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.

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...
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 Bakeman 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 (rFSH) 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/m²) | 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.

| 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 |
| 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.

| 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 | -0.330 | -3.04 | -2.015 | -1.456 |
| P-value | 0.135 | 0.050 | 0.001 | 0.011 | 0.028 | 0.041 |

All data are represented as mean ± standard deviation or percentage.

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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**

No potential conflict of interest was reported by the authors.

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