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The Influence of Low Ovarian Response on the Results of *In Vitro* Fertilization and Embryo Transfer

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Summary. **Results of *in vitro* fertilization (IVF) and embryo transfer (ET) were evaluated for different grades of ovarian response stimulated by ovulation inducers. Patients were grouped into low (L group), modest (M group) and high (H group) ovarian responders according to their serum estradiol (E_2) level on the day of HCG (human chrionic gonadotropin) administration, and their results of IVF and ET were compared.**

The number of subjected cycles totaled 149: 36 in L, 52 in M and 61 in the H group. Although more HCG was administered to the L group than to the M and H groups, the L group had significantly lower numbers of growing follicles and collected oocyte retrieval. The pregnancy rate per oocyte retrieval and per ET was significantly lower in the L group than in the M and H groups. The implantation rate in the L group was also the lowest in these three groups.

Another study was carried out with a doubly increased dosage of HMG (human menopausal gonadotropin) given to the 26 low ovarian responders. In this group, serum E_2 level on the day of HCG administration was elevated, and the cancellation rate of oocyte retrieval decreased. As a result, significant increases were observed in numbers of growing follicles, oocyte retrieval, and transferred embryos, with 5 pregnancies thus established. These results suggest that for the further improvement of results in IVF and ET, each patient's ovarian response should be diagnosed before treatment, and according to the diagnosis, methods of ovarian stimulation need to be considered individually.

INTRODUCTION

In *in vitro* fertilization (IVF) and embryo transfer (ET), an increased success rate is associated with multiple embryo replacement. Therefore, ovarian stimulation is generally performed using ovulation inducer(s) to harvest many mature oocytes *in vivo*. However, the success rate depends on the ovarian response to the stimulation. Despite the use of ovulation inducers, many failing cases have been reported in the literature in which low ovarian response[1,2] or an increased level of luteinizing hormone (LH)[3] interfered with the normal maturation of oocytes, and an adequate number of mature oocytes could not be obtained.

In this study we grouped patients into low, modest, and high ovarian responders according to the serum estradiol (E_2) level. Analyses were made on the results of IVF-ET procedures. The pregnancy rate was compared in each group. We then studied the results of IVF-ET, when doubly increased dosages of HMG were administered to the low ovarian responders.

MATERIALS AND METHODS

1. Patients and treatment

Subjects were infertile couples who underwent IVF and ET at our department from January 1989 to February 1990. Applicable infertile factors were: 1) tubal infertility; 2) male infertility, such as oligospermia and asthenozoospermia; 3) endometriosis externa; 4) immune infertility possibly involving antisperm antibodies; and 5) idiopathic factors.

The protocol of IVF and ET used in our department has been previously reported.[4] Ovarian stimulation was performed using a method of buserelin-HMG-HCG on long term administration. Namely, 300 μg of buserelin was administered nasally three times a day from the 7th day of the high phase on the basal body temperature in the previous cycle (900 μg/day). After menstruation, 300 units of HMG (HMG Nikken, Nikken Chem., Tokyo, Japan) were given on days 3 and 4 of the menstrual cycle, followed by a daily 150 units. After cycle day 8, the follicular size was measured once everyday by transvaginal ultrasound examinations. Administration of HMG was

discontinued when the dominant follicle became more than 16 mm in diameter. Thirty-four hours after the last HMG, 10,000 units of HCG (Mochida Pharm., Tokyo, Japan) was given, and 36 h after HCG, oocyte retrieval was carried out by a transvaginal ultrasound-guided approach, except for cases with less than 3 mature follicles. The collected oocytes were classified into 4 grades: immature, mature, overmature, and abnormal oocytes,[5] and pre-incubated for 4 h prior to insemination. Normal fertilization was observed 14-18 h after insemination. Forty-eight hours after oocyte retrieval, embryos, numbering five at the most, were transferred to the uterus of patients transvaginally. For luteal support, progesteron (Luteum, Teikoku Hormone, Tokyo, Japan), 50 mg on the day of oocyte retrieval and then 30 mg daily for 14 days was intramascularly injected into the patients. Fifteen days after oocyte retrieval, urinary HCG was measured (Testpak, Dinabot Co., Ill., U.S. A.). Among HCG positive cases, those resulting in live-born infants were difined as pregnancy.

Patient's blood was collected on the day of HCG administration. The serum was then cryopreserved at −20℃ until use. The E_2 level was later determined by radioimmunoassay (Japan UPC Co., Tokyo, Japan), and according to the level, the patients were grouped arbitrarily into low (L group, $E_2 < 600$ pg/ml), modest (M group, $600 \leq E_2 < 1200$ pg/ml) and high ovarian responders (H group, 1200 pg/ml $\leq E_2$). Comparisons were made regarding HMG dosage, the number of follicles, the number of collected oocytes, the number of transferred embryos and the pregnancy rate among the 3 groups.

2. Effect of double dosage of HMG on low responder group

In a total of 26 low response cases of pregnancy failure (14 cases) and cancellation of oocyte retrieval (12 cases), the dosage of HMG was doubled. Namely, 600 units of HMG was given to the patients on cycle days 3 and 4 followed by a daily 300 units. The number of growing follicles and of collected oocytes, the pregnancy rate and E_2 level on the day of HCG administration were all investigated.

3. Statistics

All statistical data were expressed as mean±SD and evaluated by Student's t-test and chi-square test.

RESULTS

During this period 97 patients entered into IVF-ET program. Ovarian stimulation was performed on a total of 190 cycles, in which oocyte retrieval was carried out in 176 cycles in the present study. The subjects were 149 cycles, because no blood for E_2 was collected in 27 cycles.

The number of cycles in L, M and H groups was 36, 52 and 61, respectively. No significant difference was found in the patient's mean ages or fertility periods among the groups (Table 1). Table 2 shows the results of IVF and ET in each group. The L group was administered with significantly more HMG than the other two groups (L v.s. M, $p<0.01$; L v.s. H, $p<0.05$). The L group also had the smallest numbers of follicles (3.9±1.4) and collected oocytes (2.5±1.7). These numbers significantly ($p<0.01$) increased for higher grades of ovarian respnders.

Regarding the fertilization rate (excluding male infertility), the M group (73.4±20.3%) had a more favorable result than the H group ($p<0.05$). The L group (60.3±37.0%) had a lower rate than the M group, but it was not significantly different. These results indicate that the L group had the smallest number of embryos per transfer, with the number increasing as the grade of the group was elevated.

With regard to the morphological estimation of embryos, the M group was significantly superior to the H group ($p<0.05$) while no significant difference

Table 1. Group characteristics.

Serum E_2 level (pg/ml)	L group $E_2<600$	M group $600\leq E_2<1200$	H group $1200\leq E_2$
No. of oocyte retrieval cycles	36	52	61
Average age	33.8±4.8	33.0±3.8	33.2±4.3
Average infertile period (yrs)	7.2±3.7	7.2±3.3	8.4±3.7

(Mean±SD)

Table 2. Results of IVF and ET.

Group	L	M	H
No. of oocyte retrieval cycles	36	52	61
HMG doses (75IU=1A)	25.6±12.5A	18.8±6.0A	19.7±7.1A
No. of follicles[a)]	3.9±1.4	6.4±2.6	9.5±4.6
No. of harvested oocytes	2.5±1.7	5.1±2.8	9.5±5.1
Fertilization rate[b)]	60.3±37.0%	73.4±20.3%	61.8±19.1%
No. of ET	25	47	50
No. of transferred embryos per ET	1.8±1.2	3.0±1.3	3.6±1.4
Morphological grade of embryos	2.8±1.0	2.9±0.7	2.6±0.7
No. of pregnancies[c)]	2	18	11
Pregnancy rate			
by oocyte retrieval	5.5%	34.6%	18.0%
by transfer	8.0%	38.3%	22.0%

(Mean±SD)

*: $p<0.05$ **: $p<0.01$

a): No. of follicles more than 10 mm in diameter on the day of HCG administration.

b): Cases with male infertility are excluded.

c): Cases that delivered live-born infants; four twins and one triplet in the M group and a twin and a triplet in the H group are included.

was observed between the L and M groups.

The number of pregnancies was 2 in the L group, 18 in the M group and 11 in the H group. The L group had the lowest pregnancy rate both per oocyte retrieval (5.5%) and per embryo transfer (8.0%).

The percentage of collected immature oocytes was significantly higher in the L group than in the M and H groups (6.7%), but no significant difference was found in overmature oocytes between the groups (Table 3).

The number of implantations, defined as number of live-born infants, was 2 in the L group, 24 in the M group, and 14 in the H group. Numbers of transferred embryos were 46 in the L group, 142 in the M, and 178 in the H group, with implantation rates accordingly being 4.3%, 16.9% and 7.9%, respectively (Table 4).

Table 5 shows the pregnancy rate for each number of transferred embryos. The L group had the lowest rate in every number.

The results of a double dosage of HMG are shown in Table 6. Serum E_2 level on the day of HCG administration increased to 1003±576 pg/ml ($p<0.01$). Moreover, the cancellation rate of oocyte retrieval significantly decreased, and numbers of growing follicles, collected oocytes, and transferred embryos significantly increased. Five pregnancies were established. The pregnancy rate was 22.7% per oocyte retrieval and 26.3% per embryo transfer. The comparison between the outcomes of conventional and double dosages is shown in Table 7. Improvement by

Table 3. Maturation status (%) of harvested oocytes.

Group	L	M	H
Total No. of harvested oocytes	89	267	582
Immature oocytes	6.7%	1.9%	1.2%
	*	* *	
Mature oocytes	89.9%	96.2%	96.4%
	*	* *	
Overmature oocytes	3.4%	1.9%	2.4%
Abnormal oocytes	0	0	0

*: $p<0.05$ **: $p<0.01$

Table 4. Implantation rates.

Group	L	M	H
No. of transferred embryos	46	142	178
No. of implantations[a)]	2	24	14
Implantation rate	4.3%	16.9%	7.9%
	*	*	

a): No. of live-born infants. *: $p<0.05$

Table 5. Results of pregnancy per ET in respect to number of transferred embryos.

Group	L	M	H
No. of transferred embryos			
1	0/14 (0%)	2/7 (28.6%)	1/7 (14.3%)
2	1/5 (24%)	4/11 (36.4%)	1/5 (20%)
3	0/3 (0%)	4/14 (35.7%)	2/5 (40%)
4	1/2 (50%)	5/9 (55.6%)	4/15 (26.7%)
more than 5	0/1 (0%)	2/6 (33.3%)	4/17 (23.5%)

more than 1 grade was observed in 65.4% (17/26), and successful cases of pregnancy belonged to both the M and H groups.

DISCUSSION

In IVF and ET, ovarian stimulation is generally performed using ovulation inducers. In cases with low ovarian response, conventional methods of ovarian stimulation, such as clomid-HMG-HCG and HMG-HCG, decrease the pregnancy rate.[1,2] In order to treat these cases, a combined method with Gn-RHa has been established.[6-8] In the present study, however, about 30% of stimulated cycles (including 14 cases of cancellation) showed low response even with this method. In this group as well, the pregnancy rate per oocyte retrieval was extremely low (5.5%).

Possible factors that influence the success of IVF and ET are: 1) the number of transferred embryos, 2) quality of oocytes, and 3) uterine acceptability for implantation. In IVF and ET, the pregnancy rate rises as the number of transferred embryos increases.[9] Compared to the M and H groups, the L group had fewer transferred embryos due to a decrease in the number of collected oocytes. This fact may be great-

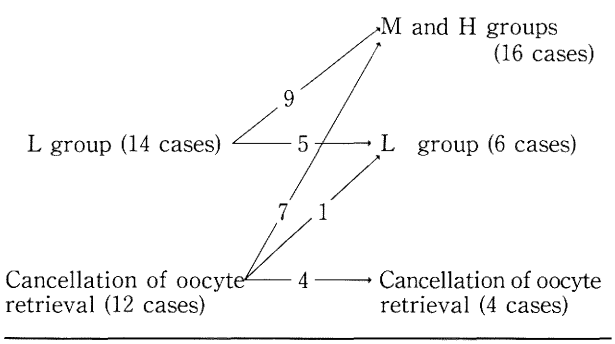
Table 6. Results by methods of conventional and double dosage.

	Conventional dosage	Double dosage	
Serum E_2 level (pg/ml)	371±313	1003±576	$p<0.01$
HMG dose (75IU=1A)	22.5±6.6A	38.3±10.5A	$p<0.01$
Cancellation rate of oocyte retrieval	46.1%	15.4%	$p<0.05$
No. of follicles[a)]	2.1±1.8	5.9±3.9	$p<0.01$
No. of harvested oocytes	1.0±0.8	5.7±4.7	$p<0.01$
No. of transferred embryos per ET	0.6±0.5	2.9±1.8	$p<0.01$
No. of pregnancies[b)]	0	5	
Pregnancy rate			
by oocytes retrieval	0%	22.7%	
by transfer	0%	26.3%	

(Mean±SD)

[a)]: No. of follicles more than 10 mm in diameter on the day of HCG administration.
[b)]: Cases that deliverd live-born infants. Two twins and a triplet are included.

Table 7. Prognosis of the cases treated by conventional or double dosage of HMG.



ly related to the decreased pregnancy rate. However, in the L group, the implantation rate per transferred embryo was significantly lower than that of the M group, and even with the same number of transferred embryos, the pregnancy rate was also lower than that of the M group. These results may thus indicate that the decreased pregnancy rate was not caused by only the decreased number of transferred embryos.

Mettler et al.[10] and Jones et al.[11] reported that the retrieval rate of immature oocytes did not increase in low ovarian responders; however, our present study showed a significant increase in the L group. IVF and ET using immature oocytes are suggested to result in a low pregnancy rate. However, in the L group, the increase in the retrieval rate of immature oocytes was about 5%, which does not seem to be the reason for the decrease in the pregnancy rate by 14-30%. Uterine acceptability for embryo implantation is possibly involved in this regard. The present study could not clarify the reason for a decreased implantation rate. Further investigation of factors inducing low uterine acceptability is necessary, by checking hormonal status in luteal phase deficiency and endometrial disorders.

Some reasons pointed out for low ovarian response are: 1) aging,[12] 2) endometriosis externa,[12,13] 3) experiences of ovarian surgery,[12,13] and 4) raised basal FSH (follicle-stimulating hormone) levels.[14-16] In the present experiment, for cases with low ovarian response and those with cancellation of oocyte retrieval, the dosage of HMG was doubled. As a result, 65.4% of the cases showed an increased E_2 level and 5 pregnancies were established. Hoffman et al.[17] also demonstrated that an increased E_2 level in poor responders, using one and a half the normal dose of pure FSH, decreased the cancellation rate of oocyte retrieval and increased the pregnancy rate. Therefore, in ovarian stimulation of IVF and ET, individualized treatment is necessary depending on each patient's background.

CONCLUSION

Analysis revealed that in IVF and ET using the Gn-RHa combined method of ovarian stimulation, the pregnancy rate was strongly associated with methods of stimulation because of the high existence of low responders. The study of the administration of

a double dosage of HMG increased the number of mature follicles and collected oocytes and transferred embryos. High rates of successful pregnancy were obtained in 22.7% per oocyte retrieval in this group.

For further improvement of the pregnancy rate, each patient's ovarian response should be diagnosed beforehand, and according to the diagnosis, individual treatment needs to be considered.

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REFERENCES

1) Garcia JE, Jones GS, Acosta AA, Write G: Human menopausal-gonadotropin/human chorionic gonadotropin follicular maturation for oocyte aspiration: phase II, 1981. *Fertil Steril* **39**: 174-179, 1983.
2) Forman, R, Belaisch-Allart J, Fries N, Hazout A, Testart J, Frydman R: Evidence for an adverse effect on elevated serum estradiol concentration on embryo implantation. *Fertil Steril* **49**: 118-122, 1988.
3) Stranger JD, Yovick JL: Reduced *in vitro* fertilization of human oocytes from patients with raised basal luteinizing hormone levels during follicular phase. *Br J Obstet Gynecol* **93**: 385-393, 1985.
4) Tani H, Sato Y, Oda K, Shichiri K, Arakawa O, Takeuchi S: Ovarian stimulation after desensitization with buserelin in *in vitro* fertilization and embryo transfer. *Jap J Fertil* **34**: 413-418, 1989.
5) Lucianda L, Veeck MLT: Morphological estimation of mature oocyte and their preparation for insemination. In: Jones HW, Jones GS, Hodgen GD, Rosenwaks Z (eds) *In vitro* fertilization. William & Wilkins, Norfork-Baltimore, 1986, p 811-820.
6) Fleming R, Coutts JRT: Induction of multiple follicular growth in normally menstruating women with endogenous gonadotropin suppression. *Fertil Steril* **45(2)**: 226-230, 1986.
7) Palermo R, Rosenwaks Z, Amedeo G, Cittadini E, Navot D: Concomitant Gonadotropin-releasing hormone agonist and menotropin treatment for the synchronized induction of multiple follicles. *Fertil Steril* **49(2)**: 290-295, 1988.
8) Neveu S, Arnal F, Heden B, Humeau C, Bringer B, Cristol P, Chincole JM, Viala JL: Ovarian stimulation by a combination of a gonadotropin-releasing hormone agonist and gonadotropins for *in vitro* fertilization. *Fertil Steril* **47(4)**: 639-643, 1987.
9) Wood C, Trounson A, Leeton J, Talbnot JM, Buttery B, Webb J, Wood J, Jessup D: A clinical assessment of nine pregnancies obtained by *in vitro* fertilization and embryo transfer. *Fertil Steril* **35**: 502-508, 1981.
10) Metter L, Tavmergen EN: Significance of oestradiol values in IVF-ET under a combined GnRH analogue-desensitization and simultaneous gonadotropin stimulation for the outcome of pregnancies. *Hum Reprod* **4**: 59-64, 1989.
11) Jones GS, Acosta AA, Garcia JE, Bernardus RE, Rosenwaks Z: The effect of follicle stimulating hormone without additional luteinizing hormone on follicular stimulation oocyte development in nomal ovulatory woman. *Fertil Steril* **43**: 696-702, 1985.
12) Jones GS, Muasher SJ, Rosenwaks Z, Acosta AA, Lui H-C: The perimenopausal patient *in vitro* fertilization: The use of gonadotropin-releasing hormone. *Fertil Steril* **46**: 885-890, 1980.
13) Hirasawa H, Tani H, Shichiri K, Oda K, Miyake T, Arakawa O, Tanaka K: The result of IVF-ET in endometriosis. *J Endometriosis Res* **11**: 299-302, 1990. (in Japanese)
14) Jones GS: Use of purified gonadotropins for ovarian stimulation in IVF. *Clin Obst Gynecol* **12**: 775-778, 1985.
15) Rosenwaks Z, Muasher SJ, Acost AA: Use of hMG and/or FSH for multiple follicle development. *Clin Obst Gynecol* **29**: 148-153, 1986.
16) Muasher SJ, Garcian JE, Rosenwaks Z: The combination of follicle stimulating hormone and human menopausal gonadotropin for the induction of multiple follicular maturation for *in vitro* fertilization. *Fertil Steril* **44**: 62-68, 1985.
17) Hoffmann GE, Toner JP, Muasher SJ, Jones GS: High-dose follicle-stimulation in low-responder patients for *in vitro* fertilization. *J IVF-ET* **6**: 285-289, 1989.