



The administration of long in-vitro fertilization protocol in adenomyosis: case series

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Background. Adenomyosis is a gynaecological condition characterized by the infiltration of endometrial glands and stroma resulting in ectopic intramyometrial, leading to the generalized enlargement of the uterus. Various cyclical regimens are employed globally to assist infertile women with adenomyosis during in-vitro fertilization (IVF) treatment because there is no consensus regarding the best protocol for managing adenomyosis.

Case: The authors reported two cases the administration of long IVF protocol in Adenomyosis with pregnancy outcome. In both cases examined were found that the levels of anti-Mullerian hormone (AMH) were below the 25th percentile. Nevertheless, in both of these cases, the number of oocytes successfully retrieved was more than 5, which may have been influenced by the use of GnRH agonists in previous cycles of long protocol. Both of them were diagnosed with intrauterine pregnancy after transfer embryo.

Conclusion. Although there is currently no consensus on the most suitable protocol for adenomyosis cases, the application of a long protocol in both of the aforementioned cases has yielded positive IVF outcomes.

Keywords: adenomyosis, case series, in-vitro fertilization, long protocol

Background

Adenomyosis is a gynaecological condition characterized by the infiltration of endometrial glands and stroma resulting in ectopic intramyometrial, leading to the generalized enlargement of the uterus. It is a common gynaecological disorder, but its underlying causes and mechanisms are not yet fully understood, necessitating further research for a comprehensive explanation. Common signs and symptoms include uterine enlargement, pelvic pain, irregular uterine bleeding, and subfertility^[1,2].

A study in 2015 with 650 000 patients estimated the annual incidence of adenomyosis around 29 cases per 10 000^[3]. This cohort study in United States which followed patients for a decade, reported an incidence of adenomyosis at 1%, with the highest incidence observed among black women^[3]. Prevalence of adenomyosis varies widely among different studies 14–57%^[4]. The exact incidence of adenomyosis in Indonesia remains uncertain. The most common symptom of adenomyosis were palpable abdominal mass, dysmenorrhoea, and abnormal

HIGHLIGHTS

- Adenomyosis patients tend to have a lower rate of live births, possibly due to disruptions in embryo invasion and placenta formation.
- The administration of GnRH agonists has significant effects in reducing angiogenesis, and inflammation, and also in inducing apoptosis.
- The expression of oestrogen receptors is higher in the endometrium affected by adenomyosis compared to the normal endometrium.
- The application of a long protocol in both of cases has positive effect in in-vitro fertilization Outcome.

bleeding. Adenomyosis was also linked to several conditions that could potentially disrupt the natural process of conception^[5,6].

Numerous studies investigating the impact of adenomyosis on in-vitro fertilization (IVF) results have shown varied outcomes. A meta-analysis of nine articles suggested that adenomyosis could potentially adversely affect IVF success, resulting in lower rates of implantation and clinical pregnancy^[7]. The incidence of early miscarriage is twice as high among individuals with adenomyosis. Adenomyosis patients who undergo IVF tend to have a lower rate of live births, possibly due to disruptions in embryo invasion and placenta formation. These disruptions processes are affected in adenomyosis^[8]. Adenomyomectomy is a surgical procedure sometimes performed in cases of adenomyosis. However, it has been observed that fewer patients who undergo adenomyomectomy (18.2%) achieve pregnancy compared to those undergoing IVF procedures (38.8%)^[9].

Various cyclical regimens are employed globally to assist infertile women with adenomyosis during IVF treatment, and one common approach is frozen-thawed embryo transfer (FET) combined with GnRH analogues^[10]. Among these regimens, the long-protocol IVF with GnRH analogues is particularly popular for managing adenomyosis. There are differences in the number

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of oocytes and pregnancy rates between short and long protocols in adenomyosis patients. Generally, the long protocol showed a better effect than the short protocol in clinical pregnancy rates. Additionally, the long protocol could achieve follicular synchronization because it started in the mid-luteal phase^[11]. The administration of GnRH agonists has been proven to have significant effects in reducing angiogenesis and inflammation linked to adenomyosis and also in inducing apoptosis in adenomyotic tissues^[12].

The long protocol is more commonly used to improve pregnancy rates in women with adenomyosis, but there is also an alternative in the form of a short protocol IVF with GnRH antagonists. Since the long protocol of GnRH agonists can initially cause a flare-up effect, a short protocol of GnRH antagonists is often considered to prevent excessive pituitary suppression, especially in cases of poor ovarian response to the long protocol^[13,14]. It's important to note that there is no consensus regarding the best protocol for managing adenomyosis^[8]. We are reporting two cases of successful pregnancies achieved through the use of the long-protocol IVF in patients with adenomyosis.

Case series

Case 1

Patient background

A 37-year-old woman, nulliparous, with a history of adenomyosis resection, myomectomy, and bilateral cystectomy for endometriosis, has been married for 2 years. The patient reported no complaints during her menstrual cycle.

Clinical presentation

The physical examination within normal limit with BMI was 29.58 kg/m².

Laboratory findings

She underwent laboratory tests which revealed an AMH level of 0.84 ng/ml (20/12/2021), estradiol level of 25.14 pg/ml (20/12/2021), and hypercoagulation as indicated by thromboelastography (22/12/2021). The patient underwent a hysterosalpingography examination (Fig. 1), which revealed that both fallopian tubes were patent. The pathological anatomical findings after the procedures (adenomyosis resection and bilateral cystectomy) which conducted on 30 March 2021, indicated the presence of uterine adenomyosis and bilateral endometriosis cysts. Her husband underwent a sperm analysis, which showed teratozoospermia with a concentration of 45 × 10⁶/ml, motility of 40%, and morphology of 3%.

In-vitro cycle

In the previous cycle, on days 10–14, the patient was administered a GnRH agonist, specifically Buserelin 0.5 mg, for a duration of 10 days, starting from 7 January 2022, until 17 January 2022. At baseline, the right ovary had 1 follicles and left ovary had 1 follicle with endometrial length 10 mm. After an evaluation on the 10th day of administering Buserelin 0.5 mg, the Buserelin dosage was reduced to 0.2 mg, and FSH 300 IU and LH 75 IU were administered on the 1st day of the following cycle, starting



Figure 1. Hysterosalpingography results indicated bilateral patent fallopian tubes.

from 19 January 2022, until 31 January 2022. On the 13th day of the cycle, an ultrasound examination was performed, revealing the presence of 10 oocytes in the right ovary and none in the left ovary. Two days later, on 2 February 2022, an ovum pick up (OPU) procedure was conducted, resulting in the retrieval of six eggs from the right ovary and one egg from the left ovary, totalling seven oocytes.

After the OPU procedure, the patient received progesterone at a dosage of 2 × 400 mg and estradiol valerate at a dosage of 3 × 4 mg. Subsequently, four embryos were obtained through the fertilization process. Three days after the OPU procedure, a fresh embryo transfer was performed, during which two embryos (excellent quality) were transferred on 5 February 2022. Following the embryo transfer, the patient was prescribed enoxaparin at a daily dosage of 0.6 mL. On 17 February 2022, a laboratory test was conducted, which revealed a beta-human chorionic gonadotropin (β-hCG) level of 381 mIU/ml. The patient was diagnosed with intrauterine pregnancy the day after. The flow chart of this case are presented in Fig. 2.

Case 2

Patient background

A 30-year-old woman, nulliparous, with a history of adenomyosis resection and two myomectomies, left cystectomy for endometriosis, and bilateral tube obstruction has been married for 7 years.

Clinical presentation

The physical examination within normal limit with BMI of 27.38 kg/m².

Laboratory findings

Laboratory tests showed an AMH level of 1.48 ng/ml, estradiol level of 13.63 pg/ml, and hypercoagulation as indicated by thromboelastography. Hysterosalpingography (Fig. 3) results indicated bilateral non-patent fallopian tubes. An anatomical pathology examination following the resection procedure revealed uterine adenomyosis. Her husband had normal sperm parameters.

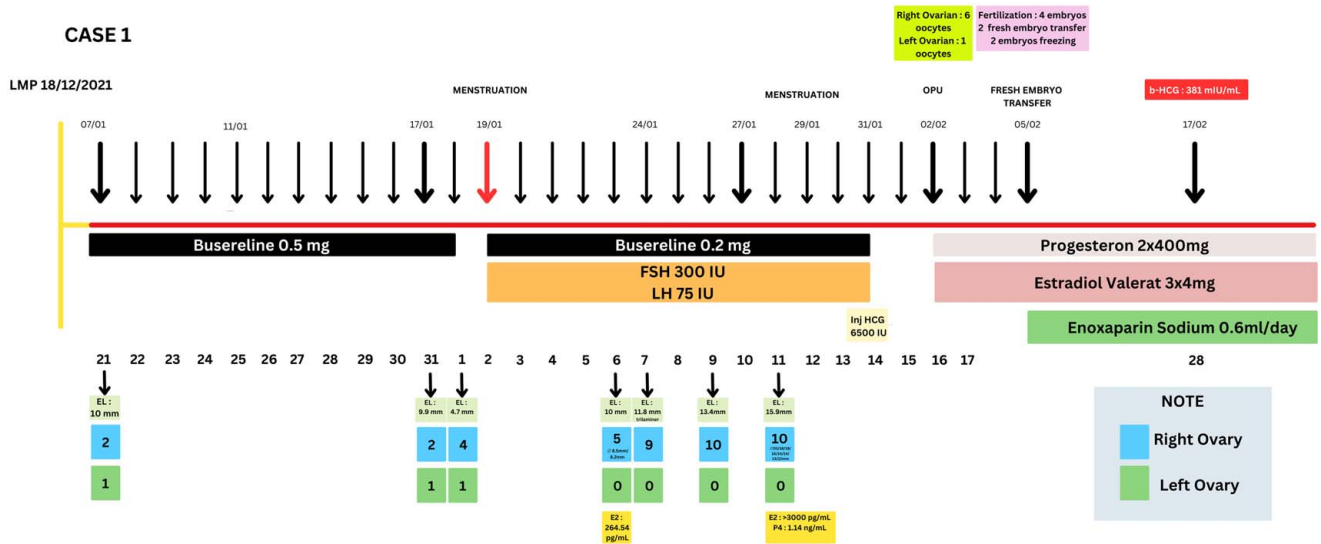


Figure 2. Chart of Case 1.



Figure 3. Hysterosalpingography results indicated bilateral non-patent fallopian tubes.

In-vitro cycle

On days 22 of the previous cycle until day 2 of the next cycle, she received Buserelin 0.5 mg as a GnRH agonist from 18 June 2021, to 27 June 2021. On day 3 of the subsequent cycle, she was given Buserelin 0.2 mg, FSH 300 IU, and LH 75 IU from 28 June 2021, to 8 July 2021. Ultrasound examination revealed 4 oocytes in the right ovary and 3 oocytes in the left ovary. On 8 July 2021, the patient received rHCG at a dose of 6500 IU. Two days later, an Ovum Pick Up procedure was performed, resulting in the retrieval of eight oocytes. After the fertilization process, five embryos with good quality were obtained. All of the embryos

were frozen. The embryo transfer process was postponed as it was considered not to meet the requirements for embryo transfer.

Six months later after ovum pick up procedure, on the second day of her menstrual cycle, she was given Buserelin 0.4 mg and estradiol valerate 4 × 2 mg in preparation for the frozen embryo transfer procedure, which took place from 29 December 2021, to 9 January 2022. On 10 January 2022 an ultrasound examination revealed an endometrial lining of 8 mm. Subsequently, the patient received progesterone at a dosage of 2 × 400 mg and estradiol valerate at a dosage of 3 × 4 mg. On 13 January 2022, the frozen embryo transfer procedure was performed. Following the procedure, the patient was prescribed enoxaparin at a dosage of 0.6 ml per day. On 26 January 2022, a laboratory test was conducted, which revealed a β-hCG level of 958 mIU/ml. The patient was diagnosed with intrauterine pregnancy. The flow chart of this case are presented in Fig. 4.

Discussion

Pathogenesis

We present two cases of adenomyosis patients undergoing IVF treatment using a long protocol. The administration of GnRH agonists in both cases effectively inhibits the natural secretion of gonadotropins during the early follicular phase. This inhibition enables antral follicles to develop to the maturation of follicles. GnRH agonists also boost the quantity of mature follicles and oocytes available for embryo transfer^[15]. However, the utilization of GnRH agonists in long protocols comes with several drawbacks for patients, including the prolonged treatment period until desensitization occurs, an elevated risk of ovarian hyperstimulation syndrome (OHSS) during the desensitization phase^[15].

GnRH receptors found in adenomyotic lesions are targeted by GnRH agonists for medical treatment, directly acting as anti-proliferative agents in the myometrium^[16]. These agonists can significantly reduce inflammatory responses and the formation of new blood vessels (angiogenesis) while promoting the

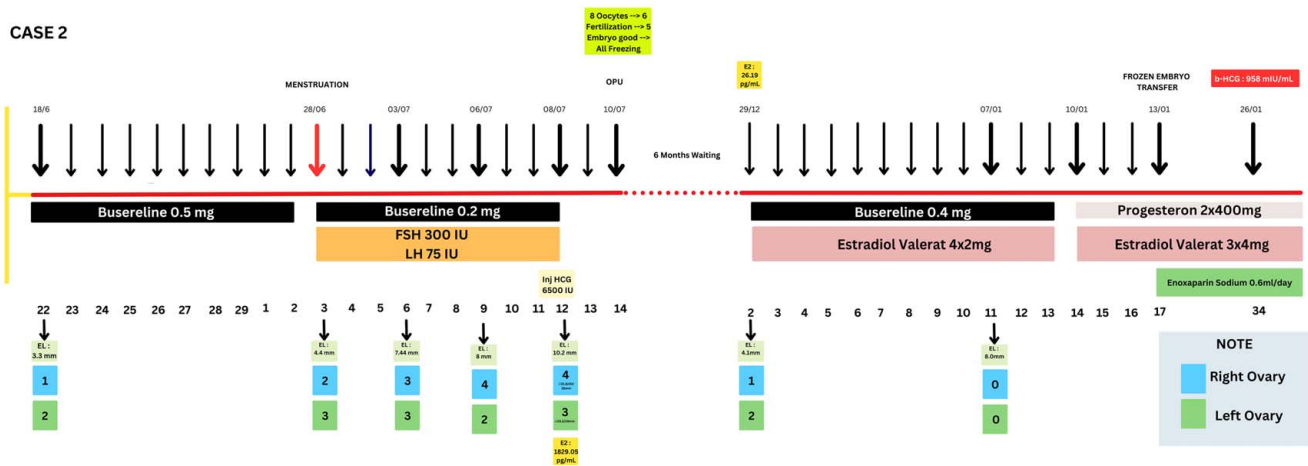


Figure 4. Chart of Case 2.

programmed cell death (apoptosis) of tissues originating from adenomyosis^[16]. Besides their direct anti-proliferative effects in the myometrium, hypoestrogenic effects might contribute to the regression of adenomyosis. The expression of oestrogen receptors is higher in the endometrium affected by adenomyosis compared to the normal endometrium, and GnRH agonists temporarily suppress the hypothalamic-pituitary-ovarian axis, inducing a state of reduced oestrogen levels^[16]. This hypoestrogenic effect leads to a reduction in uterine size and symptoms associated with adenomyosis^[16].

In the study conducted by Costello in 2011, the results showed that adenomyosis did not have a negative impact on the IVF outcomes^[9].

Clinical criteria

The number of oocytes successfully retrieved was more than 6, which may have been influenced by the use of GnRH agonists in previous cycles (both cases)^[15]. GnRH agonists play a role in regulating the reproductive cycle and can affect the ovarian response to hormonal stimulation, which may have contributed to better outcomes in terms of the number of oocytes retrieved during the IVF procedure in these two patients. However, it is important to note that adenomyosis has not been shown to have a detrimental effect on oocyte function and folliculogenesis^[15].

Pathological features

Several factors were identified that may influence infertility in adenomyosis. These factors increased of interleukin (IL-1b) and CRH expression which indicates the possible involvement of inflammatory pathways in the endometrium^[17]. This process could lead to the release of reactive oxygen by macrophages, which can affect the balance of pro-oxidant and antioxidant enzymes in the endometrium^[17]. This can impact hormone production and potentially reduce ovarian reserve, ultimately leading to conditions like amenorrhoea and infertility. In both cases examined, it was found that the levels of AMH were below the 25th percentile^[18]. AMH is a parameter used to predict the number of retrieved oocytes during the OPU procedure. The lower the AMH level, the likelihood of obtaining fewer ovarian follicles is likely to be lower as well.

Treatment approaches

In line with these findings, a retrospective study involving 5662 IVF cycles revealed a notably higher clinical pregnancy rate within the long-protocol group ($P < 0.05$). The longer protocol initiates treatment in the mid-luteal phase, allowing for an increased number and size of oocytes to be retrieved. Additionally, it promotes a more receptive endometrial environment, which is conducive to embryo implantation due to effective suppression resulting in lower serum LH levels.

However, despite the generally superior reputation of the long-term GnRH agonist protocol compared to the short-term protocol, a prospective study by Hou *et al.*^[8] in 2020 revealed a negative impact of adenomyosis on the success of IVF. Hou *et al.*^[8] discovered that the clinical pregnancy rate significantly increased by 92.5% in adenomyosis patients who underwent an ultra-long GnRH agonist protocol compared to those on a standard long protocol (odds ratio: 1.925; 95% CI: 1.137–3.250; $p < 0.015$).

The administration of anticoagulants like LMWH to patients undergoing IVF has recently sparked controversy regarding whether it should be given to all IVF patients or not. In both of the cases mentioned above, laboratory tests have revealed the presence of hypercoagulability, which justifies the use of anticoagulant treatment. However, clinical trial results have shown that the combination of aspirin with LMWH increases the rate of live births. Furthermore, anticoagulant administration does not indicate any abnormalities in placental formation.

In conclusion, we have presented two cases of adenomyosis patients undergoing IVF with a long protocol. Although there is currently no consensus on the most suitable protocol for adenomyosis cases, the application of a long protocol in both of the aforementioned cases has yielded positive IVF outcomes.

Conclusion and future directions

Although there is currently no consensus on the most suitable protocol for adenomyosis cases, the application of a long protocol in both of the aforementioned cases has yielded positive IVF outcomes. Additional research is necessary to compare the results of using ultra-long protocol and long-protocol administration in individuals with adenomyosis undergoing IVF.

Ethical approval

The institutional review board has determined that our study is exempt from ethical approval as it is a review

Informed consent statement

The patient has received a comprehensive explanation regarding the case's particulars and the images to be included in the case report. The patients have provided consent in the case series.

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Author contribution

All authors played a substantial role in this report, whether it was in conceiving the study, designing it, conducting experiments, collecting data, analyzing and interpreting findings, or in all these aspects. They were involved in drafting, revising, or providing critical feedback on the manuscript. They have granted their final approval for the version to be published and have reached a consensus on the journal where the article will be submitted. Additionally, they accept responsibility for all aspects of the work.

Conflicts of interest disclosure

The authors declare that they have no conflicts of interest.

Research registration unique identifying number (UIN)

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Guarantor

Dian Tjahyadi as the first author is the guarantor of this study.

Data availability statement

available upon reasonable request.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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