

# Incidence of birth defects after artificial insemination with frozen donor spermatozoa: a collaborative study of the French CECOS Federation on 11 535 pregnancies

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Artificial insemination using cryogenically preserved spermatozoa has been widely used in human reproduction for several decades. No evaluation of the resulting pregnancies and conceptions has been undertaken in sufficiently large study populations for minor variations to be distinguished. This study involves 11 535 pregnancies conceived by artificial insemination using donor spermatozoa and followed from the time that pregnancy was diagnosed. The pregnancies followed a normal course with, in particular, no excessive fetal losses. While the global incidence of birth defects was similar to that of natural conception, our observations raise doubts concerning trisomy 21. The frequency of trisomy 21 was somewhat elevated when compared with French malformation registries. A recruitment bias could, in part, explain this discrepancy, but donor age cannot be excluded as an influencing factor.

**Key words:** AID/birth defect/outcome of pregnancy/trisomy 21

## Introduction

Post-conception evolution and the status of infants born after using artificial insemination with donor spermatozoa (AID) have always been major concerns. It has never been proved that the procedures used (freezing, storage, ovulation induction, in-vitro manipulation) are without iatrogenic, mutagenic or teratogenic effects. Until now, data published have been unsatisfactory because they concern samples limited to a few hundred cases, and results from retrospective studies have been subjected to major recruitment biases (Swaab, 1974; Matsuoka *et al.*, 1979; Kovacs and Lording, 1980; Weller, 1980; Frazer and Forse, 1981; Trounson *et al.*, 1981; Federation Des CECOS *et al.*, 1983; Lansac *et al.*, 1983, 1984; Alfredsson, 1984; Virro and Shewchuk, 1984; Forse *et al.*, 1985; Varma and Patel, 1987; Yovich and Matson, 1988; Amazu *et al.*, 1990; Grefenstette *et al.*, 1990; Melzer, 1991). Contradictory conclusions have been drawn and, given the infrequent occurrence of malformations in the general population, it is possible that a significant rise in the number of malformations could have been missed in such small studies

For these reasons, the CECOS Federation set up a study of AID pregnancies. Preliminary results have been reported (Mayaux *et al.*, 1990). Each case has been followed from conception, making it possible to follow the pregnancy, record the outcome and evaluate the status of conceptus. Individual records from the various centres were centrally collected anonymously and analysed.

This set of infants, conceived under what might be considered experimental conditions, are of particular scientific interest to geneticists and epidemiologists. For example, the influences of paternal and maternal ages can be completely separated because, in contrast to natural reproduction, there is no a priori correlation between them. The study of gamete ageing is facilitated because the dates of insemination and fertilization can be identified during the menstrual cycle by using temperature charts. The effects of various treatments (such as hormonal therapy or ovulation induction treatments commonly used in assisted reproduction), and karyotypic variations of donors or their semen characteristics, could be investigated. All such studies would be more difficult to carry out in natural reproduction.

## Materials and methods

In France, AID is conducted almost exclusively by the CECOS Federation (Centre d'Etudes et de Conservation du Sperme et des Oeufs Humains) which unites 22 different local centres throughout the country, all of which follow the same protocol (Lansac *et al.*, 1984). This protocol defines indications for AID, methods for donor control and insemination procedures. CECOS technical commissions review contested cases and make recommendations to the local centres. Of particular interest is the Genetics Commission, which was created to define rules for the genetic control of donors using systematic karyotyping and genetic investigation (Jalbert and David, 1987; Jalbert *et al.*, 1989).

Another role of the CECOS Federation is to collect data for each year and to publish the reports on the 22 federated centres. A major advantage of this national level of organization is that it allows the analysis of a large number of AID conceptions. The number of AID infants monitored by CECOS is ~1500 per year.

The data for analysis were gathered for investigative study. This was possible because CECOS functioning requires that, following insemination, each unit returns to the centre that furnished the straws a completed form indicating the method for determination and date of ovulation, the date of insemination, the number of straws used, possible adjunctive hormonal therapy and the outcome of insemination, i.e. pregnancy or menstruation. The attribution of donors was performed by the local centre and was based on morphological, ethical and blood type criteria. The age of the recipient woman was never taken into account in the selection of a donor. The donor age was recorded on the date that the sperm specimen was collected.

Different factors were recorded for this study: first menarche age of the recipient woman, cycle length; insemination date in the conception cycle; maternal age at delivery; hormonal treatments; donor age; and sperm conservation length

Diagnosis of pregnancy was based on a persistent elevation of the temperature plateau over 21 days, and was confirmed by clinical examination and a positive immunological test for pregnancy. The investigator for the study was therefore informed of the pregnancy as soon as it was diagnosed, and remained informed of the progress of the pregnancy whether the outcome was spontaneous abortion, extra-uterine pregnancy, voluntary or medically indicated termination of pregnancy, or delivery. In cases of delivery, he was informed of the results, i.e. the number of births, the number of stillborn or live births. The status of each infant was determined by a paediatric examination in the maternity unit and recorded in the newborn baby's health record, as well as eventual karyotype results. Systematic gathering of these data permitted close follow-up of the course of each pregnancy and its outcome. Cooperation was insisted on when the required information was not furnished by the unit.

The malformations detected (including chromosomal aberrations) were either responsible for a decision for medically indicated abortion or identified at birth. They were classified with respect to the World Health Organization international classification system (British Paediatric Association, 1977; ICD 9). Congenital hip dislocations were excluded from the study because they were not taken into account in one of the French provinces (Brittany) where they are very frequent.

For quantitative variables, results were expressed using the mean  $\pm$  1 SD. They were compared using Wilcoxon's test. The Mantel-Haenszel test was used for the adjustment test on the qualitative variables. The significance threshold was fixed at 0.05.

Recruitment involved two steps. One investigation was organized in the 20 CECOS centres that existed in France between 1987 and 1989; the information collected covered 8119 pregnancies considered as a group. In the second investigation, data were collected under similar conditions from the centres covering the years before 1987. Therefore, information concerning 3689 more pregnancies was added.

The incidence of birth defects observed was compared with the French registries for the regions of Paris, Strasbourg and Marseilles, and the Central Region.

## Results

The study covers a total of 11 808 cycles concluding in confirmed conceptions. For 273 (2.3%) of these cases, the outcome is unknown despite repeated requests to the attending obstetricians for follow-up data. Our analysis therefore covers 11 535 pregnancies for which the outcome is known. A total of 9794 live births resulted.

For the 11 535 pregnancies for which the outcome is known, 2178 (18.9%) did not deliver at term. In 41 (0.4%) cases, medically indicated abortion was performed because of fetal anomalies: 13 because of chromosomal abnormalities; 12 because of a malformed fetus with normal karyotype; three for materno-fetal pathology; and 13 without an identified motive. There were 414 (4.4%) multiple births out of the 9357 pregnancies that delivered at term. Out of 9812 births, 8943 were single, i.e. 91.2% of the infants or 95.6% of the deliveries. The others were multiple, i.e. 381 twins, 26 triplets, six quadruplets and one set of quintuplets (Table I).

For single births ( $n = 8943$ ), the sex ratio was 104.4 and

Table I. Outcomes of pregnancies

Parameter	
Conception cycles	( $n = 11\ 808$ )
Information not available (%)	2.3
Pregnancies with known outcomes	( $n = 11\ 535$ )
Ectopic pregnancies (%)	0.8
Fetal losses (%)	17.7
Medical terminations (%)	0.4
Deliveries ( $n = 9357$ )	
Multiple pregnancies (%)	4.4

Table II. Incidence of birth defects from 9794 births and 35 fetuses after prenatal diagnosis

	Per thousand	$n$
Mono-malformations		
Cardiovascular	3.1	30
Limbs <sup>a</sup>	2.9	28
Urinary	1.5	15
Genital	0.4	4
Gastrointestinal	1.3	13
Central nervous	1.2	12
Respiratory <sup>b</sup>	0.4	4
Facial	1.2	12
Associated malformations (non-chromosomal)	1.0	10
Chromosomal anomalies		
Trisomy 21	2.5	25
Others	1.1	11
Not documented <sup>c</sup>	0.1	1

<sup>a</sup>Excluding congenital dislocation of the hip and hip click.

<sup>b</sup>Including diaphragmatic hernia

<sup>c</sup>Polymalformation with chromosomal anomaly not identified.

average birth weight was  $3281 \pm 491$  g. The number of babies with birth weights  $<2500$  g was 369 (4.1%). The incidence of prematurity evaluated from gestational age ( $<37$  weeks) was 4.8%. In all, 88 stillbirths (1.0%) were registered in these data.

In the singletons, 131 birth defects (1.5%) were recorded. This value remained stable for infants born of multiple pregnancies ( $n = 12$ ; 1.4%). The total frequency of defects, including 9794 infants and 35 fetuses after prenatal diagnosis, was 1.7% ( $n = 165$ ). Single malformations accounted for 1.2% ( $n = 118$ ) of the total. In order of decreasing frequency, malformations were found affecting the cardiovascular system, the limbs, the urinary system, etc. (Table II). Associated non-chromosomal malformations were noted 10 times (0.1%). A total of 36 malformed infants or fetuses demonstrated chromosomal anomalies. In 69% of the cases, trisomy 21 was recognized (0.25% incidence among 9794 children and 35 fetuses).

The group of women who gave birth to malformed children (excluding chromosomal anomalies) was compared with the group who gave birth to normal infants. Among the different factors recorded, donor age at the time of sperm collection, the duration of conservation of the spermatozoa, maternal age at menarche, average cycle length and date of insemination during the cycle did not differ between the two groups. Only maternal age at delivery was greater in the case of birth defects compared with the normal birth group (31.9 versus 31.1 years;

**Table III.** Incidence of trisomy 21 (per thousand) according to maternal and donor ages in 9794 births and 35 fetuses after prenatal diagnosis

Age (years)	Maternal age	Donor age
<35	1.6	1.4
35–39	4.9	2.3
>39	27.4	4.1
Significance	$P < 10^{-5}$	$P < 0.05$

**Table IV.** Incidence of trisomy 21 (per thousand) as a function of maternal and donor ages in 9794 births and 35 fetuses after prenatal diagnosis

Maternal age (years)	Donor age (years)	
	<38	≥38
<38	1.5	2.4
≥38	4.4	23.1

Relationship with the age of the mother when the donor age is kept constant;  $P < 0.0001$ . Relationship with the age of the donor when maternal age is kept constant;  $P < 0.05$

$P < 0.005$ ). On the other hand, the incidence of malformations did not differ according to whether the mother received hormonal treatment.

The same comparison was made for the group of women who had a product of conception exhibiting trisomy 21 and those who had no chromosomal anomaly. In this instance the mothers were also older in cases of a malformed infant or fetus (mean age 34.8 versus 31.1 years;  $P < 0.0001$ ) and the age of the donor was also significantly different (35.2 versus 34.5 years;  $P < 0.005$ ). The incidence of trisomy 21 increased with maternal age, but also increased, although less remarkably, with increased age for sperm donors (Table III).

After verifying that there was no correlation between the ages of the women and the ages of the donors (correlation coefficient  $r = 0.00$ ), adjustment tests were applied to determine the individual influence of each age on the incidence of trisomy 21. Regardless of the age of the donor below and above 38 years, there was a significant relationship with the age of the mother ( $P < 0.0001$ ). In the same way, the relationship with the age of the donor was still significant ( $P < 0.05$ ) when maternal age was kept constant (Table IV).

## Discussion

Even though the study covers two distinct periods, fusion of the data studied was performed after having verified that the results were similar in both samples. Moreover, the method of collecting data did not vary and was always carried out in a prospective mode. Data collection was performed from diagnosis all through the pregnancy and permitted follow-up on the progress of the pregnancy, its outcome and an evaluation of the status of the newborn or fetus. This procedure also offered the advantage of making recruitment relatively exhaustive compared with recruitment limited to birth declarations. This also explains why the rate of unfollowed patients was reduced to 2.3%.

The incidence of spontaneous abortion (17.7%) and of

ectopic pregnancies (0.8%) did not differ from that observed in natural procreation. In addition, the incidence of multiple pregnancies (4.4%), average birth weight, and the percentages of premature and stillbirths concurred with the rates observed in the general population. The sex ratio was balanced.

The global frequency of malformations was 1.7%, similar to that recorded in natural procreation. Moreover, apart from parental ages, none of the potential risk factors studied seemed to play a role, whether maternal (age at menarche, average cycle length, date of insemination during the cycle) or environmental factors (length of conservation of the spermatozoa, hormonal treatments, induction of ovulation). These results confirm the generally accepted, although not yet clearly documented, notion that artificial insemination with cryogenically conserved spermatozoa does not present any major deleterious risk compared with natural procreation.

The mean maternal age for conceptus affected by non-chromosomal defects was slightly increased when compared with normal births (Baird *et al.*, 1991), whereas the mean age for donors was not higher. In reflecting upon this phenomenon, it should be kept in mind that the couples in this study were, on average, older than couples who procreate naturally.

Only the incidence of trisomy 21 appeared to be elevated globally in the population studied. It was compared with that of three French registries of malformation (1988, 1989, 1990) established under conditions close to those of the study: recruitment was based on consecutive births and took induced abortions into account. After adjusting for maternal age by indirect standardization, the incidence of trisomy 21 observed in this study was compared with those found for the same years in the Paris, Marseilles and Strasbourg registries. The calculated incidence of trisomy 21 did not appear to be significantly different from that at Strasbourg or Marseilles. However, it was slightly higher than at Paris (0.25 versus 0.20%;  $P < 0.05$ ). This result requires careful interpretation, especially because the frequency of this defect rises regularly in this registry's study, from 0.14% in 1981 to 0.29% for the years 1989 and 1990 (Devigan *et al.*, 1992). It is nonetheless significant that, while the present study followed the progress of each pregnancy from the first clinical signs until birth, the registries operate using declarations made by maternity units (declaration of pregnancy is obligatory during the first 3 months of gestation) and documented medically indicated abortions. Even though the data gathered in this way are reorganized and completed by other sources (cytogenetic laboratories, paediatric and neonatology services), recording is probably not as exhaustive as pregnancy follow-up from the time of conception. No studies exist in France that operate in this way, which left no alternative but to accept these three registry series as controls. Moreover, it is worth noting that none of the variables that differentiate the use of artificial insemination from natural reproduction, such as the duration of conservation of spermatozoa or hormonal treatment, influenced the incidence of malformed and trisomic birth. Lastly, with chromosomal analysis of human spermatozoa, Martin *et al.* (1991) demonstrated no increase in disomy after a freezing-thawing procedure.

However, the growing incidence for trisomy 21 linked to

the age of the mother conforms to observations in natural procreation. Independently of maternal age, there is, in addition, an apparent effect of paternal age on the incidence of trisomy 21. The age under discussion is the age of the donors at the time of sperm collection. This factor has never been a consideration in attributing a donor to a recipient woman (anonymous donor), and there exists no correlation between the parental ages contrary to what is habitually the case in natural procreation. In the study population, a continuous rise in the incidence of trisomy 21 is nonetheless observed with increasing donor age. In other words, this incidence is higher in the group of conceptions from donors aged >38 years, whether or not the mother is > or <38 years old.

From prenatal diagnosis data, Stene *et al.* (1987a,b) concluded that paternal age was a risk factor for trisomy 21. This has been contested by Hook (Cross and Hook, 1987; Hook, 1987; Hook *et al.*, 1990). De Michelena *et al.* (1993) considered that epidemiological arguments were debatable in both these studies. The fact that 95% of trisomies 21 originate from maternal non-disjunction (Antonarakis *et al.*, 1991, 1993) does not favour a predominant role for paternal age. Moreover, with the cytogenetic analysis of spermatozoa, Martin and Rademaker (1987, 1992) and Rosenbusch *et al.* (1992) did not observe any linkage between disomy and paternal age. Nevertheless, this statistical observation, as yet without any biological explanation, must be added to the information on the still controversial question of the influence of paternal age (Friedman, 1981; Lian *et al.*, 1986; Bricarelli *et al.*, 1989; Hatch *et al.*, 1990).

Practically speaking, this observation encourages the lowering of the age limit for donors. Bordson and Leonardo (1991) have suggested that only donors aged <35 years be accepted, but that seems somewhat excessive. The 55 year limit fixed by the Human Fertilisation and Embryology Authority (HFEA) in the UK (HFEA, 1993) is not very reassuring. The American Fertility Society (1993) recommends an age <40 years. At the moment CECOS accepts only donors aged <45 years. This attitude will be re-evaluated if any strong evidence of paternal age is found. Indeed, the present situation is more theoretical than practical. The AID model allows total dissociation of maternal and paternal ages, which is not the case in natural procreation. This explains the fact that we were able to identify this influence in the study, for this tendency would be too weak to appear directly in epidemiological models; by accentuating the differences, this phenomenon shows up clearly.

### Conclusion

The number of cases studied is significant. These data show evidence that the AID procedure is not responsible for a significantly increasing rate of non-chromosomal malformations. Only a slight increased risk of trisomy 21 is recorded. Two hypotheses have to be explored: AID procedure and age of the donor. None of the variables that differentiate the AID procedure from natural conception is significantly associated with trisomy 21. For donor age, there is no other epidemiological or biological information apart from these data. Nevertheless, to unambiguously exclude any influence of paternal age,

this parameter will require further verification in a larger population. Moreover, using techniques of DNA polymorphism analysis, this study will be completed with the determination of the parental origin of trisomy 21

### References

- Alfredsson, J.H. (1984) Artificial insemination with frozen sperm *Int J. Fertil.*, **29**, 152–155.
- American Fertility Society (1993) Guidelines for gamete donation 1993 *Fertil. Steril.*, **59**, Suppl. 1.
- Amazu, B., Laxova, R. and Shapiro, S.S. (1990) Pregnancy outcome, health of children, and family adjustment after donor insemination *Obstet. Gynecol.*, **75**, 899–905.
- Antonarakis, S.E. and the Down Syndrome Collaborative Group (1991) Parental origin of the extra chromosome in trisomy 21 as indicated by analysis of DNA polymorphisms. *N Engl J. Med.*, **324**, 872–876
- Antonarakis, S., Avramopoulos, D., Blouin, J.L. *et al.* (1993) Mitotic errors in somatic cells cause trisomy 21 in about 4.5% of cases and are not associated with advanced maternal age *Nature Genet.*, **3**, 146–150
- Baird, P., Sadovnick, A. and Yee, I. (1991) Maternal age and birth defects: a population study *Lancet*, **337**, 527–530.
- Bordson, B.L. and Leonardo, V.S. (1991) The appropriate upper age limit for semen donors: a review of the genetic effects of paternal age. *Fertil. Steril.*, **56**, 397–401.
- Bricarelli, F., Pierluigi, M., Landucci, M. *et al.* (1989) Parental age and the origin of trisomy 21 — a study of 302 families. *Hum. Genet.*, **82**, 20–26.
- British Paediatric Association (1977) *British Paediatric Association Classification of Diseases* Paediatric supplement of the 9th revision of the World Health Organization international classification.
- Cross, P. and Hook, E. (1987) An analysis of paternal age and 47, +21 in 35,000 new prenatal cytogenetic diagnosis data from the New York state chromosome registry: no significant effect *Hum. Genet.*, **77**, 307–313
- De Michelena, M.I., Burstein, E., Lama, J.R. and Vasquez, J.C. (1993) Paternal age as a risk factor for Down syndrome. *Am J Med Genet.*, **45**, 679–682
- Devigan, C., Vodovar, V., Dufouil, C. and Goujard, J. (1992) Trisomy 21 and mother's age-trend in Paris, 1981–1990 *Rev. Epidém. Santé Publ.*, **40**, 369–378
- Federation Des CECOS, Mattei, J.F. and Le Marec, B. (1983) Genetic aspects of artificial insemination by donor (AID) Indications, surveillance and results. *Clin. Genet.*, **23**, 132–138
- Federation Des CECOS, Le Lannou, D. and Lansac, J. (1989) Artificial procreation with frozen donor semen: experience of the French federation CECOS. *Hum. Reprod.*, **4**, 757–761.
- Forse, R.A., Ackman, C.F.D. and Fraser, F.C. (1985) Possible teratogenic effects of artificial insemination by donor *Clin. Genet.*, **28**, 23–26.
- Frazer, F.C. and Forse, R.A. (1981) On genetic screening of donors for artificial insemination *Am. J. Med. Genet.*, **10**, 399–405
- Friedman, J.M. (1981) Genetic disease in the offspring of older fathers *Obstet. Gynecol.*, **57**, 745–749.
- Grefenstette, I., Royere, D., Barthelemy, C.L. *et al.* (1990) Devenir de 470 grossesses obtenues après insémination artificielle avec sperme congelé de donneur. *J. Gynecol. Obstet. Biol. Reprod.*, **19**, 737–744
- Hatch, M., Kline, J., Levin, B. *et al.* (1990) Paternal age and trisomy among spontaneous abortions. *Hum. Genet.*, **85**, 355–361.
- Hook, E. (1987) Issues in analysis of data on paternal age and 47, +21 implications for genetic counseling for Down syndrome *Hum. Genet.*, **77**, 303–306.
- Hook, E., Cross, P. and Regal, R. (1990) Factual, statistical and logical issues in the search for a paternal age effect for Down syndrome *Hum. Genet.*, **85**, 387–388.
- Human Fertilisation and Embryology Authority (1993) Code of Practice March 1993.
- Jalbert, P. and David, G. (1987) The genetic problems associated with artificial reproduction using donor gametes: the solutions that have been adopted by CECOS *J. Gynecol. Obstet. Biol. Reprod.*, **16**, 547–552.
- Jalbert, P., Leonard, C., Selva, J. and David, G. (1989) Genetic aspects of artificial insemination with donor semen: the French CECOS federation guidelines *Am. J. Med. Genet.*, **33**, 269–275.
- Kovacs, G.T. and Lording, D.W. (1980) Artificial insemination with donor semen. Review of 252 patients *Med J. Aust.*, **2**, 609–612

- Lansac, J, Imbault, M., Lecompte, C *et al.* (1983) Pregnancy and labor after insemination with frozen sperm from a donor *J Gynecol Obstet Biol. Reprod.*, **12**, 511–518
- Lansac, J, Le Lannou, D, Imbault, M *et al.* (1984) Pregnancy and delivery following insemination with frozen donor sperm. *Rev Fr Gynecol. Obstet.*, **79**, 565–569
- Lian, Z, Zack, M. and Erickson, D (1986) Paternal age and the occurrence of birth defects *Am. J Hum. Genet.*, **39**, 648–660
- Martin, R. and Rademaker, A (1987) The effect of age on the frequency of sperm chromosomal abnormalities in normal men *Am. J Hum. Genet.*, **41**, 484–492
- Martin, R and Rademaker, A (1992) A study of paternal age and sex ratio in sperm chromosome complements *Hum. Hered.*, **42**, 333–336
- Martin, R, Chernos, J and Rademaker, A (1991) Effect of cryopreservation of the frequency of chromosomal abnormalities and sex ratio in human sperm *Mol. Reprod. Dev.*, **80**, 159–163
- Matsuoka, T., Tanaka, M., Sunahori, K. *et al.* (1979) Results in artificial insemination with donor's semen *Acta Obstet Gynaecol Jap.*, **31**, 805–812
- Mayaux, M J, Jalbert, P and Lansac, J (1990) The quality of the conceptus obtained after AID Results of a prospective survey *Contracept Fertil. Sex.*, **18**, 581–582
- Melzer, H (1991) Artificial human donor insemination as a treatment possibility for sterile couples at the gynecologic clinic of the Chemnitz 'Friedrich Wolf' district hospital *Zentralbl. Gynäkol.*, **113**, 7–12
- Registries of Malformations (1988) (1989) (1990) Paris, Marseille, Strasbourg INSERM editions
- Rosenbusch, B, Strehler, E. and Sterzik, K (1992) Cytogenetics of human spermatozoa correlations with sperm morphology and age of fertile men *Fertil. Steril.*, **58**, 1071–1072
- Stene, E., Stene, J and Stengel-Rutkowski, S (1987a) On methodological issues regarding 47, +21 paternal age data. *Hum. Genet.*, **77**, 317
- Stene, E, Stene, J and Stengel-Rutkowski, S (1987b) A reanalysis of the New York State prenatal diagnosis data on Down's syndrome and paternal age effects *Hum. Genet.*, **77**, 299–302
- Swaab, L I (1974) Results of insemination with donor semen. *Ned. Tijdschr Geneesk.*, **118**, 493–498
- Trounson, A.O., Matthews, C D and Kovacs, G T (1981) Artificial insemination by frozen donor semen *Int. J Androl.*, **4**, 227–234
- Varma, TR and Patel, R H (1987) Outcome of pregnancy following investigation and treatment of infertility *Int J Gynecol Obstet.*, **25**, 113–120
- Virro, M.R and Shewchuk, A.B (1984) Pregnancy outcome in 242 conceptions after artificial insemination with donor sperm and effects of maternal age on the prognosis for successful pregnancy *Am. J Obstet Gynecol.*, **148**, 518–524
- Weller, J. (1980) Schwangerschafts und Geburtslauf nach 104 erfolgreichen Artifizialen Donogenen Inseminationen (ADI) mit Nativ- und Kryosperma. *Geburtshilfe Frauenheilkd.*, **40**, 269–275.
- Yovich, J L and Matson, P L (1988) Early pregnancy wastage after gamete manipulation *Br J Obstet Gynaecol.*, **95**, 1120–1127

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