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The effect of female age and ovarian reserve on pregnancy rate in male infertility: treatment of azoospermia with sperm retrieval and intracytoplasmic sperm injection

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Factors other than spermatozoa could be the major determinant of the success of assisted reproduction treatment in cases of male infertility. Our aim was to evaluate the effect of the wife's age and ovarian reserve on assisted reproduction success rates in the most severe type of male infertility, i.e. azoospermia. A total of 249 consecutive couples suffering from male infertility caused by azoospermia underwent microsurgical epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE) with intracytoplasmic sperm injection (ICSI). Of these men, 186 had irreparable obstructive azoospermia, and 63 had non-obstructive azoospermia due to testicular failure. Neither the pathology, the source, the quantity, nor the quality of spermatozoa had any effect on fertilization or pregnancy rates. Maternal age and ovarian reserve (number of eggs) had no effect on fertilization or embryo cleavage, but did dramatically affect the embryo implantation, pregnancy and delivery rates. Wives of azoospermic men who were in their 20s had a 46% live delivery rate per cycle, wives aged 30–36 years had a 34% live delivery rate per cycle, wives aged 37–39 years had a 13% live delivery rate per cycle, and wives ⩾40 years had only a 4% live delivery rate per cycle. The number of eggs retrieved also affected pregnancy and delivery rate, but to a lesser extent than age. In virtually all cases of obstructive azoospermia, and in 62% of cases with non-obstructive azoospermia caused by germinal failure, sufficient spermatozoa could be retrieved to perform ICSI, with normal fertilization and embryo cleavage. However, the pregnancy rate and the live delivery rate were dependent strictly on the age of the wife, and on her ovarian reserve. Unfortunately, exaggerated claims of high pregnancy rates can thus easily be made by manipulating, in a very simple way, selection for female factors.

Key words: age/azoospermia/fertilization/ICSI/pregnancy

Introduction

It has been known for many decades that a couple's age has a major impact on their risk for being infertile (Mosher, 1976, 1985a,b, 1987; Schwartz and Mayaux, 1991; Silber, 1990). However, it was not suggested until recently that the woman's age could be the major determinant of pregnancy rate in the most severe cases of male infertility (Silber *et al.*, 1995a). In early reports of microsurgical epididymal sperm aspiration (MESA) and testicular sperm extraction (TESE), combined with intracytoplasmic sperm injection (ICSI), for the treatment of obstructive azoospermia, neither the cause of obstruction nor the source of the spermatozoa seemed to affect the possibility of pregnancy (Silber *et al.*, 1995a). Since the results were affected by the wife's age, we suggested at that time that infertility treatment protocols might be best broken down by age of the wife in order to evaluate properly a clinic's results, or the results of any one treatment over any other treatment.

It has always been assumed that in infertile couples with male factor infertility, the more severe the oligozoospermia, the less likely it is that female factors are concurrently present (Silber, 1989). In fact, donor sperm insemination is known to give higher pregnancy rates in wives of azoospermic men than in wives of oligozoospermic men (Emperaire *et al.*, 1982). Therefore, a study of pregnancy and fertilization rates in azoospermic couples utilizing testicular sperm retrieval and ICSI could clarify further the impact of female factors, if any, in male infertility. The purpose of the present study was to analyse the fertilization, cleavage and pregnancy results (delivered) after sperm retrieval and ICSI in 186 consecutive patients with obstructive azoospermia, and in 63 consecutive patients with non-obstructive azoospermia caused by testicular failure. We wished to determine whether, in the era of ICSI, the source, the number, or the quality of the spermatozoa had any effect on treatment results for the most severe male factor infertility (i.e. azoospermia), or whether the results (assuming some spermatozoa are found) are dependent strictly on female factors, such as the wife's age, and her ovarian reserve.

Materials and methods

Patient population

A total of 186 consecutive and unselected men with irreparable obstructive azoospermia from a variety of causes were subjected to sperm retrieval procedures with ICSI. The results were analysed according to whether the spermatozoa were fresh epididymal, frozen epididymal, or testicular. Results were also analysed according to whether the cause of obstruction was congenital, inflammatory, or post-surgical, and broken down according to the number of eggs retrieved from the wife (ovarian reserve) and her age at the time of the procedure. At the same time, a group of 63 consecutive and unselected patients with non-obstructive azoospermia underwent TESE–ICSI, with the results analysed in a similar fashion (Silber *et al.*, 1996). The causes of non-obstructive azoospermia included

Table I. Obstructive azoospermia: effect of source of spermatozoa on intracytoplasmic sperm injection parameters

Source of spermatozoa	No. of cycles	No. of eggs at MII injected	No. of 2PN oocytes (% of MII eggs)	Normal cleaved embryos (% of 2PN oocytes)
Fresh epididymal (MESA)	75	927	541 (58)	365 (67)
Frozen epididymal	27	356	171 (48)	153 (89)
Testicular biopsy (TESE)	84	1051	550 (52)	388 (71)
Totals	186	2334	1262 (54)	906 (72)

MII = metaphase II; 2PN = two-pronuclear; MESA = microsurgical epididymal sperm aspiration; TESE = testicular sperm extraction.

Table II. Obstructive azoospermia: effect of source of spermatozoa on pregnancy outcome

Source of spermatozoa	No. of cycles	No. of transfers (% of cycles)	No. of clinical pregnancies (% per cycle)	No. delivered (% per cycle)	Mean no. of embryos transferred	Implantation rate % (per embryo)
Fresh epididymal (MESA)	75	73 (97)	36 (48)	26 (35)	2.46	20%
Frozen epididymal	27	27 (100)	11 (38)	10 (33)	2.91	14%
Testicular biopsy (TESE)	84	78 (93)	28 (34)	20 (24)	2.76	13%
Totals	186	178 (96)	78 (40)	56 (30)		16.2%

MESA = microsurgical epididymal sperm aspiration; TESE = testicular sperm extraction.

Table III. Obstructive azoospermia: effect of age of wife

Age of wife (years)	No. of cycles (% of total)	No. of eggs at MII	No. of 2PN oocytes (% of MII eggs)	Normal cleaved embryos (% of 2PN)	No. delivered pregnancies per cycle (% of cycles)	Implantation rate % (per embryo)
<30	50 (27)	735	392 (53)	302 (77)	22 (44)[a]	22[b]
30–36	87 (47)	1111	610 (55)	413 (68)	30 (34)[a]	19[c]
37–39	24 (13)	207	113 (55)	90 (80)	3 (12)[a]	4[d]
40+	25 (13)	281	147 (52)	101 (69)	1 (4)[a]	7[e]
Totals	186 (100)	2334	1262 (54)	906 (72)	56 (30)	16.2

[a]$P < 0.001$ between all four groups; [b,d]$P < 0.001$; [c,e]$P < 0.001$; [c,d,e]$P < 0.001$.
MII = metaphase II; 2PN = two-pronuclear.

Table IIIa. Obstructive azoospermia: effect of age of wife

Age of wife (years)	No. of cycles	No. of eggs at MII	No. of 2PN oocytes (% of MII eggs)	No. of embryos (% of 2PN)	No. delivered pregnancies (% of cycles)	Implantation rate % (per embryo)
20–25	26	408	219 (54)	149 (68)	10 (38)	18
26–29	24	327	173 (53)	153 (88)	12 (50)	26
30–32	34	501	288 (57)	194 (67)	15 (44)	24
33–36	53	610	322 (53)	219 (68)	15 (28)	15
37–38	17	137	84 (61)	62 (74)	2 (12)	5
39–40	15	187	80 (43)	60 (75)	1 (7)	8
>40	17	164	96 (59)	69 (72)	1 (6)	6

Sertoli cell-only, maturation arrest, post-chemotherapy azoospermia, cryptorchidism and post-mumps testicular atrophy.

Sperm retrieval, preparation and ICSI technique

The sperm extraction or aspiration procedures have been extensively described already (Devroey *et al.*, 1994; Silber *et al.*, 1994, 1995a,b, 1996). The husband underwent MESA if the diagnosis was congenital absence of the vas or irreparable obstruction, in which it was easy to obtain epididymal spermatozoa for both a fresh and future frozen cycles. Several years ago, this procedure was difficult and lengthy, but now it is performed under local anaesthesia in <30 min, with minimal pain, and as an outpatient. Spermatozoa were aspirated directly from the opening in the epididymal tubule with a micropipette in the proximal-most portion of the epididymis where the greatest motility was observed. Excess spermatozoa were frozen and saved for future cycles, and no further procedures were performed on the

Table IV. Obstructive azoospermia with epididymal spermatozoa: effect of age of wife. There were no significant differences between age groups for any of the parameters measured

Age of wife (years)	No. of cycles (% of total)	No. of eggs at MII	No. of 2PN oocytes (% of MII eggs)	No. of embryos (% of 2PN)	No. delivered pregnancies (% of cycles)	Implantation rate % (per embryo)
<30	36 (35)	522	273 (52)	207 (76)	16 (44)	22
30–36	49 (48)	621	361 (58)	250 (69)	17 (35)	19
37–39	10 (10)	68	44 (65)	35 (80)	2 (20)	7
40+	7 (7)	72	34 (47)	26 (76)	1 (14)	8
Totals	102 (100)	1283	697 (48)	518 (73)	36 (36)	18

Table V. Obstructive azoospermia with testicular spermatozoa: effect of age of wife

Age of wife (years)	No. of cycles (% of total)	No. of eggs at MII	No. of 2PN oocytes (% of MII eggs)	No. of embryos (% of 2PN)	No. delivered pregnancies (% of cycles)	Implantation rate % (per embryo)
<30	14 (17)	213	119 (56)	95 (80)	6 (43)[a]	23[e]
30–36	38 (45)	490	249 (51)	163 (66)	13 (34)[b]	18[f]
37–39	14 (17)	139	69 (50)	55 (80)	1 (7)[c]	3[g]
40+	18 (21)	209	113 (54)	75 (66)	0 (0)[d]	7[h]
Totals	84 (100)	1051	550 (52)	388 (71)	20 (24)	14

[a,b]$P < 0.01$; [a,d]$P < 0.01$; [b,d]$P < 0.01$; [e,g]$P < 0.01$; [e,h]$P < 0.01$; [f,g]$P < 0.01$; [f,h]$P < 0.01$.

Table VI. Obstructive azoospermia: effect of number of eggs

No. of eggs	No. of cycles	No. of eggs at MII	No. of 2PN oocytes (% of MII eggs)	Normal cleaved embryos (% of 2PN)	No. delivered pregnancies (% of cycles)	Implantation rate % (per embryo)
1–3	9	25	15 (60)	13 (87)	2 (22)	15
4–8	41	224	122 (54)	102 (84)	5 (12)	10
9–16	64	654	383 (58)	281 (73)	24 (38)	21
17–24	46	796	434 (55)	306 (71)	15 (33)	14
⩾25	26	635	308 (48)	204 (66)	10 (38)	16
Totals	186	2334	1262 (50)	906 (72)	56 (30)	16

Table VIa. Summary of overall effect of ovarian reserve

No. of eggs	Implantation rate % (per embryo)	Delivered pregnancy rate %
1–8	11	14[a]
>9	16	36[b]

[a,b]$P < 0.01$.

husband. In cases where epididymal spermatozoa could not be retrieved, or where the expectation would be that the procedure would be extremely difficult because of scarring and multiple previous procedures, spermatozoa were obtained via testicle biopsy. During the past year, all these testicle biopsies were performed under local anaesthesia as an outpatient and with minimal post-operative pain. None of the sperm retrievals were performed with needle aspiration because of the simplification of microsurgical sperm retrieval procedures in obstructed patients. In cases of non-obstructive azoospermia, the procedure was often more extensive, involving general anaesthesia and multiple testicular biopsies until sufficient spermatozoa were extracted for ICSI.

The ovarian stimulation regimens used in this study have been extensively reported already (Van Steirteghem *et al.*, 1993; Silber *et al.*, 1994; Devroey *et al.*, 1996). The female partners underwent a fairly routine induction of multiple follicular development using leuprolide acetate (Lupron; TAP Pharmaceuticals, North Chicago, IL, USA); 1 ml was administered s.c. daily until the day of follicular aspiration. After desensitization, patients receive human menopausal gonadotrophin (HMG) either in the form of Pergonal (Serono), Humegon (Organon) or Menagon (Ferring) until many follicles of ~2.0 cm diameter were noted on ultrasound. Then, 10 000 units of human chorionic gonadotrophin (HCG) were administered i.m. The patients underwent transvaginal follicle aspiration 36 h after HCG administration.

The procedures for oocyte and sperm preparation for the ICSI procedure have also been described in great detail in previous publications (Van Steirteghem *et al.*, 1993; Silber *et al.*, 1995a,b). The testicular sperm handling and ICSI procedures were somewhat different for cases of non-obstructive azoospermia than for obstructive azoospermia and have also been discussed thoroughly (Devroey *et al.*, 1995; Silber *et al.*, 1996). The only modification in the sperm handling and ICSI technique for non-obstructive azoospermia was that in very difficult cases, all of the retrieved testicular tissue (which can sometimes be extensive), rather than being minced or morselized, was first treated with collagenase and DNase, and then placed in a red blood cell lysing buffer, so as to make all of the testicular cells visible in a centrifuged specimen consisting of ~50 μl; this was then divided into 10 separate microdroplets (Ogura and Yanagimachi, 1993; Verheyen *et al.*, 1995).

Table VII. Obstructive azoospermia with <9 eggs: effect of age of wife

Age of wife (years)	No. of cycles (% of total)	No. of eggs at MII injected	No. of 2PN oocytes (% of MII eggs)	Normal cleaved embryos (% of 2PN)	No. delivered pregnancies (% per cycle)
<30	8 (16)	41	18 (44)	18 (100)	1 (12)
30–36	19 (38)	104	59 (57)	48 (81)	5 (26)
37–39	12 (24)	57	33 (58)	24 (73)	1 (8)
40+	11 (22)	47	27 (57)	25 (93)	0 (0)
Totals	50 (100)	249	137 (55)	115 (84)	7 (14)

Table VIIa. Effect of female age with low ovarian reserve (<9 eggs)

Age of wife (years)	Implantation rate % (per embryo)	Delivered pregnancy rate %
<37	16[a]	22[b]
⩾37	4[a]	4[b]

[a]$P < 0.001$; [b]$P < 0.01$.

Assessment of fertilization, embryo cleavage and pregnancy

Handling of the injected oocytes was similar in all ICSI and IVF procedures. Approximately 16–18 h after microinjection, the oocytes were observed under the inverted microscope for any sign of damage that may have been due to the microinjection, or for the presence of pronuclei and polar bodies. Fertilization was considered normal when two clearly distinct pronuclei containing nucleoli were present. The presence of one pronucleus or three pronuclei was noted, together with the presence of one or more fragmented polar bodies. If a single pronucleus was observed, a second evaluation was carried out ~4 h later to see whether the pronuclear status had changed. The embryo cleavage of the two pronuclear oocytes was evaluated after a further 24 h of in-vitro culture (day 2). The embryos were scored according to the quality, number and size of the blastomeres and the percentage of anucleate fragments. Cleaved embryos with <50% of their volume filled with anucleate fragments were eligible for transfer. Embryos with >50% of anucleate fragments were not considered to have become a cleaved embryo. Up to three, and occasionally more, embryos, depending on the age of the wife and the embryo quality, were loaded in a few microlitres of Earle's medium into a Frydman catheter (LG 4.5 Prodimed, Neuilly-En-Thelle, France) and transferred into the uterine cavity ~48 h after insemination, or microinjection.

Pregnancy was initially confirmed by detecting an increase in serum HCG concentrations on at least two occasions >12 days after embryo replacement. Clinical pregnancy was determined by observing one or more gestational sacs by means of echographic screening at 7 weeks of pregnancy, and the presence of a fetal heartbeat. The patients were followed carefully for the evolution of pregnancy and the outcome of the delivery. Clinical pregnancy was defined as the presence of one or more fetal heartbeats, and delivered pregnancy as a normal birth. All the cases reported in this series were followed to delivery.

Designation of categories for patient breakdown and analysis

All patients in the series of cases of obstructive azoospermia with sperm retrieval and ICSI were unselected, and all categorization was made afterwards via computer analysis. In the cases of obstructive azoospermia, the results were broken down according to whether the cycle utilized fresh epididymal spermatozoa (MESA), frozen epididymal spermatozoa, or testicular biopsy retrieved spermatozoa (TESE). Non-obstructive azoospermia was broken down according to the category of maturation arrest and Sertoli cell-only, as well as post-chemotherapy azoospermia and post-cryptorchidism atrophy, and whether spermatozoa were retrievable from the testis.

All of these subcategories were then broken down according to two major objective parameters in the wife: age and number of oocytes retrieved. The number of eggs retrieved in a cycle did not influence the number of embryos transferred, but nonetheless was thought possibly to be indicative of overall ovarian reserve. The age of the wife was broken down as follows: <30, 30–36, 37–39 and ⩾40 years. The number of eggs retrieved was broken down into ⩽3, 4–8, 9–16, 17–24 and ⩾25.

Statistical analysis

In summary, there was a total of 186 cycles of ICSI with sperm retrieval for obstructive azoospermia, and a total of 63 cycles of ICSI with testicular sperm retrieval for non-obstructive azoospermia. All data were analysed in Brussels using various statistical tests with an SPSS statistical package on an Inwork personal computer. Egg number and fertilization rate were compared globally by the Kruskal–Wallis test, and also by one-way analysis of variance. If significant differences were noted, then paired group comparisons were performed by means of the Mann–Whitney *U* test, and the Tukey–HSD multiple range test. Transfer fate, pregnancy rate and implantation rate were compared globally by contingency table analysis, and if significant difference occurred, then paired comparisons were performed by means of the χ^2 test between the groups.

Results

This is a cumulative report of our first 249 consecutive cycles of testicular or epididymal sperm retrieval with ICSI for obstructive and non-obstructive azoospermia, followed to delivery. Tables I–IX summarize the breakdown of those results. It is clear from Table I that the source of the retrieved epididymal or testicular spermatozoa, whether frozen or thawed, resulted in no significant difference in 2PN fertilization rate or cleavage rate. Table II demonstrates no significant difference in embryo transfer rate between the different sources of retrieved spermatozoa, or even in the ongoing and delivered pregnancy rates. At first glance, testicular biopsy (TESE) may appear to have given a lower pregnancy rate (not significant), but this was attributed to the female age distribution in the different categories of sperm source (see below).

Table III summarizes the effect of the age of the wife on the number of metaphase II eggs retrieved, the 2PN fertilization rate, normal cleavage rate, and most importantly, the ongoing and delivered pregnancy rate in the 186 cases of ICSI for obstructive azoospermia. There was no significant difference in 2PN fertilization rate or normal cleavage rate in older and

Table VIII. Obstructive azoospermia with ⩾9 eggs: effect of age of wife

Age of wife (years)	No. of cycles (% of total)	No. of eggs at MII injected	No. of 2PN oocytes (% of MII eggs)	Normal cleaved embryos (% of 2PN)	No. delivered pregnancies (% per cycle)
<30	42 (31)	694	374 (54)	284 (76)	21 (50)
30–36	68 (50)	1007	551 (55)	365 (66)	25 (37)
37–39	12 (9)	150	80 (53)	66 (82)	2 (17)
40+	14 (10)	234	120 (51)	76 (63)	1 (7)
Totals	136 (100)	2085	1125 (54)	791 (70)	49 (36)

Table VIIIa. Effect of female age with high ovarian reserve (⩾9 eggs)

Age of wife (years)	Implantation rate % (per embryo)	Delivered pregnancy rate %
<37	21[a]	42[b]
⩾37	7[a]	12[b]

[a]$P < 0.001$; [b]$P < 0.01$.

younger women. However, there was a steady decline in ongoing and delivered pregnancy per cycle as the age of the wife increased. For women <30 years of age, there was a remarkable 44% delivery rate per cycle. For women aged 30–36 years, there was still a high 34% delivery rate per cycle. However, in women aged 37–39 years, the delivery rate was only 12%, and for women aged ⩾40 years, the delivery rate was only 4%.

The effect of age on pregnancy rate and implantation rate (as demonstrated in Table IIIa) appeared not to be observable until age 32 years, and thereafter success rates began to decline. Nonetheless, in keeping with standard reporting procedures used in most in-vitro fertilization (IVF) clinics, we broke down the rest of the results according to the arbitrary divisions shown in Table III. The results did not essentially differ, whether arbitrary or yearly breakdowns were utilized. Tables IV and V show the age breakdown according to whether epididymal spermatozoa or testicular spermatozoa were used for ICSI. There was a decline in pregnancy rate with increasing age of the wife for both types that was similar to those seen in Table III, which summarizes all cases of obstructive azoospermia. Women <37 years of age had a 37% delivered pregnancy rate with testicular spermatozoa and 39% with epididymal spermatozoa. Women <37 years had an implantation rate of 19% with testicular spermatozoa and 20% with epididymal spermatozoa. Women ⩾37 years had much worse results, whether with epididymal or testicular spermatozoa. There was a higher percentage (by chance) in this series of younger women undergoing ICSI with epididymal spermatozoa as opposed to testicular spermatozoa, and this is the explanation for the apparently lower overall pregnancy rate with testicular spermatozoa. The source of the spermatozoa was not really the issue so much as the age of the wife undergoing ICSI with those spermatozoa.

Tables VI–VIII summarize the effect of the 'ovarian reserve' of the wife, which we defined as the number of eggs retrieved, on the pregnancy rate in couples with obstructive azoospermia undergoing MESA and TESE–ICSI. Once again, the 2PN fertilization rate and the cleavage rate were not significantly affected by the number of eggs retrieved (the ovarian reserve; Table VI). However, the pregnancy rate was significantly affected by the number of eggs retrieved (Table VIa). The pregnancy rate was more than 2-fold higher in women who had more than eight eggs retrieved. Since the number of embryos replaced was no different in those women who had large or small numbers of eggs retrieved, this increased pregnancy rate was not related simply to a greater number of embryos available for transfer. In fact, Tables VIa, VIIa and VIIIa reflect the relative effects of age and ovarian reserve on the implantation and pregnancy rates.

Tables VII and VIII summarize the relative effect of age versus that of ovarian reserve. In women <37 years of age, with <9 eggs retrieved (Table VIIa), there was a 22% pregnancy rate, and in women having >9 eggs, there was a 42% pregnancy rate. Women >37 years of age who had <9 eggs had only a 4% pregnancy rate, and women >37 years of age with >9 eggs had a 12% pregnancy rate. Thus, even when controlled for age, women who produced >9 eggs had an average of almost twice the pregnancy rate of women who produced <9 eggs. This difference was strictly related to an increased implantation rate per embryo transferred. The most dramatic impact (almost a 4-fold difference) in pregnancy rate was related to the age of the wife.

Table IX summarizes the overall results in a different group of azoospermic patients undergoing TESE–ICSI, i.e. those with non-obstructive azoospermia from testicular failure caused by a variety of conditions. This is a group of couples in whom the male has apparently 'absent spermatogenesis' but in whom a few spermatozoa were nonetheless located in the testicle, retrieved microsurgically, and used for ICSI (Silber *et al.*, 1996). The overall embryo transfer rate and pregnancy rate per cycle appeared to be lower in this group than for those with normal spermatogenesis and obstructive azoospermia but the pregnancy rate per cycle was no different. A breakdown according to the age of the wife of all patients with non-obstructive azoospermia revealed once again an apparently higher pregnancy rate in younger women than in older women but this was not significant. In fact, the pregnancy rate per cycle in younger women was not significantly different in patients with this severe defect in spermatogenesis than in patients with obstructive azoospermia and normal spermatogenesis.

Thus, the results of ICSI in couples with azoospermia requiring testicular or epididymal sperm retrieval indicate that the source and the quality of the spermatozoa appeared to

Table IX. Non-obstructive azoospermia (Sertoli cell only, maturation arrest, post-chemotherapy, and cryptorchidism): effect of age of wife

Age of wife (years)	No. cycles	No. cycles with sperm found (% of cycles)	No. of eggs at MII injected	No. of 2PN oocytes (% of MII eggs)	Normal cleaved embryos (% of 2PN)	No. delivered pregnancies (% per cycle)
<30	19	14 (74)	232	77 (33)	70 (91)	4 (21)
30–36	29	16 (55)	185	81 (44)	55 (68)	8 (28)
37–39	9	4 (44)	65	26 (40)	23 (88)	1 (11)
40+	6	5 (83)	37	18 (49)	15 (83)	0 (0)
Totals	63	39 (62)	519	202 (39)	163 (84)	13 (21)

have no effect on the prognosis. In our analysis of these couples, the factors that affected the prognosis with azoospermia were the age of the wife and her ovarian reserve.

Discussion

Throughout the history of IVF, overall pregnancy rates have been quoted from one clinic to another, often in an attempt to demonstrate that one programme has perhaps better results than another. Even government review organizations are requesting clinic-specific reporting of pregnancy rates in a very raw fashion as a verification of clinic quality. The categories of patients have often been related to degrees of oligozoospermia, degrees of poor sperm motility or morphology, whether the wife had endometriosis or tubal disease, etc. The only breakdown by age (whenever used) in the USA in most reporting systems has been that of 'under 40 years of age' or 'over 40 years of age.' However, most clinics, in reporting successful treatment of male factor infertility, simply quote raw pregnancy rates without any particular breakdown of patients. Most scientific papers attempting to demonstrate one approach to IVF as being superior to another, simply quote raw implantation rates or raw pregnancy rates without any breakdown by age of the male or the female.

Early studies of ICSI using ejaculated spermatozoa in oligozoospermic patients indicated that neither the severity of oligozoospermia, the morphology, nor the motility of the spermatozoa had any effect on fertilization or pregnancy rate, so long as the few spermatozoa available had any discernible movement (Nagy *et al.*, 1995a,b). We suggested that the age of the wife might be the major factor that affects pregnancy results in cases of male infertility when we reported our early results of 72 patients undergoing MESA and TESE for obstructive azoospermia (Silber *et al.*, 1995a). There was no major breakdown by age in any of the early papers (Devroey *et al.*, 1994, 1995; Silber *et al.*, 1994, 1995b). In 1995, we broke down the early results by age, and made the speculation that 'the only factor which affected success in couples undergoing MESA–ICSI or TESE–ICSI for obstructive azoospermia was the woman.' Whether the patient had congenital absence of the vas deferens, surgical obstructive azoospermia, or post-inflammatory obstructive azoospermia, and whether fresh or frozen epididymal spermatozoa or testicular spermatozoa were used, 'made no meaningful difference.' Even the cystic fibrosis genotype, sperm morphology and quality of sperm motility had no impact on those early results (Silber *et al.*, 1995a).

In 1996, Devroey *et al.* reviewed 71 cycles of ICSI with ejaculated spermatozoa in women aged >40 years, and compared them to 71 case controls aged <40 years. The delivery rate per embryo transfer in women ⩾40 years was 8.5% and in women <40 years was 25.4%. Although it was observed that on average fewer eggs were retrieved from women >40 years of age, the finding of a dramatically decreased implantation rate in women >40 compared to women <40 years argues against the number of eggs retrieved being an important factor in the decreased pregnancy rate in older women. Furthermore, the classic reports of Sauer *et al.* (1990, 1991) on egg donation demonstrated that older women have a high pregnancy rate when eggs are donated to them by younger women.

Table IXa. Effect of female age in cases of testicular failure with sperm retrievable

Age of wife (years)	Delivery rate % per cycle with spermatozoa
<37	40
⩾37	11

It is well known that the overall fecundity of a population decreases progressively with advancing age (Mosher, 1980, 1985a,b, 1987; Schwartz, 1983; Greenhall and Vessey, 1990; Silber, 1991; Lansac, 1995). The purpose of the present study was to analyse further the impact of the wife's age on pregnancy results in the most severe male infertility cases, i.e. obstructive and non-obstructive azoospermia. Furthermore, an attempt was made to determine, in addition to the age of the wife, what the impact of the overall ovarian reserve has within each age category.

The present study indicates that age of the wife is the most critical factor in evaluating pregnancy rate in couples undergoing treatment for severe male infertility, but ovarian reserve (number of eggs retrieved) also has a significant, though not necessarily independent effect. It would be easy to confuse any comparison of results of treating male infertility if they were not broken down specifically by the age of the wife and her ovarian reserve. For example, our data seemed at first to indicate a lower pregnancy rate in patients using testicular spermatozoa versus those using epididymal spermatozoa. Nonetheless, when the cases of epididymal spermatozoa and testicular spermatozoa were separately broken down by age of wife, it was apparent that there was no difference in pregnancy rates related to the source of the spermatozoa. The apparent difference was only related to the greater preponder-

ance by chance of older wives in the group undergoing TESE than in the group undergoing MESA. A multivariance analysis would be another way to demonstrate these relationships. However, the multiple breakdowns by category make it obvious that sperm origin was not important, and that the woman's age and number of eggs are highly predictive.

We defined the 'ovarian reserve' as the number of eggs retrieved rather than by the day 3 follicle stimulating hormone (FSH) concentration. This definition could be argued against, of course, but we felt that FSH is only an indirect indication of ovarian reserve, whereas the number of eggs retrieved is more direct. The number of eggs retrieved had a significant impact on pregnancy rate, though less dramatic than that of age. At first it would appear that younger women produce more eggs than older women, and that the effect of the ovarian reserve is simply secondary to the more important effect of the woman's age. However, it is possible that the ovarian reserve might be an independent variable that further affects the pregnancy rate.

Since the implantation rate was dramatically lower in older than in younger women (and since the average number of embryos transferred was similar in the various age categories, it does not appear likely that the improved pregnancy rates with greater numbers of eggs retrieved are related to the number of embryos transferred. It appears more likely that the increased pregnancy rate in women with greater ovarian reserve is related to the quality of the eggs, and that the greater number of eggs retrieved is only an indication of better egg quality. However, it is quite possible that a more modest ovarian stimulation protocol, with the retrieval of smaller numbers of eggs, might give good results in women who would have produced more eggs with a more aggressive protocol. The number of eggs may not be as important as the relationship of the ease of ovarian stimulation to pregnancy rate.

Another criticism of this study is that women who did not respond to stimulation at all, i.e. women who were found to have no eggs, were excluded. This could make the influence of the number of eggs appear to be less than it should be. There are other potential criticisms. We used HMG and not highly purified FSH for all of our stimulation cycles, and thus could not take into account the possible impact which that could have had on our impression of 'ovarian reserve' based on number of eggs retrieved (Imthurn *et al.*, 1996). Also, we did not analyse endometrial thickness because it has been shown that, at least in male factor cases, a thin endometrium has no significant effect on pregnancy or delivery rate (Rinaldi *et al.*, 1996)

In summary, it appears that most types of male infertility, even such extreme cases as maturation arrest, Sertoli cell-only, cryptorchid testicular atrophy, post-chemotherapy azoospermia, and perhaps even Klinefelter's syndrome (Staessen *et al.*, 1996), now have a possible treatment. However, the success or failure of MESA–ICSI and TESE–ICSI for male factor infertility (in those cases where spermatozoa can be retrieved) is nonetheless limited by the age, and possibly the ovarian reserve, of the wife. Thus, the future for improvement in results with the vast majority of cases of male infertility appears to relate to female more than to male factors. Furthermore, it is apparent that live delivery rates of 42% per cycle are readily obtainable when the wife is <37 years of age and nine or more eggs are retrieved (Table VIIIa). This can explain the exaggerated success some programmes can claim, if they have simply manipulated their selection criteria for female factors such as age and ovarian reserve.

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