Pregnancies, growth and development of children conceived by subzonal injection of spermatozoa

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Subzonal injection of spermatozoa (SUZI) was one of the first micromanipulation techniques efficient in treating male factor infertility and unexplained in-vitro fertilization failures. The aim of this retrospective study was to evaluate the in-vitro development of embryos conceived by SUZI, the obstetric outcome, the rate of congenital malformations and subsequent follow-up in children. Fifty-five pregnancies were obtained between 1991 and 1994 (54 after fresh embryos were transferred and one after cryopreserved embryos were transferred). Among the 50 clinical pregnancies, there were seven miscarriages (14%) and two ectopic pregnancies (4%). Among the 41 resulting evolutive pregnancies, the discovery of one anencephaly led to a medical abortion. Forty deliveries including six twin pregnancies occurred, leading to the births of 45 live neonates and one stillbirth. The gender distribution of the offspring included 17 males and 29 females (ratio 0.59:1). Birth weight, length and head circumference were within the expected ranges. Two children presented a malformation: the first one had one thumb with congenital shelf and the second a polymalformative neurological syndrome. Growth curves were normal for all these children except one (weight above the 2 SD curve). Medical follow-up detected no pathological features in these children apart from a physical disability in one girl. In this small series a 4.2% rate of malformation was observed, particularly affecting the neural tube, in SUZI offspring. However, no firm conclusions can be drawn since the study was carried out on a small cohort. SUZI is no longer performed but these observations suggest that it is necessary to collect extensive data about children conceived by microfertilization.

Key words: assisted reproductive techniques/children/congenital malformation/pregnancies/subzonal injection

Introduction

Since the birth of Louise Brown in 1978 (Steptoe and Edwards, 1978), in-vitro fertilization (IVF) has proved to be useful in alleviating long-standing infertility in couples with tubal, idiopathic or andrological infertility. Nevertheless, IVF success rates were dramatically reduced in patients with male factor infertility. Therefore, several methods of assisted fertilization have been attempted with varying degrees of success. One of them was the subzonal insemination of spermatozoa (SUZI), successfully used to obtain a pregnancy and offspring in mouse (Mann, 1988) and in human (Ng et al., 1988).

Subzonal insemination was used from 1989 to 1994 to overcome infertility related to unexplained IVF failures, severe oligoasthenoteratozoospermia, flagellar dyskinesia and sperm-associated antibodies (Fishel et al., 1990; Ng et al., 1991; Cohen et al., 1991; Wolf et al., 1992, 1993, 1995a). The major limitations of SUZI were a variable and relatively low diploid fertilization rate and a high occurrence of polyspermy when multiple spermatozoa were injected in the perivitelline space (Ng et al., 1991; Wolf et al., 1992; Palermo et al., 1993). For these reasons, SUZI has been progressively replaced by intracytoplasmic sperm injection [ICSI], a much more efficient and easier technique (Palermo et al., 1992).

Various reports have shown that growth, development and risk of malformation of children were not different when they were conceived by IVF or naturally (AILF, 1985; Morin et al., 1989; Medical Research Council, 1990; Rizk et al., 1991; Brandes et al., 1992; Epelboin, 1995; FIVNAT, 1995; Olivennes et al., 1997). But the main question when using a new technique of assisted reproduction is whether or not it leads to an increase in the frequency of known fetal pathologies and/or new malformations. After IVF, most of reported problems are linked to multiple pregnancies and prematurity. But what are the consequences of the method of conception used? More questions are raised by microassisted fertilization since most of the steps of gamete interactions are bypassed. So microinjected spermatozoa may have not achieve their normal maturation and may carry more genetic or cellular defects than naturally fertilizing spermatozoa. Also foreign material may be introduced into the egg.

Pregnancy and delivery outcomes after SUZI have been analysed in a few small series (Kola et al., 1990; Bonduelle et al., 1994; Cohen et al., 1994). The reported rate of congenital malformation was different in these studies. Moreover, until
now, there was no report concerning the growth and development of children born after SUZI. Even if this mode of fertilization is no longer used, it is important to analyse the consequences of bypassing the zona pellucida on child development or for new indications of assisted reproductive techniques.

In this study the outcome of 55 pregnancies obtained by SUZI between 1991 and 1994 and the 2–5 year follow-up of the resulting children are reported.

Materials and methods

Selection of patients and description of indications

A total of 308 couples with a history of primary or secondary infertility (mean of 6.8 years; range 1–21) participated in the SUZI programme between 1991 and 1994 and had 475 SUZI attempts. Informed consent were obtained from all patients. The programme had received the agreement of the local ethical committee.

A check-up including previous history and biological investigations in the male was systematically performed in order to determine the aetiology of infertility. Three semen analyses were performed to evaluate the mean sperm concentration, percentage of motile forms and sperm morphology assessed on a smear stained by Shorr and haematoxylin and classified (David et al., 1975). The percentage of antibody-bound motile spermatozoa was detected using anti-immunoglobulin A (IgA) and anti-IgG coated immunobeads (de Almeida et al., 1986). Autoimmunity was considered responsible for the infertility when this percentage was higher than 60% for at least one class of immunoglobulin (Wolf et al., 1995a). A selection of motile spermatozoa in B2 medium (CCD, Paris, France) was separated through a simplified Percoll gradient (95–47.5%) (Sigma, Saint-Quentin Fallavier, France). The sperm survival was considered as positive when there were at least 60% living spermatozoa at 24 h post selection (McClure et al., 1989). A cervical mucus in-vitro penetration test was performed to assess the efficiency of the sperm movement. It was considered as normal when spermatozoa displayed a good ability to penetrate a column of human ovulatory cervical mucus and to remain motile with forward progression after 4 h of contact (Kremer and Jager, 1982). Extensive sperm investigations were performed in case of negative cervical mucus penetration test. They included sperm movement parameter analysis using a computerized analysing system (CASA; Hamilton Thorn, USA) and an analysis of spermatozoa by electron microscopy in cases of abnormal sperm movement parameters (Serres et al., 1986).

According to the results of sperm analysis, several aetiologies were observed. They could be classified according to the origin of the spermatozoa which are used.

Ejaculated spermatozoa

The following pathology was individualized:

IVF failures (n = 126) when patients had at least two unexplained IVF failures involving a minimum of 10 oocytes. When sperm concentration was >40 × 10⁶/ml, with motile and normal forms >40%, patients were included in the group with ‘normal spermatozoa’ (n = 51). When one or several of these parameters were under these limits, patients were included in the group with ‘subnormal spermatozoa’ (n = 75) (Wolf et al., 1992).

Presence of sperm-associated antibodies (Wolf et al., 1995a). The mean percentage of antibody-bound spermatozoa among the 35 patients included in this group for IgG and IgA was 80.4% (range 0–100) and 65.9% (range 0–100) respectively. After motile sperm selection, it became 67.7% (0–100) and 49.4% (0–100) for the IgG and IgA respectively.

Oligoasthenoteratozoospermia (n = 62) when sperm concentration was < 20 × 10⁶/ml and/or motility and/or normal forms <20%.

Flagellar dyskinesia (n = 53) when motile spermatozoa had an abnormal movement pattern. The electron microscopy analysis of the flagella allowed us to identify four diagnoses: short flagella (n = 9), a lack of outer dynein arms (n = 19), sliding spermatozoa (n = 3) and periaxonemal abnormalities (n = 22) (Wolf et al., 1995b).

Epididymal spermatozoa

Surgically retrieved (n = 23) in cases of anejaculation or obstructive azoospermia.

Cryopreserved spermatozoa (n = 9)

Before chemotherapy or radiotherapy with not enough spermatozoa after thawing to allow in-vitro-fertilization with classical insemination. Main characteristics of patients (age, mean infertility period) are shown on Table I.

Sperm and oocytes preparations, SUZI and embryos management

Hormonal treatment of the women and oocyte retrievals were performed in five different hospitals (Saint-Vincent de Paul, Jean Verdier, Montsouris, Cochin–Port-Royal, Antoine Béclère). The oocytes were carried to the Kremlin Bicêtre Hospital for SUZI in a temperature-controlled chamber. These procedures were performed as previously described (Wolf et al., 1992). The oocytes were checked 16–18 h after SUZI for evidence of fertilization. The oocytes exhibiting two pronuclei were considered normally fertilized. Diploid zygotes were kept in culture for a further 24 h. Only regularly cleaved embryos were transferred 48 h after oocyte retrieval.

Data collection on pregnancies and follow-up of children

Outcome of pregnancies and newborn description

All pregnancies were recorded even if they were only biochemical, when a moderate and temporary rise in the human chorionic gonadotrophin (HCG) concentration occurred. Pregnancy was called clinical when a gestational sac could be detected by ultrasound scan and/or when the concentration of HCG rose above 1000 mU/ml. The implantation rate was defined as the ratio between the number of gestational sacs detected by the first ultrasound scan and the number of embryos transferred. Gestational age was recorded in theoretical weeks of amenorrhoea by adding 14 days to the difference between the day of oocyte retrieval and the date of delivery. Four pregnancy outcomes were possible: miscarriage, ectopic pregnancy, medical abortion and delivery. Miscarriages were considered as ‘early’ when they occurred before 12 weeks of amenorrhoea and as ‘late’ between 12 and 25 weeks of amenorrhoea. Pregnancy outcomes were scored as a percentage of the total number of clinical pregnancies. Data on obstetric history, mode of delivery, Apgar scores, sex, birth weight, height, head circumference and congenital malformations of newborn were retrieved from medical records. The characteristics of pregnancies and newborns, mainly concerning congenital malformations, were compared to those reported in general European or Parisian populations (Eurocat, 1991; Goujard et al., 1991). Congenital abnormalities referred to structural defects (malformations, distortions, and dysraphias), chromosome aberrations, hereditary or metabolic diseases. Malformations with surgical or functional consequences, or both, were considered major; malformations with no surgical or functional importance were regarded as minor. A minor anomaly was distinguished from a normal variation if it occurred in ≤4% of the infants in the same acial group (Holmes, 1976).
Statistical analysis was carried out using a *vitro conceived population* (Brandes et al., 1992; Olivennes et al., 1997).

Follow-up of children

Questionnaires were sent to the parents and in cases of physical or developmental malformations or abnormalities to the paediatricians. They included questions about general paediatric history, malformation discoveries, growth and mental development. Main characteristics were compared to those described for the total population or the in-vitro conceived population (Brandes et al., 1992; Olivennes et al., 1997).

Statistical analysis

Statistical analysis was carried out using a χ²-test with the Yates correction for small samples. The observed difference was considered significant for *P* < 0.05.

Results

The 475 SUZI cycles resulted in 267 fresh embryo transfers and 54 biochemical pregnancies (Table II). One more pregnancy was obtained after a transfer of cryopreserved embryos.

Five pregnancies were only biochemical. In the remaining 49 pregnancies a fetal heartbeat was detected resulting in an 18.3% clinical pregnancy rate per embryo transfer and 10.3% per attempt. The implantation rate varied between 6.9% and 25% according to the indications, with a mean of 10.5% (Table III). It was significantly higher when SUZI was performed with cryopreserved spermatozoa (25%) than in the other indications (*P* < 0.05). Nine of the 49 clinical pregnancies were terminated, after either an early miscarriage (14.3%) or an ectopic pregnancy (4.1%). No late miscarriages were noted.

Follow-up of pregnancies

Forty-one pregnancies (40 after fresh embryos and one after cryopreserved embryo transfers) were evaluable after the first quarter, six of which were multiple pregnancies (14.6%). Among them, two triplet pregnancies were reduced to twin pregnancies at 11 weeks of amenorrhoea, without any immediate consequence on the course of pregnancy. One medical termination of pregnancy was carried out for anencephaly at 22 weeks of amenorrhoea in a single pregnancy obtained with cryopreserved embryo transfers. Among them, two triplet pregnancies were reduced to twin pregnancies (89% to follow-up).

When SUZI was performed for patients with primary male factors, the pregnancy rates were slightly higher but the implantation rates were similar compared to patients with previous failed fertilization. However, in this last group, only 57.9% of the early pregnancies detected biochemically ended by a delivery versus 82.9% when SUZI was performed for male infertility.

### Table I. Main characteristics of patients according to the infertility diagnosis

<table>
<thead>
<tr>
<th>Indication</th>
<th>No. of couples</th>
<th>Women’s age (years)*</th>
<th>Men’s age (years)*</th>
<th>Infertility delay (years)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF failure with normal spermatozoa</td>
<td>51</td>
<td>35.5 ± 3.8</td>
<td>36.4 ± 6.9</td>
<td>7.6 ± 3.2</td>
</tr>
<tr>
<td>IVF failure with subnormal spermatozoa</td>
<td>75</td>
<td>32.6 ± 3.6</td>
<td>35.1 ± 5.6</td>
<td>7.1 ± 3.0</td>
</tr>
<tr>
<td>Sperm-associated antibodies</td>
<td>35</td>
<td>32.1 ± 4.5</td>
<td>35.4 ± 7.8</td>
<td>6.1 ± 2.4</td>
</tr>
<tr>
<td>Oligoasthenoteratozoospermia</td>
<td>62</td>
<td>32.2 ± 5.2</td>
<td>34.4 ± 6.1</td>
<td>6.2 ± 3.9</td>
</tr>
<tr>
<td>Flagellar dyskinesia</td>
<td>53</td>
<td>31.3 ± 4.1</td>
<td>35.7 ± 6.0</td>
<td>6.6 ± 3.9</td>
</tr>
<tr>
<td>Epididymal spermatozoa</td>
<td>23</td>
<td>31.5 ± 4.4</td>
<td>34.5 ± 5.6</td>
<td>5.8 ± 3.1</td>
</tr>
<tr>
<td>Cryopreserved spermatozoa</td>
<td>9</td>
<td>31.5 ± 2.4</td>
<td>38.3 ± 7.8</td>
<td>4.0 ± 2.8</td>
</tr>
<tr>
<td>Total</td>
<td>308</td>
<td>32.4 ± 4.2</td>
<td>34.9 ± 5.9</td>
<td>6.8 ± 3.3</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SD.

### Table II. Results of subzonal injection of spermatozoa programme

<table>
<thead>
<tr>
<th>Indication</th>
<th>No. of attempts</th>
<th>No. of microinjected oocytes</th>
<th>No. of embryos (cleavage rate, %)*</th>
<th>No. of transfers</th>
<th>Biochemical pregnancy (%)</th>
<th>Clinical pregnancy (%)</th>
<th>Deliveries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF failure with normal spermatozoa</td>
<td>80</td>
<td>525</td>
<td>100 (19.0)</td>
<td>41</td>
<td>9</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>IVF failure with subnormal spermatozoa</td>
<td>108</td>
<td>818</td>
<td>108 (13.2)</td>
<td>60</td>
<td>10</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>IVF failure (subtotal)</td>
<td>188</td>
<td>1343</td>
<td>208 (15.5)</td>
<td>101</td>
<td>19 (10.1)*</td>
<td>17 (9.0)*</td>
<td>11 (5.8)*</td>
</tr>
<tr>
<td>Sperm-associated antibodies</td>
<td>51</td>
<td>357</td>
<td>54 (15.1)</td>
<td>25</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Oligoasthenoteratozoospermia</td>
<td>90</td>
<td>575</td>
<td>85 (14.8)</td>
<td>38</td>
<td>11</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Flagellar dyskinesia</td>
<td>109</td>
<td>845</td>
<td>203 (24.0)</td>
<td>80</td>
<td>13</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Epididymal spermatozoa</td>
<td>27</td>
<td>206</td>
<td>28 (13.6)</td>
<td>16</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cryopreserved spermatozoa</td>
<td>10</td>
<td>102</td>
<td>23 (22.5)</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Primary male factor (subtotal)</td>
<td>287</td>
<td>2085</td>
<td>393 (18.8)</td>
<td>166</td>
<td>35 (12.2)*</td>
<td>32 (11.1)*</td>
<td>29 (10.1)*</td>
</tr>
<tr>
<td>Total</td>
<td>475</td>
<td>3428</td>
<td>601 (17.3)</td>
<td>267</td>
<td>54 (11.3)*</td>
<td>49 (10.3)*</td>
<td>40 (8.4)*</td>
</tr>
</tbody>
</table>

*Defined as the percentage of the ratio between the number of embryos regularly cleaved and the number of microinjected oocytes; *b*% of attempts.

Follow-up of children

Questionnaires were sent to the parents and in cases of physical or developmental malformations or abnormalities to the paediatricians. They included questions about general paediatric history, malformation discoveries, growth and mental development. Main characteristics were compared to those described for the total population or the in-vitro conceived population (Brandes et al., 1992; Olivennes et al., 1997).
Table III. Implantation rate (%) according to indications

<table>
<thead>
<tr>
<th>Indication</th>
<th>No. of transferred embryos</th>
<th>No. of implanted embryos (implantation rate, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF failure with normal spermatozoa</td>
<td>82</td>
<td>10 (12.2)</td>
</tr>
<tr>
<td>IVF failure with subnormal spermatozoa</td>
<td>102</td>
<td>10 (9.8)</td>
</tr>
<tr>
<td>IVF failure (subtotal)</td>
<td>184</td>
<td>20 (10.9)</td>
</tr>
<tr>
<td>Sperm-associated antibodies</td>
<td>48</td>
<td>4 (8.3)</td>
</tr>
<tr>
<td>Oligoasthenoteratozoospermia</td>
<td>70</td>
<td>10 (14.3)</td>
</tr>
<tr>
<td>Flagellar dyskinesia</td>
<td>173</td>
<td>12 (6.9)</td>
</tr>
<tr>
<td>Epididymal spermatozoa</td>
<td>27</td>
<td>4 (14.8)</td>
</tr>
<tr>
<td>Cryopreserved spermatozoa</td>
<td>20</td>
<td>5b (25)</td>
</tr>
<tr>
<td>Primary male factor (subtotal)</td>
<td>338</td>
<td>35 (10.4)</td>
</tr>
<tr>
<td>Total</td>
<td>522</td>
<td>55 (10.5)</td>
</tr>
</tbody>
</table>

*Defined as the ratio between the number of gestational sacs detected by ultrasound between 6 and 8 weeks of amenorrhoea and the number of transferred embryos.

**Different from other indications ($\chi^2$ test, $P < 0.05$). IVF = in-vitro fertilization.

Table IV. Weight, height and head circumference of children at birth

<table>
<thead>
<tr>
<th>Term</th>
<th>Deliveries</th>
<th>Newborn</th>
<th>Weight (g)</th>
<th>Length (cm)</th>
<th>Head circumference (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;37 WA*</td>
<td>4</td>
<td>6</td>
<td>2677 ± 619b</td>
<td>46.4 ± 2.8b</td>
<td>33.3 ± 1.9b (30–35)c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1780–3600)c</td>
<td>(43.5–50.5)c</td>
<td>(32–40)c</td>
</tr>
<tr>
<td>≥37 WA*</td>
<td>35</td>
<td>38</td>
<td>3254 ± 402b</td>
<td>49.5 ± 1.7b</td>
<td>34.8 ± 1.3b (32–40)c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2500–4120)c</td>
<td>(45–53)c</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>44</td>
<td>3177 ± 471b</td>
<td>49.1 ± 2.1b</td>
<td>34.6 ± 1.5b</td>
</tr>
</tbody>
</table>

*Weeks of amenorrhoea.

bMean ± SD.

cRange; data unknown for one child.

avoid a preterm delivery at 36 weeks + 5 days in a twin pregnancy. A placenta prævia was revealed by bleeding and led to delivery by Caesarean section. No evident repercussion of these complications on the fetuses, development was noticed.

Term and mode of delivery

The duration of pregnancies varied between 36.3 and 41.2 weeks, with a mean of 38.9 weeks (SD 1.4). The mean duration of single pregnancies was 39.3 ± 1.2 weeks (36–41.2). Five (17.2%) ended with a Caesarean and 25 (82.8%) with a vaginal delivery for which instrumental assistance was required in eight cases. The mean duration of multiple pregnancy was 37.7 ± 1.0 weeks (36.3–39). Four multiple pregnancies ended by a Caesarean section and two by a vaginal delivery. Indications for the Caesarean sections were: fetal distress (2/9), twin pregnancy (2/9), placenta prævia grade 4 (1/9), pathological pelvis (1/9) and failure to progress (1/9). Prematurity was observed for six children, four of whom were born from a twin pregnancy.

Neonatal data

Among the 46 newborn, one fetus was stillborn, at the end of a normal pregnancy and without any explanation. The gender distribution of the offspring included 17 males and 29 females (ratio 0.59:1). Birth weight (Table IV) varied between 1780 and 4120 g, with a mean of 3177 g (SD = 471). One infant was hypotrophic (1780 g at 36 weeks). No link between birthweight, maternal age and indication of SUZI was found. Mean length at birth was 49.1 cm (SD 2.1) and mean head circumference was 34.6 cm (SD 1.5).

Beside the anencephaly previously described, another neurological major malformation was observed. It was a polymalformative syndrome in a boy, associating hypotrophy, macrocephaly, small ears, 6th finger and partly recovering prepuce, which was diagnosed at 22 weeks but did not lead to a medical abortion. In this last case, the SUZI indication was linked to IVF failures with normal spermatozoa. After extensive investigations, the final diagnosis concluded 6 months after the birth to a micropolgyry with cortical malformations. The child, 5 years old, was still alive at the time of the survey and had an important delay in mental development, incompatible with a normal life.

A minor malformation, thumbs with congenital shelf, was found in one child. This abnormality was surgically corrected, leaving no further sequelae. No correlation between the indication of the SUZI and the outcome of malformations was found.

Growth and development of children

Detailed information on 43 out of 45 children was obtained (4.3% lost to follow-up). At the time of the study, 17 children were 3 years old, 20 were between 3 and 5 years old and 6 were >5 years old.

The growth curves of boys and girls were compared to the usual Lefort’s curves used in paediatric examination. Only one boy’s weight was above the 2 SD curve. No information about his parents’ weight was available.
Walking was observed between 11 and 18 months. Medical follow-up revealed problems in six children (five singletons and one twin). A physical disability with cerebellar ataxia was observed in one twin when she was 18 months old. Her neurological development always differed from her sister’s development but was improved by orthopaedic physiotherapy and compatible with a normal life, including school integration. Two children had a delay in language learning, linked with pathological adenoids for one of them and completely corrected by speech therapy. Two girls developed osteochondrisis. Another girl had talus varus feet, corrected by physiotherapy. Other children did not develop any problem and were doing well at the time of the study.

There was no correlation between children’s growth and medical or surgical pathology outcome and indication of SUZI.

Discussion

Three main concerns are usually raised by medically assisted reproduction: the health of the woman, the risk of malformations or birth defects for the child and finally the health and development of the growing child. In most studies published until now, only some of these questions were answered and very often a small proportion of the patients included in the assisted reproduction programme were followed up (Bonduelle et al., 1998). Although the series analysed here is small, it cannot be larger concerning the follow-up of children born after SUZI since this technique is no longer used. The results submitted here give information on all questions raised with a very small number lost to follow-up in the population under study.

Among the 55 pregnancies obtained by SUZI in this study, five (9.0%) were only biochemical and nine ended by miscarriage or ectopic pregnancy. The rate of miscarriage and ectopic pregnancy (18.0%) is similar to that observed with conventional in-vitro insemination, SUZI or ICSI (Cohen et al., 1994; FIVNAT, 1995; Palermo et al., 1996). This confirms the higher rate of miscarriage after IVF than after natural conception. Some factors may explain these differences: the higher accuracy of pregnancy diagnosis after IVF, the higher rate of multiple pregnancies and the older mean age of IVF patients (FIVNAT, 1995). In this small series, no difference was found in the mean maternal age when pregnancy terminated by a miscarriage (31.0 years ± 3.8) or led to a delivery (31.5 years ± 3.6). However, the percentage of miscarriage was higher when the infertility was linked to unexplained IVF failures when compared to the other indications. Significant lower fertilization rates (Gabrielsen et al., 1996) or pregnancy rates (Tomas et al., 1998) were reported after ICSI performed after previous failed fertilization. In this study, fertilization and pregnancy rates were not different, but much higher miscarriage incidence was found when SUZI was performed after previous IVF failures. This could not be explained by the age of the women or uterine factors. Therefore the data presented here reinforce the hypothesis that failed fertilization in IVF may be linked to oocyte defects that could be expressed during/after the implantation phase or to unknown genetic defects which may prevent normal embryonic development (Gabrielsen et al., 1996; Tomas et al., 1998).

In this series, a 4.2% rate of major malformations was observed among the abortion products or the newborn resulting from SUZI. This frequency is higher than in the general population or after conventional IVF or ICSI where it varies between 1.2% (FIVNAT, 1995) and 3.7% (Leppig et al., 1987). Two other studies have reported higher rates of congenital malformations in children conceived through SUZI (5.5%: Cohen et al., 1994) and ICSI (7.38%: Kurinczuk and Bower, 1997). The interpretation of these data must be approached with caution because some bias may exist in these different studies. First, the small numbers of pregnancies and births in various cohorts do not permit firm conclusions to be drawn. Indeed, these series will not become larger since SUZI has been now replaced by ICSI. Secondly, the vigilance in terms of diagnosing birth defects is not necessarily the same in the general population and among children conceived by assisted reproductive techniques. This could partly explain the over-estimation in malformation rate after SUZI or ICSI. However, using the same approaches as used for children conceived by microfertilization, e.g. in the definition of malformations and the care brought to the study of in-vitro conceived babies, none has reported an increased risk of congenital malformations in babies conceived by IVF. Thirdly, according to the definition of birth defects, the analysis of the same data may lead to different rates of malformations (Bonduelle et al., 1996a; Kurinczuk and Bower, 1997). Major malformations were defined as those causing functional impairment or requiring surgical correction and minor as the remaining abnormalities (Cohen et al., 1994 and Bonduelle et al., 1996a). Kurinczuk and Bower (1997) named congenital malformations as any defect probably of prenatal origin. In this last study, each abnormality was coded according to the five digit British Paediatric Association’s ICD-9 system (1979) and so distinguished as major or minor according to a method devised by the Centers for Disease Control. Thus, this discrepancy in the definition of congenital malformations used by Kurinczuk and Bower (1997) can explain the higher rate of abnormalities found in their report. In the present study, there is no doubt about the classification: both recorded abnormalities, anencephaly and polyformative syndrome, affected the central nervous system and were major. This rate of congenital malformation or anencephaly as observed in our series (2/47 concepti e.g. 4.2%) is higher than expected. In the European and Parisian populations, the rates of congenital malformation are 2.27% and 3.06% respectively, and the rates of anencephaly are 0.036% and 0.058% respectively (Eurocat, 1991; Goujard et al., 1991). Some teams worried that an increased prevalence of neural tube defects might exist after IVF and suggested a possible relationship between neural tube defects and ovulation induction agents (Lancaster, 1987; FIVNAT, 1995). Other data including multi-analysis did not confirm these first results and so concluded that ovulation induction was not a risk factor for neural tube defects in the offspring (Rizk et al., 1991; Van Loon et al., 1992). Other factors, such as nutritional deficiency, mainly folic acid, before or during pregnancy, have been implicated and remain under investigation. In our study, women
had apparently no nutritional deficiency. So the higher rate of neural tube defects is not explained.

As with Palermo et al. (1996), the present study failed to find any link between the occurrence of malformation and the sperm pathology (Palermo et al., 1996). Recently, in a prospective study, compiling data on 107 children born after ICSI with epididymal and testicular spermatozoa, the congenital malformation rate (2.4%) was also similar to most of the general population registries and reproductive surveys (Bonduelle et al., 1998).

Another concern raised by microfertilization is the risk of chromosomal abnormalities in the resulting child. Chromosomal abnormalities have been detected in 2% of infants conceived by ICSI and in their father (Bonduelle et al., 1996a). Recently, a prospective follow-up study of 877 children conceived by ICSI found that there was a slight increase in de-novo chromosomal aberrations which was not higher than the risk to a 37 year old women in the general population (Bonduelle et al., 1996b). Nevertheless, this study reported a higher frequency of transmitted chromosomal abnormalities and suggested that the sperm characteristics rather than the technique itself are probably a risk factor in ICSI (Bonduelle et al., 1996b). In this series, no chromosomal abnormality was detected but the chromosomal status of children was not systematically investigated.

Among the 46 children born after SUZI in this report, 29 were females and 17 were males. This imbalanced sex ratio is quite surprising and differs from other studies, whatever the type of ART (Rizk et al., 1991; Bonduelle et al., 1994, 1995; Palermo et al., 1996). It could not be related to any factor analysed. With a follow-up of 3 to 7 years after birth, no major pathological features were observed in the development of the children born after SUZI. Only one child was outside the standardized curves of >2 SD. Finally, although a physical disability was observed in one girl, the rates of medical and surgical illness observed in these children were not different from those in IVF populations (Brandes et al., 1992; Olivennes et al., 1997). It should be noted that only two children were lost to follow-up in the results analysed here (4.3%). That kind of information is very rarely available as shown by the ESHRE Task Force (1998). In their recent analysis on 877 children born after ICSI, Bonduelle and co-workers had information from only 22% of the children at the age of 1 or 2 years (Bonduelle et al., 1996b). Therefore, the follow-up of children born after microfertilization needs to be improved to draw firm conclusions.

In conclusion, the analysis of this small cohort suggests that an increased risk of births defects might exist, particularly affecting neural tube, in offspring of SUZI. Although a rise in major congenital malformations in children conceived by microfertilization has already been pointed out, several studies have reported reassuring data, even in case of extreme male infertility (Bonduelle et al., 1996b and 1998). So, it is necessary to follow-up children conceived by ICSI carefully and with long-term survey. A prospective long-term follow-up on the entire cohort of ICSI children with a matched controlled group is difficult to perform for many reasons. However, it is imperative to collect maximum data on these children in order to be better informed.

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