Comparison of Human Menopausal Gonadotrophin and Recombinant Follicle-Stimulating Hormone in In-Vitro Fertilisation and Pregnancy Outcome

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Abstract : **The study was carried out to compare the effectiveness of human menopausal gonadotrophin (HMG) and recombinant follicle-stimulating hormone (recombinant FSH) in term of in vitro fertilisation (IVF) and pregnancy outcome. A total of 238 patients who underwent IVF for infertility treatment were included in the study. The first attempt of controlled ovarian stimulation was recorded and evaluated. A long protocol of ovarian stimulation was performed with gonadotrophin releasing hormone analogue (GnRH-a) administration. Gonadotrophin, which was either HMG (group A) or recombinant FSH (group B), was administrated to each patient for ovarian stimulation. The results of this study showed no difference in the number of stimulation days, fertilised oocytes, transferred embryos and cycles with embryos available for freezing between the two groups. Although the starting doses of both gonadotrophins were similar, the total dosage of HMG was higher than that of recombinant FSH (48.8 ± 20.8 versus 42.9 ± 20.0, p = 0.03). The number of retrieved oocytes in group A was higher than that in group B (9.5 ± 4.4 versus 8.3 ± 4.3, p = 0.04). The differences in cancellation rate, fertilisation rate, pregnancy rate per cycle and per transfer, as well as implantation rate between the two groups were not statistically significant. In conclusion, patients who underwent ovarian stimulation with GnRH-a long down-regulation still benefit for HMG for their treatment. We did not find any difference in fertilisation rate or pregnancy rate as well as implantation rate between HMG and recombinant FSH. A greater number of oocytes were retrieved in patients treated with HMG. However, more ampoules of HMG were administrated to achieve ovarian stimulation, compared with recombinant FSH.**

Key words : in vitro fertilisation, HMG, recombinant FSH

เรื่องย่อ : **เปรียบเทียบประสิทธิผลของการปฏิสนธินอกร่างกายและการตั้งครรภ์ระหว่างการใช้ฮิวแมนเมโนพอสซอลโกนาโดโทรฟินและรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมน**

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การศึกษาเปรียบเทียบประสิทธิผลของการปฏิสนธินอกร่างกายและการตั้งครรภ์ระหว่างการใช้ฮิวแมนเมโนพอสซอลโกนาโดโทรฟินและรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมนในการกระตุ้นรังไข่ในผู้ป่วยที่มารับการรักษาภาวะมีบุตรยากจำนวน 238 คน ได้นำผลของการกระตุ้นรังไข่ในการรักษาเฉพาะในครั้งแรกของผู้ป่วยแต่ละคนเท่านั้นมาศึกษาผู้ป่วยทุกคนได้รับฮอร์โมนโกนาโดโทรฟินรีลีสชิงอนาลอช เพื่อเตรียมพร้อมสำหรับการกระตุ้นรังไข่ และได้รับโกนาโดโทรฟินชนิดฮิวแมนเมโนพอสซอลโกนาโดโทรฟิน (กลุ่ม 1) หรือรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมน (กลุ่ม 2) เพื่อกระตุ้นรังไข่ ผลการศึกษาไม่พบความแตกต่างระหว่างจำนวนวันของการกระตุ้นรังไข่ จำนวนไข่ที่ได้รับการปฏิสนธิ จำนวนตัวอ่อนที่ได้รับการย้ายเข้าสู่โพรงมดลูก และจำนวนรอบการรักษาที่มีตัวอ่อนเหลือสำหรับการแช่แข็งระหว่างสองกลุ่ม แม้ว่าขนาดเริ่มต้นของฮอร์โมนทั้งสองชนิดที่ใช้ในการกระตุ้นรังไข่มีขนาดใกล้เคียงกัน พบว่าขนาดของฮิวแมนเมโนพอสซอลโกนาโดโทรฟินที่ใช้ในการกระตุ้นสูงกว่ารีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมนอย่างมีนัยสำคัญทางสถิติ (48.8 ± 20.8 ต่อ 42.9 ± 20.0, p = 0.03) จำนวนของไข่ที่ได้จากกลุ่ม 1 มากกว่ากลุ่ม 2 อย่างมีนัยสำคัญทางสถิติ (9.5 ± 4.4 ต่อ 8.3 ± 4.3, p = 0.04) ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของอัตราการล้มเลิกการกระตุ้นรังไข่ อัตราการปฏิสนธิของไข่ อัตราการตั้งครรภ์ต่อการรักษาและต่อการย้ายตัวอ่อนเข้าสู่โพรงมดลูก และอัตราการฝังตัวของตัวอ่อนระหว่างทั้งสองกลุ่ม ดังนั้นผู้ป่วยภาวะมีบุตรยากยังคงได้รับประโยชน์จากการใช้ฮิวแมนเมโนพอสซอลโกนาโดโทรฟินในการกระตุ้นรังไข่ ไม่พบความแตกต่างของอัตราการปฏิสนธิของไข่ อัตราการตั้งครรภ์และอัตราการฝังตัวของตัวอ่อนในโพรงมดลูกระหว่างฮิวแมนเมโนพอสซอลโกนาโดโทรฟินและรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมนในการกระตุ้นรังไข่ จำนวนไข่ที่ได้จากการกระตุ้นรังไข่และขนาดของฮอร์โมนที่ใช้ในฮิวแมนเมโนพอสซอลโกนาโดโทรฟินสูงกว่าของรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมนอย่างมีนัยสำคัญทางสถิติ

INTRODUCTION

Several types of gonadotrophins have been introduced into infertility treatment to improve the result of assisted reproductive technology (ART). Both human menopausal gonadotrophin (HMG) and follicle-stimulating hormone (FSH) have been proven to be safe and effective in ovarian stimulation for ART.[1-3] Consequently, the highly purified FSH preparation (HP-FSH), which offers more specific FSH activity than HMG, has been introduced to prevent levels of LH that are too high and that may adversely affect fertilisation and embryo quality.[4-6]

Recombinant FSH has recently been developed using recombinant DNA technology. This product has been accepted for its high batch-to-batch consistency, greater purity and lack of LH activity, compared with previous gonadotrophin preparations.[7-9] Many studies have been performed to ensure the effectiveness of recombinant FSH and to compare this product with both highly purified FSH and HMG. Some studies have reported that recombinant FSH produces a higher pregnancy rate, compared with the HP-FSH.[10,11] In contrast, opposite results have been reported by others.[12] Therefore, this study was carried out to compare the effectiveness of HMG and recombinant FSH in term of in vitro fertilisation and pregnancy outcome in our population.

MATERIALS AND METHODS

A total of 283 patients who underwent ovarian stimulation for infertility treatment were included in this study. Their mean age was 33.9 years. The causes of their infertility were male factor (47.5%), tubal factor (21.0%), combined male and tubal factor (2.9%), ovulatory factor (10.1%), and unexplained (18.5%). Only the first ovarian stimulation cycle of each patient was included in this study.

100 µg of gonadotrophin-releasing hormone analogue (GnRH-a), which was delivered into each nostril every 6 hours, was administrated to each patient for long hypothalamopituitary down-regulation. When the long down-regulation had occurred completely, either HMG or recombinant FSH was used for ovarian stimulation. The starting doses of both gonadotrophins ranged from 3 to 6 ampoules, depending on age and basal FSH and day 2 oestradiol levels for each patient. The dose of gonadotrophin administrated each day was subsequently adjusted, depending on follicular growth monitored by serial transvaginal ultrasonography. If the ovaries did not respond to the stimulation, the treatment was eventually cancelled.

When at least 3 follicles reached a diameter of 18 mm, 10,000 IU of human chorionic gonadotrophin (hCG) was administrated to stimulate ovulation. Thirty-six hours after hCG administration, oocyte retrieval was performed under transvaginal ultrasonographic guidance. All retrieved oocytes were cultured in the IVF culture system. Intracytoplasmic sperm injection (ICSI) was only performed in cases with severe male infertility factors. The occurrence of fertilisation was determined when two pronuclei were identified in the inseminated oocyte. The embryos were transferred 48-72 hours later. All patients were given by progesterone preparations to provide luteal support. A quantitative serum β-hCG was checked 14 days after embryo transfer. Transvaginal ultrasonography for fetal viability was performed 10 days after a positive serum β-hCG. We defined a positive result of a quantitative measurement of serum β-hCG with a proven gestation sac and fetal heart activity as a clinical pregnancy. The implantation rate was calculated from the number of gestation sacs divided by the number of transferred embryos.

Age, basal FSH and day 2 oestradiol levels, number of ampoules, stimulation days, retrieved oocytes, fertilised oocytes, transferred embryos, cycles with embryos available for freezing, fertilisation rate, cancellation rate and pregnancy rate as well as implantation rate were recorded. Statistical analysis was performed using the epi-info programme (version 6.0). Student's t-test and χ^2 were used for analysis. Statistical significance was defined at a p-value of < 0.05.

RESULTS

One hundred and forty four out of 238 cycles (60.5%) were stimulated using HMG, whereas 94 of 238 (39.5%) were stimulated using recombinant FSH. No premature ovulation was detected in any cycles in this study. The comparison between HMG and recombinant FSH in terms of IVF and pregnancy outcome is demonstrated in Table 1. The mean age of the HMG and recombinant FSH groups were 33.7 and 34.1 years, respectively. This difference was not statistically significant. The mean basal FSH level and day 2 oestradiol level in the HMG group were 8.0 IU/L and 188.9 pmol/L, respectively. Meanwhile, the mean basal FSH level and day 2 oestradiol level in the recombinant FSH group were 8.6 IU/L and 180.3 pmol/L, respectively. This difference was also not statistically significant. The cancellation rates for both groups were similar, 11.8% (17/144) in the HMG group and 10.6% (10/94) in the recombinant FSH group. The difference between the pregnancy rate of the HMG (25.7%, 37/144) and the recombinant FSH group (17.0%, 16/94) was also not statistically significant.

The IVF and pregnancy outcome of the 211 cycles that achieved ovarian stimulation are shown in Table 2. The number of ampoules of recombinant FSH used for the stimulation was significantly lower than that of HMG (42.9 ± 20.0 versus 48.8 ± 20.8). The number of stimulation days needed in the two groups was, however, similar, 11.1 ± 1.3 in the HMG group and 11.1 ± 1.0 in the recombinant FSH group. The number of retrieved oocytes in the HMG group (9.5 ± 4.4) was significantly higher than that of the recombinant FSH (8.3 ± 4.3; $p = 0.04$). The number of fertilised oocytes in the HMG group (5.9 ± 3.5) was also higher than that of the recombinant FSH group (5.4 ± 3.2). However, this difference was not statistically significant. The fertilisation rates of both groups were also not significantly different, 61.1 in the HMG group and 66.5 in the recombinant FSH. Since the number of transferred embryos in the HMG group (2.5 ± 0.9) was similar to that in the recombinant

Table 1. Clinical characteristics and outcome of ovarian stimulation comparing the HMG and recombinant FSH groups (238 cycles)

Variable	Gonadotrophin		P-value
	HMG	Recombinant FSH	
No. of cycles	144	94	-
Age (years)	33.7 ± 4.4	34.1 ± 4.1	NS
Cycle day 2 FSH level (IU/L)ϕ	8.0 ± 3.0	8.6 ± 2.6	NS
Cycle day 2 oestradiol level (pmol/L)ϕ	188.9 ± 85.8	180.3 ± 55.2	NS
Cancellation rate (%)	2.7 (4/144)	4.2 (4/94)	NS
Pregnancy rate per cycle (%)	25.7 (37/144)	17.0 (16/94)	NS

ϕ Values are mean ± S.D.
NS Non-significant

FSH group (2.5 ± 0.8); the number of cycles in which embryos were available for freezing was higher in the HMG group (9.4%, 12/127) than in the recombinant FSH (5.9%, 5/84). However, the difference was not statistically significant. The pregnancy and implantation rates of the HMG group (27.5 and 12.4%) were not significantly different from those of the recombinant FSH group (17.8 and 11.2%).

Table 2. IVF and pregnancy outcome of IVF-ET of the HMG and recombinant FSH groups (211 cycles).

Variable	Gonadotrophin		P-value
	HMG	Recombinant FSH	
Numbers of ampoulesϕ	48.8 ± 20.8	42.9 ± 20.0	0.03
Numbers of stimulation daysϕ	11.1 ± 1.3	11.1 ± 1.0	NS
Numbers of retrieved oocytesϕ	9.5 ± 4.4	8.3 ± 4.3	0.04
Numbers of fertilised oocytesϕ	5.9 ± 3.5	5.4 ± 3.2	NS
Numbers of transferred embryosϕ	2.5 ± 0.9	2.5 ± 0.8	NS
Fertilisation rate (%)ϕ	61.1 ± 27.8	66.5 ± 24.3	NS
Pregnancy rate per transfer (%)	27.5 (35/127)	17.8 (15/84)	NS
Implantation rate (%)	12.4 (41/330)	11.2 (24/214)	NS
Numbers of cycles with embryos available for freezing (%)	9.4 (12/127)	5.9 (5/84)	NS

ϕ Values are mean ± S.D.
NS Non-significant

DISCUSSION

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are secreted by the pituitary gland, have been found to be responsible for the steroidogenic function of follicular growth. A low level of LH during the follicular phase stimulates theca cells to produce androgen, which is the initial substrate for oestrogen production. Granulosa cells converse this androgen to oestrogen after being trigged by FSH.[13] In assisted reproductive technology, human menopausal gonadotrophin (HMG), which contains both FSH and LH, has been usually administrated to patients to provide ovarian stimulation. Although LH takes the important role in the maturation of oocyte and ovulation, too high an LH level during the follicular and periovulatory phase may adversely affect fertilisation, cleavage and embryo quality.[4,5] A small amount of LH has been proposed to be enough for oestradiol biosynthesis in folliculogenesis.[14] Highly purified FSH (HP-FSH) was introduced to decrease LH activity and to remove unwanted protein, which was normally suspended in the former gonadotrophin preparation. This preparation offers a higher specific activity of FSH.[6] Recently, recombinant FSH has been developed using a recombinant DNA technique. A Chinese hamster ovary cell line was transfected with genes encoding human FSH.[7-9] The first report of a pregnancy and birth of a healthy baby after ovarian stimulation with this product has been reported.[15,16]

Several studies comparing recombinant FSH and other preparations have been carried out. Out, et al reported a greater number of retrieved oocytes and a smaller total administration dosage was found in the recombinant FSH group compared with the HP-FSH group. However, the difference in ongoing pregnancy rate was not statistically significant.[17] A significantly higher pregnancy rate using recombinant FSH was also found, compared with HMG and HP-FSH.[18] Daya et al[19] studied a meta-analysis and found that the pregnancy rate using recombinant FSH was significantly higher than that of HP-FSH. Raga et al[11] studied 30 poor responders and found that there were fewer total doses and stimulation days in the recombinant FSH group, compared with the HP-FSH group. Moreover, a higher number of oocytes and a larger pregnancy rate as well as implantation rate were found in the recombinant FSH group. However, some studies have reported opposite results. Jacob et al[12] compared the clinical results during cross-over from HMG to recombinant FSH in 353 consecutive IVF/ICSI treatment cycles and found the clinical pregnancy rate were 14% per cycle with recombinant FSH and 20% per cycle with HMG. Therefore, we performed this study to compare IVF and pregnancy outcome between the cycle with recombinant FSH treatment and HMG treatment in our population. Only the first attempt of ovarian stimulation in each patient was included in the study to prevent any bias that may occur from either multiple gonadotrophins in the same patient or the different numbers of attempts.

From the results of study, the characteristics of both HMG and recombinant FSH groups were similar (Table 1). We could not find any differences in cancellation rate and pregnancy rate per cycle between the groups. Further study in only IVF-ET cycles (n=211) found that the number of retrieved oocytes in the HMG group (9.5 ± 4.4) was significantly higher than that in the recombinant FSH group (8.3 ± 4.3). This result supports the study of Jacob et al[12], which reported that more follicles were aspirated and oocytes retrieved in the HMG group compared with the recombinant FSH group. It was noted in this study that more ampoules of HMG (48.8 ± 20.8) were used to achieve the stimulation, compared with the recombinant FSH group (42.9 ± 20.0). This result contradicts the study of Jacob et al that patients treated with recombinant FSH received higher doses than those treated with HMG.[12]

The numbers of fertilised oocytes and fertilisation rate were similar between the two groups (Table 2). With regard to our unit policy, no more than 3 embryos were transferred in each treatment cycle. This might result in a similar pregnancy rate between two groups. However, the pregnancy rate seemed to be in favour of the HMG group (27.5% versus 17.8%). Therefore, in our population, recombinant FSH could not significantly demonstrate a greater effectiveness compared with HMG. The lack of exogenous LH in the recombinant FSH did not improve the quality of embryo so that a difference in fertilisation capability, pregnancy rate and implantation rate could be found.

In conclusion, in our patient population, patients who underwent ovarian stimulation still benefit from using HMG for ovarian stimulation. Although some studies have suggested that the exogenous LH from HMG might adversely affect the quality of embryo and pregnancy outcome, we did not find any difference between recombinant FSH and HMG in fertilisation rate and pregnancy rate as well as implantation rate. Patients with diminished ovarian reserve, however, might gain more benefit in improving the quality of their embryos than in "normal" infertile patients. Therefore, further comparative studies between recombinant FSH and HMG in poorly responding patients would be useful and would provide more information for the treatment of such patients.

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