International Journal of Gynecology and Obstetrics xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Gynecology and Obstetrics

journal homepage: www.elsevier.com/locate/ijgo

www.figo.org

CLINICAL ARTICLE

The effects of growth hormone on clinical outcomes after frozen–thawed embryo transfer

Wang Xue-mei, Jiang Hong *, Zhang Wen-xiang, Li Yang

Reproductive Medicine Centre, 105th Hospital of People's Liberation Army, Hefei, China

ARTICLE INFO

Article history:
Received 4 July 2015
Received in revised form 20 October 2015
Accepted 25 February 2016

Keywords:
Clinical outcomes
Endometrial receptivity
Frozen–thawed embryo transfer
Growth hormone
Hormone-replacement therapy

ABSTRACT

Objective: To evaluate the effects of recombinant human growth hormone (rhGH) on clinical outcomes of frozen–thawed embryo transfer (FET). *Methods:* A prospective study was conducted among 240 patients (aged ≤38 years) who underwent FET cycles at a center in Hefei, China, between November 2011 and October 2012. Patients were divided into three groups on the basis of visit order: those in group A received hormone-replacement therapy (HRT) for endometrial preparation, those in group B received HRT plus simultaneous rhGH, and those in group C received rhGH on day 8 of HRT. *Results:* Ten cycles were cancelled; 230 FET cycles were analyzed (77 in group A, 77 in group B, 76 in group C). The rates of clinical pregnancy, embryo implantation, and live birth were significantly higher in group B than in group A, as were the serum levels of estradiol and insulin-like growth factor-1 ($P \leq 0.033$ for all comparisons). Endometrial thickness and serum levels of vascular endothelial growth factor were significantly higher in group B than in groups A and C, whereas pulsatility index, resistance index, and peak systolic velocity/end diastolic velocity of the uterine arcuate artery were significantly lower ($P \leq 0.017$ for all comparisons). *Conclusion:* Simultaneous administration of rhGH with HRT could improve clinical outcomes after FET by increasing endometrial blood perfusion and expression of cytokines related to endometrial receptivity.

1. Introduction

In the past three decades, frozen–thawed embryo transfer (FET) has become a widely used and cost-effective adjunct to in vitro fertilization (IVF) and embryo transfer. FET maximizes embryo utilization rates and increases cumulative pregnancy rates, effectively preventing complications such as ovarian hyperstimulation syndrome [1]. Furthermore, FET is a less invasive procedure than IVF and embryo transfer, and reduces financial costs for patients. Protocols for FET are simpler than are those for cycles of IVF and embryo transfer during controlled ovarian stimulation, with the primary aim limited to adequate preparation of the endometrium to receive the thawed embryo. However, although pregnancy rates following FET have gradually increased with time, the results remain varied and unsatisfactory [2,3]. To improve the outcomes of FET, efforts have been made to optimize the freezing techniques and preparation of the endometrium.

Growth hormone (GH) is a peptide hormone secreted by the anterior pituitary gland in a pulsatile manner; it has important roles in cell growth and metabolism throughout the body. The GH receptor is present in the cumulus cells and oocytes of several species, including human beings [4]. The addition of GH during ovulation induction could optimize the clinical pregnancy rate of IVF and embryo transfer by increasing the number of oocytes retrieved and improving the quality of both eggs and embryos [5–7]. The activity of GH might also influence luteal function, either directly or indirectly, via insulin-like growth factor-1 (IGF-1) [8]. By contrast, its effects on endometrial receptivity and implantation remain inconclusive. In fresh cycles of IVF and embryo transfer, clinical outcomes are affected by many factors, such as oocyte quality, the protocol used for ovulation induction, and embryo quality [9]. Other than age and embryo factors, endometrial receptivity and synchronization between endometrial and embryonic development could be the crucial factors affecting embryo implantation in FET cycles. Whether administration of GH could improve endometrial receptivity is uncertain, with the available data limited to a small number of animal studies [10].

The aim of the present study was to evaluate the effects of recombinant human GH (rhGH) on clinical outcomes after FET.

2. Materials and methods

A prospective study was conducted among women who underwent FET cycles at the Reproductive Medicine Centre of the 105th Hospital of People's Liberation Army, Hefei, China, between November 1, 2011, and October 31, 2012. Inclusion criteria were age 38 years or younger, freezing of whole embryos in the fresh cycle or usable surplus embryos after fresh transfer, FET performed at least two menstrual periods after oocyte retrieval, receipt of hormone-replacement therapy (HRT) for endometrial preparation, embryos frozen by vitrification within the

* Corresponding author at: Reproductive Medicine Centre, the 105th Hospital of People's Liberation Army, 424 West Changjiang Road, Hefei 230031, Anhui, China. Tel.: +86 551 65966361; fax: +86 551 5132818.
E-mail address: jiangh105@sina.com (J. Hong).

http://dx.doi.org/10.1016/j.ijgo.2015.10.020
0020-7292/

previous 2 years, and at least two embryos frozen per patient. Exclusion criteria were congenital or acquired uterine malformations, endometrial polyps and submucosal fibroids, intrauterine adhesion, severe endometriosis or adenomyosis, and systemic diseases, such as diabetes mellitus or abnormalities of blood clotting. The protocol was approved by the ethics committee of the study center; all patients provided written informed consent.

The participants were divided into three groups according to the visit order. Patients assigned to group A received HRT only for endometrial preparation, which was initiated on the third day of menstruation by administration of oral estradiol valerate at a daily dose of 4–10 mg, with the amount modified according to the thickness and morphology of the endometrium. When endometrial thickness reached at least 7 mm under continuous ultrasonographic observation, 40 mg progesterone was administrated intramuscularly once daily for 3 days. Patients assigned to group B received 4 IU of rhGH daily by subcutaneous injection that commenced simultaneously with HRT from day 3 of the menstrual cycle until the day of progesterone injection. Patients assigned to group C received 4 IU of rhGH daily from day 8 of HRT until the day of progesterone injection.

Embryos were classified as grade I (equal size of blastomeres, free of fragmentation), grade II (unequal size of blastomere, fragmentations <20%), grade III (unequal size of blastomere, fragmentations 20%–50%), and grade IV (unequal size of blastomere, fragmentations >50%). Grade I–III embryos that comprised at least six cells on day 3 of oocyte retrieval were defined as usable; two or three usable embryos were transferred in fresh cycles, with the surplus frozen by vitrification and used in any subsequent FET.

Embryos were thawed on day 3 of progesterone injection and their quality reassessed. Survival was defined as the retention of at least 50% of intact cells; embryos comprising at least six cells and with less than 20% intracellular fragmentation were defined as good quality. Usable thawed embryos were cultured for 2 hours, and then transferred into the uterine cavity under ultrasonographic guidance (2–3 embryos per procedure).

All patients received 40 mg progesterone daily after FET, administered intramuscularly, in addition to the original dose of estradiol valerate. Ultrasonography was performed on days 30–35 after FET, and clinical pregnancy defined as the presence of a gestational sac, with or without a fetal heartbeat. Luteal support with estradiol valerate and progesterone was continued until 10 weeks of pregnancy. Embryonic developmental arrest or spontaneous abortion at less than 12 weeks of pregnancy was defined as early abortion.

Blood samples were collected at room temperature on the day of embryo transfer. The fresh serum samples were used to measure the levels of progesterone and estradiol (E2) by chemiluminescence (BHP9507, Bio-Ekon Biotechnology, Beijing, China). The remaining samples were centrifuged at 3000 *g* for 10 minutes, the supernatant divided into aliquots, and stored at −70°C for subsequent measurement of vascular endothelial growth factor (VEGF) and IGF-1. The levels of VEGF and IGF-1 were evaluated using commercially available enzyme-linked immumosorbent assay kits (R&D Biological Engineering, Shanghai, China) within 6 months of sample collection.

Endometrial pattern and thickness, pulsatility index (PI), resistance index (RI), and peak systolic velocity/end diastolic velocity (S/D) of the uterine arcuate artery were detected by color Doppler ultrasonography (Logiq 5 Pro, GE Healthcare, Gyeonggi, South Korea) with a 6–10-MHz multifrequency transvaginal probe by W.X-m. on the day of progesterone administration. Endometrial thickness was measured as the maximum distance between the myometrial and endometrial interfaces in the central longitudinal axis of the uterus [11]. Blood flow velocity waveforms of the uterine arcuate artery were obtained when abundant color signals were obtained from the middle of the myometrial region. A color Doppler window was then positioned in the thickest area of the endometrium and the highest color intensity identified from the endometrial–subendometrial area. The PI, RI, and S/D were measured when at least five consecutive stable waveforms were obtained.

The present study was designed to have sufficient power to detect an absolute difference of 20% in the clinical pregnancy rate. Thus, approximately 80 cycles were required in each of the three groups to detect a difference of 20% with 80% power and a 5% significance level.

The data were analyzed using SPSS version 16.0 (SPSS Inc, Chicago, IL, USA). Comparisons were performed using one-way analysis of variance, the χ^2 test, or the Fisher exact test, as appropriate. $P<0.05$ was considered statistically significant.

3. Results

A total of 240 women were included. Ten cycles were cancelled because of a thin endometrium (<7 mm), monilial vaginitis, or poor embryo quality after thawing. Consequently, a total of 230 FET cycles were analyzed in the present study: 77 in group A, 77 in group B, and 76 in group C.

Table 1 summarizes the characteristics and clinical outcomes of the present study cohort. The rates of clinical pregnancy, embryo implantation, and live birth per FET were significantly higher in group B than in

Table 1
Patient characteristics and clinical outcomes.[a]

Variable	Group A (HRT)	Group B (HRT + simultaneous rhGH)	Group C (HRT + rhGH on day 8)	*P* value
Transferred cycles	77	77	76	NA
Age, y	30.3 ± 4.1	31.3 ± 5.0	30.7 ± 4.3	0.430 [b,c]
Body mass index [d]	21.3 ± 2.6	21.3 ± 2.2	21.5 ± 2.6	0.861 [b,c]
Duration of infertility, y	5.0 ± 2.1	5.1 ± 2.1	4.9 ± 2.0	0.857 [b,c]
Estradiol dose, mg	70.8 ± 16.4	69.4 ± 10.2	71.1 ± 13.7	0.706 [b,c]
Duration of estradiol treatment, d	11.7 ± 1.8	11.4 ± 1.5	11.6 ± 1.3	0.505 [b,c]
No. of embryos thawed per cycle	2.9 ± 0.7	2.9 ± 0.5	3.0 ± 0.5	0.699 [b,c]
No. of embryos survived	2.8 ± 0.5	2.8 ± 0.5	2.8 ± 0.4	0.953 [b,c]
No. of good-quality embryos	2.5 ± 0.7	2.5 ± 0.6	2.5 ± 0.6	0.964 [b,c]
No. of embryos transferred	2.7 ± 0.5	2.7 ± 0.5	2.8 ± 0.4	0.713 [b,c]
Clinical pregnancy	25/77 (32.5)	38/77 (49.4)	30/76 (39.5)	0.033 [e,f]
Implantation	30/210 (14.3)	47/207 (22.7)	36/209 (17.2)	0.027 [e,f]
Early abortion	4/25 (16.0)	5/38 (13.2)	4/30 (13.3)	0.943 [c,e]
Live birth	19/77 (24.7)	32/77 (41.6)	26/76 (34.2)	0.026 [e,f]

Abbreviations: HRT, hormone-replacement therapy; rhGH, recombinant human growth hormone; NA, not applicable.
[a] Values are given as number, mean ± SD, or number/total number (percentage), unless indicated otherwise.
[b] One-way analysis of variance.
[c] Comparison of all three groups.
[d] Calculated as weight in kilograms divided by the square of height in meters.
[e] χ^2 test.
[f] Group B vs group A; other between-group comparisons not significant.

Please cite this article as: Xue-mei W, et al, The effects of growth hormone on clinical outcomes after frozen–thawed embryo transfer, Int J Gynecol Obstet (2016), http://dx.doi.org/10.1016/j.ijgo.2015.10.020