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Favourable pregnancy results with insemination of *in vitro* matured oocytes from unstimulated patients

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BACKGROUND: The purpose has been to develop an *in vitro* oocyte maturation (IVM) method for a wide range of patients. METHODS: A total of 239 cycles with immature oocyte retrieval (IOC) were carried out without hormonal priming. Patients with regular cycles and normal or polycystic ovaries (PCO) and anovulatory cycles with PCOS were included. Insemination or intracytoplasmic sperm injection (ICSI) according to sperm quality was alternatively used in fertilization of the matured oocytes. RESULTS: A total of 971 immature oocytes (mean 8.0 ± 5.2) were collected in 122 IVM-IVF cycles and 851 oocytes (mean 7.3 ± 4.4) in 117 IVM-ICSI cycles. The oocyte maturation and fertilization rate was 62.6% and 37.7% after insemination, and 53.9% and 69.3% after ICSI, respectively. The mean number of embryos transferred was 1.6. Clinical pregnancy rate per IOC was 23.8% in IVM-IVF and 17.1% in IVM-ICSI (ns). Implantation rate was higher in IVM-IVF (24.2%) than in IVM-ICSI (14.8%) ($P < 0.05$). CONCLUSIONS: Insemination of IVM oocytes functions well, resulting in comparable pregnancy rates per IOC between IVM-IVF and IVM-ICSI. Good pregnancy results can be achieved both in patients with regular cycles and with PCO(S) by transferring only one or two embryos at a time.

Key words: ICSI/*in vitro* maturation/IVF/PCOS/regular cycles

Introduction

Immature oocyte retrieval from unstimulated ovaries in combination with *in vitro* oocyte maturation (IVM) and fertilization is an assisted reproductive technology of growing interest. *In vitro* maturation of oocytes has potentially many advantages over conventional *in vitro* fertilization (IVF). Benefits of IVM include simple and less time-consuming protocols, no or minimal use of fertility drugs, and reduced cost of the treatment. The inherent risk of ovarian hyperstimulation syndrome (OHSS) is entirely avoided. Reduced treatment intervention and avoidance of adverse drug effects make the treatment an attractive option for infertility patients.

The ability of immature oocytes to mature spontaneously when removed from the follicle was first shown in animals by Pincus and Enzmann (1935) and in humans by Edwards (1965). The embryos resulting in the birth of the first human IVM babies were derived from oocytes originating from excised ovaries (Cha *et al.*, 1991). A few years later live births after transvaginal immature oocyte collection (IOC) were reported (Trounson *et al.*, 1994; Barnes *et al.*, 1995; Russell *et al.*, 1997).

Success rate of the clinical IVM was modest at first as only sporadic live births were reported (Trounson *et al.*, 1994; Russell *et al.*, 1997). During the last 5 years, pregnancy rates have reached 25–35%, implying substantial improvement of the method (Mikkelsen *et al.*, 1999; Chian *et al.*, 2000; Lin *et al.*, 2003). Currently, ~300 healthy infants have been born worldwide (Chian *et al.*, 2004). The majority of the published work has focused on the use of IVM among patients with polycystic ovaries (PCO) or polycystic ovary syndrome (PCOS) (Cha *et al.*, 2000; Chian *et al.*, 2000, 2003; Child *et al.*, 2001; Mikkelsen and Lindenberg, 2001; Lin *et al.*, 2003). Far fewer cycles have been carried out in women with regular menstrual cycles (Barnes *et al.*, 1996; Mikkelsen *et al.* 1999, 2000, 2001; Suikkari *et al.*, 2000; Child *et al.*, 2001). A low number of oocytes retrieved, and subsequently only a few embryos available for transfer, have been thought to reduce the applicability of IVM in this group of women (Child *et al.*, 2001).

Thus far, most investigators have used hormonal pre-treatment either with follicle stimulating hormone (FSH) or priming with human chorionic gonadotrophin (HCG) 36 h before IOC (Trounson *et al.*, 1998; Mikkelsen *et al.*, 1999; Chian *et al.*, 2000; Suikkari *et al.*, 2000; Child *et al.*, 2001; Mikkelsen and Lindenberg, 2001; Lin *et al.*, 2003). The conclusions of these studies have been controversial. It seems that there may be a beneficial effect of FSH priming on

oocyte maturation (Wynn *et al.*, 1998) with no effect on clinical outcome in regularly cycling women (Trounson *et al.*, 1998; Mikkelsen *et al.*, 1999; Suikkari *et al.*, 2000). According to Mikkelsen and Lindenberg (2001), a short FSH pre-treatment in the follicular phase may improve maturation of the oocytes and implantation rate (IR) of the embryos in PCOS patients. In addition, HCG priming has been demonstrated to hasten the maturation time of the oocytes in PCOS patients (Chian *et al.*, 2000). In a recent study, Lin *et al.* (2003) did not observe any additional beneficial effect of FSH stimulation on HCG primed PCOS women undergoing IVM. Although HCG priming has been used in patients with regular cycles (Child *et al.*, 2001) the outcome has not been compared to non-HCG cycles.

At the Infertility Clinic of the Family Federation of Finland, Helsinki, IVM was started as an experimental procedure in 1998. Over the past 6 years our objective has been to develop a clinically manageable IVM method and to be able to offer our patients a less demanding alternative, compared with conventional IVF/ICSI. In our first preliminary IVM study we used FSH priming before immature oocyte retrieval and fertilization with ICSI (Suikkari *et al.*, 2000). In the present study, the majority of IVM cycles were carried out in natural cycles without any hormonal priming in women with regular menstrual cycles and normal ovaries. To the best of our knowledge, this is exceptional, since most investigators thus far have concentrated on the effect of various hormonal priming protocols in PCO(S) women.

Intracytoplasmic sperm injection (ICSI) has been regarded as necessary for fertilization of *in vitro* matured oocytes even in conditions where sperm parameters are not impaired. We decided to test the possibility of using insemination of the *in vitro* matured oocytes whenever microinjection was not required. Since 2002 we have used either insemination or ICSI in the IVM programme according to the same clinical laboratory criteria used in conventional treatments.

The aim of this study is to report our experience of IVM carried out as a practical clinical programme. The majority of our IVM patients are regularly cycling women with normal ovaries. We present our results with insemination of the *in vitro* matured oocytes and compare the outcome to that of microinjection both in patients with normal and polycystic ovaries and PCOS.

Materials and methods

Subjects and study protocol

Between November 1999 and April 2004, a total of 218 women went through 275 cycles with immature oocyte retrieval. Participation in the IVM treatment was suggested to couples coming for their first, second or third IVF or ICSI due to male factor, tubal or unexplained infertility as an alternative to conventional approaches. In addition, women with PCO or PCOS were offered IVM. Further inclusion criteria were female aged <38 years and a baseline serum FSH concentration <10 IU/l. All couples were thoroughly counselled for known and unknown issues involved in the new technique. Because we performed IOCs only from Monday to Thursday, patients were carefully informed that there would be a risk that more than one cycle had to be monitored before an optimal date for IOC was achieved. Children born were offered a developmental examination by a paediatrician at 6 months and 12 months and a neuropsychological evaluation at 2 years of age. A written informed consent to participate was obtained before starting the treatment. The IVM protocol was approved by the local Ethics Committee.

All IVM-IVF cycles ($n = 122$) and the majority (117/153) of all IVM-ICSI cycles were carried out without hormonal priming before IOC. Comparisons of all data have been carried out between IVM-IVF and IVM-ICSI patients without hormonal priming. The patients were divided into three groups as follows, group 1: patients with normal ovaries and regular cycles ($n = 191$); group 2: patients with PCO ovaries on ultrasound scan and with follicular selection ($n = 20$); and group 3: patients with PCO ovaries, oligo- or anovulation, and no follicular selection (PCOS; $n = 28$). Subjects with normal ovaries on ultrasound scan were classified as regularly cycling if the length of the menstrual cycle was between 24 and 35 days. Polycystic ovaries and PCOS were defined as recently stated in a joint ASRM/ESHRE meeting (Balen *et al.*, 2003). The characteristics of the patients are presented in Table I.

In 2001, an additional 36 regularly cycling subjects received HCG 5000 IU (Profasi, Ares-Serono, Europe, London, UK) 36 h before oocyte collection. As this protocol was a part of the development of our IVM programme, the data will shortly be presented separately from the other results.

Cycle monitoring

A baseline ultrasound scan was performed on cycle day 3–4 to exclude cysts and to confirm the presence of at least four small antral follicles in both ovaries. A second ultrasound scan was performed on cycle day 6–8 and thereafter a daily, or every second day, ultrasound scan was carried out to monitor the selection of the dominant follicle. Once a leading follicle was seen, IOC was

Table I. Patient characteristics

	Regular IVF	ICSI	PCO IVF	ICSI	PCOS IVF	ICSI
No. of patients	91	100	13	7	18	10
Age (years)	32.1 ± 3.8	31.3 ± 3.8	33.5 ± 3.6	32.3 ± 2.4	30.8 ± 4.2	31.7 ± 5.7
BMI (kg/m^2)	21.6 ± 2.7	21.8 ± 2.8	23.3 ± 3.4	20.9 ± 2.5	23.8 ± 4.1	23.1 ± 6.2
Duration of infertility (months)	42.5 ± 27.3	44.9 ± 28.3	50.5 ± 22.6	58.7 ± 36.1	48.1 ± 23.1	43.5 ± 24.0
Previous no. of IVF/ICSI treatments	0.2 ± 0.7	0.5 ± 1.0[a]	0.7 ± 0.8	1.0 ± 1.0	0.4 ± 0.8	0.9 ± 1.0
Primary infertility (%)	54.9	52.0	61.5	71.4	55.6	80.0
Diagnosis						
Tubal only	15	9	0	0	0	0
Other female only	10	6	6	2	8	1
Male factor	21	51	1	1	1	0
Multiple causes	14	22	5	4	8	9
Unexplained	31	12	1	0	1	0

[a]IVF vs ICSI $P < 0.05$.

scheduled for the next day. In anovulatory patients, IOC was scheduled once the endometrium had reached at least 5 mm in thickness and there was no follicle larger than 10 mm. A serum sample for estradiol measurement was obtained at every ultrasound visit.

Immature oocyte collection

Oocytes were recovered by ultrasound-guided transvaginal aspiration of all visible follicles. A 17 gauge aspiration needle (Swemed, Sweden), a Hitachi EUB-415 ultrasound and a 5 MHz vaginal probe was used for oocyte recovery. The aspiration pressure was approximately one third of the pressure used for a standard oocyte pick-up for IVF. Immature oocytes were collected into 10 ml Falcon tubes containing 2 ml of Dulbecco's phosphate-buffered saline (PBS, GibcoBRL, Life Technologies, Paisley, Scotland) containing heparin. Isolation of oocytes was carried out by washing the follicular aspirate in PBS through an Em-Con embryo concentrating filter (Veterinary Concepts, Spring Valley, WI). Oocytes were identified in the filter retentant under a stereo dissecting microscope in 100 × 15 mm petri dishes.

Oocyte maturation and fertilization

Oocyte-cumulus masses were matured either in Tissue Culture Medium 199 (Gibco, Life Technologies Inc., Paisley, Scotland) supplemented with 10% patient serum, 0.075IU /ml rec-FSH (Gonal-F, Ares-Serono, Europe, London, UK), 0.1 IU/ml hCG (Profasi, Ares-Serono, Europe, London, UK), pyruvate, penicillin and streptomycin (Gibco), or in Medi-Cult IVM medium (Medi-Cult, Denmark) supplemented with 10% patient serum, 0.075 IU/ml rec-FSH (Gonal-F, Ares-Serono, Europe, London, UK) and 0.1 IU/ml hCG (Profasi, Ares-Serono, Europe, London, UK). Oocytes were cultured for 24–36 h at 37 °C in 5% CO_2 in air. In 1999–2001, ICSI was routinely used in fertilization of the IVM oocytes. Since 2002, IVF or ICSI was alternatively used in fertilization of the matured oocytes according to sperm quality criteria applied in conventional treatments at our clinic. The maturation of the oocytes for IVM-ICSI was assessed 30 h after IOC prior to ICSI and ~48 h after IOC, i.e. 18 h after insemination for IVM-IVF.

Embryo culture, transfer and freezing

Fertilized oocytes were transferred into IVF Universal Media (Medi-Cult, Denmark). The cleaved embryos were scored as regards to the number and regularity of blastomeres, the amount of fragmentation and the presence of multinucleated blastomeres (Scott *et al.*, 1991). Embryo transfer (ET) was carried out on day 2 or 3 following IVF/ICSI. A maximum of two morphologically normal cleaved embryos were transferred at a time. All good quality supernumerary embryos were frozen using 1,2-propanediol and sucrose as cryoprotectants in PBS as previously described (Lassalle *et al.*, 1985).

Endometrial priming and pregnancy ultrasound

The priming of endometrium was done as previously described by Mikkelsen *et al.* (1999). The women used oral 17β-estradiolvalerate (6 mg/day) (Progynova, Schering, Finland) immediately after IOC to enhance endometrial proliferation. Vaginal micronized progesterone (600 mg/day; Lugesteron, Leiras, Finland) was commenced on the evening after IOC. This luteal support was continued until the day of the pregnancy test. If serum HCG was positive, hormonal support was continued until 9 weeks of gestation with decreasing dosages over the last 2 weeks. Transvaginal ultrasound examination was done ~5 weeks after ET to confirm gestation sac or fetal heartbeat.

Statistics

Statistical analysis was carried out using the χ^2 and Mann–Whitney U tests where appropriate. Data in the tables were expressed as the mean ± SD. A difference of $P < 0.05$ was considered as significant.

Results

Within the patient groups 1–3 there were no statistically significant differences in age, BMI, duration of infertility, or

Table II. Comparison of outcomes between IVM-IVF and IVM-ICSI in all patients without hormonal priming (summary of groups 1–3)

	IVM-IVF	IVM-ICSI	Significance
No. of immature oocyte collection cycles (IOC)	122	117	
No. of follicles cd 3–4	17.5 ± 7.4	14.7 ± 6.7	$P < 0.01$
Cycle day of IOC	9.4 ± 3.1	9.3 ± 2.7	NS
Endometrium at day of IOC (mm)	5.6 ± 1.3	5.4 ± 1.4	NS
No. of oocytes collected			
Total	971	851	
Mean	8.0 ± 5.2	7.3 ± 4.4	NS
No. of oocytes matured (%)	608 (62.6)	459 (53.9)	
No. of matured oocytes fertilized (%)	229 (37.7)	318 (69.3)	
No. of fertilized oocytes cleaved (%)			
Total	193	259	$P < 0.01$
Mean	1.6 ± 1.5	2.2 ± 1.6	
No. of embryo transfer (ET) cycles (%)	84 (68.9)	100 (85.5)	$P < 0.01$
No. of cycles with more than two embryos to choose for transfer (%)	24 (19.7)	42 (35.9)	$P < 0.01$
No. of embryos transferred			
Total	128	159	
Mean	1.5 ± 0.5	1.6 ± 0.5	NS
Clinical pregnancy rate per ET (%)	34.5 (29/84)	20.0 (20/100)	$P < 0.05$
Clinical pregnancy rate per IOC (%)	23.8 (29/122)	17.1 (20/117)	NS
No. of implantations (%)	31 (24.2)	22 (14.8)	$P < 0.05$
No. of deliveries/ongoing pregnancies (% per IOC)	20 (16.4)	16 (13.7)	NS
No. of miscarriages (%)	9 (31)	4 (20.0)	NS
No. of cycles with cryopreservation of excess good quality embryos (%)	13 (10.6)	19 (16.2)	NS

Table III. *In vitro* maturation (IVM) outcome in women with presumed ovulatory, regular cycles (group 1)

	IVM-IVF	IVM-ICSI	Significance
No. of immature oocyte collection cycles (IOC)	91	100	
No. of follicles cd 3–4	15.2 ± 5.7	13.4 ± 5.1	$P < 0.05$
Cycle day of IOC	8.6 ± 1.6	8.8 ± 2.0	NS
Endometrium at day of IOC (mm)	5.7 ± 1.3	5.4 ± 1.5	NS
No. of oocytes collected			
Total	574	653	
Mean	6.3 ± 3.4	6.5 ± 3.6	NS
No. of oocytes matured (%)	384 (66.9)	356 (54.5)	
No. of matured oocytes fertilized (%)	138 (35.9)	239 (67.1)	
No. of fertilized oocytes cleaved (%)	117 (84.8)	205 (85.8)	
Total	117	205	$P < 0.001$
Mean	1.3 ± 1.3	2.05 ± 1.5	
No. of embryo transfer (ET) cycles	58	86	
No. of embryos transferred			
Total	84	133	
Mean	1.4 ± 0.5	1.5 ± 0.5	NS
Clinical pregnancy rate per ET (%)	31.0 (18/58)	21.0 (18/86)	NS
Clinical pregnancy rate per IOC (%)	19.8 (18/91)	18 (18/100)	NS
No. of implantations (%)	19 (22.6)	20 (15.0)	NS
No. of deliveries/ongoing pregnancies (% per IOC)	12 (13.2)	15 (15.0)	NS
No. of miscarriages (%)	6 (33.3)	3 (16.7)	NS

percentage of primary infertility between patients undergoing IVM-IVF and IVM-ICSI (Table I). Subjects with regular cycles undergoing IVM-ICSI had had more previous IVF/ICSI treatments compared with those in the IVM-IVF group.

A total of 971 immature oocytes were collected in 122 IVM-IVF cycles and 851 oocytes in 117 IVM-ICSI cycles (Table II). Of all initiated IVM monitoring cycles, 28% were cancelled because of logistic reasons, i.e. the pick-ups were done only from Monday to Thursday. For oocyte maturation, TCM 199 based medium was used in 92.8% of the IVM-IVF cycles and in 91.5% of the IVM-ICSI cycles. In the rest of the cycles the Medi-Cult IVM medium was used. The maturation rate was 58.9% (945/1604) for TCM 199-based medium and 56% (122/218) for Medi-Cult IVM medium. There were no statistically significant differences in the outcome between the two media.

Comparison of outcomes between IVM-IVF and IVM-ICSI in all patients without hormonal priming is summarized in Table II. The fertilization rate after insemination was 37.7% (229/608). The numbers represent the fertilized oocytes over all oocytes used for insemination, excluding immature oocytes at the time of fertilization check. The fertilization rate after ICSI was 69.3% (318/459). The numbers represent the fertilized oocytes over matured oocytes used for microinjection. The clinical PR per IOC was similar in IVM-IVF and IVM-ICSI subjects (23.8% vs 17.1%). However, both the clinical PR per ET and the IR were higher in IVM-IVF (34.5%; 24.2%) than in IVM-ICSI patients (20.0%; 14.8%) ($P < 0.05$).

Detailed data of the three groups of patients are shown in Tables III–V. In group 1, the mean number of cleaved embryos was statistically lower among the IVM-IVF patients compared with that in the IVM-ICSI group, whereas such

Table IV. *In vitro* maturation (IVM) outcome in women with PCO-ovaries and follicular selection (group 2)

	IVM-IVF	IVM-ICSI	Significance
No. of immature oocyte collection (IOC) cycles	13	7	
No. of follicles cd 3–4	24.6 ± 5.9	18.7 ± 5.6	$P < 0.05$
IOC day	10.2 ± 2.2	11.0 ± 1.3	NS
Endometrium thickness IOC day (mm)	5.3 ± 1.0	5.0 ± 0.7	NS
No. of oocytes			
Total	132	59	
Mean	10.2 ± 4.2	8.4 ± 4.0	NS
No. of oocytes matured (%)	80 (60.6)	29 (49.2)	
No. of matured oocytes fertilized (%)	28 (35.0)	21 (72.4)	
No. of fertilized oocytes cleaved (%)	24 (85.7)	13 (61.9)	
Total	24	13	
Mean	1.8 ± 1.6	1.9 ± 1.3	NS
No. of embryo transfer (ET) cycles	9	5	
No. of embryos transferred			
Total	15	10	
Mean	1.7 ± 0.5	2.0 ± 0	NS
Clinical pregnancy rate per ET (%)	22.2 (2/9)	0 (0/5)	NS
Clinical pregnancy rate per IOC (%)	15.4 (2/13)	0 (0/7)	NS
No. of implantations (%)	2 (13.3)	0	NS
No. of deliveries/ongoing pregnancies (% per IOC)	2 (15.4)	0	NS
No. of miscarriages (%)	0	0	NS

Table V. *In vitro* maturation (IVM) outcome in women with PCOS (group 3)

	IVM-IVF	IVM-ICSI	Significance
No. of immature oocyte collection (IOC) cycles	18	10	
No. of follicles cd 3–4	23.6 ± 8.9	27.8 ± 9.4	NS
IOC day	12.9 ± 5.9	13.4 ± 5.2	NS
Endometrium thickness IOC day (mm)	5.5 ± 1.0	6.1 ± 0.5	$P < 0.05$
No. of oocytes			
Total	265	139	NS
Mean	14.7 ± 7.0	13.9 ± 6.0	
No. of oocytes matured (%)	144 (54.3)	74 (53.2)	
No. of matured oocytes fertilized (%)	63 (43.8)	58 (78.4)	
No. of fertilized oocytes cleaved (%)	52 (82.5)	41 (70.9)	
Total	52	41	NS
Mean	2.9 ± 2.1	4.1 ± 1.4	
No. of embryo transfer (ET) cycles	17	9	
No. of embryos transferred			
Total	29	16	NS
Mean	1.7 ± 0.5	1.8 ± 0.4	
Clinical pregnancy rate per (ET) (%)	52.9 (9/17)	22.2 (2/9)	NS
Clinical pregnancy rate per IOC (%)	50.0 (9/18)	20 (2/10)	NS
No. of implantations (%)	10 (34.5)	2 (12.5)	NS
No. of deliveries/ongoing pregnancies (% per IOC)	6 (33.3)	1 (10)	NS
No. of miscarriages (%)	3 (33.3)	1 (50)	NS

differences were not found in groups 2 and 3. Within the different groups of patients, pregnancy rates (PRs) per IOC and per ET were similar between IVM-IVF and IVF-ICSI. The highest PR (52.9%/ET) was achieved in PCOS patients after insemination of the oocytes (Table V).

In 2001, 36 women with regular cycles were given HCG 5000 IU subcutaneously 36 h before IOC. This dose was chosen because it is routinely used in our standard IVF programme. The patient characteristics and the outcome of the HCG-primed regular cycles were similar to those in group 1 (data not shown) with the exception of a slightly thicker endometrium at IOC day (6.5 mm). A mean of 5.7 oocytes was collected on cycle day 10. The maturation rate was 46.6% (95/204) and fertilization rate after ICSI was 66.3% (63/95). An average of 1.7 oocytes was replaced and the clinical PR was 16.7% (6/36) per IOC and 25.0% (6/24) per ET. Four pregnancies resulted in a delivery and two ended in miscarriage.

Until September 2004, fresh embryo transfers after IVM have resulted in the birth of 43 infants (37 singletons, three sets of twins). In addition, transfers with frozen-thawed embryos have resulted in the birth of two singleton babies. The mean birth weight of the singleton infants ($n = 39$) was 3532 ± 432 g (2575–4250 g) and the mean birth length 50.4 ± 2.5 cm (46–55 cm). The mean birth weight of the twins was 2622 ± 95 g (2400–2850 g). Follow-up of the health and development of the IVM children will be presented elsewhere.

Discussion

This retrospective analysis of an IVM programme includes all cycles carried out in a clinical setting over the last four and half years. A clinical PR of 20% per aspiration and 26% per ET was achieved with only one or two embryos transferred at a time. Thus far, patients with PCO have been regarded the prime candidates for IVM because of their high risk of OHSS in conventional IVF, and good immature oocyte yield. We offered IVM to a wide range of patients as we thought it is important to expand the method to patients with various histories of infertility.

We observed a better overall PR per ET in IVM-IVF (35%) than in IVM-ICSI (20%). This difference was even more pronounced when the different patient groups were analysed separately (Tables III–V). Equal numbers of oocytes were collected per IOC despite higher antral follicle count (AFC) at baseline in the IVM-IVF group. Because we did not want to disturb the cumulus–oocyte complexes by stripping the cumulus cells at the time of insemination, it was not possible to assess the maturity of IVM-IVF oocytes at 30 h of maturation time as was done with the IVM-ICSI oocytes. Because the time points for assessing maturity were innately different, the maturation and fertilization rates between IVM-IVF and IVM-ICSI cannot be directly compared. From the start of the current programme until late 2001 only IVM-ICSI was used. Thereafter, the proportion of IVM-IVF cycles increased steadily and comprises now approximately two thirds of all IVM cycles, representing a similar ratio between IVF and ICSI cycles observed in our conventional IVF programme. The results have remained consistent over time (data not shown). Exclusion of IVM-ICSI cycles carried out before IVM-IVF was started did not change the results on the outcome of IVM-IVF and IVM-ICSI (data not shown).

Other factors could have influenced the poorer outcome in IVM-ICSI compared with IVM-IVF. Women in group 1 undergoing IVM-ICSI had had more previous IVF/ICSI cycles than IVM-IVF patients. As expected, there were more couples with male infertility in the ICSI group, and a possible influence of sperm quality on the results cannot be ruled out. Furthermore, a possible impact of the fact that in patient groups 1–2, the AFC on baseline ultrasound scan was lower in the IVM-ICSI group compared to the IVM-IVF group, has

to be considered. However, the mean number of oocytes collected was similar for IVM-IVF and IVM-ICSI in groups 1 and 2 (Tables III and IV).

In our hands, the mean number of oocytes retrieved from women with PCO was comparable to that reported in other studies in which FSH or HCG priming has been used (Cha *et al.*, 2000; Chian *et al.*, 2000; Mikkelsen and Lindenberg, 2001). Although the number of oocytes collected in regularly cycling women with normal ovaries was lower than that in women with PCO, it was higher than that reported earlier (Trounson *et al.*, 1994; Barnes *et al.*, 1996; Child *et al.*, 2001; Mikkelsen *et al.*, 2001). We did not find any fundamental differences in the oocyte pick-up technique used by us and other groups (personal communication). The relatively high oocyte number per retrieval may partly explain the good outcome in spite of only one or two embryos transferred.

The maturation rate of the immature oocytes was somewhat lower than reported by other investigators (Child *et al.*, 2001; Lin *et al.*, 2003). In the study by Chian *et al.* (2000), 10 000 IU HCG was administered subcutaneously 36 h prior to IOC to improve maturation, fertilization and competence of the embryos. Although they could not find significant differences in fertilization, cleavage and pregnancy rates between HCG-primed and non-primed patients, the maturation time was hastened in the HCG primed cycles. In our small group of patients receiving HCG before follicle aspiration, we did not find any beneficial effect of HCG priming on the number of oocytes collected, maturation, fertilization or cleavage rates compared with non-primed patients undergoing IVM-ICSI. Therefore we chose not to use HCG priming in our IVM programme.

Currently, ICSI has been regarded the best alternative for fertilization of *in vitro* matured oocytes even in conditions in which sperm parameters are normal. Reports of insemination of human *in vitro*-matured oocytes are scarce. In the study by Barnes *et al.* (1996), 43% of mature oocytes from normal ovaries and 26% of *in vitro* matured oocytes from PCOS women fertilized after insemination. The reason for poor fertilization rates after standard insemination has been thought to depend on altered characteristics of zona pellucida as a result of the longer culture time before insemination (Nagy *et al.*, 1996). By using ICSI, the fertilization rate of human IVM oocytes has been reported to be 70–80% (Mikkelsen *et al.*, 2000; Child *et al.*, 2001).

Our results show that, although the fertilization rate after insemination per all matured oocytes is low (38%), the developmental potential of the embryos is high. Among other factors discussed above, the low fertilization rate can at least partly be explained by the fact that the maturity of oocytes used for IVM-IVF is assessed after and not before insemination as is done in the case of microinjection. Although the patients in the IVM-ICSI group underwent ET significantly more often than patients in the IVM-IVF group, and had more embryos available for transfer compared with IVM-IVF patients, the clinical PR per IOC was similar between the groups. The better IR of embryos after IVM-IVF embryos compared with that after IVM-ICSI suggests that the developmental competence of the fertilized oocytes is better after IVF than after ICSI.

In women with PCO or PCOS, pregnancy rates between 23 and 35% per ET and implantation rates between 7 and 12% have been reported (Cha *et al.*, 2000; Chian *et al.*, 2000; Child *et al.*, 2001; Chian, 2003; Lin *et al.*, 2003). Most investigators have used HCG priming and routinely transferred 3–4, even 6, embryos at a time (Cha *et al.*, 2000). We have shown a PR of 42% per ET and an IR of 27% in our PCOS patients by transferring fewer than two embryos at a time. Furthermore, an IR of 35% was achieved in PCOS patients after IVM-IVF. Interestingly, such good IR has only been achieved after transferring blastocysts produced from IVM oocytes at one centre (Chian, 2003). The reason for high IR of IVM-IVF in PCOS patients needs further investigation.

Where the results in PCOS women were excellent, the outcome in PCO women with follicular selection was less favourable. We wanted to separate these two groups of patients, as they behave endocrinologically differently due to the presence or absence of ovulation. In PCO patients, only two pregnancies started in 20 cycles, both after IVM-IVF. However, the PCO group was small and the fact that there were fewer follicles seen on baseline ultrasound scan in IVM-ICSI than in IVM-IVF patients might explain the poorer outcome of the IVM-ICSI group.

It has been suggested that women with PCO have intraovarian endocrine abnormalities, which might compromise the quality of the oocytes (Russell, 1999). By applying IVM to women with normal ovaries these putative unfavourable effects on oocyte quality are circumvented. Thus far, there have been only a few IVM studies in women with normal ovaries. Mikkelsen *et al.* (1999) reported five pregnancies in 20 FSH pre-treated regularly cycling patients. The pregnancy rates in these non-PCO women have been low; between 4 and 18% per ET (Mikkelsen *et al.*, 2000, 2001; Child *et al.*, 2001). A PR of 25% per ET in 191 subjects shows that IVM is a good alternative also in non-PCO patients.

Adequate preparation of the endometrium is of crucial importance in the IVM cycle as the truncated follicular phase in most cases results in asynchrony between endometrium and the developing embryo. An attempt to overcome this problem was made by freezing all embryos and transferring them back in a natural or hormone replacement frozen–thaw cycle (Suikkari *et al.*, 2000). This strategy proved to be inefficient due to the poor cryosurvival of IVM embryos. In this study we used the same hormone replacement therapy (HRT) for endometrial priming as used by several groups, with minor modifications (Barnes *et al.*, 1995; Mikkelsen *et al.*, 1999; Chian *et al.*, 2000). Studies in oocyte donation (OD) cycles indicate a positive correlation between endometrial thickness and implantation (Check *et al.*, 1993; Friedler *et al.*, 1996; Hoffman *et al.*, 1996). Endometrial thickness of 7 mm has usually been considered appropriate for implantation (Friedler *et al.*, 1996). Altogether, 12 clinical pregnancies started among 66 regularly cycling women with an endometrium thinner than 5 mm on the day of IOC. The thinnest endometrium with an ongoing pregnancy was 3 mm on

the day of IOC. This suggests that the HRT commenced after oocyte retrieval is sufficient for embryo implantation even in situations with a very thin endometrium.

The miscarriage rate of 27% was somewhat higher than that of 21–24% after conventional IVF/ICSI over the last 3 years at our clinic. The reason for this is unclear. No positive correlation between the embryo quality and the miscarriage rate was found (data not shown). Possible explanations might be failures in embryo development or insufficient endometrial priming. In OD recipients, the early miscarriage rate is significantly higher in recipients with short compared with long estrogen priming, indicating an adverse affect of the short cycle on endometrial receptivity (Navot *et al.*, 1991). This conclusion may apply for IVM cycles also.

Immature oocyte collection combined with IVM is an easy and convenient option for the infertile couple compared to conventional approaches. Favourable pregnancy results can be achieved both in patients with regular cycles and PCO(S) by transferring only one or two embryos at a time. This is important as it is in line with our general embryo transfer policy. Our experience shows that insemination of IVM oocytes functions well, leading to a similar pregnancy rate per IOC as with IVM-ICSI. The IVM method has in a short time progressed from a research level into clinical practice, producing live births at a consistent rate. Patients need to be carefully counselled regarding this new method and follow-up of children will be necessary.

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