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Option in treating male factor infertility in the absence of intracytoplasmic injection (ICSI)

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ABSTRACT

The Department of Obstetrics and Gynaecology, University Kebangsaan Malaysia (UKM) started the IVF-ET (in-vitro fertilization-embryo transfer) service in 1998. High sperm insemination concentration (HIC) was utilized from the onset since the patient population consisted of a high percentage of male factor infertility (81.6%). To further improve the outcome of IVF, a short incubation protocol was used to minimize the exposure of the oocytes to toxins originating from the high sperm concentration. A retrospective analysis of data was done after 18 months to review the results of these modifications in the IVF protocol. To date, 49 cycles have been carried out resulting in 25 ET. A biochemical pregnancy rate of 20.4% per cycle and 40% per ET with a resultant clinical pregnancy rate of 6.1% per cycle and 12% per ET was achieved. The use of HIC has enabled a fertilization rate of 45.8% in a population with a mean 3.6 $\pm$ 4.5% normal morphology. Among the factors that positively contributes towards a higher fertilization rate were higher number of inseminated sperms and better seminal parameters respectively. Good embryo morphology is associated with a higher pregnancy rate although not statistically significant. Attributes associated with the low pregnancy rate and IVF-ET outcome includes severe teratozoospermia of the male patients, the age of female patients and the low number of cycles performed.

Keywords: In-vitro fertilization embryo transfer, male infertility, high insemination concentration, short incubation time

INTRODUCTION

The Department of Obstetrics and Gynaecology, Universiti Kebangsaan Malaysia commenced the IVF-ET (in vitro fertilization-embryo transfer) service in 1998 to provide treatment for infertile couples with more severe forms of subfertility not amenable to ovarian stimulation and/or intrauterine sperm insemination. From our regular semen analysis evaluation, we identified from early on that our population of subfertile patients consists of a high percentage of male factor infertility (89%) especially teratozoospermia [Ong Fee Bee et al, 1998].

Hence in our new IVF set up, we utilized wholly the HIC (high sperm insemination concentration) coupled with the short insemination protocol as we do not have ICSI (intracytoplasmic injection) service. The rationale for the HIC is to increase the fertilization rate by increasing the number of normal sperms available [Oehninger et al, 1988] as zona pellucida binding is impaired in cases of severe teratozoospermia [Franken et al, 1990]. The short insemination protocol has been shown to improve the outcome of in vitro fertilization with higher embryo quality and better implantation rate [Dirnfeld et al, 1999]. The hypotheses was that

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the short insemination time minimizes exposure of the oocytes to toxins originating from the high number of sperms which may be detrimental to their development and embryo implantation [Swenson et al, 1999].

We thus review our results in view of these modifications in our IVF protocol.

MATERIALS AND METHODS

This is a retrospective analysis of medical records from subfertile patients who underwent IVF-ET at the Medically Assisted Conception Unit, Department of Obstetrics and Gynaecology, University Kebangsaan Malaysia, a tertiary academic institution infertility referral unit between 1998 and August 1999. These 49 cycles represent our first efforts of assisted conception since the setting up of the IVF laboratory.

Patient selection

Most of the couples (with patent tubes) had undergone at least three ovarian stimulation cycles and intrauterine insemination before proceeding on the IVF-ET. Some with long standing infertility were referred from other government hospitals. No exclusion criteria was used in the patient selection process.

Ovulation induction

Patients were down regulated with GnRH-a (Buserelin or Suprefact, 300ug t.i.d) beginning in the mid-luteal phase of the previous menstrual cycle (long protocol) or on the beginning of menses of the current menstrual cycle (short protocol) before the initiation of gonadotrophin injections. A baseline ultrasound examination is taken before gonadotrophin therapy was begun on day 1. Gonadotrophins used for ovulation induction included Humegon, Metrodin, Pergonal and Puregon. Dosages were individualized according to the patient's ovulation history and response to previous stimulation cycles. Dosages were adjusted based on ultrasound findings. HCG were administered when there were four or more follicles >/= to 18 mm in diameter.

IVF-ET

Transvaginal ultrasound guided oocyte retrieval was performed 32 to 36 hours after hCG administration. Oocytes were incubated for 3–6 hours in Universal IVF media (Medicult, U.K.) at 37°C, 5% CO_2 in air before IVF (in-vitro fertilization) with husband's sperm.

Semen was obtained from the husband on the morning of oocyte retrieval and processed via a two-gradient density column (Puresperm, Sweden; Sil-Select, Belgium; Ixaprep, U.K). A semen analysia according to WHO criteria [1992] was completed for all the patients. Two slides were made from the semen sample and later evaluated for sperm morphology according to strict criteria [Kruger et al, 1988].

The short insemination protocol was used whereby the oocytes were transferred out of the sperm microdroplet into a new culture droplet after 60–80 min. The oocytes are denuded mechanically to check for fertilization 16–18 hours later.

Embryos were monitored daily and a media change is done at every stage. Just before transfer, embryos were graded according to morphology (Grade 1–4, with 4 being the best) and an embryo score was produced by multiplying the cell number and grade [Steer etal. 1992]. Thriving embryos were returned into the patient's uterine cavity 46–52 hours after oocyte retrieval via ultrasound guided replacement with an embryo transfer catheter (Cooke, Aust; Wallace, U.K). Patients were started on 400 mg progesterone (Utrogestan) after oocyte retrieval. 2000 IU hCG was given prior to embryo transfer and every three days until the determination of biochemical pregnancy on day 16 after ET (embryo transfer) as determined by a positive urinary beta-hCG.

Statistical analysis

Statistical analysis was performed with the use of ANOVA, Student's t test and χ^2 test. A $p < 0.05$ was considered as statistically significant.

RESULTS

Table 1 shows the age of the coupes, semen parameters and ethnic background of the women who underwent IVF-ET. The seminal parameters demonstrated mean progressive motility of 36% which is well below the WHO criteria of 50% and mean sperm morphology of less than 4%. In this study, a progressive motility of 35% and less and normal sperm morphology of less than 15% was used to define male factor subfertility.

Female factor infertility made up 16.3% (8/49) of the patients while male factor infertility accounts for 18.4% (9/49). Majority of the patients (63.2%) demonstrated combine infertility factors (Table 2). Sixty seven percent (33/49) have primary infertility while the rest have secondary infertility. Overall, 81.6% (40/49) of the couples had a male factor component. Table 3 shows the breakdown of sperm abnormalities in the male infertility patients (n = 40). The most frequently seen abnormalities are teratozoospermia and asthenoteratozoospermia which altogether make up 87.5% of the male factor patients or 71.4% (35/49) of all the men.

Altogether 49 cycles of oocyte retrieval were carried out and in 10.2% of the cycles no eggs were retrieved (Table 4). The five women in which no oocytes were recovered were significantly older, 38.20 ± 2.95, $p < 0.05$ as compared to the rest, 35.04 ± 3.66. Twenty-nine cycles had fertilization but only 25 had viable embryos for transfer. Of the four cycles without embryo transfer, three had one or two fertilized (2PN) oocytes, which did not undergo cleavage. The fourth had seven eggs of which five underwent polyspermy (≥ 4 PN) and two of the normally fertilized ones did not cleave.

In Figure 1, normal motile sperm density was divided into 4 groups and tabulated against the fertilization rate.Normal motile sperm/ml were calculated based on the following formula: % normal morphology x fresh sperm density/ml x% forward motility. There was a significant increase in fertilization if 25,000 or more normal motile sperm were present per ml of seminal fluid ($p = 0.002$). The fertilization rate of > 25,000 normal motile sperm/ml group is threefold that of the tenth percentile group, < 2,500 normal motile sperm/ml. Twelve of 19 cycles in the tenth percentile group (< 2500) demonstrated complete fertilization failure. For cycles with 10,000–25,000 normal sperm density/ml, a fertilization rate of 56.5% was seen and none with complete fertilization failure.

When the cases were divided into those which had fertilization and those with complete fertilization failure, there were significant differences in the number of sperms inseminated, the fresh sperm density and the progressive motility (Table 5).

When the embryo morphology and cell numbers were tabulated into a cumulative embryo score derived by adding up the scores of transferred embryos versus pregnancy, a higher pregnancy rate was seen when the cumulative embryo score was ≥ 36 as compared to those with lower scoring embryos < 36 with a pregnancy rate of 70% (n = 10) and 20% (n = 15) respectively, which did not achieve statistical significance due to the small number of cases.

TABLE 1
Biodata and cycle variables of couples undergoing IVF

Variables	mean	±	sd
Age of wife (years)	35.4	±	3.7
Age of husband (years)	37.6	±	4.9
Sperm density ($\times 10^6$/ml)	60.7	±	57.1
Seminal volume (ml)	3.0	±	1.4
Total motility (%)	36.0	±	17.8
Sperm morphology by strict criteria (%)	3.6	±	4.5
Ethnicity	n		(%)
Malay	30		(61.2)
Chinese	16		(32.7)
Indian	2		(4.1)
Others	1		(2.0)
Total	49		(100.0)

TABLE 2
Infertility diagnosis of couples undergoing IVF

Infertility factor	n	%
Endometriosis	5	(10.2)
Tuboperitoneal	3	(6.1)
Idiopathic	1	(2.0)
Male factor	9	(18.4)
Endometriosis + Male factor	13	(26.5)
Tuboperitoneal + Male factor	18	(36.7)

TABLE 3
Sperm defects in male factor patients

Sperm defect	n	(%)
Single defect		
Asthenozoospermia (A)	1	(2.5)
Teratozoospermia (T)	20	(50.0)
Oligozoospermia (O)	1	(2.5)
Double defect		
AT	15	(37.5)
OT	1	(2.5)
Triple defect-OAT	2	(5.0)
Total	40	(100.0)

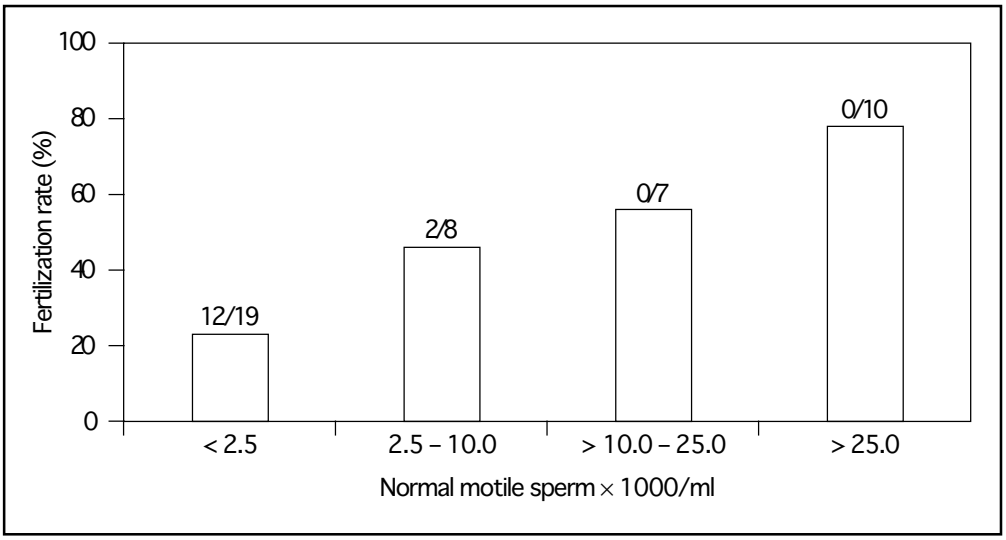
TABLE 4
Outcome variables of patients undergoing IVF-ET (n = 49)

Variables	n		(%)
No. oocyte retrievals	49		(100.0)
No. IVF cycles	44		(89.8)
No. of embryo transfer cycles	25		(51.0)
Cycles with no oocytes retrieved	5		(10.2)
	mean	±	sd
No. follicles per cycle	11.0	±	6.7
No. oocytes recovered per cycle	5.6	±	4.7
No. motile sperms inseminated per oocyte ($\times 10^5$)	3.7	±	2.1
No. oocytes fertilized per cycle	2.9	±	3.7
No. embryos viable per cycle	2.0	±	2.5
No. embryos transferred per cycle	2.4	±	0.9
Fertilization rate per cycle	45.8	±	39.7%
3PN formation rate (4/126)	3.2%		
≥ 4PN formation rate (5/126)	4.0%		
Biochemical pregnancy rate per cycle (10/49)	20.4%		
Biochemical pregnancy rate per embryo transfer (10/25)	40.0%		
Clinical pregnancy rate per cycle (3/49)	6.1%		
Clinical pregnancy rate per embryo transfer (3/25)	12.0%		

TABLE 5
Fertilized cycles versus non-fertilized cycles

	Fertilized cycles	Non fertilized cycles	P
	(n = 29)	(n = 15)	
	Mean ± sd	Mean ± sd	
No. sperms inseminated/egg ($\times 10^5$)	4.18 ± 2.14	2.64 ± 1.74	0.02
Sperm density ($\times 10^6$/ml)	76.12 ± 61.77	26.45 ± 62.37	0.005
Progressive motility (%)	41.62 ± 16.67	24.71 ± 15.03	0.002

Fig. 1. Normal motile sperm density and fertilization rate



The ratio on top of each column indicates the number of non fertilized cycles over the total number of cycles in the particular group.

DISCUSSION

High insemination concentration (HIC) have been used from the onset of the IVF program and this has achieved a fertilization rate of 45.8% for a population of men with a mean of less than 4% normal morphology. In comparison, Fishel et al [1995] obtained a fertilization rate of 7% utilizing HIC in men with less than 5% normal morphology. In this study, fertilization is associated with better semen parameters and hence better sperm recovery and a higher number of sperms used in insemination.

A clinical pregnancy rate of 6% per cycle and 12% per embryo transfer was achieved although there were no live births. The longest pregnancy progressed to about nine weeks. We suspect a number of circumstances may have contributed to this outcome.

A number of studies have shown that severely abnormal sperm morphology affects early embryonic development and implantation [Parinaud et al, 1993]. Teratozoospermia had been associated with a higher frequency of chromosomal abnormalities and aneuploidy [Moonsani et al, 1994; Bernadini et al, 1997]. It is manifested in a much lower rate of embryo development affecting both cleavage rate and cellular morphology, with more fragmentation seen [Fishel et al, 1995]. However, it was the observation of this laboratory that the majority of the viable embryos are of good quality (95%) albeit by a morphological scoring system.

A comparative analysis of embryo implantation potential in patients with severe teratozoospermia showed that in cases of severe teratozoospermia (< 5%) no pregnancies occur. In cases of moderate teratozoospermia, although fertilization approaches normal, the conception rate remains low [Ombelet et al, 1994; Oehninger et al, 1996]. The fact that over 70% of the patients had teratozoospermia may have contributed to a poor pregnancy outcome.

A few studies have suggested the presence of toxic sperm factors that may attach to the zona pellucida or the oocyte membrane during fertilization leading to implantation failure. Associated with teratozoospermia and asthenozoospermia is the formation of reactive oxygen species (ROS) resulting from lipid peroxidation during in vitro culture. The formation rate of ROS is a good predictor of the lifespan (up to complete loss of motility) of a fresh semen sample [Alvarez et al, 1987]. ROS have been implicated in causing cellular membrane damage and loss of function [Alvarez & Storey, 1988].

Although a short insemination protocol with a shorter incubation time was used to avoid a high concentration of sperm toxic factors, the possibility exists that toxic sperm factors may be present due to the high insemination concentration. Therefore shortening the insemination time maay not be enough to overcome the abnormality [Swensen et al, 1999].

Another contributing factor is the patient population which consist of couples with long standing infertility

(6–16 years) and the age of women, average 35.4 ± 3.7 years old as no exclusion criteria was used in patient selection (range 29–42). Many studies have shown that the pregnancy rate is lower for women above 35 years old due to a poorer implantation rate and higher levels of spontaneous abortion [Agarwal & Buyalos, 1996; Pantos et al, 1999]. There is also an aging oocyte factor which may contribute towards a lower quality embryo. Fifty percent of women between the ages of 35–39 and 95% of women age 40 and above have chromosomal degeneration of oocytes [Lim & Tsakok, 1999].

Most unsuccessful IVF cycles are attributed to embryo implantation failure. Factors influencing IVF implantation can be divided into three categories: (1) embryonic, (2) endometrial and (3) method of embryo transfer [Noyes et al, 1999], involving the laboratory and clinical component. Our present data implicates implantation failure for poor IVF outcome as only 12% of the transferred embryos went on to become viable pregnancies although the biochemical pregnancy per embryo transfer was 40%. In addition, the very low number of cycles performed i.e. 49 cycles in 18 months has impeded progress in gaining experience and optimization of the IVF program.

The fertilization rate (78.4 ± 24.0%) in men with more than 25,000 normal motile sperms/ml approaches those seen in other IVF units [Fishel et al, 1995; Oehninger et al, 1996; Swensen et al, 1999]. Although HIC was used, the tripronuclear (3 PN) formation rate of 3.2% was low with an overall polyspermy fertilization rate of 7.1%. We observed that embryo formation and development has not been compromised as the majority of embryos were of good quality. In this study, a higher embryo score is associated with a higher pregnancy rate although not statistically significant, an observation that has been corroborated by many published studies [Cooperman et al, 1995; Hu et al, 1999].

In the review of data, an important diagnostic information has been obtained whereby if > 25,000 normal motile sperms/ml were available, the fertilization rate approaches normal utilizing the HIC protocol. The corresponding seminal parameters would be 2.5% normal morphology by strict criteria, sperm density 20×10^6/ml with 50% forward motility. The fertiliztion rate falls to below 50% if there were < 10,000 normal motile sperms present which is equivalent to 1% normal morphology, 20×10^6ml sperm and 50% forward motility. This information may be used to counsel the patient accordingly as to the fertilization outcome.

In summary, HIC coupled with the short insemination protocol has provided an acceptable fertilization rate in the absence of other methods. The clinical pregnancy rate of 6.1% per cycle and 12% per embryo transfer respectively is encouraging although there is no ongoing pregnancy and no live birth. In the patient's context, non-fertilization of a cycle is more damaging psychologically. Early embryo formation and development has not been compromised although there is no ongong pregnancy, the reasons for which we have yet to elucidate fully.

For future development, with the degree of severe male defects seen in the patient population, a move has been made towards micromanipulation and assisted fertilization to better help those with such abnormalities.

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