Comparison of Effectiveness between Recombinant Follicle Stimulating Hormone and Human Menopausal Gonadotrophin Administration in those with Diminished Ovarian Reserve

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Abstract : **The study was carried out to compare the effectiveness of recombinant follicle stimulating hormone (recombinant FSH) and human menopausal gonadotrophin (HMG) in those with diminished ovarian reserve. A total of 106 ovarian stimulation cycles from 88 poor responders were included in this study. Either recombinant FHS or HMG was administered in order to stimulate the ovary for each cycle. The pregnancy rate of the recombinant FSH group (22.5%) was higher than that of the HMG group (9.1%). The cancellation rate of the recombinant FSH group (10.0%) was lower than that of the HMG group (19.7%). In in vitro fertilisation-embryo transfer (IVF-ET) cycles, the fertilisation rate of the recombinant FSH group (62.8%) was higher than that of the HMG group (51.8%). The pregnancy rate and the implantation rate of the recombinant FSH group (23.0 and 9.1%, respectively) were higher than those of the HMG group (13.6 and 5.9%, respectively). Although this did not achieve statistical significance, only the recombinant FSH group achieved pregnancies using gamete intrafallopian transfer (GIFT). In conclusion, recombinant FSH is probably more effective than HMG in improving the IVF and pregnancy rate in poor responders.**

Key words: Human menopausal gonadotrophin, recombinant follicle stimulating hormone, poor responder

เรื่องย่อ : **เปรียบเทียบประสิทธิผลระหว่างการใช้ฮิวแมนเมโนพอสซอลโกนาโดโทรฟินและรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมนในผู้ป่วยรังไข่ตอบสนองต่อการกระตุ้นลดลง**

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การศึกษาเปรียบเทียบประสิทธิผลระหว่างการใช้ฮิวแมนเมโนพอสซอลโกนาโดโทรฟินและรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมนในผู้ป่วยรังไข่ตอบสนองต่อการกระตุ้นลดลง ได้ทำการศึกษาในรอบการกระตุ้นรังไข่จำนวน 106 รอบจากผู้ป่วยรังไข่ตอบสนองต่อการกระตุ้นลดลงจำนวน 88 ราย ใช้ฮิวแมนเมโนพอสซอลโกนาโดโทรฟินหรือรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมนในการกระตุ้นรังไข่ของแต่ละรอบการรักษา ผลการศึกษาพบว่า อัตราการตั้งครรภ์ของกลุ่มรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมน (ร้อยละ 22.5) สูงกว่ากลุ่มฮิวแมนเมโนพอสซอลโกนาโดโทรฟิน (ร้อยละ 9.1) อัตราการยกเลิกการกระตุ้นรังไข่ของกลุ่มรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมน (ร้อยละ 10.0) ต่ำกว่าของกลุ่มฮิวแมนเมโนพอสซอลโกนาโดโทรฟิน (ร้อยละ 19.7) ในรอบการรักษาด้วยการปฏิสนธินอกร่างกายและย้ายตัวอ่อนสู่โพรงมดลูกพบว่า อัตราการปฏิสนธิของกลุ่มรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมน (ร้อยละ 62.8) สูงกว่าของกลุ่มฮิวแมนเมโนพอสซอลโกนาโดโทรฟิน (ร้อยละ 51.8) อัตราการตั้งครรภ์และการฝังตัวของตัวอ่อนของกลุ่มรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมน (ร้อยละ 23.0 และ 9.1 ตามลำดับ) สูงกว่ากลุ่มฮิวแมนเมโนพอสซอลโกนาโดโทรฟิน (ร้อยละ 13.6 และ 5.9 ตามลำดับ) เฉพาะกลุ่มรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมนเท่านั้นที่สามารถตั้งครรภ์ได้จากวิธีนำเซลล์สืบพันธุ์มาใส่รวมในท่อนำไข่ สรุปรีคอมบิแนนท์ฟอลลิเคิลสติมูเลติงฮอร์โมนอาจมีประสิทธิภาพดีกว่าฮิวแมนเมโนพอสซอลโกนาโดโทรฟินในการปรับปรุงอัตราการปฏิสนธินอกร่างกายและอัตราการตั้งครรภ์ให้สูงขึ้นในผู้ป่วยรังไข่ตอบสนองต่อการกระตุ้นลดลง

INTRODUCTION

In controlled ovarian stimulation (COH) of assisted reproductive technology (ART), patients with diminished ovarian reserve, the so called "poor responders", produce a smaller number of oocytes and thus have lower fertilisation rates, pregnancy rates and implantation rates, compared with "normal" infertile patients.[1] To achieve pregnancy in such patients has been accepted as a crucial challenge in ART. A variety of hormonal parameters have been introduced for predicting ovarian reserve in infertile women. The results of these hormonal tests help clinicians make an appropriate decision about an ovarian stimulation regimen. The basal follicle stimulating hormone (FSH) and oestradiol levels have been suggested as useful predictors of ovarian reserve and pregnancy outcome.[2-4] Recently, the gonadotrophin-releasing hormone agonist (GnRH-a) test has been reported as an accurate predictor of ovarian reserve.[5] The patient whose day 2 FSH level more than 9.5 IU/L and/or the difference in oestradiol levels from day 3 to day 2 less than 180 pmol/L has been diagnosed as a poor responder by the GnRH-a test.

In 1990, using recombinant DNA technology, recombinant FHS (recombinant FSH) was developed for use in ovarian stimulation in ART.[6,7] Many studies have been performed to investigate the effectiveness of recombinant FSH and to compare this product with other forms of gonadotrophins used in infertile patients.[8-10] To our knowledge, there has been no study comparing the effectiveness of recombinant FSH and human menopausal gonadotrophin (HMG) in those with diminished ovarian reserve. Therefore, the purpose of this study was to compare the effectiveness of recombinant FSH and HMG in terms of in-vitro fertilisation (IVF) and pregnancy outcome in the diminished ovarian reserve diagnosed by GnRH-a test.

MATERIALS AND METHODS

A total of 106 ovarian stimulation cycles from 88 patients undergoing ovarian stimulation for infertility treatment at the Assisted Conception Unit, University College Hospital, were included in this study. All patients were diagnosed as poor responders by the GnRH-a test.[5] GnRH-a (as buserelin nasal

spray) was administrated to each patient at the midluteal phase of the previous cycle and was discontinued on the first day of menstruation. Either HMG or recombinant FSH was started for ovarian stimulation on the first day of menstruation in each patient with randomization. Follicular growth was monitored by transvaginal ultrasonography. The dosage of gonadotrophin was subsequently adjusted, depending on the follicular growth monitored by ultrasonography.

When at least 3 leading follicles reached a diameter of 18 mm, 10,000 IU of human chorionic gonadotrophin (hCG) was administered to induce ovulation. Oocyte retrieval was performed 36 hours after the hCG administration under transvaginal ultrasonographic guidance. Intracytoplasmic sperm injection (ICSI) was performed in cases with severe male infertility. The occurrence of two pronuclei in the oocyte was defined as fertilisation. Embryo transfer was performed 48-72 hours later. Nineteen patients underwent gamete intrafallopian transfer (GIFT) after the oocyte retrieval as a result of a small number of fertilised oocytes. Progesterone preparation was given to provide luteal support. A quantitative serum β-hCG was checked 14 days after embryo transfer. We defined a positive result of a quantitative measurement of serum β-hCG with a proven gestation sac as a clinical pregnancy. The implantation rate was calculated from the number of gestation sacs divided by the number of transferred embryos.

Numbers of gonadotrophin ampules, stimulation days, retrieved oocytes, fertilised oocytes, transferred embryos, cycles with frozen embryos, the fertilisation rate, cancellation rate and pregnancy rate as well as the implantation rate were evaluated. Statistical analysis was performed using the epi-info programme (version 6.0). Student's t-test, chi-square and Fisher's exact test, if needed, were used for analysis. Statistical significance was defined at a P-value of < 0.05.

RESULTS

A total of 106 ovarian stimulation cycles from 88 patients were included in the study. Sixty-six out of 106 cycles (62.3%) were stimulated using HMG, whereas 40 (37.7%) were stimulated using recombinant FSH. The comparison between HMG and recombinant FSH in terms of IVF and pregnancy outcome is shown in Table 1. The mean age of the HMG and recombinant FSH groups was 36.6 and 36.1 years, respectively. The basal FSH level, day 2 and 3 oestradiol levels in the HMG group were 8.9 ± 2.9 IU/L, 199.8 ± 109.1 pmol/L and 402.5 ± 186.5 pmol/L, respectively. Meanwhile, the basal FSH level, day 2 and 3 oestradiol levels in the recombinant FSH group were 9.8 ± 3.9 IU/L, 180.0 ± 38.1 pmol/L and 368.2 ± 149.2 pmol/L, respectively. The difference in the day 2 and 3 oestradiol levels of the HMG and recombinant FSH groups were 202.6 ± 139.5 and 188.2 ± 134.8 pmol/L, respectively. There was no significant difference between these variables. The cancellation rate of the HMG group (19.7%) was almost twice of that of the recombinant FSH group (10.0%). This difference was not statistically significant. The pregnancy rate of the recombinant FSH group (22.5%) was also more than twice of that of the HMG group (9.1%). The difference, however, was not statistically significant.

The IVF and pregnancy outcomes of the 70 IVF-ET cycles are shown in Table 2. The starting dose of the HMG group (5.7 ± 1.6) was similar to that of the recombinant FSH group (5.2 ± 1.2). The number of ampules used of the recombinant FSH group (58.8 ±14.8) was lower than that of the HMG group (64.3 ± 20.1). The number of stimulation days required in the two groups was similar, 11.1 ± 0.9 in the HMG group and 11.1 ± 0.4 in the recombinant FSH group. There was no significant difference in oestradiol levels produced on hCG administration between the two groups. The oestradiol levels of the HMG and the recombinant FSH groups were 7728.5 ± 3876.2 and 7678.6 ± 3150.1 pmol/L, respectively. The number of oocytes retrieved from the HMG group (7.2 ± 3.7) was also similar to that of the recombinant FSH group (7.2 ± 3.7). The number of cycles with ICSI of the HMG group (38.6%) was similar to that of the recombinant FSH group (42.5%). Interestingly, the number of fertilised oocytes obtained in the recombinant FSH group (4.5 ± 2.4) was higher than that of the HMG group (4.0 ± 3.0), resulting in a difference in fertilisation rates between the two groups (recombinant FSH group 62.8%, HMG group

Table 1. Clinical characteristics and outcome of ovarian stimulation comparing the HMG and recombinant FSH groups (106 cycles).

Variables	Gonadotrophin		P-value
	HMG	Recombinant FSH	
No of cycles	66	40	-
Age (years)ø	36.6 ± 3.7	36.1 ± 3.8	NS
Cycle day 2 FSH level (IU/L)ø	8.9 ± 2.9	9.8 ± 3.9	NS
Cycle day 2 oestradiol level (pmol/L)ø	199.8 ± 109.1	180.0 ± 38.1	NS
Cycle day 3 oestradiol level (pmol/L)ø	402.5 ± 186.5	368.2 ± 149.2	NS
Difference in oestradiol levels from day 3 to day 2 (pmol/L)ø	202.6 ± 139.5	188.2 ± 134.8	NS
Cancellation rate (%)	19.7 (13/66)	10.0 (4/40)	NS
Pregnancy rate per cycle (%)	9.1 (6/66)	22.5 (9/40)	NS

ø Values are mean ± S.D.
NS Not significant

51.9%). However, the difference was not statistically significant. The number of embryos transferred in the HMG group (2.2 ± 1.0) was not significantly different from that of the recombinant FSH group (2.5 ± 0.8). Only the recombinant FSH group produced good quality embryos available for freezing after embryo transfer (7.7%, 2/26). The pregnancy and implantation rates of the recombinant FSH group (23.0%, 9.1%) were obviously higher than those of the HMG group (13.6% and 5.9%). However, the difference was not statistically significant.

The characteristics and pregnancy outcome of the 19 GIFT cycles are shown in Table 3. The starting dose of the two groups was not significantly different, 5.2 ± 1.0 in the HMG group versus 5.0 ± 1.0 in the recombinant FSH group. The number of ampoules used and the number of stimulation days of the HMG group (58.7 ± 9.2, 11.2 ± 0.8) were similar to those of the recombinant FSH group (58.1 ± 12.4, 11.1 ± 0.5). The oestradiol level on hCG administration of the HMG group (5,605.0 ± 3,767.5) was not significantly different from that of the recombinant FSH group (4,311.6 ± 1,676.9). Only the recombinant FSH group could achieve pregnancy (30.0%, 3/10). Meanwhile, there was no pregnancy in the 9 GIFT of the HMG group.

DISCUSSION

A variety of ovarian stimulation regimens and newly developed medication have been introduced in ART to achieve higher pregnancy rates. GnRH-a, which can prevent premature luteinization, has been used in many ovarian stimulation regimens.[11-16] Most IVF centres prefer to start GnRH-a administration from the midluteal phase of the previous cycle and continue until the succession of ovarian stimulation using gonadotrophin.[17] This protocol has been found to produce a high number of retrieved oocytes, resulting in an appropriate number of embryos available for transfer.[18]

Poor responders, who have diminished ovarian reserve, produce a small number of oocytes available for retrieval, resulting in a low pregnancy rate.[1] Faber et al have reported that the administration of GnRH-a initiated in the midluteal phase and discontinued at the onset of menstruation could improve the IVF and pregnancy outcome in these patients.[16] Therefore, GnRHa was stoped on the first day of menstruation in the 106 ovarian stimulation cycles from the 88 poor responders diagnosed by the GnRH-a test in this study. There was no premature ovulation in any cases in our population. This is supported by many studies which suggest that GnRH-

Table 2. IVF and pregnancy outcome of IVF-ET of the HMG and recombinant FSH groups (70 cycles).

Variables	Gonadotrophin		P-value
	HMG	Recombinant FSH	
Number of cycles	44	26	
Starting dose of gonadotrophin (ampules)ø	5.7 ± 1.6	5.2 ± 1.2	NS
Number of ampoulesø	64.3 ± 20.1	58.8 ±14.8	NS
Number of stimulation daysø	11.1 ± 0.9	11.1 ± 0.4	NS
Oestradiol level on hCG administration (pmol/L)ø	7,728.5 ± 3,876.2	7,678.6 ± 3,150.1	NS
Number of retrieved oocytesø	7.2 ± 3.7	7.2 ± 3.7	NS
Number of cycles with ICSI (%)	38.6 (17/44)	42.5 (11/26)	NS
Number of fertilised oocytesø	4.0 ± 3.0	4.5 ± 2.4	NS
Number of transferred embryosø	2.2 ± 1.0	2.5 ± 0.8	NS
Fertilisation rate (%)ø	51.8 ± 25.7	62.8 ± 26.6	0.10
Pregnancy rate per transfer (%)	13.6 (6/44)	23.0 (6/26)	0.31
Implantation rate (%)	5.9 (6/101)	9.1 (6/66)	0.31
Cycles with embryos available for freezing (%)	0.0 (0/44)	7.7 (2/26)	NS

ø Values are mean ± S.D.
NS Not significant

Table 3. Characteristics and pregnancy outcome of GIFT of the HMG and recombinant FSH groups (19 cycles).

Variables	Gonadotrophin		P-value
	HMG	Recombinant FSH	
Starting dose of gonadotrophin (ampules)ø	5.2 ± 1.0	5.0 ± 1.0	NS
Number of ampoulesø	58.7 ± 9.2	58.1 ± 12.4	NS
Number of stimulation daysø	11.2 ± 0.8	11.1 ± 0.5	NS
Oestradiol level on hCG administration (pmol/L)ø	5,605.0 ± 3,767.5	4,311.6 ± 1,676.9	NS
Pregnancy rate per GIFT (%)	0.0 (0/9)	30.0% (3/10)	-

ø Values are mean ± S.D.
NS Not significant

a can be discontinued safely for a period of time without a premature LH surge due to the return of gonadotrophin secretion to normal levels which may take a few weeks after stopping.[13, 19-20] The results of Ranieri et al[5], show that poor responders can produce less than 5 follicles following conventional GnRHa administration. In this study, the patients produced more retrieved oocytes by ceasing GnRH-a administration on the first day of menstruation.

The factors influencing implantation and pregnancy rate are not only the number of oocytes retrieved, but also the quality of embryos obtained from IVF treatment.[21] Hormonal status during the period preceding oocyte retrieval has been shown to influence fertilisation, implantation and embryonic development.[22] Although luteinizing (LH) takes an important role in the maturation of oocytes and follicular rupture, an elevated LH level during the follicular and periovulatory phase may adversely affect fertilisation, cleavage and embryo quality.[23-25] A more purified gonadotrophin may produce a higher pregnancy rate by improving the quality of embryos from IVF. Urinary highly-purified FSH has been introduced and is claimed to be more effective than HMG.[26] Recently, recombinant FSH has been introduced and has been shown to be more effective than HMG or urinary highly purified FSH in "normal" infertile patients.[8-10] In this study, in poor responders, we found no significant difference in the cancellation rate and pregnancy rate between the two groups (Table 1). However, the cancellation rate and pregnancy rate of the recombinant FSH group (10.0% and 22.5%, respectively) were better than those of the HMG group (19.7% and 9.1%, respectively). This difference might be accepted in clinical practice. There was no difference in the starting doses and numbers of ampules used as well as the duration of stimulation between the two groups in the IVF-ET cycles (Table 2). Although the numbers of retrieved oocytes were similar between the two groups, the number of fertilised oocytes and fertilisation rate were in favor of the recombinant FSH group. Unfortunately, the difference was not statistically significant. Furthermore, only the recombinant FSH group produced embryos of good enough quality for freezing after embryo transfer. This might be the result of the difference in the quality of the oocytes retrieved. These findings are consistent with the study of Mercan et al which showed that patients who received FSH had higher pregnancy rates than those receiving FSH/HMG treatment as a result of the difference in oocyte quality between the two groups.[27]

Although there were only 19 GIFT cycles in our study, this procedure seemed to be an alternative treatment in these patients producing a high pregnancy rate (15.7%). Recombinant FSH has been shown to be more effective than HMG in this group. Only the patients in the recombinant FSH group achieved any pregnancies (pregnancy rate 30%). However, more cases should be studied in order to draw a more definite conclusion.

In this study, we found no difference in oestradiol levels on the day of hCG administration between the recombinant FSH and HMG groups in both IVF-ET and GIFT cycles. Therefore, the endogenous LH in the recombinant FSH group was enough to produce oestradiol biosynthesis in the ovaries. This result confirms that a small amount of LH is enough for oestradiol biosynthesis in folliculogenesis.[28] However, this might also be because of the small number of stimulated follicles in poor responders. The difference in the amount of oestradiol produced from such a small number of follicles between the two groups did not reach statistical significance.

In conclusion, to achieve a good number of oocytes retrieved and good embryo quality in poor responders, ceasing GnRH-a administration on the first day of menstruation may benefit those patients with a low risk of premature LH surge. Recombinant FSH seemed to be more effective than HMG in improving the IVF and pregnancy outcome in such patients. The increased fertilisation rate and pregnancy rate, as well as implantation rate of the cycles stimulated with recombinant FSH might be the result of improved embryo quality and an appropriate LH level after the gonadotrophin was used.

ACKNOWLEDGEMENT

The authors gratefully thank Paul Serhal and Massimo Ranieri from the Assisted Conception Unit, University College Hospital, for their help and allowing of the using of data for publication.

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