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Original Article

Analysis of embryo quality with luteal phase ovarian stimulation after failed in vitro fertilization-embryo transfer with long or ultra-long protocol

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ABSTRACT

Objective: To investigate the effect of luteal phase ovarian stimulation on embryo quality after failed in vitro fertilization-embryo transfer with long or ultra-long protocol.
Methods: In patients who underwent luteal phase ovarian stimulation after failed in vitro fertilization-embryo transfer with long or ultra-long protocol in the reproductive center between January 2015 and October 2017, self-control observations and statistical analyses were carried out for the number of oocytes retrieved, the rate of fertilization, the rate of D3 high-quality embryos, the rate of transplantable blastocyst formation, and the pregnancy rate of transfer cycle between long or ultra-long protocol and luteal phase ovarian stimulation.
Results: The rate of fertilization and blastocyst were significantly increased after luteal phase ovarian stimulation ($P < 0.05$). However, the difference of the number of oocytes retrieved and the rate of D3 high-quality embryos was not statistically significant ($p > 0.05$).
Conclusions: The patients who failed with long protocol or ultra-long protocol due to low quality embryos, the protocol changed over to luteal phase ovarian stimulation. Can significantly improve the rate of fertilization oocytes and the transplantable blastocyst, and improve the outcome of clinical pregnancy. It provides an alternative ovarian stimulation protocol in patients with IVF-ET failure.

Introduction

Currently, the conventional ovarian stimulation protocol mostly used during in vitro fertilization-embryo transfer (IVF-ET) is gonadotropin-releasing hormone agonist protocol such as long or ultra-long protocol in mid-luteal phase [1]. In some of the patients, IVF-ET failed due to poor number/quality of oocytes and poor number/quality of embryos [2]. In recent years, the follicular wave theory has been proposed. There may be 23 follicular waves in the same menstrual cycle. After the dominant follicles ovulate, other follicles in the luteal phase are not necessarily locked, but may be re-raised. Foreign scholars have confirmed that the luteal phase can also obtain mature eggs and can be successfully fertilized. It shortens the course of treatment and can take eggs twice in a menstrual cycle, shortening the waiting time of patients. The disadvantage is that the fresh cycle cannot be transplanted due to the uneven development of the endometrial. Therefore, the embryos need to be frozen after taking the eggs [3–5]. Between October 2017 and January 2015, a total of 40 cases of IVF-ET failed after long protocol/ultra-long protocol ovarian stimulation due to factors of poor embryo quality. The embryo quality was improved significantly and good pregnancy outcome was achieved after the protocol luteal phase ovarian stimulation.

Methods

Patients who underwent IVF-ET in our reproductive center from January 2015 to October 2017, and failed in fertility treated with the mid-luteal phase long or ultra-long protocol, were changed over to luteal phase ovarian stimulation. Patients with endometrial factor-induced embryo implantation difficulties and male factors requiring intra-cytoplasmic sperm injection (ICSI) were not included in the study, e.g.①endometrial depression induced by adenomyoma and hysteromyoma. ② Endometrial ≤ 7.0 mm, the echo of endometrial was inhomogeneous on the day of HCG or the day of transfer. ③ Uterine cavity effusion resulted from any factor on the day of HCG or the day of transfer. ④ Patients underwent ICSI

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for male factors. All patients had normal function of thyroid and no significant complications after surgery. 27 cases of them, received long protocol, average age: 35.27 ± 5.74 years old, FSH: 6.53 ± 1.47 U/L, LH: 4.94 ± 1.83U/L, E_2: 141.96 ± 47.92 pmol/L, P: 1.73 ± 0.68 nmol/L.13 cases received ultra-long protocol, average age: 31 ± 4.12 years old, FSH: 6.91 ± 1.39 U/L, LH: 5.19 ± 2.17U/L, E_2: 136.58 ± 51.36 pmol/L, P: 1.59 ± 0.57 nmol/L. Luteal phase ovarian stimulation was carried out after IVF-ET failure in both groups, and frozen embryo transfer was performed.

Long protocol: GnRH-a (long-acting GnRH-a 0.9–1.85 mg, or short acting GnRH-a 0.05 mg/d until the HCG day) was injected in the mid-luteal phase of previous cycle. Gn was given to start after it had been confirmed that the criteria of pituitary down-regulation were met.

Ultra-long protocol: a full dose of long-acting GnRH-a was injected in the mid-luteal phase of previous cycle. Gn was injected 28 days later.

Luteal phase ovarian stimulation: After the day of natural-cycle oocyte retrieval or the day of follicular rupture under the monitoring with B-ultrasonography, Gn225−300 IU/d was given, growth hormone 4–6 IU/d and letrozole 2.5 mg/d was added for 8 days. The follicular size was monitored by B-ultrasonography after 5–6 days and the drug was adjusted until there was more than 2 mature follicles ≥18 mm on both ovaries. GnRHa 0.1 mg and HCG 1000 IU was given at night. Oocytes were retrieved after 34 h, and medroxyprogesterone 10 mg/d was added to prevent menstruation in advance, and affecting oocyte retrieval.

Self-control observation was conducted for the number of oocytes retrieved, the rate of fertilization, the rate of D3 high-quality embryos, the rate of transplantable blastocyst, and the pregnancy rate of transfer cycle.

SPSS16.0 software was used to carry out the statistical analysis of the self matched data. The measurement data were tested by *t* test. The enumeration data were tested by χ^2 test. The difference was statistically significant when $p < 0.05$.

Results

Comparison of laboratory indicators between long protocol and luteal phase ovarian stimulation

The comparison of various laboratory indicators in 27 patients underwent long protocol ovarian stimulation and luteal phase ovarian stimulation is shown in Table 1 below. The rate of fertilization and the rate of blastocyst in the long protocol group were significantly lower than those in luteal phase ovarian stimulation, the differences were statistically significant ($p < 0.05$); the number of oocytes retrieved and the rate of D3 high-quality embryos were slightly higher than those in luteal phase ovarian stimulation, the differences were not statistically significant ($p > 0.05$). In the luteal phase ovarian stimulation group, frozen-thawed cycle transfer was performed, and the clinical pregnancy rate was 38.89%.

Comparison of laboratory indicators between ultra-long protocol and luteal phase ovarian stimulation

13 patients underwent ultra-long protocol ovarian stimulation and luteal phase ovarian stimulation. Comparison of laboratory indicators is shown in Table 2 below. The rate of fertilization and the rate of blastocyst in the ultra-long protocol group were significantly lower than those in the luteal phase ovarian stimulation group, the differences were statistically significant ($p < 0.05$); the number of oocytes retrieved and the rate of D3 high-quality embryos were slightly lower than those with luteal phase ovarian stimulation, the differences were not statistically significant ($p > 0.05$). In the luteal phase ovarian stimulation group, frozen-thawed cycle transfer was performed, and the clinical pregnancy rate was 60.00%.

Discussion

Is to compare the embryo quality of luteal phase ovarian stimulation to long or ultra-long protocol by self-control comparison after excluding the influence of uterine and male factors. The results indicated that the fertilization rate of oocytes and blastocyst significantly increased after the luteal phase ovarian stimulation, the number and the quality of transplantable embryos were significantly increased. Satisfactory clinical pregnancy rate was achieved after frozen-thawed transfer.

The quality of oocyte and embryo is one of the two key factors affecting the clinical pregnancy in IVF-ET [6]. Since the 1980s, GnRH-a have been used in controlled ovarian hyper stimulation protocol. Long and ultra-long protocol has been proven to be able to obtain sufficient number of oocytes and high quality embryos, and to achieve high clinical pregnancy rate [7,8]. They have become conventional ovarian stimulation protocol in most reproductive centers. In order to obtain high quality oocytes and embryo after IVF-ET failure, the ovarian stimulation protocol has been altered, and increasingly diversified and individualized [9,10]. The current consensus is that: the failure of IVF-ET is mainly due to less oocytes, low oocyte quality which result in low fertilization rate, and low quality embryos after excluding male and uterine factors, and the improvement of the number and the quality of ovum can be achieved by improving ovarian function and ovarian stimulation protocol. Exist in conventional long and ultra-long protocol, inconsistent sensitivity of patient to GnRH-a, different inhibition degree of pituitary down-regulation, slow ovarian response and low luteinizing hormone (LH) occurred and result in decreased number of oocytes retrieved, low quality of ovum, reduced number of transplantable embryos, and increased rate of cycle cancellation [11]. In recent years, many protocols of antagonist have been used in order to improve the ovarian response and avoid above-mentioned adverse factors. However, the dose and the start time of gonadotropin releasing hormone antagonists are difficult to be controlled. Therefore, delayed follicular development and decreased estradiol are likely to occur in the late phase

Table 1
Comparison of laboratory indicators between long protocol and luteal phase ovarian stimulation.

Group	No. of cases	No. of oocytes retrieved	Rate of fertilization	Rate of D3 high-quality embryos	Rate of blastocyst formation	Pregnancy rate of transfer cycle
Long protocol group	27	10.97 ± 2.05	51.20%(149/291)	53.02%(79/149)	37.58%(56/149)	0.00%(0/13*)
Luteal phase ovarian stimulation group	27	8.37 ± 1.94	63.89%(161/252)	49.07%(79/161)	49.69%(80/161)	38.89%(7/18*)
t/P value		0.947/0.343	8.872/0.003	0.484/0.487	4.605/0.032	

**In 27 patients underwent long protocol, 13 patients had transplantable blastocyst, and 14 ones had no one. After luteal phase ovarian stimulation after failed long protocol, 23 patients had frozen blastocyst to be transferred, and 4 patients had no any transplantable blastocyst.

Table 2
Comparison of laboratory indicators between ultra-long protocol and luteal phase ovarian stimulation.

Group	No. of cases	No. of oocytes retrieved	Rate of fertilization	Rate of D3 high-quality embryos	Rate of blastocyst formation	Pregnancy rate of transfer cycle
Ultra-long protocol group	13	11.23 ± 6.76	43.06%(62/144)	38.71%(24/62)	32.26%(20/62)	0.00%(0/7*)
Luteal phase ovarian stimulation group	13	15.38 ± 10.54	64.65%(128/198)	44.53%(57/128)	49.22%(63/128)	60.00%(6/10*)
t/P value		6.533/<0.001	15.740/<0.001	0.579/0.447	4.884/0.027	

**In underwent ultra-long protocol group, 7 patients and 6 patients had no one. In luteal phase ovarian stimulation group, 10 patients had transplantable blastocyst, 2 patients had no.

of ovarian hyper stimulation, leading to inconsistent evaluation of its effect [12].

Some scholars have proposed the theory that there may be 23 follicular waves in the same menstrual cycle. The other follicles in the luteal phase are not locked after ovulation of the dominant follicle; they can be recruited again and are not affected by LH [3,4]. A number of studies have reported that oocytes obtained by ovarian stimulation in different phases of the menstrual cycle have the same ability of fertilization and potential of embryonic development in patients with fertility preservation [13,14]. With the clinical use of urinary gonadotropin in luteal phase ovarian stimulation, the luteal phase ovarian stimulation has become a hot topic of ovarian stimulation protocol in recent years. Its efficacy is especially better in patients with poor ovarian reserve. Enhanced number and quality of transplantable embryos can be obtained by the use of it [15,16]. Theoretically, the luteal phase ovarian stimulation, can avoid the excessive use of Gn, the decreased number of ova, and poor quality of embryo due to excessive suppression of down-regulation and excessive low of LH by long and ultra-long protocol. This self-control study confirmed that the luteal phase ovarian stimulation to long or ultra-long protocol, the fertilization rate of oocytes and the rate of blastocyst formation were significantly enhanced, and the clinical pregnancy outcome was improved. This provides an alternative option for ovarian stimulation in patients with failed IVF-ET. The limitation of this study is its small sample size. Multicenter and large sample studies are required.

Author contributions

Q.-C.L. and Y.-J.G. planned the project. Q.-C. L., H.-L.Z., D.-H.L., and C.L. conceived of and designed the study. Q.-C.L., X.-T.C., and Y.-J.G. analyzed the data and drafted the manuscript. All authors reviewed the manuscript and approved the final version.

Conflicts of interest

The authors have no competing interests to declare.

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