Effects of artificial cycles with and without gonadotropin-releasing hormone agonist pretreatment on frozen embryo transfer outcomes in patients with adenomyosis

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Research Article

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Abstract

Gonadotropin-releasing hormone agonist(GnRH-a) is generally added to improve pregnancy outcomes of adenomyosis based hormone replacement therapy cycle. Our objective in this study is to investigate whether adding GnRH-a can obtain better pregnancy outcomes. In this retrospective analysis, a total of 341 patients with adenomyosis complicated in vitro fertilization-embryo transfer(IVF-ET) of the frozen embryo transfer (FET). The control group was only treated by hormone replacement therapy cycles to prepare emdometrium, and the study group was added GnRH-a before using hormone to adjust menstruation period. Based on the similar baseline values and embryological data, there was no significantly difference about their clinical pregnancy rates (40.63% vs 42.54%, P=0.72) and live birth rates (23.75% vs 23.75%, P=0.74) between the control group and the study group. Other secondary outcomes including clinical miscarriage rates, ectopic pregnancy rates, preterm pregnancy rates and term pregnancy rates did not show significant difference between the two groups. Compared with using hormone replacement therapy cycle alone, GnRH-a down-regulation based on hormone replacement therapy cycle may not increase the rates of clinical pregnancy and live birth rates in IVF-ET of FET for infertile patients with adenomyosis.

Introduction

Adenomyosis is defined as endometrium-like epithelium and stroma outside the endometrium invading the myometrium. Some studies showed that patients with adenomyosis had lower implantation rates, clinical pregnancy rates and ongoing pregnancy rates but high level of miscarriage rates compared with non-adenomyosis patients[1-2]. At the same time, patients with adenomyosis benefits less from in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI)[3-4]. Considering adverse effect of adenomyosis, many methods have been tried in order to obtain better reproductive outcomes.

It is still disputable whether GnRH-a downregulation can help patients with endometriosis obtain reproductive outcomes. A meta-analysis suggested that Gonadotropin-releasing hormone agonist (GnRH-a) can effectively elevate the clinical pregnancy rates for patients with endometriosis[5]. In assisted reproductive technology(ART), GnRH-a had been widely used in patients with endometriosis for frozen or fresh embryo transfer in order to improve the pregnancy outcomes[6-9]. Another study found only fertilization rates but not clinical pregnancy rates involving fresh and frozen embryo transfer(FET) benefited from GnRH-a for women with endometriosis[10]. There were also two studies which suggested GnRH-a down-regulation did not work well in patients of endometriosis undergoing IVF[11-12].

As for the application of GnRH-a in adenomyosis, this drug was used to carry out pituitary down-regulation in order to ameliorate the not positive reproductive situations in ART. Studies showed the potential efficacy of adding GnRH-a on pregnancy outcomes in women who undergo the IVF/ICSI and supported using GnRH-a down-regulation can improve the success rates of IVF/ICSI involving fresh embryo transfer or FET[13-15].

However, considering the controversial role of GnRH-a in patients with endometriosis, we analyzed the pregnancy outcomes aiming whether the GnRH-a down-regulation would be beneficial in the endometrial preparation protocols of adenomyosis with the FET based on hormone replacement therapy cycle.

Materials And Methods

We analyzed the data from the hospital electronic database for patients undergoing FET between 2013 and 2018. This retrospective cohort study was conducted at the Reproductive Hospital Affiliated to Shandong University. Inclusion criteria included patients were no more than 45 years old and were diagnosed as adenomyosis mainly by two-dimensional ultrasound, which were depicted that (1) subjective enlargement of uterine corpus, (2) asymmetrically thickened myometrium between anterior and posterior walls, (3) heterogeneity of myometrium/hypoechoic striations, (4) poor definition of endometrio-myometrial junction[16]. This article only analysed the first cycle of FET. The day of embryo transfer was respectively day 3, day 5 and day 6 and the number of transferring embryos was no more than two. Patients with donor oocytes were excluded. Other exclusion criteria were malformations of reproductive system without therapy, hydrosalpinx, polycystic ovary syndrome(PCOS), endometriosis, malignant diseases of reproductive system and chromosome abnormality or disease genes existing in one part of couples. The patients in group A were treated by the dual treatment of hormone replacement cycle and GnRH-a, and group B were treated by hormone replacement cycle.

Procedures

For the hormone replacement therapy cycle regimen, the endometrium prepared with oral estradiol valerateat a dose of 4mg daily was started on days 2–4 of the menstrual cycle for 5-6 days. and then 6 mg for the following 5-6 days. Endometrium thickness was monitored after 10–12 days of medication by transvaginal ultrasound along with the serum levels of FSH, LH, estradiol (E2) and progesterone (P). Thereafter the dose of estradiol valerate , which was 8mg/d maximally, was modulated according to the endometrium thickness and the E2 levels. Progesterone capsule 200 mg/day and oral dydrogesterone 40 mg/day as luteal phase support were added when the endometrial thickness reached to 7 mm or more and FET was carried out in 6 days. Estradiol valerate at the dose for endometrium preparation was continued until the day of the serum hCG test, 2 weeks after embryo transfer. If pregnancy was achieved, estradiol valerate stopped gradually at 7-8 weeks of gestation; progesterone capsule and oral dydrogesterone was continued until 12 weeks of gestation.

In the study group, GnRH-a long-acting injection including triptorelin and leuprorelin was used at a

dose of 3.75mg during menstration. After a follow-visit in 30 days, patients would start hormone replacement cycles. Some patients had a larger uterus due to adenomyosis and would receive multiple GnRH-a injections at the 3.75mg dose once every month.

Outcome measures

One primary outcomes of this study are clinical pregnancy rates and live birth rates. Secondary outcomes include clinical miscarriage rates, ectopic pregnancy rates, preterm pregnancy rates and term pregnancy rates. Clinical pregnancy is defined as a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy. Apart from intra-uterine pregnancy, it includes a clinically documented ectopic pregnancy. Live birth is defined as the complete expulsion or extraction from a woman of a product of fertilization, after 22 completed weeks of gestational age; which, after such separation, breathes or shows any other evidence of life, such as heart beat, umbilical cord pulsation or definite movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is attached. A birth weight of 500 grams or more can be used if gestational age is unknown. Live births refer to the individual newborn; for example, a twin delivery represents two live births. Secondary outcomes are defined according to our previous published paper[17].

Statistical analysis

Relevant data was analyzed by SPSS 22.0.Values. $P<0.05$ was considered to indicate statistical significance. Mean ± standard deviation was used to show continuous variables of normal distribution by two-sample t-test. Variables were tested for normality. We expressed categorical variables as percentage, and make inter-group comparisons by the chi-square test or Fisher's exact test. Binary logistic regression was used to analyze the relationship between the dichotomous dependent variable and independent variable.

Ethics approval

The study protocol was approved by the Ethics Committee of the Reproductive Hospital Affiliated to Shandong University and adhered to relevant ethical guidelines.

Results

Totally, 341 women were analyzed in this study. One hundred and sixty female in Group A were carried out with GnRH-a down-regulation treatment based on the hormone replacement therapy cycle, the other one hundred and eighty-one patients in Group B were only treated with hormone replacement therapy cycle.

The baseline values of patients are presented in Table 1. There was no significant difference between two groups including age, height, weight and body mass index(BMI)(34.56±4.49 vs 35.25±4.95, P=0.18; 162.63±5.57cm vs 161.56±5.29cm, P=0.08; 64.99±11.04kg vs 63.76 ±9.42kg, P=0.27; 24.56±3.87 vs 24.43± 3.42, P=0.73). Types of infertility and baseline hormone values including FSH, LH, E2 and PRL all were comparable between the two groups(6.54±1.89IU/L vs 6.32± 2.04IU/L, P=0.31; 4.39±2.05IU/L vs 4.85±2.57IU/L, P=0.07; 42.38±33.86pg/ml vs 48.08 ±76.54pg/ml, P=0.39; 19.87±35.15ng/ml vs 16.76±19.48pg/ml, P=0.31). The percentage of primary and secondary infertility were also comparable between the two groups(40.62% vs 33.70%, 59.38% vs 66.30%, P=0.19). The endometrial thickness of

patients in group B was slightly thinner than that in Group A(0.96±0.20mm vs 0.91±0.18mm，P=0.02), but mean values both were more than 8mm.

Embryological data was shown in the Table 2. Time of embryo transfer was divided as three parts which are respectively cleavage stage embryos and blastocyst embryos. Cleavage stage embryo was defined embryos beginning with the 2-cell stage and up to, but not including, the morula stage. Blastocyst embryo was defined as the preimplantation embryo development that occured around day 5–6 after insemination or ICSI. The blastocyst contained a fluid filled central cavity (blastocoele), an outer layer of cells(trophectoderm) and an inner group of cells (inner cell mass). The number of transferring embryos was generally no more than two. Time of embryo transfer and the number of transferring embryos did not differ obviously between the two groups. The percentage of day 3, day 5 or day 6 and the percentage of one or two embryo did not show any significant difference between two groups(1.25% vs 2.76%, 72.5% vs 75.14%, 26.25% vs 22.10%, P=0.44; 89.83% vs 88.40%， 10.62% vs 11.60%，P=0.78)

We respectively compared the pregnancy outcomes of infertility patients with adenomyosis between the two groups in Table 3. Compared with the direct hormone replacement group, the clinical pregnancy rates (40.63% vs 42.54%, P=0.72) and the live birth rates (23.75% vs 23.75%, P=0.74) do not differ from each other. The clinical miscarriage rates(41.5% vs 44.2%, P=0.754), biochemical pregnancy rates(13.75% vs 11.05%, P=0.45) and ectopic pregnancy rates (3.08% vs 3.90%, P=1) were also similar with each other.

Binary logistic regression in Table 4 indicated that age, BMI, endometrial thickness on HCG trigger day, baseline hormone values of FSH, LH, PRL, and E2 and types of infertility were not related to clinical pregnancy rates. However, it was the day of embryo transfer that was the only independent variables associated with clinical pregnancy rates(OR 1.67, 95% CI 0.27 to 10.14 for women having a day 5 embryo transfer compared with women having a day 3 embryo transfer; OR 2.99, 95% CI 1.62 to 5.52 for women having a day 6 embryo transfer compared with women having a day 3 embryo transfer). For live birth rates, binary logistic regression in Table 5 showed that BMI, types of infertility, baseline hormone values of FSH, LH, PRL, and E2 and endometrial thickness on HCG trigger day were not associated with that. Age and the day of embryo transfer were the related independent variables to the live birth rates for patients with adenomyosis (OR 0.92, 95% CI 0.87 to 0.98 for live birth rates for age; OR 4.42 95% CI 0.60 to 32.81 for women having a day 5 embryo transfer compared with women having a day 3 embryo transfer; OR 3.64, 95% CI 1.60 to 8.27 for women having a day 6 embryo transfer compared with women having a day 3 embryo transfer).

Time of GnRH-a down-regulation in study group was shown in Table 6. 73.75% and 15.625% of patients respectively underwent once and twice GnRH-a down-regulation and only 10.625% of patients underwent no less than three times GnRH-a down-regulation.

Discussion

This respective study did not find obvious effecy of GnRH-a in pregnancy outcomes for patient with adenomyosis. The result of comparable clinical pregnancy rates seemed conflicted with GnRH-a

increasing the chance of pregnancy for female with adenomyosis[13-14]. Preterm rates and full-term rates happened almost in the same level between the two groups, which was different from the previous epidemiological studies[18].

Meta-analysis explored the association between the endometrium thickness and pregnancy outcomes, thinner endometrium(≤7mm) resulting in negative ongoing pregnancy rates and live birth rates[19-20]. In this study, the difference of endometrium thickness between two groups had statistical significance; however, mean values of both were more than 9mm. So it was debatable to detect whether the different endometrium thickness impaired pregnancy outcomes in this study.

A randomized controlled trial indicated that embryo transfer conducted at day 5 was more likely to obtain higher ongoing or cumulative pregnancy rates compared with the result at day 3[21]. Another meta-analysis demonstrated that clinical pregnancy rates and live birth rates were significantly higher following day 5 compared to day 6 blastocyst transfers[22]. Both Group A and Group B covered the day 3, day 5 and day 6 of embryo transfer and the variation between two groups was no statistical significance even if the proportion of day 3 was relatively low. The embryos of day 5 covered the vast majority in both groups, which can improve the pregnancy rates in a way.

The proportion of primary infertility in Group A was more than that of Group B, although the difference of the ratio of primary and secondary infertility between two groups did not have statistical significance. Studies demonstrated that pregnancy performance of the secondary infertility was significantly better than that of the primary infertility[23-24]. The higher percentage of primary infertility in group A may be a key factor to decreasing rates.

As for the number of transferring embryos, a study indicated that two elective single embryo can obtain better reproductive outcomes than one double embryo transfer using blastocysts[25]. In our study, single embryo transfer protocol was also regarded as the major protocol to ensure the better pregnancy outcomes. In addition, it was easy to find the total clinical pregnancy rates and live birth rates of patients still stayed at low level regardless what measures had been taken.

In this study, GnRH-a did not show obvious effect of improving pregnancy outcomes. We came up with several reasons to explain this phenomenon. Firstly, the current cycles of GnRH-a was not enough for patients with adenomyosis so that the effects of the drug did not work. We failed to get the information of the degree of adenomyosis between the two groups, maybe many patients did not suffer from severe adenomyosis, and it may be another reason leading to the negative result in GnRH-a improving pregnancy outcomes. Furthermore, we can consider whether patients was not sensitive to GnRH-a resulting in uterus not changing much, or GnRH-a did not work at all.

One advantage of our study was that the number of women with adenomyosis was relatively large, including patients in a time span of six years. In addition, according to 2018 specialist consensus published in Journal of Reproductive Medicine, the number of embryo transfer was no more than two under 40 years and single embryo transfer was recommended. In our study, single embryo transfer

constitutes the vast majority and the others were double embryo transfer, which was consistent with consensus. This two advantages made this study applicative for more population with adenomyosis. Apart from that, we also found that good reproductive outcomes still stayed at a low level although different measures had been taken, which meant many new explorations needed to be done to improve reproductive outcomes for women with adenomyosis.

Two obvious defects existed in this study. Firstly, this study did not analysis the duration of GnRH-a for women with adenomyosis. If patients could get enough duration of GnRH-a down-regulation, the attachment of high quality follicles will be affected as most of our patients only underwent only once GnRH-a down-regulation. In addition, the severity of adenomyosis did not be discussed, which may be influence therapeutic effect in a way. These questions both needed to be studied in the future.

Although many studies thought that administrating GnRH agonists was very beneficial for patients of adenomyosis in improving reproductive outcomes of IVF-ET, our study did not demonstrate advantages of adding GnRH agonists based on hormone replacement therapy cycle. Similiar negative conclusions were also shown in endometriosis[10-12]. In addition, Movahedi S et al. did a study that Endometrial preparation for FET using GnRH agonists appeared to be as effective as FET without administrating these agonists[26]. The other two articles also did not think that GnRH-a down-regulation showed any significant advantages of IVF-ET[27-28]. Those all provided some supportive evidence for our study result.

In conclusion, patients with adenomysis carried out GnRH-a down regulation or not based on hormone replacement therapy cycle had similar reproductive outcomes. Adding GnRH-a based on hormone replacement therapy cycle may not increase the rates of clinical pregnancy and live birth.

Declarations

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Author Contribution

Muzi Li took the responsibility of project development, data collection, data analysis,and manuscript writing. And Heng Zhao, Lihong Xu, Yanbo Du and Lei Yan also took part in the work project development. In addition, Lei Yan also finished part of manuscript writing and amending.

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Author Contribution: Muzi Li: project development, data collection, data analysis, manuscript writing; Heng Zhao: project development; Lihong Xu: project development; Lei Yan: project development, manuscript writing.

Data availability statement

The datasets generated during and/or analyzed during the current study are available by request.

Ethics declarations

The studies involving human participants were reviewed and approved by research ethics committee of Reproductive Hospital affiliate d to Shandong University. The patients/participants provided their written informed consent to participate in this study.

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Tables

Table 1 Comparison of baseline data between two groups

	GROUP A(n=160)[1]	GROUP B(n=181)[2]	Two-tailed P value
	Mean(SD)	Mean(SD)	
Age(SD)(year)	34.56±4.49	35.25±4.95	0.18
Height（SD）(cm)	162.63(5.57)	161.56(5.29)	0.08
Weight（SD）(kg)	64.99(11.04)	63.76(9.42)	0.27
BMI(SD)	24.56(3.87)	24.43(3.42)	0.73
Laboratory Tests			
	Mean(SD)	Mean(SD)	
FSH(SD)（IU/L）	6.54(1.89)	6.32(2.04)	0.31
LH(SD)(IU/L)	4.39(2.05)	4.85(2.57)	0.07
E2(SD)(Pg/ml)	42.38(33.86)	48.08(76.54)	0.39
PRL(SD)(ng/ml)	19.87(35.15)	16.76(19.48)[3]	0.31
Infertility			0.19
Primary Infertility— no./total no. (%)	65/160(40.62)	61/181(33.70)	
Secondary Infertility— no./total no. (%)	95/160(59.38)	120/181(66.30)	
Endometrial thickness on hCG trigger day(mm)（	0.96（0.20）[4]	0.91（0.18）	0.02

[1]the study group (n = 160) was added GnRH-a based on GnRH-a before using hormone to adjust menstruation period.

[2]The control group (n = 181) was only treated by artificial hormone cycle to prepare emdometrium.

[3]One value was missing.

[4]Five values were missing.

Table 2 Comparison of embryological characteristics between two groups

	GROUP A(n=160)[1]	GROUP B(n=181)[2]	Two-tailed P value
Time of embryo transfer — no./total no. (%)			
D3	2/160(1.25)	5/181(2.76)	0.44
D5	116/160(72.5)	136/181(75.14)	
D6	42/160(26.25)	40/181(22.10)	
No. of embryos transferred			0.78
One embryo — no./total no. (%)	143/160(89.38)	160/181(88.40)	
Two embryos — no./total no. (%)	17/160(10.62)	21/181(11.60)	

[1]the study group (n = 160) was added GnRH-a based on GnRH-a before using hormone to adjust menstruation period.
[2]The control group (n = 181) was only treated by artificial hormone cycle to prepare emdometrium.

Table 3 Comparison of clinical pregnancy outcomes between two groups

	GROUP A(n=160)[1]	GROUP B(n=181)[2]	Two-tailed P value
Clinical outcome(%)			
clinical pregancy	65/160(40.63)	77/181(42.54)	0.72
biochemical pregnancy rate	22/160(13.75)	20/181(11.05)	0.45
clinical miscarriage rate	27/65(41.54)	34/77(44.16)	0.75
ectopic pregnancy	2/65(3.08)	3/77(3.90)	1[3]
live birth rate	38/160(23.75)	43/181(23.75)	0.74
preterm pregnancy	5/160(3.12)	5/181(2.76)	0.84
full-term pregnancy	33/160(20.63)	38/181(20.99)	0.95

[1]the study group (n = 160) was added GnRH-a based on GnRH-a before using hormone to adjust menstruation period.
[2]The control group (n = 181) was only treated by artificial hormone cycle to prepare emdometrium.
[3]This value was calculated by Fisher’s exact test.

Table 4 Binary logistic regression of influence factors of pregnancy rates between two groups

	OR（95%CI）	Two-tailed P value
the day of embryo transfer		
DAY3	1	P=0.00
DAY5	1.67(0.27-10.14)	
DAY6	2.99(1.62-5.52)	

Table 5 Binary logistic regression of influence factors of live birth rates between two groups

	OR（95%CI）	Two-tailed P value
age(year)	0.92(0.87-0.98)	P=0.01
the day of embryo transfer		
DAY3	1	P=0.01
DAY5	4.42(0.60-32.81)	
DAY6	3.64(1.60-8.27)	

Table 6 Time of GnRH-a down-regulation in Group A

	GROUP A(n=160)
time of GnRH-a down-regulation(%)	
1	118/160(73.75)
2	25/160(15.625)
no less than 3	17/160(10.625)