

Study on Several Factors Involved in IVF-ET of Human Beings

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Abstract: The experiment was designed to study the several relative factors in order to improve the effects of IVF-ET in human beings. 108 infertile couples were included in IVF-ET treatment and clinic data were statistically analyzed. The effects of both long and short superovulating protocols were researched on oocyte maturation and embryo quality. The relationships were investigated respectively among woman's age, insemination method, embryo quality, the number of embryos transferred, embryonic culture condition and clinical pregnancy rate. The results show that there are no statistically differences between both superovulating protocol in the number of oocyte retrieved, metaphase-II stage oocytes, fertilization rate, cleaved rate, high quality embryos rate and clinical pregnancy rate. Women of the short superovulating protocol group needed less gonadotropin ampoules compared with those in long one. The clinical pregnancy rate with patient's age ≥ 35 was remarkably lower and woman's age affected IVF-ET. Fertilization rate and clinical pregnancy rate were not statistically significantly different between routine IVF group and ICSI group. The numbers of 4-cell embryos transferred and high quality embryos were significantly higher in the pregnancy group than non-pregnancy one and embryo quality was related to the pregnancy rate in IVF-ET. [Nature and Science. 2005;4(1):47-53].

Keywords: human being; IVF; embryo transfer

1. INTRODUCTION

Assisted reproductive technology (ART) have made great progresses since the first test-tube baby was born in 1978^[1-4]. In vitro fertilization and embryo transfer (IVF-ET) have been proved to be an effective alternative for couples who would be unable to achieve pregnancy^[5-6]. Even so, there are still some factors which have not well been researched in IVF process, such as superovulating protocol, female age, fertilizing method, uterine receptivity, quality and number of embryos transferred, culture conditions and so on. Therefore the several factors were clinically chosen in order to verify their effects on IVF and

provide the practical and theoretic data for IVF of human beings.

2. MATERIALS AND METHODS

108 patients with age of 24-44 underwent IVF programmes were accepted for the study.

The long and the short protocols were used in IVF and intracytoplasmic sperm injection (ICSI) procedures with Gonadotropin Releasing Hormone agonist (GnRHa), Follicle Stimulating Hormone (FSH), Human Chorionic Gonadotropin (HCG). The long protocol began on the day 21 of the mid-luteal phase with GnRHa, gonadotropin (Gn) was provided

three days later until HCG was administered. The short protocol commenced with GnRHa at day 2 and Gn at day 3. When two or more follicles of more than 18 mm in diameter appeared, HCG was administered at a dose of 10000 IU i.m and oocyte retrieval followed at 35-36h with transvaginal ultrasound-guided puncture. For both IVF and ICSI, Fertilization of ova with cumulus cells lasted for 18h and determined by confirmation of two pronuclei under the microscope. Embryos were cultured for 48h and then selected for transfer on the basis of morphology and score on day 2. One to four embryos were transferred per cycle. Excess embryos with more than 2 cells and less than 50% fragmentation were frozen. After rapid-freezing and slow-thawing, embryos with more than 50% survival cells were regarded as viable embryo and could be transferred after 2 h in vitro culture.

Statistical analysis: All data were analysed using SPSS version 10.0.

3. RESULTS AND DISCUSSION

3.1 The effect of superstimulation protocol on oocyte maturation, embryo quality and pregnancy rate.

The results showed in table1 that Gn dose was significantly lower and stimulating time was less in the short protocol compared with that in the long protocol one ($P < 0.05$). However, there was no significant difference between the long and short protocol in numbers of retrieved oocyte and mature oocyte, fertilization rate, cleavage rate, high quality embryonic rate and pregnancy rate ($P > 0.05$).

GnRHa of superovulating protocol improved oocyte quality, synchronized follicle development, increased follicular recruitment and improved IVF effects. The long protocol initiated in the mid-luteal phase with GnRHa resulted in more prompt and profound suppression so that the doses of exogenous Gn had to be increased and the stimulation period was prolonged. The short protocol takes advantage of the “flare up”

initially and then the pituitary desensitization effect with GnRHa. With the reduced doses of Gn required and less stimulation periods, the cost of IVF cycle also reduced^[7]. San's reported that retrieved oocyte was fewer and clinical pregnancy rate was lower with the short protocol compared with that of the long protocol^[8]. However Our experimental results were quite different. Bstandig^[9] found that short protocol with low dose of GnRHa stimulated the development of follicles in the early follicular phase effectively and prevented premature LH surge, as did the long protocol, which especially benefits “poor responders”. This study demonstrates that short protocol can achieved similar effect of hyperovulation as did the long protocol.

3.2 The effect of female age on IVF outcome

A total of 108 patients were divided into three age group: young group (aged ≤ 29 years), moderate group (range 30-34 years) and aging group (aged ≥ 35 years). As shown in Table 2, with the age growing, the clinical pregnancy rate were significantly lower (46.4%, 35.0% and 10.0% respectively). There was no difference about M \square oocyte among aging, young and middle age group. The ovarian reservation decreased with advancing age, while a parallel decrease in the number of mature oocytes and decline in oocyte quality was present. Degressive oocyte quality does not affect fertilization potential^[10]. Therefore, there was no significant difference in terms of fertilization rate, cleavage rate and high quality embryo rate in the three group.

It has been noticed that the pregnancy rate for the aging group is 10%. Investigations demonstrated that waning oocyte quality and declining uterine receptivity were the main factors responsible

for the age-related reduction of female fecundity. Oocyte quality declines, due in part to increased aneuploidy because of oxidative damage at meiosis I stage, which cause pregnancy rate decreased while fertilization rate and high quality embryo rate seems normal. Declining uterine receptivity is attributed to a series of endometrial changes in morphology and function, such as

decreased content of DNA in matrix cell, reduced estrogen and progesterone receptor, declined vascular perfusion of the endometrium, and some pathological alternation of the uterus. Thus, age is negatively correlated with pregnancy rate in IVF cycles. Female age is an important predictor of pregnancy rate.

Table 1. The effect of both protocol on oocyte maturation, embryonic quality and pregnancy rate

G	cycle	Gn Dose (ampoule)	Stimulating periods (day)	Number of retrieved oocyte	Number Of MII oocyte	Fertilizing Rate (%)	Cleavage rate (%)	High quality embryo rate (%)	Pregnancy rate (%)
L	42	31.4±13.8	12.6±2.0	13.7±7.2	11.4±6.9	71.4±19.8	91.3±13.6	68.5±18.8	33.3
S	66	25.7±8.5	11.7±1.6	13.3±6.0	11.1±5.9	70.4±19.9	93.4±14.3	63.5±24.3	33.3
P		P<0.05	P<0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

G: group; L: Long protocol; S: Short protocol

Table 2. The effect of age on IVF-ET outcome

G	cycle	Number of MII oocyte	Fertilization Rate (%)	Cleavage rate (%)	High quality embryo rate (%)	Pregnant (case)	Pregnancy rate (%)
Y	28	11.4±6.0	70.6±20.3	88.8±17.5	65.1±22.3	13	46.4**
M	60	12.2±6.6	72.2±20.1	93.7±12.9	65.9±21.3	21	35.0*
A	20	8.2±4.9	66.8±18.3	94.6±11.3	64.6±26.5	2	10.0
P		P>0.05	P>0.05	P>0.05	P>0.05		

G: group ;Y: Young ;M: Middle; A: Aging;

**compared with aging group P<0.01; *compared with aging group P<0.05;

3.3 The impact of fertilization method on IVF outcome

108 cycles were divided into two groups by two methods of conventional IVF and ICSI.

Table 3. The effect of fertilizing method on IVF-ET outcome

Group	Cycle	Fertilization rate (%)	Cleavage rate (%)	High quality embryo rate (%)	Pregnancy (case)	Pregnancy rate (%)
IVF	51	71.9±18.7	93.1±14.1	63.0±23.7	16	31.4%
ICSI	57	69.6±21.0	92.0±14.0	68.1±20.7	20	35.1%
P		P>0.05	P>0.05	P>0.05		P>0.05

As shown in Table 3, there was no significant difference between the two groups in fertilization rate, cleavage rate, high quality embryo rate and pregnancy rate. It would be concluded that microinjection is a safe procedure for oocyte manipulation and ICSI could achieve satisfactory fertilization rate and clinical pregnancy rate in severe male-factor infertility, such as severe oligozoospermia, asthenozoospermia, positive antisperm antibody, azoospermia, and failure with conventional IVF.

There has no evidence so far excluding that ICSI procedure itself is related to chromosome aberration. Up to now, the two explanation has been reasonable to explain possibility that microinjection lead to offspring malformation. The first is that injection is done with exogenous DNA or contaminative granules into oocyte cytoplasm and further results in parthenogenetic reproduction, cytoplasmic disorder of

physiology or biochemistry, and the second one is selection of sperm with abnormal chromosome. So it is important to normalize the manipulation and avoid injecting of exogenous DNA or contaminative granule into oocyte cytoplasm. Mammal oocyte and embryo have the ability to repair DNA damage, which can correct the cellular injury caused by micromanipulation before rounding into embryo developmental abnormalities. ICSI is a safe and effective means for severe forms of male infertility, complying with manipulation regulations.

3.4 The relationship between the quality and number of embryos transferred and pregnancy outcome respectively

The quality and number of embryos transferred in pregnancy group and non-pregnancy group are listed in Table 4.

Table 4. T embryonic quality, numbers transferred and pregnancy outcome

Group	case	Number of 4-cell embryos transferred	Number of high quality embryos transferred	Number of embryos transferred
Pregnancy	36	2.28±0.78	2.44±0.61	2.97±0.45
Non-pregnancy	72	1.89±0.94	1.88±0.87	2.82±0.68
P		P<0.05	P<0.05	P>0.05

Outcome of IVF cycles is significantly correlated with the quality and number of embryos transferred. It

has been shown that embryo quality is an important predictor of pregnancy rates. Embryo developmental

stage, proportion of anuclear fragments and even state of blastomere are the main parameters to assess embryo quality. Normal embryos usually develop to 4-cell stage 48h after retrieval^[11]. Giorgetti^[12] found that 4-cell embryos gave rise to significantly higher rates of implantation than did other cleaving stage embryos in the study of embryo morphology with single embryo transfer cycles. It has been demonstrate that 4-cell embryos are more easy to implant compared with early- and late-cleaving embryos on day 2. Pregnancy rate increased with the increasing of 4-cell embryos transferred^[13]. Our study also found that 4-cell stage is an optimal cleavage status, with more 4-cell embryos transferred in pregnancy group than that in non-pregnancy group, indicating that the number of 4-cell embryos transferred is correlated with pregnancy. It is also infered from the Table 4 that the total number of good quality embryos is related with pregnancy rate positively.

According to the report by Elsner^[14], pregnancy rate was significantly higher when 3 embryos were transferred with increasing embryo number and did not, however, increase further more when transfer 4-6 embryos. Most patients were transferred with 3 embryos and no more than 4. Statistical analysis showed no significant difference in number of embryo transferred in the two groups.

This study demonstrates that the quality of embryo transferred has a close relationship with pregnancy rate. Selecting good quality embryo on the basis of morphology and cleavage stage help to raise pregnancy rate, reduce number of embryo transferred

and reduce multiple pregnancy as a result.

3.5 The effect of embryo culture condition on frozen-thawed embryo transfer (FET) outcome

From January 2003 to October 2003, and July 2004 to December 2004, 54 FET cycles were divided into non-controlled environment group and controlled environment group. Embryos were manipulated at super-clean bench in ordinary lab and laminar flow room, respectively. Cryopreservation were performed at super-clean bench in ordinary lab with non-controlled environment.

The thawing principle is that three embryo be thawed if frozen embryo >3, and thawing the remaining embryos until get 3 survival embryos to be transferred if embryo dead in the procedure. Frozen embryo should be thawed at the same time when it ≤3 and all survival embryos should be transferred. This study showed that the number of embryos thawed and dead is significantly lower in controlled environment group than that in non-controlled environment group. The pregnancy rate is, however, significantly higher than that of non-controlled environment group. With decreasing of dead embryo, the number of FET cycles increased and pregnancy chance increased as a result. Culture conditions play an important role in the outcome of IVF. In vitro culture of embryos should be performed at laminar flow room.

Table 5. The effect of embryonic culture condition on frozen embryo transfer result and pregnancy rate

group	cycle	Number of embryos thawed	Number of dead embryos	Pregnancy (case)	Pregnancy rate(%)
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non-controlled environment	30	3.53 ± 0.94	0.67 ± 0.88	8	26.7
controlled environment	24	2.96 ± 0.55	0.25 ± 0.44	13	54.2
P		P < 0.05	P < 0.05		P < 0.05

4. CONCLUSION

The long and short protocols of IVF-ET have the similar effects in numbers of retrieved oocyte and MII oocyte, fertilization rate, cleavage rate, high quality embryo rate and pregnancy rate, but using dose of Gn is much lower in the short protocol.

The clinical pregnancy rate of IVF become significantly lower in the women aged ≥ 35 years.

IVF and ICSI can produce the same effects in fertilization rate, cleavage rate, high quality embryo rate and pregnancy rate.

Number of 4-cell embryos and quality of embryos transferred is positively correlated with pregnancy rate.

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