Original Article

Comparison of Vaginal Gel and Intramuscular Progesterone for *In vitro* Fertilization and Embryo Transfer with Gonadotropin-Releasing Hormone Antagonist Protocol

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Abstract

Background: Luteal support is a key to patients undergoing *in vitro* fertilization and embryo transfer (IVF-ET) with gonadotropin-releasing hormone (GnRH)-antagonist protocol. This study aimed to compare the effect between vaginal progesterone (VP) and intramuscular progesterone (IMP) with GnRH-antagonist protocol after IVF-ET.

Methods: A total of 1760 patients (18 years ≤ age ≤35 years) undergoing IVF-ET with GnRH-antagonist protocol were studied retrospectively between September 2014 and August 2015 in Peking University Third Hospital. In the patients, 1341 patients received VP (VP group) and 419 patients received IMP (IMP group) as luteal support. We compared clinical outcomes between these two groups. The primary objective of the study was the live birth rate. Measurement data between the two groups were conducted using independent samples *t*-test. The variables in line with non-normal distribution were expressed as median (p25 and p75) and were compared using nonparametric Mann–Whitney *U*-test.

Results: Live birth rate in VP group was 38.55%, significantly higher than that in the IMP group, which was 30.79% (χ^2 = 8.287, P = 0.004). The clinical intrauterine pregnancy rate and implantation rate in VP group were also significantly higher than those in the IMP group (clinical intrauterine pregnancy rate 47.35% vs. 41.29%, χ^2 = 4.727, P = 0.030; implantation rate 30.99% vs. 25.26%, χ^2 = 14.546, P < 0.001). Any statistically significant differences in ectopic pregnancy and abortion rates between two groups were not observed.

Conclusion: Luteal support with VP had better clinical outcomes for young women undergoing IVF-ET with GnRH-antagonist protocol.

Key words: Gonadotropin-Releasing Hormone Antagonist; Intramuscular Progesterone; Undergoing *In vitro* Fertilization and Embryo Transfer; Vaginal Progesterone

Introduction

A successful *in vitro* fertilization and embryo transfer (IVF-ET) pregnancy is well-known to primarily depend on two factors: embryo quality and endometrial receptivity. Affected by the degree of synchronization between endometrium and sex hormone level, endometrial receptivity is the prerequisite for the complex process of embryo implantation. Luteal insufficiency is popular in IVF process, and it may reduce endometrial receptivity, so luteal support is essential to improve the endometrium status, and thus, improve the pregnancy rate.[1-3]

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Luteal support is necessary for patients undergoing IVF-ET with Gonadotropin-releasing hormone (GnRH)-antagonist protocol. Accumulating lots of evidence have confirmed

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that as luteal support drugs the effect of progestogens is superior to that of placebo and comparable to that of hCG, which also can avoid the risk of hCG-induced severe ovarian hyperstimulation syndrome complications.[2] Vaginal progesterone (VP) and intramuscular progesterone (IMP) are gradually becoming the drug of the first choice for IVF-ET luteal support.[4] However, whether there are differences in curative effects, such as clinical pregnancy rate and live birth rate, between VP and IMP is yet controversial. Some prospective or retrospective studies speculated comparable effects of these two methods.[5,6] Nevertheless, a recent study revealed that for patients <35 years old, the clinical pregnancy rate in VP group was significantly increased compared to that in the IMP group.[7] Fertility is known to gradually decrease with increasing age in women. The age of 35 years old is an inflection point when the fertility is significantly declined, and the abortion rate is increased.[8,9] Therefore, we only enrolled patients aged ≤35 years in this study. Most of these studies focused on GnRH-agonist protocol, how about GnRH-antagonist protocol? There is little study to elucidate it, especially patients <35 years old. The aim of the present large-scale trial was to evaluate the efficiency of VP gel versus IMP for luteal support in GnRH-antagonist cycles with patients <35 years old.

Methods

Ethical approval

This study was approved by the Institutional Ethics Committee of the Peking University Third Hospital, and written informed consent was obtained from each subject.

Subjects and study design

We enrolled patients who did IVF-ET or intracytoplasmic sperm injection and embryo transfer (ICSI-ET) between September 2014 and August 2015 in Peking University Third Hospital. The inclusion criteria were as follows: 18 years ≤ age ≤35 years; in the fresh cycle of ovulation-promoting; patients undergoing IVF-ET with GnRH antagonist protocol; previous ovulation-promoting cycles ≤2 times; normal uterus pattern in B-ultrasound or hysterosalpingography. The exclusion criteria were as follows: patients did not undergo embryo or blastocyst transfer; patients were provided luteal support in combination with other drugs such as estrogen and a variety of progestogens; patients suffered from endometrial polyps, hydrosalpinx, and recurrent abortion.

A total of 1760 infertile patients undergoing IVF-ET or ICSI-ET between September 2014 and August 2015 were enrolled, including 1341 patients receiving VP (VP group) and 419 receiving IMP (IMP group). The patients opted for VP or IMP self-willingly and were not provided other medication guidance.

Ovulation-promoting methods

The cycle was initiated at the 2nd day of natural period or 1 month after pretreatment with contraception pills. The initial dose of follicle-stimulating hormone (FSH) (Gonal F, Serono, Germany), ranging from 150 to 450 IU, was selected according to antral follicle count, hormone level, age, and other factors. Human menopausal Gn (hMG, Livzon Pharmaceutical Group Inc. China) was added appropriately based on the ultrasound monitoring of follicular development. After continuous injection of FSH, 0.25 mg GnRH antagonist (Cetrotide, Serono, Germany) was injected at the 7th–8th day of the menstrual cycle or the follicle diameter was >14 mm. More than 2 follicles diameters ≥18 mm, patients were administered hCG trigger by injecting rhCG 250 μg (Eiser, Serono, Germany). Simultaneously, the endometrial thickness was recorded, and oocytes were retrieved after 34–36 h. On the day of oocyte retrieval, sperm was collected from the male counterpart. The natural fertilization or ICSI was determined based on the semen quality. A maximum of two embryos was transferred on the 3rd day after oocyte retrieval.

Luteal support protocols and follow-up

From the day of oocyte retrieval, patients were given luteal support, 90 mg/d progesterone (Crinone, Serono) for patients in the VP group and 60 mg/d for IMP group. Until the 14th day after embryo transfer, the serum hCG level was detected to determine pregnancy, wherein a serum hCG level ≥30 IU/ml referred to a biochemical pregnancy positive. Then, the patients were continuously administered luteal support until the 30th day after embryo transfer and were subjected to B-ultrasound, wherein visible fetal sac referred to continuous pregnancy positive clinically. If the fetal sac was inside the uterus with fetal heartbeat, the patients were continuously provided luteal support until 10 weeks of intrauterine pregnancy, and then they were followed up by phone until delivery. However, if the fetal heartbeat was not detected, patients underwent an ultrasound review at an alternate week, wherein they might be diagnosed as spontaneous abortion, and were discontinued luteal support. Moreover, if the fetal heartbeat were detected, they would be given the treatment mentioned above. If the fetal sac was outside the uterus, patients were designated as ectopic pregnancy and discontinued luteal support, followed by surgical or conservative treatment.

Outcome variables

The primary objective of the study was the live birth rate. The secondary objectives included ongoing pregnancy rate, spontaneous abortion rate, ectopic pregnancy rate, and implantation rate.

Statistical analysis

Statistical analyses were performed using SPSS 18.0 software (SPSS Inc., USA). All data were assimilated from the information database of the hospital. Measurement data for the normal distribution variables were expressed as mean ± standard deviation (SD), and comparisons between the two groups were conducted using independent samples *t*-test. The variables in line with non-normal distribution were expressed as median (p25, p75), and were compared using nonparametric Mann–Whitney *U*-test. The numerical

data were expressed as *n* (%) and were compared using Chi-square test or Fisher's exact test. A difference with $P < 0.05$ (two-sided) was considered statistically significant.

Results

General information of patients

As shown in Table 1, no statistically significant difference was observed in the average age between the VP and IMP groups (29.97 ± 3.36 vs. 30.22 ± 3.37 years old, $P = 0.184$). Furthermore, there were insignificant differences in the duration of infertility, causes of infertility, the number of pregnancies, body mass index, antral follicle count, baseline FSH, luteinizing hormone, E_2, prolactin, and T levels between the two groups.

Ovulation-promoting and laboratory results

As shown in Table 2, there was no significant difference in the days of Gn application, the total amount of Gn, a number of retrieved oocytes, good-quality embryos, embryo transferred number while the endometrial thickness on the day of the hCG trigger was comparable between the two groups.

Clinical outcomes

The embryo implantation rate, clinical pregnancy rate, and live birth rate in the VP group were 30.99%, 47.35%, and 38.55%, respectively, which were significantly higher than those of 25.26%, 41.29%, and 30.79%, respectively in the IMP group ($P < 0.001$, $P = 0.030$, and $P = 0.004$, respectively). However, the differences in spontaneous abortion rate and ectopic pregnancy rate between the two groups were statistically insignificant [Table 3].

Discussion

In this study, we retrospectively analyzed 1760 patients undergoing IVF-ET or ICSI-ET with GnRH-antagonist protocol and compared the clinical efficiency of different routes of administration of progesterone. We found that the embryo implantation rate, clinical pregnancy rate, and live birth rate were significantly higher in patients of the VP group as compared to those in the IMP group. To the best of our knowledge, this is the first study encompassing more than 1500 individuals for the comparison of the clinical efficiency of VP and IMP for GnRH-antagonist protocol, which is a significant guidance for diverse infertile patients.

Table 1: Comparison of general information between IMP and VP groups

Items	IMP (n = 419)	VP (n = 1341)	$t/Z/\chi^2$	P
Age (years), mean ± SD	30.22 ± 3.37	29.97 ± 3.36	1.329	0.184
Infertility (years), median (p25, p75)	4.00 (2.00, 6.00)	4.00 (2.00, 6.00)	0.932	0.290
Number of pregnancies (*n*), median (p25, p75)	0.00 (0.00, 1.00)	0.00 (0.00, 1.00)	0.037	0.923
BMI (kg/m^2), mean ± SD	22.50 ± 3.31	22.50 ± 3.67	0.053	0.988
Antral follicle count (*n*), median (p25, p75)	6.00 (4.00, 8.00)	6.00 (4.00, 8.00)	0.974	0.570
Baseline FSH (mIU/ml), mean ± SD	6.91 ± 2.23	6.90 ± 2.94	0.056	0.989
Baseline E2 (pmol/L), median (p25, p75)	142.00 (109.00, 180.00)	139.00 (106.00, 182.00)	0.528	0.553
Baseline PRL (ng/ml), median (p25, p75)	11.90 (8.67, 15.90)	11.90 (8.82, 16.50)	0.324	0.846
Baseline LH (mIU/ml), median (p25, p75)	3.80 (2.60, 5.50)	3.54 (2.47, 5.03)	1.238	0.105
Baseline T (nmol/L), median (p25, p75)	0.70 (0.69, 0.99)	0.69 (0.69, 1.00)	1.876	0.097
Baseline A (nmol/L), median (p25, p75)	7.10 (5.00, 10.00)	7.05 (5.00, 9.50)	0.811	0.395
Primary diagnosis (*n* (%))			1.493	0.828
Tubal factor	213 (50.84)	654 (48.77)		
Endometriosis	27 (6.44)	92 (6.86)		
Anovulation	38 (9.07)	128 (9.55)		
Male factor	128 (30.55)	410 (30.57)		
Unexplained	13 (3.10)	57 (4.25)		

IMP: Intramuscular progesterone; VP: Vaginal progesterone; SD: Standard deviation; BMI: Body mass index; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; PRL: Prolactin.

Table 2: Comparison of ovulation-promoting and embryo results between IMP and VP groups

Items	IMP (n = 419)	VP (n = 1341)	t/Z	P
Gn time (days), mean ± SD	10.49 ± 1.98	10.59 ± 1.90	0.927	0.344
Gn amount (IU), mean ± SD	2230.28 ± 915.01	2227.30 ± 960.55	0.062	0.955
Endometrial thickness (mm), mean ± SD	10.78 ± 1.56	10.83 ± 1.51	0.592	0.557
hCG day serum E_2 (pmol/L), median (p25, p75)	7281.5 (5066, 12,724)	7136.0 (4853, 11,927)	1.569	0.133
Number of retrieved oocytes (*n*), mean ± SD	12.28 ± 5.83	12.19 ± 5.83	0.276	0.763
Transferrable embryos (*n*), median (p25, p75)	3 (2, 9)	3 (2, 9)	0.805	0.443
Good-quality embryos (*n*), median (p25, p75)	5 (2, 7)	4 (2, 8)	0.182	0.909
Embryo transferred number (*n*), mean ± SD	1.96 ± 0.32	1.94 ± 0.28	1.236	0.206

IMP: Intramuscular progesterone; VP: Vaginal progesterone; SD: Standard deviation; hCG: Human chorionic gonadotropin; Gn: Gonadotropin.

Table 3: Comparison of clinical outcomes between IMP and VP groups

Items	IMP (n = 419)	VP (n = 1341)	χ^2	P
Embryo implantation (n (%))	219 (25.26)	841 (30.99)	14.546	<0.001
Clinical pregnancy (n (%))	173 (41.29)	635 (47.35)	4.727	0.030
Spontaneous abortion (n (%))	16 (3.82)	60 (4.47)	0.332	0.564
Ectopic pregnancy (n (%))	14 (3.34)	26 (1.94)	2.827	0.093
Live birth (n (%))	129 (30.79)	517 (38.55)	8.287	0.004

IMP: Intramuscular progesterone; VP: Vaginal progesterone.

In another study comparing the VP and IMP for GnRH-antagonist protocol, Kahraman *et al*. divided 426 patients into two groups: one group received 90 mg Crinone, twice daily, and another group received 100 mg progesterone daily, both from the first day after oocyte retrieval. The clinical outcomes showed no significant differences in the implantation rate, clinical pregnancy rate, and ongoing pregnancy rate between the two groups.[5] These results differed from the current findings, which might be due to different luteal support time. In this study, the luteal support was applied from the day of oocyte retrieval. However, Fanchin *et al*. postulated that the uterine contraction frequency on the day of embryo transfer would significantly decrease if VP were used from the day of oocyte retrieval, whereas applying VP in advance might exert a better relaxing effect on the uterine muscle, thereby increasing the pregnancy rate.[10,11] Ayoubi *et al*. also reported that the uterine contraction frequency was significantly decreased after applying VP for 3 days.[12] Further evidence has shown that the application of VP gel once daily is prone to provide sufficient luteal support for new cycle IVF-ET patients.[13] However, whether it is correlated with the initial time of progesterone application is subject to further studies.

Progestogen promotes the proliferation and differentiation of glandular cells and stromal cells in the endometrium, which creates an appropriate endometrial environment for implantation.[14] The degree of synchronization between endometrium and embryos is the prime factor for ensuring a successful implantation of the embryos.[15] Our study found that the embryo implantation rate, clinical pregnancy rate, and the live birth rate of the VP group were significantly higher than those in the IMP group. Consistent results were also reported by Ho *et al*. that for patients undergoing IVF-ET/ICSI with luteal support, although the VP group showed a lower serum progesterone level, it exhibited a significantly higher implantation rate and ongoing pregnancy rate compared to the IMP group.[16] Progesterone administrated through vagina can be locally absorbed by cervical cells, and rapidly transported to the endometrial cells, thereby achieving a high progestogen concentration in the uterine cavity and preferable conditions for implantation of embryos.[17] The histochemical analyses of endometrial biopsy found that progestogen concentration in endometrial cells inpatients of the VP group was significantly higher than that in IMP patients and the luteal phase concentration in untreated patients. Vaginal administration can enhance the absorption of progesterone, promote histological changes of the endometrium, and thus, enable improved synchronization of the implantation of embryos.[18] In addition, the histological maturity of endometrium in patients with the intramuscular administration was 2–3 days later than the natural cycle.[19] In addition, the topical vaginal administration could not only act on the endometrium, but also the localized high concentration progestogen in uterine can be quickly circulated to the ovary and induce the positive feedback of the endocrine. This can prevent corpus luteum from atrophy and autolysis, thereby extending its functionality.[20] Several studies support that the effects of VP on the maturity of the endometrium are superior to those of IMP. A similar conclusion was obtained in the present clinical study, which showed that the implantation rate and clinical pregnancy rate of the VP group were better than those of the IMP group.

We were unable to investigate the patients' subjective perception due to a large number of participants. Furthermore, this is a retrospective study rather than a strict randomized controlled clinical cohort study. However, it can reflect the actual clinical conditions of a certain population due to the large scale.

In summary, this study aimed to compare the efficiency of VP gel versus IMP for luteal support in GnRH-antagonist cycles. The results demonstrated that the embryo implantation rate, clinical pregnancy rate, and live birth rate were higher in patients aged ≤35 years in the VP group compared to those in the IMP group. Thus, we recommended VP as the drug of the first choice for luteal support in patients undergoing IVF-ET with GnRH-antagonist protocol in young women.

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Conflicts of interest

There are no conflicts of interest.

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阴道用黄体酮凝胶与肌注黄体酮在选用促性腺激素释放激素拮抗剂方案进行体外受精胚胎移植的比较研究

摘要

背景：黄体支持是体外受精-胚胎移植成功的关键因素，本研究的目的是比较使用促性腺激素释放激素拮抗剂方案进行体外受精—胚胎移植时，不同黄体支持药物即阴道用黄体酮凝胶与肌注黄体酮之间有无临床差异。

方法：选取2014年9月和2015年8月之间在北京大学第三医院使用促性腺激素释放激素拮抗剂方案进行体外受精—胚胎移植的患者共1760例，年龄在18-35岁之间，其中1341例患者接受阴道用黄体酮凝胶，419例在肌注黄体酮组，比较两组的临床结局，主要结果为活产率。两组之间数据采用独立样本t检验，非正态分布的变量表示为中位数（P25，p75），并采用非参数Mann-Whitney U检验比较。

结果：阴道用黄体酮凝胶组的活产率为38.55%，显著高于肌注黄体酮组的30.79% (χ^2=8.287, p=0.004)。阴道用黄体酮凝胶组临床妊娠率和种植率也显著高于IMP组（临床妊娠率47.35% vs. 41.29%，χ^2=4.727, p＝0.030，种植率30.99% vs. 25.26%，χ^2=14.546, p＝0.001）。两组间的异位妊娠和自然流产率无明显差异。

结论：年轻女性若使用促性腺激素释放激素拮抗剂方案进行体外受精胚胎移植，阴道用黄体酮凝胶的有更好的临床妊娠结局。