underwent their first attempt *in vitro* fertilisation/intracytoplasmic sperm injection (IVF/ICSI) were identified. The data were retrieved and classified using revised American Society Reproductive Medicine (rASRM) either into early disease (stage I-II) or advanced disease (stage III-IV).

Women who had received recombinant gonadotrophin stimulation, had elevated day-2 FSH level (>15 IU/l), were >40-year-old were excluded. Severe male factors defined as total spermatozoa count of <5 million per ejaculate were also excluded. Figure 1 shows the flow of patient selection and reasons for exclusion.

*Ovarian stimulation, oocyte retrieval, and intracytoplasmic sperm injection procedures*

All patients had an ovulation stimulation with daily subcutaneous injections of human menopausal gonadotropin (Menopur, Feriing, Germany) at appropriate doses between 225 and 300IU. The ovarian response to treatment was tracked by transvaginal ultrasound. Gonadotrophin releasing hormone (GnRH) antagonist was not used routinely and if was used, it was started once the leading follicle reaches 14mm. Once the dominant follicle reached 18 mm in diameter, 10,000IU human chorionic gonadotropin (Pregnyl®, Merck Sharp & Dohme Limited, UK) were injected to trigger ovulation.

Oocyte retrieval was performed after 34-36 hour under sedation and later fertilised. Intracytoplasmic sperm insemination (ICSI) was implemented for all mature oocytes three hours after retrieval. The oocytes were examined for fertilisation on the following day and cultured in a 20µL of G-TL (Vitrolife, Sweden) enclosed in 3mL of Ovoi (Vitrolife, Sweden) under effect of 5.5% $CO_2$ and 5.0% $O_2$.

At day-3, the embryos were evaluated based on the number of the blastomeres, shape and fragmentation. Cultivated embryos were graded and recorded according to their morphology and cleavage stage according to Gardner classification.

*Fresh embryo transfer and luteal phase support*

A single or double embryo transfer (ET) was performed 3-5 days after oocytes aspiration. The decision was based on the discussion between the clinician and couple, which included previous IVF outcomes, patients age and multiple pregnancy and cost concerns. One or two embryos were immersed in 1mL of embryoglue (Vitrolife, Sweden) and placed for 15 minutes. Later, a transfer catheter (Kitazato, Shizuoka, Japan) which was loaded with the embryos was used under transabdominal ultrasound guidance. All patients received luteal phase support with a combination of oral and vaginal progesterone for at least two weeks.

*Assessment of hCG positive, clinical pregnancy and implantation rates*

The serum hCG concentrations of patients were recorded 14 days after embryo transfer to confirm pregnancy. Biochemical pregnancy referred to as elevated serum b-HCG levels (>5IU/mL) taken 14 days after ET. Women with positive biochemical pregnancy had a serial ultrasound monitoring, to determine the gestational sac(s) with foetal cardiac activity at four weeks after embryo transfer and considered as clinical pregnancy.

The primary outcome of interest in the study was to determine the clinical pregnancy rate, defined as the presence of gestational sac on vaginal ultrasonography. Secondary outcomes included the number of oocytes obtained, fertilisation rate, number of blastocysts cultured, biochemical pregnancy and ectopic pregnancy, and miscarriage.

The oocytes response, embryo grade and pregnancy outcome, including biochemical pregnancy rate and clinical pregnancy rate, were analysed. Only data from first cycle of fresh transfer were included.

*Statistical analysis*

Statistical analyses were carried out with SPSS version 26.0 software (SPSS Inc., Chicago, IL, USA). Shapiro-Wilks test was used to evaluate the distribution of the data. The Continuous data were presented as mean±standard deviation (SD). Statistically significant differences in groups were compared with one-way analysis of variance (ANOVA) with Bonferroni adjustment or Kruskal-Wallis test as appropriate. Categorical variables were presented as percentages and numbers. Differences between proportions or rates were evaluated with the chi-square test.

## RESULTS

A total of 74 women with endometriosis who fulfilled the inclusion and exclusion criteria were identified. The characteristics of the patients are shown in Table I. Women with advanced endometriosis were younger but had a longer infertility duration than patients with mild endometriosis. The type of subfertility and body mass index (BMI) were comparable between the two groups. In mild endometriosis, two third of subjects had their day-2 basal serum CA-125 elevated more than 35U/mL but majority (97.1%) of advanced endometriosis had an elevated basal CA-125.

In our study, all patients had a fresh embryo transfer, either on day-3 or day-5, and no cancellation of cycle was observed. The IVF laboratory parameters and IVF outcomes are presented in Table II. Patients with mild endometriosis had more oocytes retrieved but had a lower fertilisation rate compared to advanced disease. The quality of the embryo of both groups at day-3 was comparable and statistically significant. In early disease, 22 patients (56.4%) had embryo transfer using day-3 embryo and 17 patients (43.6%) had

**Table I: Baseline characteristics of the endometriosis patients who underwent HMG stimulation according to rASRM stages**

| | ARSM staging | | p-value |
|---|---|---|---|
| | I/II (n = 39) | III/IV (n = 35) | |
| Age (mean±SD) | 32.7±3.5 | 30.2±3.3 | p=0.954 |
| Body Mass Index (mean±SD) | 22.6±3.1 | 22.7±3.1 | p=0.196 |
| Duration of subfertility (mean years±SD) | 3.4±1.3 | 4.3±4.3 | p<0.05a |
| Subfertility (%) | | | |
| Primary | 26 (66.6)[b] | 31 (68.6) | p<0.001[c] |
| Secondary | 13 (33.4)[b] | 4 (31.4)[b] | |
| Day 2 basal serum CA125 (U/mL) (%) | | | |
| ≤35 | 13 (33.3) | 1 (2.9) | p=1.000 |
| ≥36 | 26 (66.6) | 34 (97.1) | |

a<0.05. Data presented in mean±SD and subjected to Mann-Whitney test; b<0.001. Data presented in percentage and subjected to Chi-Square test; c<0.001. Comparison between group using Pearson Chi-Square test.
Note: SD, Standard Deviation

**Table II: The overall outcomes of women with mild and advanced endometriosis who underwent IVF-ICSI cycles with hMG stimulation and fresh embryo transfer**

| | ARSM staging | | p-value |
|---|---|---|---|
| | I / II (n=39) | III / IV (n=35) | |
| No. oocytes retrieved per cycle (mean±SD) | 5.3±5.8 | 4.4±5.9 | p=0.554 |
| No. of fertilized (mean±SD) | 3.3±2.4 | 4.3±3.1 | p=0.266 |
| Fertilization rate (%) | 50.2 % | 56.8 % | |
| Embryo grade on transfer (%) | | | |
| I | 29 (74.4) | 22 (62.9) | p<0.001[c] |
| II | 7 (17.9)[b] | 8 (22.9)[b] | |
| III | 3 (7.7)[b] | 5 (14.3)[b] | |
| Day of embryos transferred (%) | | | |
| Day 3 | 22 (56.4) | 19 (54.3)[b] | p<0.001d |
| Day 5 | 17 (43.6) | 16 (45.7)[b] | |
| Biochemical pregnancy rate (%) | 27 / 39 (69.2) | 11 / 35 (31.4) | p=0.074 |
| Clinical pregnancy rate (%) | 18 / 39 (46.1) (17.1) | 6 / 35 | p=0.016[c] |

Note: SD, Standard Deviaiton
b<0.001. Data presented in percentage and subjected to Chi-Square test; c<0.05. Comparison between group using Pearson Chi-Square test; d<0.001. Data presented in percentage and subjected to Chi-Square test.

embryo transfer with day-5 embryo. Meanwhile, in advanced endometriosis, 19 patients (54.3%) had embryo transfer using day-3 embryo and 16 patients (45.7%) had embryo transfer with day-5 embryo.

Biochemical pregnancy was achieved in 69.2% of women with a mild disease but only 31.4% in advanced disease. Clinical pregnancies were 46.1% and 17.1% for mild and advanced endometriosis, respectively.

## DISCUSSION

Among the patients recruited in this study, even though it was not significant difference, we noticed that patients with advanced stages of endometriosis were younger than the ones with mild disease. This is possible because patients with severe endometriosis could have presented earlier for other symptoms like dysmenorrhea, leading to an early laparoscopic diagnosis and IVF treatment. Most of the patients with advanced disease had an elevated basal serum CA-125 compared with those with mild disease. Our finding is consistent with previous studies in which high level of serum CA-125 directly correlates with advanced endometriosis.[10] However, it has limited role in IVF/ICSI as it was considered as nonspecific marker and poor predictor for IVF outcomes.[11]

In our study, it was observed that various aspects of IVF were badly affected by advanced stage endometriosis, especially the number of oocytes obtained, embryo quality, implantation, and clinical pregnancy rate. Sonja et al. (2014) also reported these findings, which showed the success rate for IVF was also reduced as the disease severity progresses.[12] The low response could be due to the nature of the disease or the type of previous ovarian surgery.

Highly purified menotropin gonadotrophin is perceived to perform lower than the recombinant FSH, although a recent meta-analysis proved that hMG achieved a similar result compared with recombinant FSH as an ovarian stimulation.[13] In one study in which recombinant FSH was used for IVF stimulation in endometriosis, the author also showed that advanced endometriosis had a worse prognosis for IVF treatment than mild stage.[14] Similarly, when hMG was used in this study, the findings consistently showed that advanced disease had more unsatisfactory IVF performance than mild disease. This study had shown that severe or advanced endometriosis had a detrimental effect on IVF outcome using HMG. This outcome maybe similar irrespective of the type of gonadotropin used as demonstrated in meta-analysis by Bordewijk EM et al.[13]

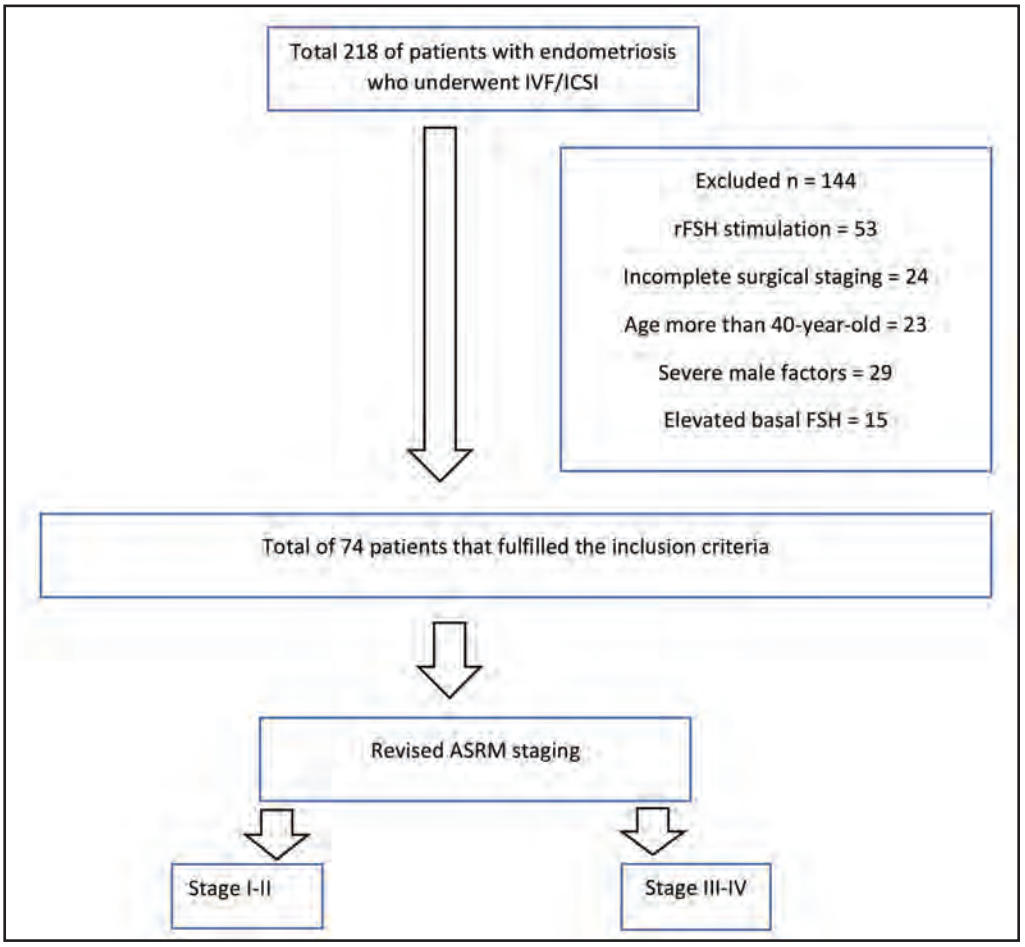


**Fig. 1:** Flow chart showing participants excluded patients excluded from the study.
Note: rFSH = recombinant FSH

It is also important to note that a lower fertilisation rate was recorded in mild endometriosis in our study. This could have resulted from the ongoing secretions by the active glands seen in the mild group, as a previous study showed that peritoneal fluid of women with active lesions has a higher chemotactic activity.[15] However, 74.4% of the fertilised embryos were of excellent quality at day-3 compared with the advanced disease despite a lower fertilisation rate. This explained the higher rate of both biochemical and clinical pregnancy rates in mild disease than the advanced disease.

The current findings also have several clinical importance. Firstly, the rASRM classification is considered reliable in predicting the IVF outcome of infertility treatment, which can be useful for counselling. Advanced endometriosis translates a worse prognosis for IVF outcomes compared to milder stages. Secondly, the usage of hMG as ovarian stimulation in endometriosis is not inferior compared with recombinant FSH, making it a suitable alternative medication. Lastly, it can be concluded that fertilisation rates are not impaired in all endometriosis stages.

It is important to note that there was no cycle cancellation in our study and only pregnancy outcomes of fresh embryo transfer in endometriosis were analysed. The outcomes could be different in a case of frozen embryo transfer. Endometrial receptivity, on the other hand, could be negatively affected in patients with endometriosis. More studies are required to support this point of view.

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