[原著]Clinical outcome of in vitro fertilization-embryo transfer in patients over 40 years from a single institution in Guangdong, China

Clinical outcome of *in vitro* fertilization-embryo transfer in patients over 40 years from a single institution in Guangdong, China

Lin Tong[1,2], Keiko Mekaru[1], Chiaki Yagi[1], Kozue Asato[1], Tadatsugu Kinjyo[1], Hitoshi Masamoto[1], Zhang Xiulan[2] and Yoichi Aoki[1]

[1]*Department of Obstetrics and Gynecology, Graduate School of Medical Science, University of the Ryukyus, Okinawa, Japan, and*
[2]*Center for Reproductive Medicine and Infertility, Guangdong Armed Police General Hospital, Guangdong, China*

(Received on December 16, 2013, accepted on January 14, 2014)

ABSTRACT

Purpose: We retrospectively analyzed the clinical outcome of patients over 40 years who received *in vitro* fertilization-embryo transfer (IVF-ET) or intracytoplasmic sperm injection (ICSI) at a single center, and compared four different ovarian stimulation protocols to determine the most suitable method for use in these women. The aim of the study is to provide valuable information for use in the counseling of prospective IVF patients. **Patients and Methods:** The clinical records of 272 patients (aged ≥41 years) who received IVF-ET/ICSI at our institution between January 2008 and September 2011 were retrospectively analyzed. Four ovarian stimulation protocols were used: Group A received GnRH agonist long protocol with short-acting drugs, Group B received GnRH agonist long protocol with long-acting drugs, Group C received GnRH antagonist protocol, and Group D received microdose GnRH agonist protocol. **Results:** The mean age was 42.5 years (range, 41-49 years), and the median duration of infertility was 8.2 years (range, 0.5-22 years). The overall pregnancy rate, and live-birth rate per transfer were 20.3%, and 9.1%, respectively, and the abortion rate was 46.8%. The live-birth rate per transfer was decreased as follows: 15.6% at 41 years, 6.4% at 42 years, 6.3% at 43 years, and 4.5% over 44years. A significantly lower live-birth rate per transfer ($p = 0.0165$) was observed in age group ≥42 years than in 41 years of age. The age ≥42 years and the number of oocyte retrieval (≤4) were found to be an independent unfavorable factor for both pregnancy and live birth by a multivariate analysis. Groups A and B received the highest dosage of HMG injection and the longest HMG treatment, and had the highest oocyte retrieval, whereas group D received the lowest HMG dosage over the shortest period, but had the highest cycle cancelation rate and the fewest oocytes ($p < 0.01$). Group C had a lower HMG dosage and fewer HMG medication days than Groups A and B ($p < 0.01$). However, no significant differences were observed in the pregnancy rates among patients among the four groups. Conclusions: Age, in particular ≥42 years, and the less oocyte retrieval are found to be a predictive factor for the treatment failure. For the decreased medication costs and patient discomfort, use of GnRH antagonist or microdose GnRH agonist for ovarian stimulation seem to be better in patients over 40 years. *Ryukyu Med. J., 32(3,4) 79～88, 2013*

Key words: infertile patients over 40 years, IVF-ET/ICSI, controlled ovarian stimulation, microdose GnRH agonist, GnRH antagonist

INTRODUCTION

The first Chinese test-tube baby was born in 1988 at the Center of Reproductive Medicine, Third School of Clinical Medicine, Peking University. According to statistics provided by the China Population Association, over 40 million Chinese people are found to be infertile in 2012, accounting for 12.5% of the country's reproductive population. Twenty years ago, this rate was only 3%[1]. The increasing prevalence of an unhealthy lifestyle (obesity, inappropriate diet, high alcohol intake, smoking, and insomnia), the tendency to marry late in life, and the everyday stress of work probably contribute to the rapid rise in infertility. Another important reason for this is the increasing environmental pollution, which leads to increase in spermatogenic cell disorders and lower sperm quality, manifesting as azoospermia, oligospermia, and asthenozoospermia[2].

Although there have been many developments in assisted reproductive technology (ART), the management of older patients has been one of the most difficult challenges, and most of the solutions proposed have yielded disappointing results. Spandorfer et al. showed that the rate of embryo implantation diminishes annually by 2.77% in a woman after the age of 35 years[3]. Aging of the ovaries seems to play a key role in this process, comprising both follicle reduction and oocyte quality deterioration[4].

The pregnancy rate of infertile women treated with in vitro fertilization-embryo transfer (IVF-ET) is approximately 35%[1]. However, in a retrospective series of 428 cycles of IVF in women aged ≥41 years in China, Yang et al. reported that the clinical pregnancy rate per transfer was 19.4%[5]. Wang et al. retrospectively analyzed the IVF outcome for women in different age groups and found the clinical pregnancy rate per transfer to be 13.9% in women aged ≥40[6]. In a retrospective database analysis of 2,705 ART cycles, Klipstein et al.[7] reported that the overall live birth rate per initiated cycle was 9.7% and the overall abortion rate was 32.6% for women at aged ≥40 years. In 2000, Ron-El et al.[8] reported that the cycle retrieval rate was 87%, pregnancy rate per oocyte retrieval was 12.4%, and live birth rate per oocyte retrieval was 4.5% in women aged ≥41 years. In addition, they reported no live births for women aged ≥44 years, and no pregnancies were observed in women aged ≥45 years. Serour et al.[9] retrospectively analyzed 1,645 women aged ≥40 years who underwent 2004 fresh non-donor IVF/ICSI cycles; they found that the overall live-birth rate per initiated cycle was 6.7%, and that the live-birth rate per initiated cycle in women aged <43 years was significantly higher than that in women aged ≥43 years. These findings, including the finding of the present study, indicate that pregnancy and live-birth rates fall with concurrent rise miscarriage rates in women >40 years of age. The age of woman is therefore the most important factor in determining the pregnancy success rate, even after ART. In August 2013, the Fertility Clinic Report Displays 2011 ART results, and success rates for individual U.S. fertility clinics, showed the range of birth rate per transfer as being 8.8%-35% (aged 41-42 years of age)[10]. Women aged ≥40 years have a reduced ovarian reserve, and various treatment protocols have been proposed aiming to increase this ovarian response.

In the present study, we retrospectively analyzed the clinical outcome of 272 patients over 40 years who received IVF-ET or intracytoplasmic sperm injection (ICSI) at a single center. In addition, we compared four different ovarian stimulation protocols to determine the most suitable method for use in these women. The aim of the study is to provide valuable information for use in the counseling of prospective IVF patients.

PATIENTS and METHODS

Patients

We retrospectively analyzed the clinical records of 272 patients (aged ≥41 years) who received IVF-ET/ICSI at the Center for Reproductive Medicine and Infertility, Guangdong Armed Police General Hospital between January 2008 and September 2011 for their initiated treatment cycles. This study was conducted according to the principles stated in the Declaration of Helsinki (1964) and all subsequent revisions, and was approved by the Institutional Review Board of Guangdong Armed Police General Hospital and University of the Ryukyus (#198). Several different ovarian stimulation protocols were used, and the regimes were thus categorized into four

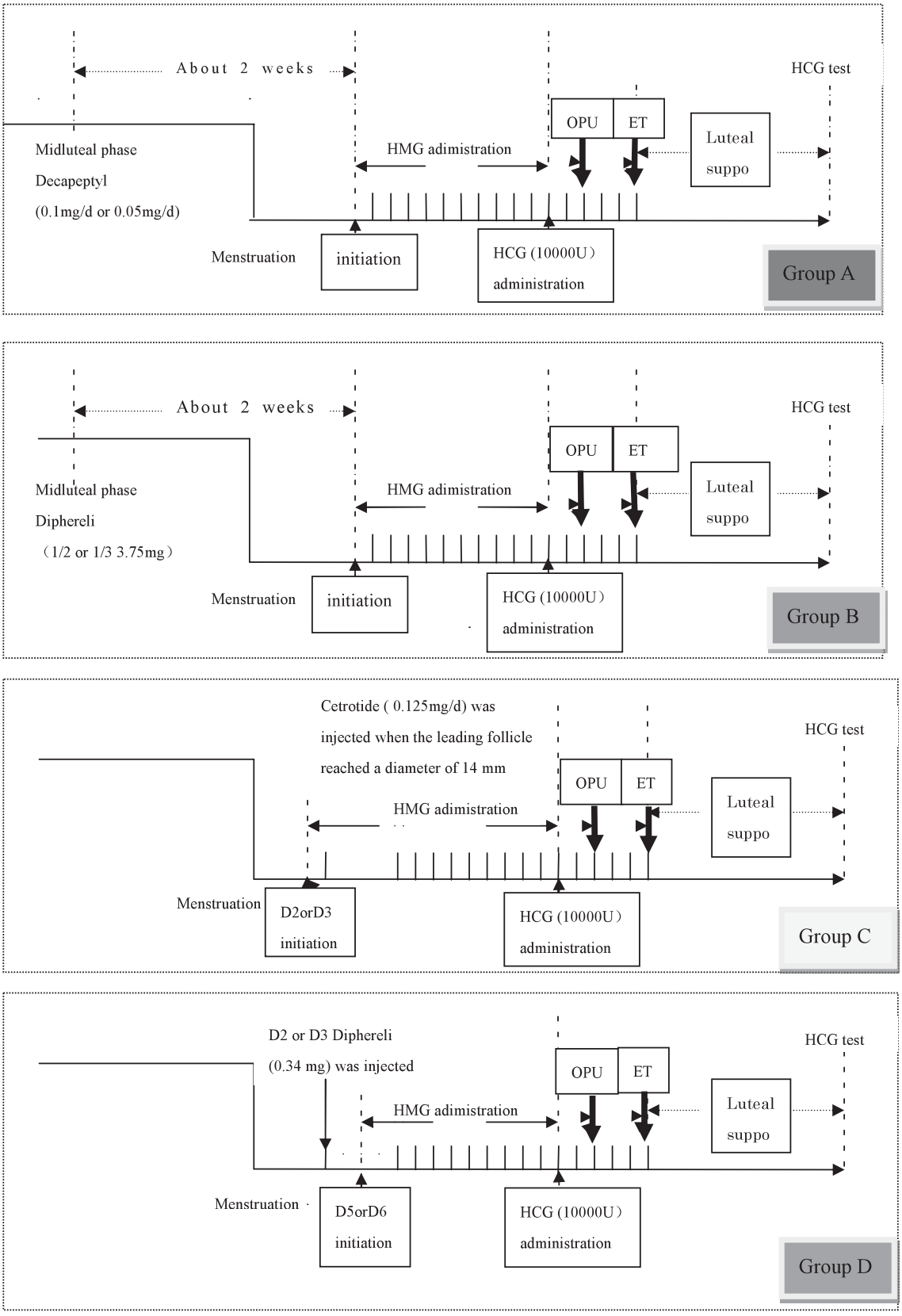

Fig. 1 **Ovarian stimulation protocols.** Group A received GnRH agonist long protocol with short-acting drugs, Group B received GnRH agonist long protocol with long-acting drugs, Group C received GnRH antagonist protocol, and Group D received microdose GnRH agonist protocol.

groups (Figure 1): Group A received gonadotropin releasing hormone (GnRH) agonist long protocol with short-acting drugs (77 cycles), Group B received GnRH agonist long protocol with long-acting drugs (30 cycles), Group C received GnRH antagonist protocol (72 cycles), and Group D received microdose GnRH agonist protocol (93 cycles).

Ovarian stimulation protocols (Fig. 1)

Group A: GnRH agonist (Decapeptyl®; Ferring GmbH Ltd, Wittland, Germany) was subcutaneously injected (0.1 mg/day or 0.05 mg/-day) everyday from the midluteal phase until the day of human chorionic gonadotropin (HCG; Livzon Pharmaceutical Co. Ltd, Guangdong, China) administration. The standard of down-regulation was defined as follows: luteinizing hormone (LH) < 5 IU/L, estradiol (E2) < 50 pg/ml, endometrium thickness ≤ 5 mm, and maximum follicle diameter < 10 mm. When this standard was reached, the follicle-stimulating hormone (FSH; Gonal-f; Merck Serono Ltd, London, United Kingdom) or human menopausal gonadotropin (HMG; Menotrophin, Livzon Pharmaceuticals Co. Ltd, Guangdong, China) treatment was initiated (150-400 IU) and administered daily until the day of HCG administration. During this period, HMG dosage was adjusted depending on the results of transvaginal ultrasound examination and serum hormone levels.

Group B: In the preceding midluteal phase, a GnRH agonist (Diphereli ; Beaufour Ipsen

Pharmaceutical Ltd., Paris, France) was subcutaneously injected at one-half to one-third the dose of 3.75 mg. When the standard of down-regulation was reached, HMG (150-400 IU) treatment was initiated and administered daily until the day of HCG administration. During this period, HMG dosage was adjusted as described for Group A.

Group C: On day 2-3 of menstruation, when the endometrium thickness was ≤5 mm, maximum follicle diameter was <10 mm, and E2 was <50 pg/ml, HMG (150-400 IU) treatment was initiated and administered daily until the day of HCG administration. When the leading follicle reached a diameter of 14 mm, a GnRH antagonist (Cetrotide®; Merck Serono Ltd., Switzerland) was injected subcutaneously at a dosage of 0.125 mg daily until the day of HCG administration. During this period, the HMG dosage was adjusted depending as described for Group A.

Group D: On days 2-3 of menstruation, when the endometrium thickness was ≤5 mm, maximum follicle diameter was <10 mm, and E2 was <50 pg/ml, GnRH agonist (Diphereli; Beaufour Ipsen Pharmaceutical Ltd., Paris, France) was subcutaneously injected (dosage of 0.34 mg). On days 4-6 of menstruation, HMG (225-400 IU) treatment was initiated and administered daily until the day of HCG administration. During this period, HMG dosage was adjusted as described for Group A.

These four groups were administered HCG (10,000 IU) when the leading follicles reached a mean diameter of 18 mm (as detected by transvaginal ultrasound), and transvaginal oocyte retrieval was scheduled at 34-36 h after HCG injection. Depending on the quality of sperm obtained, conventional IVF or ICSI was performed to fertilize the eggs (WHO Laboratory Manual for the Examination and Processing of Human Semen)[5]. ET with good quality embryos was performed under ultrasound guidance on day 3. At our institution, no more than two embryos at a time are transferred in women <35 years of age, and 2 or 3 embryos are transferred in women ≥ 35 years of age. Luteal support was provided with intramural progesterone (Progeston; General Pharmaceutical Co. Ltd, Shanghai, China) in patients whose embryos were transferred. Chemical pregnancy was determined using HCG test 14 days after oocyte retrieval, and clinical pregnancy was defined as the presence of a gestational sac accompanied by fetal heartbeat on ultrasound examination 4 weeks after ET.

Statistical Analyses

Statistical analyses were performed using the Software JMP (ver. 10.0.2; SAS Institute Inc., Cary, NC, U.S.). The results were expressed as mean ± standard deviation or rate (%). Fisher's exact test was used for rate comparisons. Normally distributed outcome variables were analyzed with the Student's t-test and Tukey-Kramer HSD test, while non-normally-distributed outcome variables were analyzed with the Kruskal-Wallis and Steel-Dwass tests as appropriate. Multiple logistic regression analysis was used for infertility in

relation to age, infertility duration, serum E2 level, serum FSH/LH ratio and oocytes retrieval. P < 0.05 was considered statistically significant.

RESULTS

Table 1 summarizes patient characteristics. The mean age was 42.5 years (range, 41-49 years), and the median duration of infertility was 8.2 years (range, 0.5-22 years). Infertility was attributed to several different factors: disorders of pelvic cavity and fallopian tube (65.1%), poor ovarian reserve function and ovulation failure (5.9%), endometriosis (2.2%), problems in the male partner (35.3%), and unknown factors (9.6%). Baseline FSH levels were 8.0 mIU/ml (range, 0.25-48.4 mIU/ml). Ovarian stimulation cycle was initiated in 272 patients were initiated and 41 cycles were cancelled. Eventually, embryo transfer was performed in 231 women and 47 patients became pregnant. The overall pregnancy rate, and live-birth rate per transfer were 20.3%, and 9.1%, respectively, and the abortion rate was 46.8%. When all the initiated cycles were divided by age, the live-birth rate per transfer decreased as follows: 15.8% at 41 years, 6.4% at 42 years, 6.3% at 43 years, and 4.5% over 44years. There was a trend of decreased live-birth rate when compared patient group of 41 years with that of 42 years and that of ≥44 years (p = 0.0572 and 0.0577, respectively) (Fig. 2). A significantly lower live-birth rate per transfer (p = 0.0165) was observed in age group ≥42 years than in 41 years of age.

The patient characteristics and clinical outcomes in both the pregnant and non-pregnant groups are summarized in Table 2. The mean age was significantly lower in the pregnant group (p = 0.0078). The number of retrieved oocytes and the rate of IVF fertilization in the pregnant group were higher than those in the non-pregnant group (p <0.0001 and p = 0.003, respectively). However, no significant intergroup differences were found in the following parameters: duration of fertility, baseline serum FSH level, baseline serum FSH/LH ratio, baseline serum E2 level, HMG dosage and HMG medication days, MII rate, ICSI fertility rate, high quality rate, and ovarian hyperstimulation syndrome (OHSS) rate. When these variables were analyzed by a logistic regression analysis, the age (≥ 42) (OR 2.534, 95% CI, 1.287-5.032, p = 0.0073 for pregnancy, and OR 2.715, 95% CI, 1.071-7.103, p = 0.035

Table 1 **Patient characteristics and overall cycle outcomes in 272 initiated cycles in women aged over 40 years**

Variable	
Age (years) mean (range)	42.5 (41-49)
Duration of fertility (years) median (range)	8.2 (0.5-22)
Infertility factor	
Pelvic cavity and fallopian tube problems	177 (65.1%)
Poor ovarian reserve function and ovulation failure	16 (5.9%)
Endometriosis	6 (2.2%)
Unknown factor	26 (9.6%)
Male factor	96 (35.3%)
Baseline FSH (mIU/ml) (range)	8.0 (0.25-48.8)
Baseline FSH/LH (range)	3.0 (0.06-28.2)
Baseline E2 (pg/dl) (range)	37.0 (0.17-105)
Cycles initiated	272
Cancellation	41 (15.1%)
Embryo transfer	231 (84.9%)
Cinical pregnancy	47
(rate per initiated cycle, transfer)	(17.3%, 20.3%)
Abortion	22 (46.8%)
Live birth	21
(rate per initiated cycle, transfer)	(7.7%, 9.1%)
Ectopic pregnancy	1
Lost follow up	3

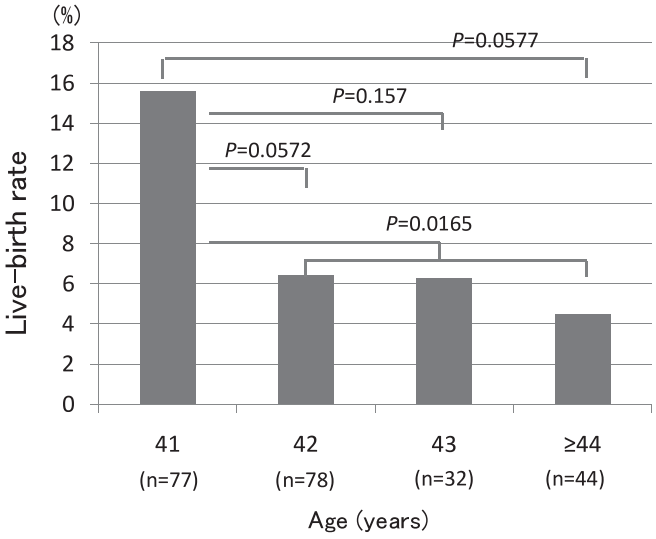

Fig. 2 **Live-birth rate per initiated cycle by age.** When all the initiated cycles were divided by age, the live-birth rate per transfer decreased as follows: 15.6% at 41 years, 6.4% at 42 years, 6.3% at 43 years, and 4.5% ≥44years. There was a trend of decreased live-birth rate when compared patient group of 41 years with that of 42 years and that of ≥44 years (p = 0.0572 and 0.0577, respectively). A significantly lower live-birth rate per transfer (p = 0.0165) was observed in age group ≥42 years than in 41 years of age.

for live birth) and the number of oocyte retrieval (≤ 4) (OR 4.944, 95% CI, 2.404-10.91, p <0.0001 for pregnancy, and OR 5.401, 95% CI, 1.891-19.48, p = 0.0011 for live birth) were found to be an independent unfavorable factor for both pregnancy and live birth (Table 3).

Table 4 shows the clinical characteristics and IVF-ET/ICSI outcomes for patients following the four different ovarian stimulation protocols. No significant intergroup differences were found in the following parameters: age, duration of infertility, basal serum FSH level, serum FSH/LH ratio, and basal serum E_2 level. Groups A and B received the highest dosage of HMG injection and the longest HMG treatment, and had the highest oocyte retrieval, whereas group D received the lowest HMG dosage over the shortest period, but had the highest cycle cancelation rate and the fewest oocytes (p < 0.01). Group C had a lower HMG dosage and fewer HMG medication days than Groups A and B (p < 0.01). However, no significant differences were observed in the pregnancy rates among patients among the four groups.

Table 2 **Patient characteristics and overall cycle outcomes in the pregnant group and nonpregnant group**

	Pregnant (n=47)	Non-pregnant (n=225)	p-value*
Age (mean ± SD)	42.0 ± 1.4	42.6 ± 1.7	0.0078
Duration of infertility (years)	9.0 ± 5.9	8.0 ± 5.4	0.296
Baseline serum FSH level (mIU/ml)	7.5 ± 2.1	8.1 ± 4.5	0.394
Baseline serum FSH/LH ratio	2.7 ± 1.4	3.1 ± 2.4	0.352
Baseline serum E2 level (pg/dl)	36.2 ± 16.9	37.2 ± 20.4	0.761
HMG dosage (x75IU)	26.7 ± 10.6	30.0 ± 11.2	0.066
HMG medciation days (days)	8.8 ± 2.5	9.4 ± 2.5	0.117
No. of oocyte retrieval	7.4 ± 3.9	5.3 ± 5.2	<0.0001
MII rate (%)	83.4	83.9	0.433
IVF fertility rate (%)	86.8	70.2	0.003
ICSI fertility rate (%)	87.7	84.8	0.182
High quality rate (%)	57.6	57.6	0.521
OHSS rate (%)	0.8	0.76	0.681

*Normally distributed outcome variables were analyzed with the Student's t test, while non-normally-distributed outcome variables were analyzed with Kruskal-Wallis test.
HMG: human menopausal gonadotropin, MII: metaphase II, IVF: in vitro fertilization, ICSI: intracytopoasmic sperm injection,
OHSS: ovarian Hyperstimulation syndrome

Table 3 **Multivariate analysis predicting infertility**

Variables	Pregnancy			Live birth		
	OR	95% CI	p-value	OR	95% CI	p-value
Age (≥42)	2.534	1.287-5.032	0.0073	2.715	1.071-7.103	0.035
Infertility period (≥12Mo)	0.548	0.262-1.167	0.117	1.237	0.416-4.555	0.717
No. of oocytes (≤4)	4.944	2.404-10.91	<0.0001	5.401	1.891-19.48	0.0011
Serum E2 level (≥33.9 pg/dl)	1.073	0.547-2.108	0.838	1.267	0.499-3.267	0.618
Serum FSH/LH ratio (≥2.54)	0.894	0.450-1.771	0.747	0.734	0.283-1.878	0.517

OR: odds ratio. CI: confidence interval

Table 4 **Patient characteristics and overall cycle outcomes in four different ovarian stimulation groups**

	Group A GnRH agonist long protocol with short-acting	Group B GnRH agonist long protocol with long-acting	Group C GnRH antagonist protocol	Group D microdose GnRH agonist protocol
Cycles	n=77	n=30	n=72	n=93
Age	42.4 ± 1.7	42.1 ± 1.3	42.4 ± 1.7	42.7± 1.6
Duration of fertility	8.0 ± 5.4	7.7 ± 5.3	7.4 ± 5.1	9.1 ± 6.0
Baseline FSH (mIU/ml)	7.9 ± 3.9	7.0 ± 2.0	7.5 ± 2.4	8.9 ± 5.7
Baseline FSH/LH	3.2 ± 3.2	2.7 ± 1.5	2.8 ± 1.3	3.1 ± 2.3
Baseline E2 (pg/dl)	36.5 ± 19.9	39.4 ± 16.1	34.7 ± 19.6	38.4 ± 21.0
HMG dosage (x75 IU)	36.4± 8.6*#	35.4±10.2*#	28.4± 8.2*	22.6± 11.0
HMG medication days	10.1± 1.9*#	10.9± 2.0*#	8.9 ± 1.8*	8.4 ± 3.1
Oocyte retrieval	6.1 ± 3.9*@	10.6 ± 9.0*$	5.9 ± 4.4*	3.5 ± 2.8
MII rate (%)	84.9	83.7	81.1	85.8
IVF fertility rate (%)	75.0	69.4	60.9	68.0
ICSI fertility rate (%)	82.0	82.3	79.2	80.0
High quality rate (%)	59.2	52.9	56.3	61.5
Implantation rate (%)	9	11.6	8.2	12.8
Cycle cancellation rate (%)	10.4*	13.3*	8.3*	24.7
Clinical pregnancy rate (%)	17.4	23.1	16.7	25.7
Abortion rate (%)	50	50	36.4	50
OHSS rate (%)	0.7	1.0	0.8	0.6
Live birth rate (%)	7.2	11.5	10.6	8.6

*p < 0.01; vs. Group D, #p < 0.01; vs. Group C, $ p < 0.05; vs. Group C, @p < 0.05; vs. Group B. Normally distributed outcome variables were analyzed with Tukey-Kramer HSD test, while non-normally-distributed outcome variables were analyzed with Steel-Dwass test.
HMG: human menopausal gonadotropin, MII: metaphase II, IVF: in vitro fertilization, ICSI: intracytopoasmic sperm injection, OHSS: ovarian Hyperstimulation syndrome

DISCUSSION

As expected, our findings revealed that patients with poor outcomes were older (in particular, ≥42 years), and had lower oocyte retrieval. Many studies have investigated factors affecting successful ART outcomes for infertile women aged ≥40 years[5-9, 11, 12]. The literature reviews were summarized in Table 5. The clinical pregnancy rates were 7.3 to 17.3% per initiated cycle and 17.9 to 29.4% per transfer, and the live-birth rates were 4.7 to 16.0% per initiated cycle and around 9% per transfer. Furthermore, the abortion rates were very high as indicated as 26.7 to 46.8%. A poor ovarian response could be caused by either idiopathic factors or other factors relevant

Table 5 **Literature review of artificial reproductive technology outcomes in women aged over 40 years**

Author (year)[ref]	Treatment period	Age (years)	No. of cycles /patients	Clinical pregnancy rate (%)	Abortion rate (%)	Live birth rate (%)
Yang et al. (2012)[5]	2008.1～2011.12	≥41	428/NA	19.4 /transfer	36.1	NA*
Wang et al. (2005)[6]	2002.1～2005.5	≥40	56/NA	29.4 /transfer	26.7	NA
Klipstein et al. (2005)[7]	1999.1～2002.6	≥40	2705/1263	14.8 /initiated cycle	32.6	9.7 initiated cycle
Tsafrir et al. (2007)[11]	1995～2004	≥40	1217/381	7.3 /initiated cycle	33	4.7 /initiated cycle
Fujimoto et al. (2009)[12]	2001～2006	≥40	119/119	NA	NA	16 /initiated cycle
Ron-EI et al. (2000)[8]	1993～1998	≥41	431/376	12.4 /oocyte retrieval	NA	4.5 /oocyte retrieval
Serour et al. (2010)[9]	2003.1～2008.9	≥40	2386/1645	13.4 /initiated cycle 17.9 /transfer	44.8	6.7 /initiated cycle 8.8 /transfer
Current study	2008.1～2011.9	≥41	272/272	17.3 /initiated cycle 20.3 /transfer	46.8	7.7 /initiated cycle 9.1 /transfer

* NA: not assessed

to the condition of the patient's health such as age, diminished ovarian reserve, endometriosis, and prior ovarian surgery. These findings, including the finding of the present study, indicate that pregnancy and live-birth rates fall with concurrent rise miscarriage rates in women >40 years of age. The age of woman is therefore the most important factor in determining the pregnancy success rate, even after ART.

At present, the management of infertile women with a poor ovarian response (including older women) is very challenging. Ovarian stimulation protocols aim to enhance follicular recruitment and avoid spontaneous ovulation. The enhancement of sensitivity for patients in response to controlled ovarian hyperstimulation is a pivotal factor associated with successful clinical pregnancy during the IVF-ET treatment[13]. We compared four different ovarian stimulation protocols to determine the most suitable one for use in this group of women. In traditional GnRH agonist protocols, the GnRH agonist is administered in the luteal phase, awaiting down-regulation prior to initiation of gonadotropins. The GnRH antagonist leads to immediate, rapid gonadotropin suppression by competitive blocking of GnRH receptors in the anterior pituitary gland, thereby preventing endogenous GnRH from inducing LH and FSH release from the pituitary cells[14-16]. In the GnRH agonist microdose protocol, the dose of GnRH agonist is reduced to the lowest dose that can successfully induce endogenous gonadotropin release[17].

The use of GnRH antagonists in ovarian stimulation for older patients has given clinicians a new perspective. Several studies comparing antagonist protocols with the traditional protocols have reported no differences in implantation or clinical pregnancy rates, although the number of embryos available for transfer is higher when using the antagonist protocols[18-20]. In our study, the implantation and pregnancy rates were similar among the four protocols, but the dosage of HMG and the number of cycles canceled in women receiving the antagonist protocol was lower than those in women receiving the traditional GnRH agonist protocols.

Traditional GnRH agonist regimens cause significant increases in follicular-phase serum progesterone and androgen levels; however, these increases may exert deleterious effects on follicular development and oocytes quality[21, 22]. To minimize this effect while maintaining the benefits of stimulating the release of endogenous gonadotropins, the administration of lower doses of GnRH agonist has been proposed[23]. A recent study reported that a microdose GnRH agonist treatment attenuated the endometrial expression of endothelial nitric oxide synthase, suggesting that this effect might improve the endometrial

receptivity[24]. The high implantation rate achieved using microdose GnRH agonist may be caused by a reduction in the levels of endothelial nitric oxide synthase, leading to an improved implantation rate for poor responders in IVF procedures. In the present study, trends toward higher implantation and clinical pregnancy rates were noted for those who were given the microdose GnRH agonist protocol (i.e., Group D) compared with those administered other protocols (i.e., Groups A-C). In addition, the procedure is superior to others because of the total reduction in the HMG dose. However, this approach could potentially cause an increased incidence of spontaneous LH surges and premature ovulation[25]. In our study, the cancelation rate under the microdose flare protocol was significantly higher than that of the remaining protocols, possibly due to the advanced age of the patients.

In comparison with the traditional GnRH agonist protocols, there was no significant difference in the clinical pregnancy rate and birth rate in the GnRH antagonist group and microdose GnRH agonist group. However, a decreased HMG dose and HMG injection period were observed in the latter two groups in our study, thereby leading to decreased medication costs and decreased patient discomfort. Nevertheless, in the absence of a sufficient number of adequately designed prospective trials with consistent inclusion criteria, it is extremely difficult to conclusively demonstrate the superiority of any single protocol for all poor responders.

REFERENCES

1) English.news.cn: China Focus, Increasing infertility concerns Chinese couples.12: 18, 2012.
2) Wang J.P.: The investigation report of the current state of Chinese infertility. Chinese Infertility Summit Forum. 10: 16, 2009.
3) Spandorfer S.D., Chung P.H., Kligman I., Liu H.C., Davis O.K. and Rosenwaks Z.: An analysis of the effect of age on implantation rates. J Assist Reprod Genet. 17: 303-306, 2000.
4) Faddy M.J., Gosden R.G., Gougeon A., Richardson S.J. and Nelson J.F.: Accelerated disappearance of ovarian follicles in mid-life: implication forecasting menopause. Hum Reprod. 7: 1342-1346, 1992.
5) Yan J., Wu K., Tang R., Ding L. and Chen Z.J.: Effect of maternal age on the outcome of *in vitro* fertilization and embryo transfer (IVF-ET). Sci China Life Sci. 55: 694-698, 2012.
6) Wang J.X., Sun H.X., Hu Y.L., Wang B., Zhang N.Y. and Chen H.: Influence of female age on the pregnancy and obstetric outcome in *in vitro* fertilization and embryo transfer. Zhonghua Nan Ke Xue. 11: 900-903, 2005.
7) Klipstein S., Regan M., Ryley D.A., Goldman M.B., Alper M.M. and Reindollar R.H.: One last chance for pregnancy: a review of 2705 *in vitro* fertilization cycles initiated in women age 40years and above. Fertil Steril. 84: 435-445, 2005.
8) Ron-EI R., Raziel A., Strassburger D., Schachter M., Kasterstein E. and Friedler S.: Outcome of assisted reproductive technology in women over the age of 41. Fertil Steril. 74: 471-475, 2000.
9) Serour G., Mansour R., Serour A., Aboulghar M., Amin Y., Kamal O., Al-Inany H. and Aboulghar M.: Analysis of 2,386 consecutive cycles of *in vitro* fertilization or intracytoplasmic sperm injection using autologous oocytes in women aged 40 years and above. Fertil Steril. 94: 1707-1712, 2010.
10) http://www.cdc.gov/art/ART2011/PDFs/ART_2011_Clinic_Report-Full.pdf
Assisted Reproductive Technology: 2011 Fertility Clinic Success Rates Report. U.S. Department of Health and Human Services. August 2013.
11) Tsafrir A., Simon A., Revel A., Reubinoff B., Lewin A. and Laufer N.: Retrospective analysis of 1217 IVF cycles in women aged 40 years and older. Reprod Biomed Online. 14: 348-355, 2007.
12) Fujimoto A., Fujiwara T., Oishi H., Hirata T., Yano T. and Taketani Y.: Predictive factors of successful pregnancy after assisted reproductive technology in women aged 40 years and older. Reprod Med Biol. 8: 145-149, 2009.
13) Templeton A., Morrian J.K. and Parslow W.: Factors that affect outcome of *in vitro* fertilization treatment. Lancet. 348: 1402-1406, 1996.
14) Gordon K. and Hodgen G.D.: GnRH agonists and antagonists in assisted reproduction.

Baillieres Clin Obstet Gynaecol. 11: 247-265, 1992.
15) Borm G. and Mannaerts B.: Treatment with the gonadotropin releasing hormone antagonist ganirelix in women undergoing ovarian stimulation with recombinant follicle stimulating hormone is effective, safe and convenient: results of a controlled, randomized, multicentre trial. The European Orgalutran Study Group. Hum Reprod. 15: 1490-1498, 2000.
16) Al-Inany H. and Aboulghar M.: GnRH antagonist in assisted reproduction: a Cochrane review. Hum Reprod. 17: 874-885, 2002.
17) Scott R.T. and Navot D.: Enhancement of ovarian responsiveness with microdoses of gonadotropin-releasing hormone agonist during ovulation induction for *in vitro* fertilization. Fertil Steril. 61: 880-885, 1994.
18) Fasouliotis S.J., Laufer N., Sabbagh-Ehrlich S., Lewin A., Hurwitz A. and Simon A.: Gonadotropin-releasing hormone (GnRH)-antagonist versus GnRH-agonist in ovarian stimulation of poor responders undergoing IVF. J Assisted Reprod Genet. 20: 455-460, 2003.
19) Mohamed K.A., Davies W.A., Allsopp J. and Lashen H.: Agonist "flare-up" versus antagonist in the management of poor responders undergoing *in vitro* fertilization treatment. Fertil Steril. 83: 331-335, 2005.
20) Mekaru K., Asato K., Yagi C. and Aoki Y.: Comparison between the gonadotropin-releasing hormone antagonist protocol and the gonadotropin-releasing hormone agonist long protocol for controlled ovarian hyperstimulation in the first *in vitro* fertilization-embryo transfer cycle in an unspecified population of infertile couples. Reprod Med Biol. 11: 79-83, 2012.
21) San Roman G.A., Surrey E.S., Judd H.L. and Kerin J.F.: A prospective randomized comparison of luteal phase versus concurrent follicular phase initiation of gonadotropin-releasing hormone agonist for *in vitro* fertilization. Fertil Steril. 58: 744-749, 1992.
22) Gelety T.J., Pearlstine A.C. and Surrey E.S.: Short-term endocrine response to gonadotropin-releasing hormone agonist initiated in the early follicular, mid luteal or late luteal phase in normally cycling women. Fertil Steril. 64: 1074-80, 1995.
23) Schoolcraft W.B., Surrey E.S., Minaret D.A., Stevens J.M. and Gardner D.K.: Management of poor responders: can outcomes be improved with a novel gonadotropin releasing hormone antagonist/letrozole protocol? Fertil Steril. 89: 151-156, 2008.
24) Tarlatzis B.C., Zepiridis L., Grimbizis G. and Bontis J.: Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum Reprod Update. 9: 61-76, 2003.
25) Surrey E.S.: Management of the poor responder: the role of GnRH agonists and antagonist. J Assisted Reprod Genet. 24: 613-619, 2007.