Intracytoplasmic sperm injection, results in women older than 39, according to age and the number of embryos replaced in selective or non-selective transfers

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The aim of this study was to assess the results of intracytoplasmic sperm injection (ICSI) in a large cohort of women older than 39 according to age and to embryo transfer policy. In all, 736 ICSI cycles were analysed retrospectively. In 576 (78.3%) cycles an embryo transfer was carried out. The embryo transfer was defined as non-selective when all the available embryos were transferred, and as selective when fewer than the available number of embryos were replaced. A statistically significant gradual decrease in the number of embryos available for transfer, the number of good or excellent quality embryos available for transfer, the pregnancy rates, the clinical pregnancy rates, the implantation rates and the viable pregnancy rates was found with advancing age. No viable pregnancies ensued in women from 45 years old onwards. There was a statistically significant gradual increase in the pregnancy rates, the clinical pregnancy rates, the implantation rates and the viable pregnancy rates from non-selective to selective transfers. The results were similar in women with five or more embryos available, irrespective of the embryo transfer policy. It seems, therefore, that the ovarian reserve and the chances for a successful pregnancy decrease gradually with advancing age, and it is pointless to treat women from 45 years old onwards. A subgroup of patients with better ovarian response and more embryos available for transfer have higher chances of conception. Conception and implantation rates depend mainly on the quality of the transferred embryos. However, the implantation capacity of the embryos is generally lower irrespective of their good morphology.
Key words: embryo quality/embryo transfer/female age/implantation/intracytoplasmic sperm injection

Introduction

Fertility, defined as the ability to achieve a pregnancy, declines gradually over a woman's reproductive lifespan. Although this decline seems to begin from the age of 30 years, it is more obvious between 35 and 40 and increases dramatically thereafter. This phenomenon has been demonstrated in couples attempting natural conception and occurs despite the continuation of apparently normal ovulatory cycles (Menken et al., 1986). Data from therapeutic donor insemination programmes have also documented a decline in fertility with maternal age (CECOS, 1982). Moreover, this age-dependent decrease in female fertility has been confirmed by the lower success rates with advancing age in women undergoing various assisted reproduction techniques (Tan et al., 1992; Devroey et al. 1996; Hull et al. 1996; Marcus and Brinsden 1996; Templeton et al., 1996). In addition to the impaired ability to become pregnant, the risk of miscarriage is also increased in older women, further decreasing the chances of a successful delivery (Abdalla et al., 1993).

The decline in female reproductive potential correlates with ovarian factors (Navot et al., 1991; Scott and Hoffmann, 1995; Wallach, 1995; Marcus and Brinsden, 1996; Templeton et al., 1996), although a slight contribution from reduced uterine receptivity cannot be totally excluded (Marcus and Brinsden, 1996; Templeton et al., 1996). The ovarian reserve decreases with advancing age, as indicated by the constant and unrelenting reduction in the numbers of oocytes (Faddy and Gosden, 1996). Furthermore, there is a concomitant decrease in the quality of the oocytes, as indicated also by the increased incidence of oocyte aneuploidy (Munné et al., 1995).

However, an important aspect of the diminished ovarian reserve and of the associated decline in reproductive potential is that the timing of its onset is highly variable, since it is a biological and not a chronological phenomenon (Scott and Hofmann, 1995; Wallach, 1995; Marcus and Brinsden, 1996). Ovarian response to stimulation seems to be a reliable indicator of the ovarian reserve (Scott and Hofmann, 1995; Marcus and Brinsden, 1996). Thus, women >40 years old with adequate follicular development may represent a subgroup of patients with higher chances of conception (Scott et al., 1995; Roest et al., 1996; Tarlatzis et al., 1996; Vandervorst et al., 1997), especially when combined with a more aggressive embryo transfer policy (Widra et al., 1996; Adonakis et al., 1997).

The aim of this study was to assess the results of intracytoplasmic sperm injection (ICSI) in a large cohort of women >39 years old. It is an extension of our previous study (Adonakis et al., 1997) in an increased number of cycles, examining also the effect of age and the numbers of embryos replaced in selective or non-selective transfers.

Materials and methods

A total of 736 ICSI cycles, which were performed in 443 women >39 years old from 1 October 1991 until 31 December 1996, has been retrospectively analysed. The indications for ICSI were: (i) total

absence or <5% of normal fertilization after standard IVF and (ii) <500 000 progressively motile spermatozoa in the whole ejaculate. Ovarian stimulation was induced by human menopausal gonadotrophin (HMG; Humegon®; Organon, Oss, The Netherlands or Pergonal®; Serono, Geneva, Switzerland) in association mainly with the gonadotrophin-releasing hormone analogue (GnRHa) buserelin (Suprefact®; Hoechst, Frankfurt, Germany). The ovarian stimulation protocols have been described in detail elsewhere (Smitz et al., 1988; Staessen et al., 1989). At an appropriate time in the stimulation, 10 000 IU of human chorionic gonadotrophin (HCG; Pregnyl®; Organon, Oss, The Netherlands or Profasi®; Serono, Geneva, Switzerland) were injected; 36 h later, oocyte retrieval was carried out under vaginal ultrasound guidance.

Oocyte handling and ICSI procedures have been extensively described elsewhere (Van Steirteghem et al., 1993, 1995). Approximately 16 h after ICSI procedure, fertilization was confirmed by the presence of two distinct pronuclei. Embryo cleavage as well as the evaluation of embryo quality were assessed after a further 24 h of culture. The cleaved embryos were classified into three categories. Type A, or excellent quality, embryos were defined as embryos in which all blastomeres have equal or unequal size without anucleate fragments. Type B, or good quality, embryos have blastomeres of equal or unequal size and a maximum of 20% of the volume of the embryo filled with anucleate fragments. In the third category, type C, or fair quality, embryos, anucleate fragments are present in 20–50% of the volume of the embryo (Staessen et al., 1992).

Embryos were transferred 48 h after oocyte retrieval. The embryo transfer was defined as non-selective when all the available embryos were transferred and as selective when the number of embryos transferred was fewer than the number of embryos available for transfer. This was a team proposal and a combined decision with the patient. The following groups of embryo transfers were studied: non-selective transfer of one embryo, non-selective transfer of two embryos, non-selective and selective transfers of three embryos, non-selective and selective transfers of four embryos, and transfer of five or more embryos. In the latter group, both non-selective and selective transfers of five or six embryos were included, since in only 10 out of 98 cycles embryos available were seven or more. Moreover, in women with five or more embryos available, two different groups of embryo transfers were studied: selective transfer of three or four embryos and transfer of five or six embryos.

The luteal phase was supplemented with progesterone (Utrogestan®; Piette, Brussels, Belgium) administered vaginally or with HCG administered i.m. Pregnancy was confirmed by detecting increasing serum HCG concentrations on two occasions, 4 days apart, at least 12 days after embryo transfer. Clinical pregnancy was defined as the presence of at least one gestational sac at 7 weeks of gestation on ultrasound examination together with a positive HCG test. The implantation rate was defined as the ratio of the number of sacs and the number of embryos transferred (Hull et al., 1996). Viable pregnancy was defined as the presence of at least one living fetus over 12 weeks of gestation.

Prenatal diagnosis was proposed by chorionic villus sampling at 9–10 weeks of gestation or by amniocentesis at 16 weeks of gestation. Genetic counselling was given in view of the prenatal diagnosis and for the planning of a prospective follow-up study of the children born after ICSI. The referring gynaecologist and the patients were asked to provide detailed information on the evolution of the pregnancy and the outcome of the delivery (Wisanto et al., 1996; Bonduelle et al., 1996).

Statistics

The correlation between the number and quality of the embryos available for transfer with the patient's age was examined using polynomial regression analysis. The correlation between the number and quality of the available embryos was examined using linear regression analysis. The differences between the pregnancy and the implantation rates were compared using the χ^2 test or the χ^2 test for overall trend.

Results

In 576 (78.3%) out of the 736 ICSI cycles analysed here, an embryo transfer was carried out. In 160 cycles there was no embryo available for transfer. In 33 cycles no mature oocyte was retrieved, in 17 cycles no spermatozoa were available for ICSI, in 75 cycles none of the injected oocytes fertilized normally and in the remaining 35 cycles none of the fertilized oocytes cleaved. In all, 108 pregnancies ensued. In 83 of these, at least one gestational sac was detected (clinical pregnancies), while 53 pregnancies continued after 12 weeks of gestation (viable pregnancies). Forty-one women delivered, two had a second trimester abortion, while the final outcome is not known at the time of writing for 10 pregnancies.

The pregnancy rates, the clinical pregnancy rates, the implantation rates and the viable pregnancy rates per started cycle and per cycle with embryo transfer according to age are shown in Table I. A statistically significant gradual decrease ($P < 0.0001$) in the percentage of cycles with embryo transfer was observed with advancing age. Moreover, there was a statistically significant gradual decrease in the pregnancy rates ($P = 0.002$), the clinical pregnancy rates ($P < 0.05$), the implantation rates ($P = 0.01$) and the viable pregnancy rates ($P < 0.05$) with advancing age. No ongoing pregnancy has been observed in women from 45 years old onwards. The proportion of preclinical and clinical abortions was also high (Table I).
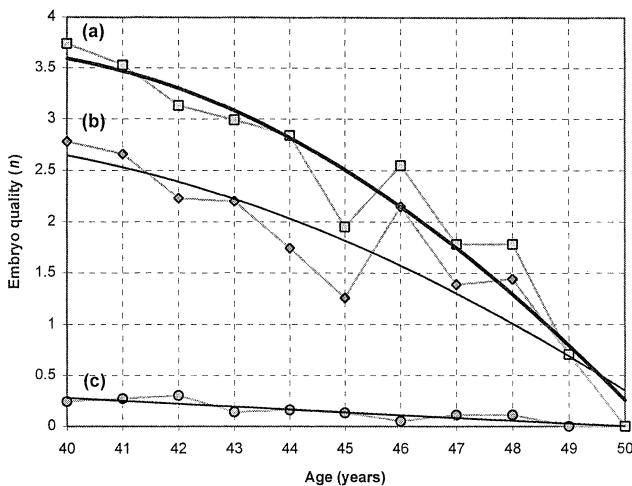
Regression analysis of the mean numbers of the available embryos ($y = –0.0233x^2 – 0.0543x + 3.6707$, $r^2 = 0.938$), the mean numbers plus the standard deviation of the available embryos ($y = –0.0714x^2 + 0.235x + 6.5539$, $r^2 = 0.954$), and the mean numbers minus the standard deviation of the available embryos ($y = 0.0248x^2 – 0.3436x + 0.7875$, $r^2 = 0.476$) according to age are shown in Figure 1. A significant negative correlation ($P < 0.001$) between the number of available embryos and the age of the patient was found.

A regression analysis of the mean numbers of the embryos available ($y = –0.0233x^2 – 0.0543x + 3.6707$, $r^2 = 0.938$), the mean numbers of type A (excellent quality) embryos available ($y = –0.0127x^2 – 0.0774x + 2.7359$, $r^2 = 0.844$) and the mean numbers of type A plus B (excellent or good quality) embryos available ($y = 0.0001x^2 – 0.0291x + 0.3056$, $r^2 = 0.813$) according to age is given in Figure 2. A gradual decrease in the number of good or excellent quality embryos was found with advancing age.

The distribution of the cycles according to the number of embryos available is shown in Figure 3. A gradual decrease in the number of cycles as the number of embryos increased was observed. A linear regression analysis of mean numbers of type A (excellent quality) embryos available ($y = 0.0812x – 0.1516$, $r^2 = 0.524$), and mean numbers of type A plus B (excellent or good quality) embryos available ($y = 0.81x$
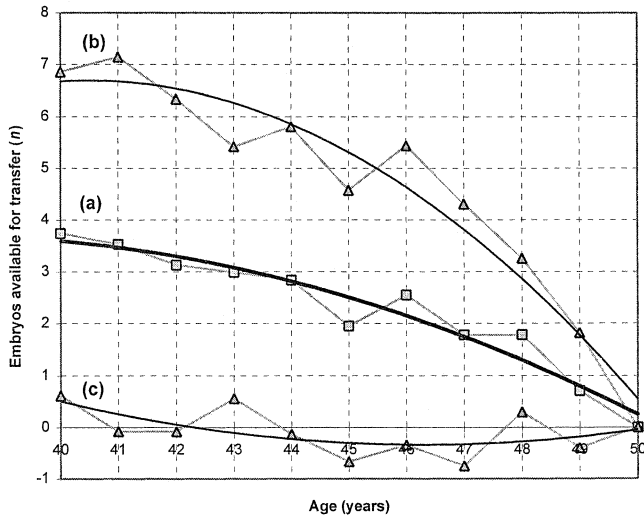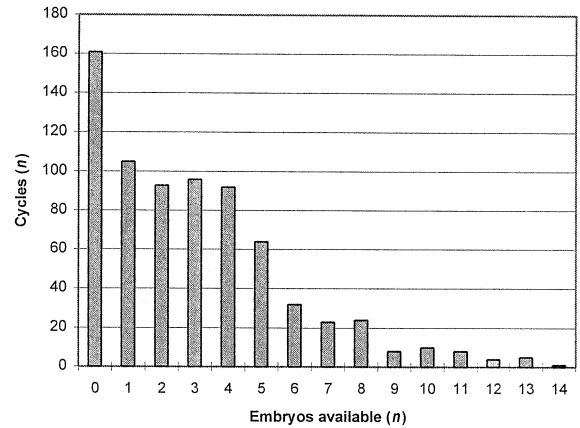
Figure 1. Regression analysis of (**a**) the mean numbers of the available embryos (y = $-0.0233x^2 - 0.0543x + 3.6707$, $r^2 = 0.938$), (**b**) the mean numbers plus the standard deviation of the available embryos (y = $-0.0714x^2 + 0.235x + 6.5539$, $r^2 = 0.954$) and (**c**) the mean numbers minus the standard deviation of the available embryos (y = $0.0248x^2 - 0.3436x + 0.7875$, $r^2 = 0.476$) according to age.
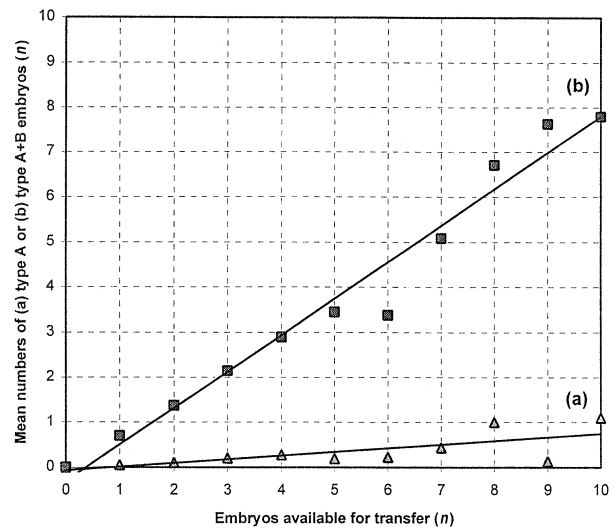

Figure 2. Regression analysis of (**a**) the mean numbers of embryos available (y = $-0.0233x^2 - 0.0543x + 3.6707$, $r^2 = 0.938$), (**b**) the mean numbers of type A and B (excellent or good quality) embryos available (y = $0.0001x^2 - 0.0291x + 0.3056$, $r^2 = 0.813$) and (**c**) the mean numbers of type A (excellent quality) embryos available (y = $-0.0127x^2 - 0.0774x + 2.7359$, $r^2 = 0.844$) according to age.

$- 1.1164$, $r^2 = 0.968$) according to the total number of embryos available for transfer is given in Figure 4.

The results according to the groups of embryos transferred are shown in Table II. There was a statistically significant gradual increase in the pregnancy rates ($P < 0.0001$), the clinical pregnancy rates ($P < 0.0001$), the implantation rates ($P < 0.01$) and the viable pregnancy rates ($P < 0.0001$) in the following order: non-selective transfer of one embryo, non-selective transfer of two or three embryos, non-selective transfer of four or five or more embryos and selective transfer of three or four embryos. Moreover, the viable pregnancy rates correlated more closely with the implantation rates (Table II).

Figure 3. Distribution of the cycles according to the number of embryos available for transfer.

Figure 4. Linear regression analysis of the mean numbers of (**a**) type A (excellent quality) embryos available (y = $0.0812x - 0.1516$, $r^2 = 0.524$) and (**b**) the mean numbers of type A and B (excellent or good quality) embryos available (y = $0.81x - 1.1164$, $r^2 = 0.968$) according to the total number of embryos available for transfer.

In all, 16 multiple pregnancies were achieved: 15 twins and one triplet (non-elective transfer of four embryos). Six (40%) out of 15 continued as singleton viable pregnancies (vanishing twins) and one ended as a miscarriage. No difference was found between the different types of embryo transfer in the clinical and the viable multiple pregnancy rates, expressed either per transfer (Table II) or per clinical and per viable pregnancy.

The results according to the groups of embryos transferred in women with five or more embryos available are shown in Table III. The pregnancy rates, the clinical pregnancy rates, the implantation rates, the viable pregnancy rates and the multiple pregnancy rates were similar between women with selective embryo transfer of three or four embryos and women with embryo transfer of five or six embryos, although a trend towards better results was observed in women with selective embryo transfer of three or four embryos.

Table I. ICSI results according to the age of the patients

		Age (years)							Total	P value
		40	41	42	43	44	45	46		
Cycles started	(n)	222	166	110	80	62	40	56	736	
Transfer	(n)	191	131	90	61	46	24	33	576	
per started cycle		86	78.9	81.8	76.2	74.2	60	58.9	78.3	< 0.0001 (χ^2 for trend)
Pregnancies	(n)	46	33	12	11	3	2	1	108	
per cycle	(%)	20.7	19.9	10.9	13.7	4.8	5.0	1.8	14.7	
per transfer	(%)	24.1	25.2	13.3	18.0	6.5	8.3	3.0	18.7	0.002 (χ^2 for trend)
Preclinical abortions	(n)	11	8	4	2	0	0	0	25	
Clinical pregnancies	(n)	35	25	8	9	3	2	1	83	
per cycle	(%)	15.8	15.1	7.3	11.2	4.8	5.0	1.8	11.3	
per transfer	(%)	18.3	19.1	8.9	14.7	6.5	8.3	3.0	14.4	< 0.05 (χ^2 for trend)
Implantation rate	(%)	6.7	7.7	3.8	4.8	1.9	3.0	0.9	5.4	0.01 (χ^2 for trend)
Abortions	(n)	12	7	1	5	2	1	1	29	
Ectopics	(n)	0	0	0	0	0	1	0	1	
Viable pregnancies	(n)	23	18	7	4	1	0	0	53	
per cycle	(%)	10.4	10.8	6.4	5.0	1.6	0.0	0.0	7.2	
per transfer	(%)	12.0	13.7	7.8	6.7	2.2	0.0	0.0	9.2	< 0.05 (χ^2 for trend)

Table II. ICSI results in women ≥40 years old according to the number of embryos transferred

		1 Non-select.	2 Non-select.	3 Non-select.	4 Non-select.	≥5[a] Select. & non-select.	3 Select.	4 Select.	Total	P value
Cycles	(n)	105	101	94	73	98	50	55	576	
Pregnancies	(n)	2	10	14	20	26	14	22	108	
per transfer	(%)	1.9	10.0	14.9	27.4	26.5	28.0	40.0	18.7	< 0.0001 (χ^2 for trend)
Preclinical abortions	(n)	0	3	4	4	5	2	7	25	
Clinical pregnancies	(n)	2	7	10	16	21	12	15	83	
per transfer	(%)	1.9	7.0	10.6	21.9	21.4	24.0	27.3	14.4	< 0.0001 (χ^2 for trend)
Implantation rate	(%)	1.9	3.5	3.5	6.4	5.0	10.7	7.7	5.4	< 0.01 (χ^2 for trend)
Abortions	(n)	1	2	3	6	9	2	6	29	
Ectopics	(n)	0	0	1	0	0	0	0	1	
Viable pregnancies	(n)	1	5	6	10	12	10	9	53	
per transfer	(%)	0.9	5.0	6.4	13.7	12.3	20.0	16.4	9.2	< 0.0001 (χ^2 for trend)
Clinical multiple pregnancies	(n)	0	0	0	3[b]	7	4	2	16[b,c]	
per transfer	(%)	0.0	0.0	0.0	4.1	7.1	8	3.6	2.8	NS (χ^2)
Viable multiple pregnancies	(n)	0	0	0	2[b]	3	2	2	9[b]	
per transfer	(%)	0.0	0.0	0.0	2.7	3.1	4	3.6	1.6	NS (χ^2)

[a]Non-selective (mainly) or selective transfer of five or six embryos, [b]one triplet, [c]one miscarriage and six vanishing twins (ongoing as singleton), NS: not significant.

Discussion

The growing trend in delayed childbearing in Western countries for a variety of socio-economic reasons has increased the demand for therapy from infertile women >40 years old (Bopp et al., 1995; Marcus and Brinsden 1996; van Kooij et al., 1996). More insight will help physicians to counsel their infertile couples appropriately, especially when an advanced and expensive treatment such as ICSI is needed. The present study examines the results of ICSI in a cohort of women >39 years old, the largest to our knowledge from one single centre. It is limited to ICSI results and cannot be extrapolated to IVF, since more chromosomally abnormal embryos may be present in the ICSI population due to possible male abnormality. In IVF, with possibly more chromosomally normal embryos, more ongoing pregnancies may be expected. The findings of

this study confirm the generally accepted decrease in pregnancy rates with advancing age in women treated with various forms of assisted reproductive techniques (Tan et al., 1992; Abdelmassih et al., 1996; Devroey et al., 1996; Dor et al., 1996; Hull et al., 1996; Templeton et al., 1996; Widra et al., 1996). Despite the application of an advanced therapy, the chance of a successful pregnancy in this group appears limited due to the intrinsic reduced fertility coupled with a higher risk of fetal loss (Hull et al., 1996; Widra et al., 1996). Moreover, it seems that treatment of patients from 45 years old onwards is pointless, as also observed by Widra et al. (1996).

In our study population, a statistically significant gradual decrease in the mean number of the available embryos per started cycle was observed with advancing age. This finding reflects the diminished ovarian reserve with advancing age,

Table III. ICSI results in women 40 years old and over with five or more embryos available according to the number of embryos transferred

		≤4[a] selective	≥5[b] selective & non-selective	Total	*P* value
Cycles	(*n*)	93	98	191	
Pregnancies	(*n*)	31	26	57	
per transfer	(%)	33.3	26.5	29.8	NS (χ^2)
Preclinical abortions	(*n*)	7	5	12	
Clinical pregnancies	(*n*)	24	21	45	
per transfer	(%)	25.8	21.4	23.6	NS (χ^2)
Implantation rate	(%)	8.4	5.0	6.3	NS (χ^2)
Abortions	(*n*)	6	8	14	
Ectopics	(*n*)	0	0	0	
Viable pregnancies	(*n*)	18	12	30	
per transfer	(%)	19.4	12.3	15.7	NS (χ^2)
Clinical multiple pregnancies	(*n*)	4	7	11	
per transfer	(%)	4.3	7.1	5.8	NS (χ^2)
Viable multiple pregnancies	(*n*)	3	3	6	
per transfer	(%)	3.2	3.1	3.1	NS (χ^2)

[a]Selective transfer of three or four embryos, [b]non-selective (mainly) or selective transfer of five or six embryos, NS: not significant.

since fertilization rates after ICSI do not depend on sperm quality and are similar in women >40 years and in younger patients (Devroey *et al.*, 1996; Hull *et al.*, 1996). A parallel gradual decrease in the available embryos of good or excellent quality was also found. However, the number of the embryos available for transfer may differ from patient to patient, as is shown in Figures 1 and 3. Some women of even older age may have a better response to stimulation and thus more embryos available for transfer. These patients seem to represent a subgroup with better chances of conception (Roest *et al.*, 1996; Tarlatzis *et al.*, 1996; Vandervorst *et al.*, 1997). Moreover, a more aggressive embryo transfer policy has been proposed in order further to increase pregnancy rates, since implantation rates are lower in older women and thus the risk of multiple pregnancies limited (Azem *et al.*, 1995; Widra *et al.*, 1996; Adonakis *et al.*, 1997). It has therefore been proposed to transfer up to six embryos, rather than four, in women >40 years old (Widra *et al.*, 1996; Adonakis *et al.*, 1997). Cryopreservation of embryos in women >40 years could be an alternative. However, we were not able to obtain acceptable pregnancy rates with frozen–thawed embryos in women ≥40 years of age.

The routine application of pre-implantation genetic diagnosis (PGD) in older women and the subsequent elective transfer of euploid embryos have been recently proposed as an alternative in order to improve the pregnancy rates, to reduce the abortion rates, to avoid high-order multiple pregnancies and to prevent the birth of children with common trisomies (Verlinsky and Kuliev, 1996). Two approaches have become available for PGD of aneuploidies on the basis of fluorescent in-situ hybridization (FISH), i.e. the cleavage-stage biopsy and FISH analysis of one or two blastomeres or the FISH analysis of polar bodies removed from the oocytes (Verlinsky and Kuliev, 1996). However, there is still debate concerning the safety, the accuracy and the necessity of this approach (Reubinoff and

Shushan, 1996) in the absence of controlled randomized studies. Moreover, PGD is available only in a limited number of centres all over the world.

The variation in the numbers of embryos available for transfer, as well as the different embryo transfer policies, are reflected in the different groups of embryo transfers. A statistically significant gradual increase was found in pregnancy rates, clinical pregnancy rates, implantation rates and viable pregnancy rates when the number of the embryos available for transfer increased in cases of non-selective transfer as well as with the selective transfer of fewer than the available number of embryos in women with adequate ovarian response. It therefore seems that the achievement of pregnancy depends mainly on the quality of the transferred embryos, since success rates were higher in the selective embryo transfers. The increased pregnancy rates with the transfer of more embryos in the non-selective transfers may simply be due to the parallel increase in the number of good or excellent quality embryos replaced.

However, even in the selective transfers, the implantation rates never reached high values and these values were far from the implantation rates achieved in younger women (Staessen *et al.*, 1992). This is probably due to a decrease in the implantation capacity of the embryos irrespective of their excellent or good morphology. Age-related oocyte factors commonly lead to aneuploidy in the cleaving embryo (including mosaicism), without interfering with fertilization and early stages of embryo development. Expression of the embryonic genome would occur only after the four-cell to eight-cell stage and might impair implantation and normal evolution of the pregnancy despite apparently normal fertilization and embryo morphology (Munné *et al.*, 1995; Adonakis *et al.*, 1997).

The lower implantation capacity of the embryos in women >40 years old is also expressed in the lower than expected multiple-pregnancy rates irrespective of the group of embryo transfer (Azem *et al.*, 1995). Moreover, most of the multiple pregnancies involved were twins, as also observed by other investigators (Azem *et al.*, 1995). On the other hand, there is an increased possibility of a higher-order multiple pregnancy (Walters, 1996). However, this seems to be associated with the transfer of three or more embryos (even non-selective) and not only to the transfer of five or more embryos, since the triplet pregnancy was achieved with a non-selective transfer of four embryos. This is important when embryo culture conditions are improved or duration of embryo culture is increased. In this condition a more conservative embryo transfer policy could be adopted.

Approximately half of the twin pregnancies continued as viable singletons. This may be an advantage of the more aggressive embryo transfer policy, since at least a viable ongoing pregnancy ensued from the replacement of higher numbers of the embryos available.

The effectiveness of a more aggressive embryo transfer policy in women >39 years old with five or more embryos available was evaluated by comparing two different groups of embryo transfer. The similar results obtained either by an selective transfer of three or four embryos or by the transfer of five or six embryos are probably due to the selection of the

best embryos in cases of selective transfer. This also confirms that the achievement of pregnancy depends on embryo quality. Thus, in order to benefit from an aggressive embryo transfer policy, five or more good quality embryos need to be transferred. As shown in Figure 5, this can be achieved when more than seven embryos are available for transfer, but this is possible in a limited number of patients because usually fewer oocytes are available.

In conclusion, it seems that the ovarian reserve and the chances of a successful pregnancy decrease gradually with advancing age in women from 40 years old onwards. Moreover, it is pointless to treat women from 45 years old onwards. A subgroup of patients with adequate ovarian response and more embryos available for transfer have greater chances of conception. Conception and implantation rates depend mainly on the quality of the transferred embryos. However, the implantation capacity of the embryos is generally lower irrespective of their good morphology. Thus, until the full elucidation of the validity of routine PGD, a more aggressive embryo transfer policy in women >40 years old may improve pregnancy rates, since the risk of high-order pregnancies seems to be limited. The effectiveness of this policy seems also to be related to the quality of the embryos available for transfer.

Acknowledgements

References

Abdalla, I.H., Burton, G., Kirkland, A. *et al.* (1993) Age, pregnancy and miscarriage: uterine versus ovarian factors. *Hum. Reprod.*, **8**, 1512–1517.

Abdelmassih, R., Sollia, S., Moretto, M. and Acosta, A.A. (1996) Female age is an important parameter to predict treatment outcome in intracytoplasmic sperm injection. *Fertil. Steril.*, **65**, 573–577.

Adonakis, G., Camus, M., Joris, H. *et al.* (1997) The role of the number of replaced embryos on the intracytoplasmic sperm injection outcome in women over the age of 40. *Hum. Reprod.*, **12**, 2542–2545.

Azem, F., Yaron, Y., Amit, A. *et al.* (1995) Transfer of six more embryos improves success rates in patients with repeated *in vitro* fertilization failures. *Fertil. Steril.*, **63**, 1043–1046.

Bonduelle, M., Wilikens, A., Buysse, A. *et al.* (1996) Prospective follow-up study of 877 children born after intracytoplasmic sperm injection (ICSI), with ejaculated, epididymal and testicular spermatozoa and after replacement of cryopreserved embryos obtained after ICSI. *Hum. Reprod.*, **11**, 131–155.

Bopp, L.B., Alper, M.M., Thompson, E.I. and Mortola, J. (1995) Success rates with gamete intrafallopian transfer and *in vitro* fertilization in women of advanced maternal age. *Fertil. Steril.*, **63**, 1278–1283.

CECOS Federation, Schwartz, D., Mayaux, J.M. (1982) Female fecundity as a function of age: results of artificial insemination in 2193 nulliparous women with azoospermic husbands. *N. Engl. J. Med.*, **306**, 404–406.

Devroey, P., Godoy, H., Smitz, J. *et al.* (1996) Female age predicts embryonic implantation after ICSI: a case-control study. *Hum. Reprod.*, **11**, 1324–1327.

Dor, J., Seidman, S.D., Ben-Shlomo, I. *et al.* (1996) Cumulative pregnancy rates following *in vitro* fertilization: the significance of age and infertility aetiology. *Hum. Reprod.*, **11**, 425–428.

Faddy, M.J. and Gosden, R.G. (1996) A model conforming the decline in follicle numbers to the age of menopause in women. *Hum. Reprod.*, **11**, 1484–1486.

Hull, G.R.M., Fleming, F.C., Hughes, O.A. and McDermott, A. (1996) The age-related decline in female fecundity: a quantitative controlled study of implanting capacity and survival of individual embryos after *in vitro* fertilization. *Fertil. Steril.*, **65**, 783–790.

Marcus, S.F. and Brinsden, P.R. (1996) In-vitro fertilization and embryo transfer in women aged 40 years and over. *Hum. Reprod. Update*, **2**, 459–468.

Menken, J., Trussell, J. and Larsen, U. (1986) Age and infertility. *Science*, **233**, 1389–1394.

Munné, S., Alikani, M., Tomkin, G. *et al.* (1995) Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. *Fertil. Steril.*, **64**, 382–391.

Navot, D., Bergh, A.P., Williams, M.-A. *et al.* (1991) Poor oocyte quality rather than implantation failure as a cause of age related decline in female fertility. *Lancet*, **337**, 1375–1377.

Reubinoff, B.E. and Shushan, A. (1996) Preimplantation diagnosis in older patients: to biopsy or not to biopsy? *Hum. Reprod.*, **11**, 2071–2076.

Roest, J., van Heusden, A., Mous, H. *et al.* (1996) The ovarian response as a predictor for a successful *in vitro* fertilization treatment after the age of 40 years. *Fertil. Steril.*, **66**, 969–973.

Scott, T.R. and Hofmann, E.G. (1995) Prognostic assessment of ovarian reserve. *Fertil. Steril.*, **63**, 1–11.

Scott, T.R., Opsahl, S.M., Leonardi, R.M. *et al.* (1995) Life table analysis of pregnancy rates in a general infertility population relative to ovarian reserve and patient age. *Hum. Reprod.*, **10**, 1706–1710.

Smitz, J., Devroey, P., Camus, M. *et al.* (1988) The luteal phase and the early pregnancy after combined GnRH agonist/hMG treatment for superovulation in IVF and GIFT. *Hum. Reprod.*, **3**, 585–590.

Staessen, C., Camus, M., Khan, I. *et al.* (1989) An 18-month survey of infertility treatment by in-vitro fertilization, gamete and zygote intrafallopian transfer and replacement of frozen-thawed embryos. *J. In Vitro Fert. Embryo Transfer*, **6**, 22–29.

Staessen, C., Camus, M., Bollen, N. *et al.* (1992) The relationship between embryo quality and the occurrence of multiple pregnancies. *Fertil. Steril.*, **57**, 626–630.

Tan, S-L., Royston, P., Campbell, S. *et al.* (1992) Cumulative conception and livebirth rates after in-vitro fertilisation. *Lancet*, **339**, 1390–1394.

Tarlatzis, B.C., Grimbizis, G., Bontis, J. *et al.* (1996) The number of follicles developed after stimulation can better predict the IVF outcome than the woman's age. *Hum. Reprod.*, **11**, 146.

Templeton, A., Morris, K.J. and Parslow, W. (1996) Factors that affect outcome of in-vitro fertilization treatment. *Lancet*, **348**, 1402–1406.

Vandervorst, M., Joris, H., Van Steirteghem, A. and Devroey, P. (1997) Correlation between ongoing pregnancy rates and the number of cumulus–oocyte complexes retrieved in agonist-HMG-stimulated ICSI cycles. *Hum. Reprod.*, **12**, 168–169.

van Kooij, J.R., Looman, W.N.C., Habbema, D.F.J. *et al.* (1996) Age-dependent decrease in embryo implantation rate after *in vitro* fertilization. *Fertil. Steril.*, **66**, 769–775.

Van Steirteghem, A., Nagy, Z., Joris, H. *et al.* (1993) High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum. Reprod.*, **7**, 1061–1066.

Van Steirteghem, A., Joris, H., Liu, J. *et al.* (1995) Protocol for intracytoplasmic sperm injection. *Hum. Reprod. Update*, **1**, 302–327.

Verlinsky, Y. and Kuliev, A. (1996) Preimplantation diagnosis of common aneuploidies in infertile couples of advanced maternal age. *Hum. Reprod.*, **11**, 2076–2077.

Wallach, E.E. (1995) Pitfalls in evaluating ovarian reserve. *Fertil. Steril.*, **63**, 12.

Walters, D.E. (1996) The statistical implication of the 'number of replacements' in embryo transfer. *Hum. Reprod.*, **11**, 10–12.

Widra, A.E., Gindoff, R.P., Smotrich, B.D. and Stillman, J.R. (1996) Achieving multiple-order embryo transfer identifies women 40 years of age with improved *in vitro* fertilization outcome. *Fertil. Steril.*, **65**, 103–108.

Wisanto, A., Bonduelle, M., Camus, M. *et al.* (1996) Obstetric outcome of 904 pregnancies after intracytoplasmic sperm injection. *Hum. Reprod.*, **11**, 121–130.