Assisted Reproductive Technology and Related Clinical Factors

Zuan-chong FENG, Zhong-mei LI
Shanghai Institute of Planned Parenthood Research, Shanghai 200032, China

Key Words: ART; IVF-ET; clinical factors

As a widely-applied clinical therapy for infertility and sterility, ART has its own merits compared to other techniques and operations; however, the outcome of ART is influenced by various factors ranging from age, endometrial receptivity, reproductive system condition (e.g. uterus, fallopian tube and pelvic factors, etc.), immune system and so on. From our clinical experience and the literatures reviewed, it is strongly recommended that the situation of every infertile couple be evaluated thoroughly before applying therapy.

Age

More and more attention was paid to the relationship between advanced female reproductive age (or old age) and ART. The decrease of female fecundity began in the 30’s, becoming more pronounced after 40. There is an approximately 50% decrease in the fecundity rate of women attempting pregnancy at the age of 40 or over compared with younger women, and a twofold to threefold increase in the rate of spontaneous abortions. In addition to the pelvic organ diseases and endocrine disorders, the main cause of ART failure is the aging of oocyte and granulosa cell, and then is the reduction of endometrium receptivity, which may be related to the blood flow of uterus [1]. Ovarian reserve tests, e.g. the basal level of FSH, E2, and inhibin, FSH/LH ratio and real-time stimulation test (e.g. CC, GnH or GnRH test), should be done in women who are more than 35 years old; and transvaginal sonography (TVS) should also be done to evaluate the ovarian volume, follicular development, blood flow, etc. Some authors suggest that the basal level of FSH is more effective in prospecting the rate of cycle canceling and pregnancy in IVF program than age [2].

Increasing age of patient is associated with poor ovarian response, as represented by smaller ovarian volume, lower antral follicle count, and poor stromal vascularity. Three-dimensional power Doppler ultrasonography can help to individualize IVF in patients regardless of age [3].

Corresponding author: Zhong-mei Li; E-mail: lizhongmei2003@yahoo.com.cn
Of the parameters tested, total antral follicle count showed the best correlation with women's age and declined linearly at a rate of 3.8% per year.  

Countermeasure It is most important for women of advanced reproductive age to evaluate ovary reserve before ART. The prognosis of ART can be improved by increased dosage, prolonged course of ovarian stimulation, co-culture, preimplantation genetic diagnosis (PGD), and so on. Oocyte donation, nuclear or cytoplasmic transfer and oocyte cryopreservation should be further studied. Patients who have insufficient blood flow in uterus and ovaries need medicines administration to promote blood supply and endometrium development. For premature ovarian failure, 3-6 cycles of hormone replacement treatment can improve symptoms and decrease the level of serum FSH and LH, which is possibly helpful to making the ovulation induction successful in some cases (ovarian resistance syndrome). In ART clinical practice, relationship between age and pregnancy rate is well documented.

Uterine factors

Uterine factors include gross or slight pathological changes, endometrial receptivity, etc. It is valuable to enquire about all suspected endometrial lesion or hypoplasia history, including intrauterine surgery, infection, tuberculosis, anomalies (e.g. incomplete uterine septum), submucous myoma, polyp and their previous therapeutic results. TVS and hysteroscopy examination are useful to finding intrauterine gross and slight (e.g. localized endometrial hyperplasia or hypoplasia, mild intrauterine adhesions, endometritis, etc.) pathological changes, and they should be the routine examination before ART. TVS is used to measure endometrial thickness. At oocyte retrieval, the thickness of endometrium should not be less than 8 mm. Retrospective studies showed the relationship between pregnancy rate and hysteroscopy in "implantation window" stage in ART program. The result shows that early miscarriage rate in women with ring-type glandular openings and well-developed vascular networks on the endometrial surface is significantly lower than that of women with dot and/or punctate-type glandular opening and poor vasculature. If necessary, endometrial biopsy should be taken for evaluation during luteal phase.  

Countermeasure TVS and hysteroscopy should be performed within 1-3 cycles before ART. If possible, endometrium in "implantation window" and luteal peak phase should be evaluated. Endometrium dysplasia should be given treatments, including different type, dosage and program estrogen-progestin stimulation therapy for endometrial development, and improvement of uterine artery blood flow, e.g. aspirin, viagra, and so on. It is reported that 4 cases with uterine artery blood flow deficiency and endometrium dysplasia (endometrial thickness less than 8 mm), after repeated IVF failure, a vaginal viagra suppository were administrated. The result showed blood flow increased in all cases during ovulatory phase. Three of the four patients conceived successfully after IVF-ET, whose endometrial thick-
ness reached 10 mm; the failed case had a history of intrauterine synechiae. If IVF-ET fails for 3 times, excluding laboratory technology and other clinical factors, hysteroscopy examination should be considered to find slight pathological changes (such as localized endometrial hyperplasia, polyp and adhesion), which are possibly caused by superovulative stimulation and repeated intrauterine procedure, and after treatment pregnancy rate of IVF can be improved.

Pelvic and/or tubal factors

According to different pathological conditions, e.g., the position, degree of tubal blocking, tubal macroscopic appearance and tubal wall thickness, pelvis and fallopian tube (e.g. the tenderness, thickness, utica intima destruction of fallopian tube, hydrosalpinx and the density, position and range of peritubo-ovarian adhesion), surgeons’ skill and patients’ desire, combined laparoscopy and hysteroscopy reconstructive surgery or IVF-ET should be performed to give patient a chance of fecundity. For serious pathological changes, fecundity can hardly be recovered after difficult surgeries. IVF may be the first choice for this kind of patients. Laparoscopy or laparotomy, such as adhesion dissection, especially involucral adhesion of peritubo-ovarian areas (it always binds to and blocks multiple follicular development after ovarian stimulation therapy), sometimes can only facilitate the following IVF. In practice, we met some repeated IVF failure cases because of pelvic involucral adhesions. After lysing the adhesions and freeing ovaries by laparoscopic surgery, they achieved pregnancy after IVF procedure. For hydrosalpinx, it is still debatable whether it is suitable to perform salpingostomy or not before IVF. Retrospective data supported the salpingectomy before IVF procedure, which can improve the IVF success rate; but prospective studies showed that resection didn’t have benefit and/or may have bad effect on ovarian reserve of the same side. However, further research is still needed to know the value and surgical indications for hydrosalpinx, how to evaluate the potential function of fallopian tube, and to understand which surgical interventions (such as needle aspiration of hydrosalpinx fluid, laparoscopic proximal tubal occlusion and laparoscopic salpingostomy or salpingectomy) for women with hydrosalpinges are better. Countermeasure With more than 20 years of clinical experience, we have established the indications, guidelines and processes of hysteroscopic tubal catheterization and hydrotubation, laparoscopic surgery combined with hysteroscopy and ART, which are based on the hysterosalpingography (HSG) assessment. If possible, we prefer the first and second therapy for their lower cost to ART. In addition, we had many cases in which the patients who had suffered from IVF failure got pregnant after hysteroscopic tubal catheterization and hydrotubation or laparoscopic surgery combined with hysteroscopy. Removal of hydrosalpinx by salpingectomy before IVF-ET can improve the result and shows no effects on the
ovary response to superovulation [15], but there are different opinions [16].

**Endometriosis**

Up to now, endometriosis is still a mysterious disease and a relative infertile factor. Except for the fact that anatomic structural destroy of fallopian tube, adhesia and pelvis was induced by severe endometriosis, the mechanism of endometriosis-induced infertility remains unclear. Hypotheses concerning subtler forms of endometriosis suggests that infertility is impaired due to disruption of ovum transport, interference with hormone support, ovulation dysfunction, detrimental effects on gametes and/or reduced granulosa cell steroidogenesis, which has been noted with diminished ovarian reserve [17].

Peritoneal macrophages have been activated and cytokines may be detrimental to gamete fertilization. This has been one of many reasons why patients with endometriosis undergo IVF [18]. Several studies have suggested that patients with endometriosis had lower pregnancy and implantation rate [19] and fewer oocytes retrieved during IVF, as compared with controls (tubal disease, oocyte donation [20], intracytoplasmic sperm injection [21]), while the fertilization rate had no difference between women with or without endometriosis. One study indicates that autoantibodies, as a factor related to endometriosis, may have adverse effect on embryo implantation; but the effect can be overcome by administration of corticosteroids [22].

**Countermeasure** It is necessary to explain all possibilities clearly to the patients with pelvic endometriosis before performing IVF-ET. We encountered some cases, who had mild endometriosis and experienced repeated IVF failure, achieved pregnancy without any therapy; or some cases, whose tubes were diagnosed as partially patent by HSG, got gestation after undergoing hydrotubation. In addition, the etiological factors were treated. But whether laparoscopic surgery has the value of improving the pregnancy rate for the cases with the mild pelvic endometriosis before IVF is still controversial [23].

**Immune factors**

Autoimmune antibodies (especially anticardiolipin antibody, ACA) are always regarded as negative factors for IVF. Pregnancy rate of IVF with autoimmune antibody positive is significantly lower than that of antibody negative [24-26]. Autoimmune antibodies (or autoimmune phenomena) are not only found in systemic autoimmune diseases, e.g. systemic lupus erythematosus and chronic nephritis, but also in pelvic organic disease (endometriosis, chronic pelvic inflammatory disease, pelvic adhesion), unexplained infertility, recurrent spontaneous abortion and repeated IVF failure. It is reported that IVF pregnancy rates in patients with ACA significantly increased [27] if aspirin and heparin were administrated from embryo transfer day. The possible mechanisms interfering with reproduction of autoimmune phenomena include that ACA inhibits prostacyclin production with resultant thrombocyte preponderance,
inverts the prostacyclin/thromboxane ratio, and increases platelet aggregation and cytokine dysfunction, at last making embryo implantation difficult or decreasing the blood supply of blastoderm[28].

**Countermeasure** With suspicious case of autoimmune disease or phenomena, patients should check autoimmune antibodies before IVF or after repeated IVF failure. If the results are positive, the cases should be treated reasonably.

**Unexplained infertility**

The conception of unexplained infertility is still not clear. With the development of science and technology, its definition is still continuously revised. That some unexplained infertility couples will be pregnant after controlled ovarian stimulation treatment alone partially explains the pregnancy rate of gamete intrafallopian transfer (GIFT). For this reason, ovarian stimulation should be considered in unexplained infertility before more elaborate forms of assisted reproduction are used[28]. IVF failure is related to embryo quality and endometrial receptivity. It was reported that IVF failure rate of unexplained infertility was significantly higher than that of fallopian infertility. It is also reported that uterine artery pulsatility index was >3.0 (normal is <2.5) during about 35% of IVF failure women[30]. In this condition, embryos should be cryopreserved for transfer in subsequent cycles. Blood supply deficiency is found in patients with positive autoimmune antibody, such as ACA, lupus anticoagulant, and antinuclear antibodies (ANA), and negative autoimmune antibody. A total of 96 women undergoing IVF treatment were examined by transvaginal ultrasonography with colour and pulsed Doppler ultrasound on d 22 of the menstrual cycle preceding IVF. The authors assessed endometrial thickness, endometrial morphology, myometrial echogenicity, subendometrial vascularization, the uterine artery pulsatility index, protodiastolic notch and end diastolic blood flow in order to define a uterine score which could be correlated with the pregnancy rate. No pregnancy occurred if the score was between 0 and 10; with a score of 11-15 there was a 34.7% chance of pregnancy, and score >16 had a 42% chance of pregnancy. The uterine score calculated prior to IVF cycle appears to be a useful predictor of implantation[31]. Recently, the changes in endometrial vascularity throughout the normal menstrual cycle with three-dimensional power Doppler angiography were reported[32]. A study found that vitamin B12 deficiency was associated with infertility and recurrent spontaneous abortion. After vitamin B12 supplement, fecundity recovered in some patients[33].

**Countermeasure** Couples with unexplained infertility should choose ART with caution. If there are indications for ART, factors such as the quality of zygote and embryos, endometrial development, blood supplement, immunity, infection, etc. should also be considered[34]. For improvement of endometrial blood flow, the doses of aspirin have been discussed[35].
Artificial insemination

Refractory cervical infertility and sub-clinical male infertility are the capital indications of intrauterine insemination (IUI). In patients with cervical stenosis, cervical dilation can be performed during the cycle before IUI. It should be emphasized that the premise for IUI is the tubal patency. In some cases patients failed to be pregnant after repeated IUI because of “partially patent tubes”. The signs of diagnosis of partially patent tubes by HSG were: a) iodized oil passes the fallopian tube slowly; b) there was iodized oil remaining in tube lumen 24 h after HSG, as shown in despite of the spill of X-ray contrast in pelvic cavity. Partially patent tubes are one of the important factors which lead to infertility and tubal pregnancy\(^{[36]}\). It is more difficult for American and European physicians to make the diagnosis of partially patent tubes with water-soluble contrast medium for HSG. Oil-soluble radiopaque was found used for tubal insufflations in world literature\(^{[37]}\). With more than 20 years of the experiences, we have concluded the sequential steps for management of ovulatory infertile women with uterine, tubal and/or pelvic diseases, including HSG, hysteroscopic tubal catheterization and hydrotubation under abdominal sonographic monitoring, laparoscopic surgeries (reconstructive surgery) combined with hysteroscopy or promoting IVF and so on\(^{[10]}\). A recent prospective, randomized and double-blind study showed that the use of vaginal misoprostol (400 \(\mu\)g) may improve the chance for pregnancy in women having IUI in a wide variety of cycle types. The possible mechanisms are the known effects of PGE on increasing myometrial contractility, potential relaxation of tubal isthmus, improving spermatozoon-oocyte binding/penetration and attenuation of the female immune response to spermatozoa, which is beneficial to fertilization and embryo development\(^{[38]}\).

IVF-ET during natural cycle

The first successful IVF-ET birth in the world was achieved in a natural cycle. Since then, in order to improve pregnancy rate, ovarian stimulation has been widely adopted in assisted reproduction programs all over the world. But IVF-ET also has many risks, e.g. ovarian hyperstimulation syndrome (OHSS), multiple pregnancy, decreased endometrial receptivity, and increased cost, which hindered its popularization, especially in poverty-stricken areas. For these reasons, there has been renewed interest in natural cycles. Despite of its low single pregnancy rate, IVF-ET during natural cycle was accepted for its accumulated pregnancy rate. Natural cycle IVF-ET offers a low-cost alternative to patients with infertility. In addition, natural cycle IVF-ET provides good chances to study the follicular fluid and peri-implantation endometrium in their un-stimulated, natural state\(^{[39]}\).

As a high-technique, ART gives a good prospect for infertility treatment, such as intracytoplasmic sperm injection (ICSI), pre-implantation genetic diagnosis (PGD), nuclear or plasmal transfer, blastocyst culture (co-culture, sequence-culture) and so on. But from
the aspect of clinical practice, we should clearly know the condition and power of China. We
should take every aspect and multiple factors into our consideration to work out better individu-
also, solutions for every infertile couples. Recently, some scholars have considered ART,
which is also called new reproductive technologies (NRTs), not perfect, even in developed
countries[4]. When it is “transferred” into poor-resource areas or developing countries, lower
quality laboratory and lack of qualified paramedical personnel may magnify its negative
effects. We must pay attention to ART, which should be regulated and standardized.

References

2. Trout SW & Seltzer DB. Do women with unexplained recurrent pregnancy loss have higher day 3 serum E2
4. Ng BY, Yeung WSBB, Fong DYF, et al. Effects of age on hormonal and ultrasound markers of ovarian reserve
intratrunal lesions in infertile patients in an assisted fertilization programme. Gynaecol Endoscopy, 2001,
7. Masamoto H, Nakama K & Karazawa K. Hysteroscopic appearance of the mid-secretory endometrium:
8. Sher G & Flach JD. Vaginal sildenafil (Viagra): a preliminary report of a novel method to improve uterine
806-9.
10. Feng ZC. Hysteroscopic tubal catheterization and hydrotubation or laparoscopic surgery combined with
13. Dechaud H. Hydrosalpinx and A.RT: Hydrosalpinges suitable for salpingectomy before IVF. Hum Reprod,
14. Johnson NP, Mui W & Sowter MC. Laparoscopic salpingectomy for women with hydrosalpinges enhances
15. Liu QJ, Long XL, Chen YB, et al. Significance of removing hydrosalpinx by salpingectomy before in vitro
17 (1):37-8.
17. Hock LX, Shariff K, Dagostino LW, et al. Contribution of diminished ovarian reserve to hypofertility associ-