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ARTICLE

Comparison of luteal phase ovulation induction and ultra-short gonadotropin-releasing hormone agonist protocols in older patients undergoing *in vitro* fertilization

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ABSTRACT

Many undergoing *in vitro* fertilization-embryo transfer (IVF-ET) procedures treatments have been tried for older infertile patients, but still can not reverse the aging effect on oocyte, and infertility treatment is expensive, even for people in developed countries. The study aimed to compare outcomes following the application of luteal phase ovulation induction (LPOI) and ultra-short gonadotropin-releasing hormone agonist (GnRH-a) protocols in patients aged more than 40 years undergoing IVF-ET and to examine the effectiveness and feasibility of LPOI. A total of 266 IVF-ET cycles in 155 patients aged 40 years and over were retrospectively analyzed. Of these patients, 105 underwent the ultra-short GnRH-a protocol (GnRH-a group) and 50 underwent LPOI (LPOI group). Various clinical outcomes were compared between these two groups using either *t*-tests or the chi-square test. The study showed patients in the LPOI group required a higher dosage of human menopausal gonadotropin and a lower dosage of recombinant follicle stimulating hormone than those in the GnRH-a group. Furthermore, though the total dosage of gonadotropin was higher in the LPOI, its cost was lower. Finally, fertilization rates were higher and high-quality embryo rates were lower in the LPOI group, and the live birth rate of LPOI group is higher than (GnRH-a group) . These between-group differences were all significant ($P < 0.05$). Compared with the ultra-short GnRH-a protocol, LPOI may enable higher 2-pronuclear embryo fertilization rates and lower gonadotropin costs to be achieved, indicating that LPOI might be an ideal choice for older patients undergoing IVF-ET.

KEYWORDS

Lutealphase ovulation induction; poor ovarian response; ultrashort GnRHa protocol; *in vitro* fertilization-embryo transfer (IVF-ET)

1. Introduction

With the release of the second child policy in China, there is an increasing requirement for women of more advanced age (i.e. at least 40 years) to reproduce. However, declining ovarian function, as can be indicated by a reduction in the number of follicles and/or a decline in oocyte quality, insensitivity to exogenous drug stimulation, and an increasing rate of poor ovarian response (POR), can all lead to unsatisfactory ovulation results and low pregnancy rates [1]. To overcome these challenges and achieve better clinical outcomes, fertility physicians have tried various ovulation stimulating protocols to improve pregnancy rates in older patients undergoing *in vitro* fertilization-embryo transfer (IVF-ET) procedures. The ultra-short gonadotropin-releasing hormone agonist (GnRH-a) protocol has generally been conventionally used for POR patients, though in recent years, the luteal phase ovulation induction (LPOI) protocol has gradually gained the approval of increasing numbers of clinicians. All in all, the two protocols are often used in elderly IVF patients, but there are relatively few comparative studies on the two protocols at present, and came to no conclusion on which ovulation promotion scheme is more suitable for elderly patients. In this study, we retrospectively analyzed 266 older IVF-ET patients who underwent either the LPOI or ultra-short GnRH-a protocol to explore the application value of LPOI in patients who are at least 40 years of age.

2. Materials and methods

The study design is following: First of all, patients using LPOI or ultra-short GnRH-a protocol were selected. Next, the treatment outcomes of patients were recorded. Last, the treatment outcomes were analyzed by SPSS and then the pros and cons of difference treatment programs were discussed.

2.1. Patient recruitment

From October 2015 to June 2018, 155 IVF cases involving the fallopian tube conditions were selected from

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the Affiliated Hospital of Shandong University of Traditional Chinese Medicine in collaboration with the Reproductive & Genetic Centre. The requirement for informed consent was waived due to the retrospective nature of this study.

Either the LPOI or ultra-short GnRH-a protocol was used in each of these patients over a total of 266 cycles. Of these patients, 105 underwent the ultra-short GnRH-a protocol (GnRH-a group) and 50 underwent LPOI (LPOI group). Patients were recruited if they satisfied at least two of the following criteria: basal follicle stimulating hormone (bFSH) >10 U/L, antral follicle count ≤5, and age ≥40 years [2–4]. Patients were excluded if they had any of the following: endocrine disease, adenomyosis, endometrial dysplasia, premature ovarian failure, chromosome abnormalities, and so on. Patients were allocated into either GnRH-a or LPOI group according to the stimulation protocol they underwent. All hormones were detected using the Bakerman chemiluminescence assay.

2.2. Protocols

2.2.1. The ultra-short GnRH-a protocol

An ultrasound scan was performed on day 2 of menstruation to ensure that there were no ovarian cysts, to record the size and quantity of antral follicles, and to test that whether bFSH was <20 U/L. A single 0.1-mg dose of triptorelin acetate (GnRH-a) was then given as a subcutaneous injection. From day 3 of menstruation, recombinant follicle stimulating hormone (r-FSH) and human menopausal gonadotropin (HMG) (75 IU/ampoule, Zhuhai Livzon Pharmaceutical Group Co. Ltd., Zhuhai, China) was administered at doses of 150 to 225 IU/day. The amount of gonadotropin (Gn) given was based on the growth follicular shown on ultrasound scans. Once patients were seen to have one dominant follicle ≥18 mm in diameter, or two dominant follicles ≥17 mm in diameter in both ovaries, the administration of Gn was stopped and a 10000-IU intramuscular injection of human chorionic gonadotropin (HCG) (5000 IU/ampoule, Zhuhai Livzon Pharmaceutical Group Co. Ltd., Zhuhai, China) was given. Egg retrieval could then be carried out 36 h later. After 72 h of IVF or intracytoplasmic sperm injection (ICSI), the embryos either underwent fresh embryo transfer (ET) or were frozen for storage (if cleavage embryos had >4 cells and were above stage 2) and then select the appropriate opportunity for frozen-thawed ET (FET).

2.2.2. Luteal phase ovulation induction

The injections of HMG were started at an initial dosage of 150–225 IU/day after egg retrieval during the follicular phase or during natural cycle ovulation. After 2–3 days, follicle growth was monitored with ultrasound and Gn dosage was adjusted accordingly. When one follicle ≥18 mm was detected, 10000 IU of HCG was injected and the egg retrieval procedure was carried out 36 h later. Frozen embryos could then be carried out after 72 h in accordance with the criteria for freezing as mentioned above.

2.3. Embryo transfer

After the ultra-short GnRH-a protocol, ET was carried out. Briefly, after egg retrieval, ET was performed on the 3rd day following endometrial transformation if endometrial thickness was ≥8 mm and blood progesterone was <1.5 ng/mL. Progesterone support was then provided until 10–12 weeks after ET.

On the other hand, FET was performed after the LPOI protocol. Briefly, the ovaries and basal hormones were checked on day 2 of the first menstrual cycle after egg retrieval, after which hormone replacement was carried out to establish an artificial cycle. Hormone replacement was achieved with administration of 4–6 mg/day of progynova (estradiol valerate tablets; Bayer Pharmaceuticals, Berlin, Germany) from day 3 of the menstrual cycle. Subsequently, the endometrium was monitored with ultrasound. When the uterus lining reached ≥8 mm and was in a good state, 40 mg/day of progesterone was intramuscularly injected to induce the endometrium to enter the secretion phase. After 3 days, FET was performed and patients were continuously supported with progesterone and progynova. Alternatively, ovulation during the natural rather than artificial cycle could be monitored before FET was carried out.

2.4. Monitoring pregnancy outcomes

Serum HCG levels were measured 14 days after transfer, with beta HCG >5 IU/mL indicating pregnancy. Ultrasound was also conducted after 2 weeks to confirm the pregnancy. Presence of a fetal heartbeat in the intrauterine gestational sac was considered the clinical manifestation of pregnancy.

2.5. Observation targets

Dosage and cost of Gn, serum estradiol (E2) levels on the day HCG was administered, oocyte quantity, fertilization rate (number of fertilized eggs/number of mature eggs), 2-pronuclear embryo (2PN) cleavage rate (2PN cleavage number/number of fertilized eggs), available embryo and high-quality embryo rates (available embryo rate = number of available embryos/2PN cleavage number; high-quality embryo rate = number of high-quality embryos/2PN cleavage number), clinical pregnancy rate (cumulative pregnancy rate), and abortion rate were compared between the GnRH-a and LPOI groups.

2.6. Statistical analysis

Statistical analysis was carried out using statistical product and service solutions (SPSS) software version 22.0 (IBM, New York, USA). For data expressed as mean ± standard deviation, between-group comparisons were carried out using *t*-tests for two independent samples or *t*-tests with the Bonferroni correction. For data expressed in terms of rate, between-group comparisons were instead carried out using the chi-square test. *P*-values *<0.05* were considered to indicate a significant difference.

3. Results

3.1. Patient demographics and clinical characteristics

There were no significant differences between the GnRH-a and LPOI groups in terms of age; years of infertility; body mass index (BMI); or basal follicle stimulating hormone (bFSH), basal luteinizing hormone (bLH), and basal estradiol (basal E2) (*P > 0.05*) (Table 1) levels.

3.2. Drug administration and protocol outcomes

Although Gn and HMG dosages were significantly greater in the LPOI than GnRH-a group, Gn cost was significantly lower in the former (*P < 0.05*). The dosage of (follicle stimulating hormone) FSH was also lower in the LPOI than GnRH-a group (*P < 0.05*), while the number of days over which Gn was given did not significantly differ between groups (*P > 0.05*). These results are shown in Table 2.

In terms of ovulation outcomes, there were no significant between-group differences in egg retrieval number or available embryo quantity (*P > 0.05*). There were also no significant between-group differences in terms of 2PN cleavage rate or transferable embryo rate (*P > 0.05*). In contrast, 2PN fertilization rate was significantly higher in the LPOI than GnRH-a group, while high-quality embryo rate was significantly higher in the GnRH-a than LPOI group (*P* < 0.05) (Table 3).

3.3. Clinical outcomes

There were no significant differences in clinical pregnancy and miscarriage rates between the GnRH-a and LPOI groups (*P > 0.05*), while live birth rate was significantly higher in the LPOI GnRH-a group than GnRH-a group (*P* < 0.05) (Table 4).

4. Discussions

As women age, their number of eggs gradually declines; a woman is born with about 500,000–1,000,000 egg cells, but only about 1% ever mature fully to be released

Table 1. Between-group comparison of demographic and clinical characteristics.

Group	Age (years)	Years of infertility	BMI (kg/m^2)	bFSH (IU/L)	bLH (IU/L)	bE2 (pg/mL)
GnRH-a	43.00 ± 2.07	4.95 ± 4.52	23.08 ± 3.95	11.003 ± 3.01	4. 26 ± 2.90	55.01 ± 68.57
LPOI	42.78 ± 2.40	3.62 ± 2.44	24.12 ± 2.50	11.09 ± 6.02	5.03 ± 1.87	53.44 ± 26.02
t-statistic	0.193	1.181	-1.11	-0.710	0.397	0.301
P-value	0.734	0.309	0.396	0.765	0.784	0.183

All data are represented as mean ± standard deviation.
GnRH-a, gonadotropin-releasing hormone agonist; LPOI, luteal phase ovulation induction; BMI, body mass index; bFSH, basal follicle stimulating hormone; bLH, basal luteinizing hormone; bE2, basal estradiol

Table 2. Between-group comparison of drug administration dosages.

Group	Gn usage (days)	Gn dosage (IU)	FSH dosage (IU)	HMG dosage (IU)	Gn cost (yuan)
GnRH-a	8.16 ± 2.03	2976.56 ± 1066.22	405.96 ± 362.39	2897.59 ± 711.09	2177.41 ± 2024.73
LPOI	9.95 ± 3.05	3895.24 ± 1299.13	9.52 ± 43.64	3935.809 ± 1267.17	1484.87 ± 463.80
GnRH-a	9.45 ± 2.07	2966.87 ± 1046.22	415.96 ± 372.69	2896.891 ± 709.09	2159.98 ± 2027.32
LPOI	10.00 ± 3.05	3898.14 ± 1269.13	8.99 ± 23.56	3938.804 ± 1234.05	1489.76 ± 450.40
t-statistic	-1.330	-3.304	2.015	-4.565	2.345
P-value	0.369	0.039	0.009	0.001	0.005

All data are represented as mean ± standard deviation.
GnRH-a, gonadotropin-releasing hormone agonist; LPOI, luteal phase ovulation induction; Gn, gonadotropin; FSH, follicle stimulating hormone; HMG, human menopausal gonadotropin

Table 3. Between-group comparison of ovulation outcomes.

Group	Number of eggs retrieved	Number of available embryos	2PN fertilization rate	2PN cleavage rate	Transferable embryo rate	High-quality embryo rate
GnRH-a	2.97 ± 2.37	1.45 ± 1.34	62.09%	78.00%	74.98%	26.45%
LPOI	4.76 ± 3.70	1.99 ± 1.36	77.01%	76.98%	70.30%	14.37%
t-statistic	-1.541	1.044	-	-	-	-
χ2	-	-	10.300	0.028	1.081	4.515
P-value	0.135	0.050	0.011	0.857	0.259	0.041

All data are represented as mean ± standard deviation or percentage.
GnRH-a, gonadotropin-releasing hormone agonist; LPOI, luteal phase ovulation induction; 2PN, 2-pronuclear embryo

Table 4. Between-group comparison of clinical outcomes.

Group	Pregnancy rate	Miscarriage rate	Live birth rate
GnRH-a	20.95% (22/105)	22.72% (4/22)	54.45% (12/22)
LPOI	26.0% (13/50)	23.07% (3/13)	61.54% (8/13)
$\chi 2$	0.039	0.035	0.037
P-value	0.686	0.715	0.049

GnRH-a, gonadotropin-releasing hormone agonist; LPOI, luteal phase ovulation induction

during the menstrual cycle; the rest degenerate overtime [5–8]. As such, reproductive function gradually declines as women age. In women aged 40 years and over, this decline in the quality and quantity of eggs may manifest as irregular menstruation, while women aged 50 and over may experience menopause [9,10]. Thus, choosing a better stimulation protocol to improve clinical pregnancy rates in older IVF patients is of great importance.

The ultra-short GnRH-a protocol has been widely used in clinical settings. In this protocol, GnRH-a is used only once on day 2 of menstruation, after which Gn is started on day 3 and maintained until the administration of HCG. This protocol utilizes the flare-up effect of GnRH-a, which can act to enhance follicle recruitment, shorten follicle maturation times, and reduce Gn usage. Furthermore, since the GnRH-a is injected only once, pituitary function before and after HCG administration is not affected, and there is only a small effect on endogenous luteinizing hormone (LH) levels during the luteal phase. Thus, the GnRH-a protocol is more suitable for POR patients or those with few antral follicles. However, there has been researched indicating that downregulation of GnRH via a negative feedback mechanism caused by the ultra-short protocol might have an adverse effect on egg quality due to increased E2 levels caused by follicle stimulation. In addition, the transient effect of downregulation might cause an early endogenous LH peak [11], leading to premature luteinization of follicles and oocytes and in turn, lower fertilization rates [12]. Our study appears to confirm this, with a significantly lower fertilization rate in the GnRH-a.

An alternative to the GnRH-a protocol is the LPOI protocol, which has gradually been gaining acceptance and has been increasingly used in clinical settings. Recent studies have found that there are 2–3 follicular development waves per menstrual cycle [13,14], and that LPOI outcomes are more satisfactory than those following the ultra-short protocol. The possible mechanism by which these improved outcomes are attained is twofold. First, the LPOI protocol may improve the chances of gaining more oocytes of higher quality in older POR patients. This is because an additional follicular wave after ovulation may result in enhanced steroid hormone synthesis on a larger scale during the luteal phase. This would offer a higher level of serum E2 and higher expression of LH receptor mRNA in granule cells and follicular fluid [15,16]. Second, studies have indicated that though follicles grow in a high progesterone state during the luteal phase, LPOI does not affect follicle maturation. Thus, there is a lower chance of a premature LH surge with the LPOI than GnRH-a protocol. This could act to prevent ovum escape, which occurs commonly in POR patients [17,18]. In turn, more oocytes of higher quality can be obtained, making it possible to achieve higher fertilization rates and embryo numbers with LPOI [19]. As such, the application of LPOI may improve clinical pregnancy rates in older IVF patients.

Overall, our findings appear to support this conclusion and were as follows: (1) the LPOI group used more HMG, but less r-FSH, required a higher total Gn dosage but at a lower cost, and had a higher rate of fertilization than the GnRH-a group. This may be related to the low sensitivity of follicles to Gn during the luteal phase, meaning LPOI patients needed more HMG to promote follicular maturation [20]. (2) The LPOI group had a significantly lower high-quality embryo rate than the GnRH-a group. This result might have been due to the small sample size of this study, or the lack of follicles that could achieve stimulation during the luteal phase in some patients. There are also studies showing that the LPOI protocol may cause follicular dysplasia, luteinized unruptured follicles, or preovulation of unmatured follicles, which can all lead to unsatisfactory clinical outcomes [20]. (3) The LPOI group had better egg endings and fertilization outcomes, and more available embryos than the GnRH-a group, which may have been related to the low LH levels and increased synchronous development of follicles afforded by the LPOI protocol. (4) The LPOI group showed a higher clinical pregnancy rate and lower abortion rate than the GnRH-a group. This may be related to the synchronous development of the endometrium and embryo in the FET cycle.

In summary, by comparing outcomes between the ultra-short GnRH-a and LPOI protocols in older IVF patients, we found that the LPOI protocol allowed patients to achieve higher fertilization rates, live birth rate ($P < 0.05$), and a higher pregnancy rate [although there is no statistical difference between two groups ($P > 0.05$)] at a lower Gn cost, indicating that the LPOI protocol may be a better choice for POR treatment. Considering the small sample size of this study, however, further prospective clinical research is needed to provide more substantial evidence for this conclusion.

Disclosure statement

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References

[1] Li XL, Wang L, Lv F, Huang XM, et al. The influence of different growth hormone addition protocols to poor ovarian responders on clinical outcomes in controlled ovary stimulation cycles: A systematic review and meta-analysis. Medicine (Baltimore). 2017;96(12):e6443.
[2] Gingold JA, Lee JA, Whitehouse MC. Maximum basal FSH predicts reproductive outcome better than cycle-specific basal FSH levels: waiting for a "better" month conveys limited retrieval benefits. Reprod Biol Endocrinol. 2015;13:91.
[3] Younis JS, Ben-Ami M, Ben-Shlomo I. The Bologna criteria for poor ovarian response: a contemporary critical appraisal. J Ovarian Res. 2015;8:76.
[4] Baird DT, Collins J, Egozcue J, et al. Fertility and ageing. Hum Reprod Update. 2005;11:261–276.
[5] Hansen KR, Knowlton NS, Thyer AC, et al. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. Hum Reprod. 2008;23:699–708.
[6] Wallace WH, Kelsey TW. Human Ovarian reserve from conception to the menopause. Plos One. 2009;51: e8772.
[7] Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev. 1996;17:121.
[8] Pelosi E, Simonsick E, Forabosco A, et al. Dynamics of the Ovarian reserve and impact of genetic and epidemiological factors on age of menopause. Biol Reprod. 2015;92:22–22.
[9] Ge ZJ, Schatten H, Zhang CL, et al. Oocyte ageing and epigenetics. Reproduction. 2015;149:R103.
[10] Nelson SM, Telfer EE, Anderson RA. The ageing ovary and uterus: new biological insights. Hum Reprod Update. 2013;19:67–83.
[11] Gelety TJ, Pearlstone AC, Surrey ES. Short-term endocrine response to gonadotropin-releasing hormone agonist initiated in the early follicular, midluteal, or late luteal phase in normally cycling women. Fertil Steril. 1995;64:1074–1080.
[12] Mascarenhas M, Kamath MS, Chandy A, et al. Progesterone/Estradiol ratio as a predictor in the ART cycles with premature progesterone elevation on the day of hCG trigger. J Reprod Infertil. 2015; 16:155–161.
[13] Randolph JF Jr, Harlow SD, Helmuth ME, et al. Updated assays for inhibin B and AMH provide evidence for regular episodic secretion of inhibin B but not AMH in the follicular phase of the normal menstrual cycle. Hum Reprod. 2014;29:592–600.
[14] Mori T. Regulatory principles of follicular development. In: Allahbadia G, Morimoto Y, editors. Ovarian stimulation protocols. Vol. 6. New Delhi: Springer; 2016. p. 12–14.
[15] Droesch K, Muasher SJ, Brzyski RG, et al. Value of suppression with a gonadotropin-releasing hormone agonist prior to gonadotropin stimulation for in vitro fertilization. Fertil Steril. 1989;51:292–297.
[16] Miura R, Haneda S, Lee HH, et al. Evidence that the dominant follicle of the first wave is more active than that of the second wave in terms of its growth rate, blood flow supply and steroidogenic capacity in cows. Anim Reprod Sci. 2014;145:114–122.
[17] Lamazou F, Fuchs F, Grynberg M, et al. Cancellation of IVF-ET cycles: poor prognosis, poor responder, or variability of the response to controlled ovarian hyper stimulation? An analysis of 142 cancellations. Gynecol Obstet Biol Reprod. 2012;41:41–47.
[18] Kuang Y, Hong Q, Chen Q, et al. Luteal-phase ovarian stimulation is feasible for producing competent oocytes in women undergoing in vitro fertilization/ intracytoplasmic sperm injection treatment, with optimal pregnancy outcomes in frozen-thawed embryo transfer cycles. Fertil Steril. 2014;101:105–111.
[19] Durnerin CI, Erb K, Fleming R, et al. Effects of recombinant LH treatment on folliculogenesis and responsiveness to FSH stimulation. Hum Reprod. 2008;23: 421–426.
[20] Maman E, Meirow D, Brengauz M, et al. Luteal phase oocyte retrieval and in vitro maturation is an optional procedure for urgent fertility preservation. Fertil Steril. 2011;95:64–67.