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Effects of age, gravidity and male infertility status on cumulative conception rates following artificial insemination with cryopreserved donor semen: analysis of 2998 cycles of treatment in one centre over 10 years

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The effects of age, gravidity and male infertility status on cumulative conception rates after donor insemination were investigated in an analysis of 2998 treatment cycles undertaken on 443 patients. It was found that the cumulative conception rates affer 3, 6 and 12 cycles of treatment were 21, 40 and 62% respectively for patients <30 years of age compared with 17, 26 and 44% for those aged ≥30 years ($P = 0.008$). There was also a significant difference ($P < 0.001$) in results depending on course of treatment and the cumulative conception rates were 19% after 3 cycles, 33% after 6 cycles and 54% after 12 cycles of treatment in the first course of treatment compared with 40, 67 and 79% respectively in those who returned for subsequent courses of treatment after having achieved a donor insemination pregnancy in the first treatment course. Gravidity and male infertility status (azoospermia or oligozoospermia/asthenozoospermia) did not significantly affect the cumulative conception rates.

Key words: age/cumulative conception rate/donor insemination/parity

Introduction

Sperm dysfunction is one of the biggest and most elusive problems in infertility practice today. Not only is it a major cause of infertility but diagnosis and treatment are largely ineffective (Wardle *et al.*, 1989) so that donor insemination remains the most effective treatment for a major proportion of cases. Although this is an established and widely practised form of infertility therapy, it is psychologically stressful (Harvey and Harvey, 1977) especially if the duration of treatment becomes protracted in order to achieve pregnancy. It is, therefore, incumbent on clinicians to counsel their patients and provide them with a realistic prognosis in terms of the duration and outcome of treatment.

In this regard, the optimal method of determining success of any infertility therapy is to calculate the cumulative conception rates using life-table methods, which allow for variable periods of follow-up and losses to follow-up (Cramer *et al.*, 1979). Moreover to optimize its usefulness for counselling, the cumulative conception rates should be related to the major factors that affect the probability of success following treatment with donor insemination. With this in mind, all consecutive treatment cycles in a single unit were assessed to determine the effects of the recipient's age and gravidity, and the degree of her partner's impaired fertility, on the cumulative conception rates following donor insemination.

Materials and methods

The case records were reviewed of all consecutive patients who were referred for donor insemination at the Fertility Unit of the Middlesex Hospital in London between September 1981 and September 1991. All patients in the study were evaluated prior to the commencement of donor insemination to detect the presence of concomitant infertility factors that could affect the outcome of treatment. Following clinical history taking, physical examination and counselling, the occurrence of ovulation was established (biphasic body temperature (BBT) charts and mid-luteal serum progesterone > 30 nmol/l) and patients with ovulatory dysfunction were treated with clomiphene citrate (50 to 100 mg daily from day 2–6 of the menstrual cycle) or tamoxifen (20 to 40 mg day 2–5). Patients who had hyperprolactinaemia were administered bromocriptine (Tan and Jacobs, 1985) while those who had clomiphene resistant anovulation were treated with human menopausal gonadotrophin or pulsatile luteinizing hormone releasing hormone therapy (Armar *et al.*, 1987). Investigation of tubal patency was generally performed by laparoscopy and dye hydrotubation but sometimes by hysterosalpingography. These tests were performed before donor insemination was initiated if there was suspicion of pelvic pathology (history of pelvic inflammatory disease, clinical suspicion of endometriosis, previous ectopic pregnancy, abnormal pelvic examination or failure to conceive after three to six cycles in other treatment programmes). In the absence of clinical suspicion of pelvic pathology, laparoscopy or hysterosalpingography was deferred until the patient had failed to conceive after three to six cycles.

Only cryopreserved semen was used for insemination. Donors were healthy volunteers who were free from sexually transmitted diseases (syphilis, gonorrhoea, chlamydia, human immunodeficiency virus) and transmissible genetic disorders, and who had good quality semen (minimum sperm concentration of 60×10^6/ml with 50% good progressive motility and 70% normal forms). Donors were screened for hepatitis and matched to the legal fathers by gross physical characteristics (racial origin, skin, hair and eye colour, build and height) and, where possible,

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by blood type (except in the case of Rhesus incompatibility).

Donors produced semen samples by masturbation in a special room adjacent to the laboratory. The specimens were allowed to liquefy and an aliquot was taken for analysis. The remaining sample was mixed with an equal volume of egg yolk/glycerol cryoprotectant medium. The semen was then stored in ampoules, each containing 1 ml, which were then cooled by two-staged freezing over nitrogen vapour followed by immersion into liquid nitrogen. The frozen semen was then stored in liquid nitrogen in storage tanks (Mahadevan and Trounson, 1980).

Insemination was timed using the patient's cycle length and temperature charts and/or ultrasound measurement of follicular growth. In the last 3 years, almost all patients had been using urinary luteinizing hormone (LH) sticks (Clearplan, Unipath, Bedford or Ovuquick, Medimar Laboratories, Gerrards Cross, Buckinghamshire, UK) to time their inseminations. In general, two inseminations were performed around the time of presumed ovulation. All inseminations were performed intra-cervically, either by the gynaecologist or research sister, using a 2 ml syringe and Kwill filling tube (Universal Hospital Supplies Ltd, Southgate, London, UK). The patients were asked to rest for 5 to 10 min after the procedure. Only one doctor was involved in the donor insemination procedures in the entire series who was also responsible for reviewing all patients after every one or two cycles.

Each patient's age was taken on the first day of the menstrual cycle immediately preceding her first cycle of donor insemination. The patients were classified by gravidity into two groups, namely nulligravida and multigravida, and by their partner's degree of infertility into two groups, namely azoospermia, and oligozoospermia and asthenozoospermia. Cumulative conception rates were analysed by the life-table approach (Cramer *et al.*, 1979; Cooke *et al.*, 1981) with analysis being confined to 12 cycles of treatment. Statistical significance of differences in the cumulative conception rates between different groups was determined by the log-rank test (Kahn and Sempos, 1989). Analysis of the effects of age on cumulative conception rates was performed using Cox proportional hazards survival analysis (Peto *et al.*, 1977).

Table I. Characteristics of patients undergoing treatment with donor insemination

	First course of treatment *n* (%)	Second or further courses *n* (%)
Number of women	443	65
Indication for treatment		
Azoospermia in partner	291 (65)	46 (71)
Oligozoospermia in partner	149 (34)	19 (29)
Genetic indications in partner	3 (1)	0 (–)
Female age at time of first cycle		
<30 years	211 (48)	19 (29)
>30 years	232 (52)	46 (71)
Mean age (range)	30 (19–42)	31 (22–41)
Gravidity before first treatment		
No previous pregnancies	306 (69)	0 (–)
One or more previous pregnancies	137 (31)	65 (100)

Results

A total of 443 patients underwent donor insemination for one or more cycles. Of those, 378 patients had one course of treatment only, 52 had a second course of treatment after having achieved a pregnancy in the first course, 11 had three courses and two had four courses. A total of 2998 cycles were undertaken, resulting in 215 pregnancies. The indications for treatment and patient characteristics are shown in Table I.

The patients' mean age at first treatment was 30 years (range 19 to 42) for the first course of treatment and 31 for subsequent

Table II. Cumulative conception rates with number of treatment cycles and for various patient characteristics

	Number women at start	% cumulative conception rate (95% CI)		
		3 cycles	6 cycles	12 cycles
First treatment course	443	18.9 (15.5–22.9)	32.5 (28.1–37.4)	53.5 (46.4–60.9)*
Later treatment courses	65	40.1 (29.1–53.5)	67.0 (54.8–78.8)	79.2 (66.8–89.3)*
Azoospermia in partner[a]	291	20.9 (16.6–26.2)	35.4 (29.9–41.6)	55.0 (46.3–64.1)
Oligozoospermia in partner[a]	149	15.2 (10.3–22.2)	27.3 (20.4–36.0)	51.5 (39.9–64.4)
<30 years[a]	211	20.8 (15.8–27.0)	40.1 (33.4–47.7)	62.3 (52.2–72.5)**
30 or more[a]	232	17.2 (12.8–22.8)	25.6 (20.2–32.2)	44.1 (35.3–53.9)**
Nulligravida[a]	306	19.6 (15.5–24.7)	33.9 (28.5–39.9)	53.6 (44.7–63.2)
Multigravida[a]	137	17.2 (11.8–24.7)	29.5 (22.3–38.5)	53.0 (42.9–63.8)

95% CI = 95% confidence interval.
*$P < 0.001$, **$P = 0.008$.
[a]First course of treatment only.

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courses. They underwent a mean of six cycles of treatment (range, one to twelve) in the first course of treatment and four in subsequent courses. The cumulative conception rates were 33% after six cycles and 54% after twelve cycles in the first course of treatment compared with 67 and 79% respectively in subsequent courses (Table II, Figure 1). The differences were statistically significant ($P < 0.001$).

Within the first course of treatment, 291 of the men were azoospermic, while 149 were severely oligozoospermic (sperm concentration $<5 \times 10^6$/ml)/asthenozoospermic (total sperm

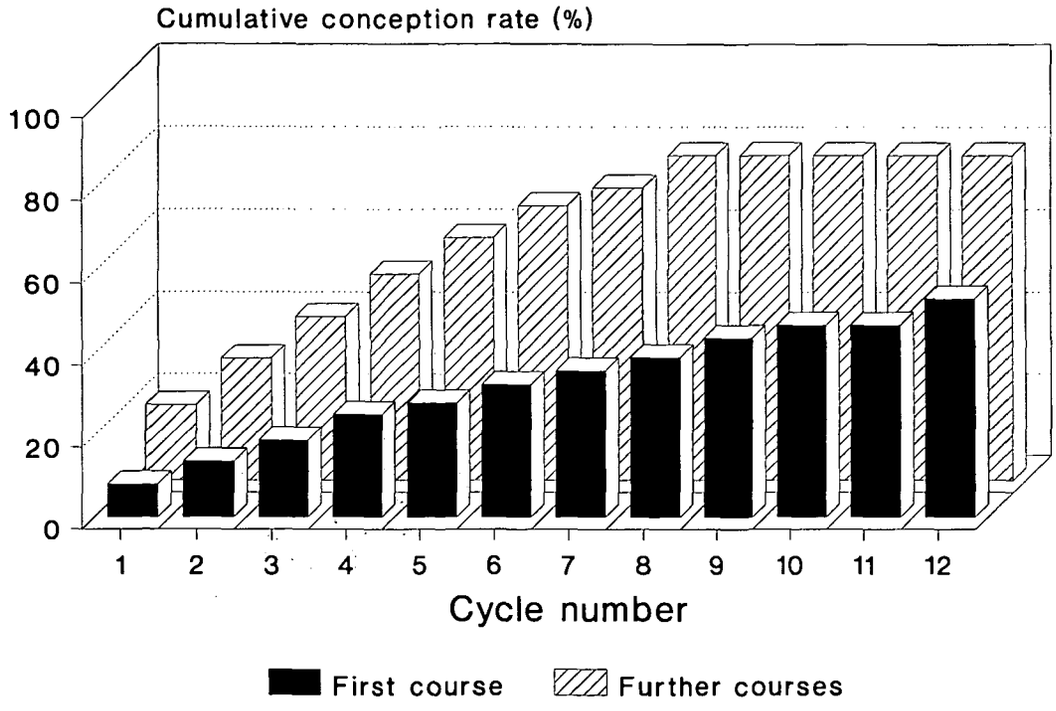

Fig. 1. Cumulative conception rates by course of treatment.

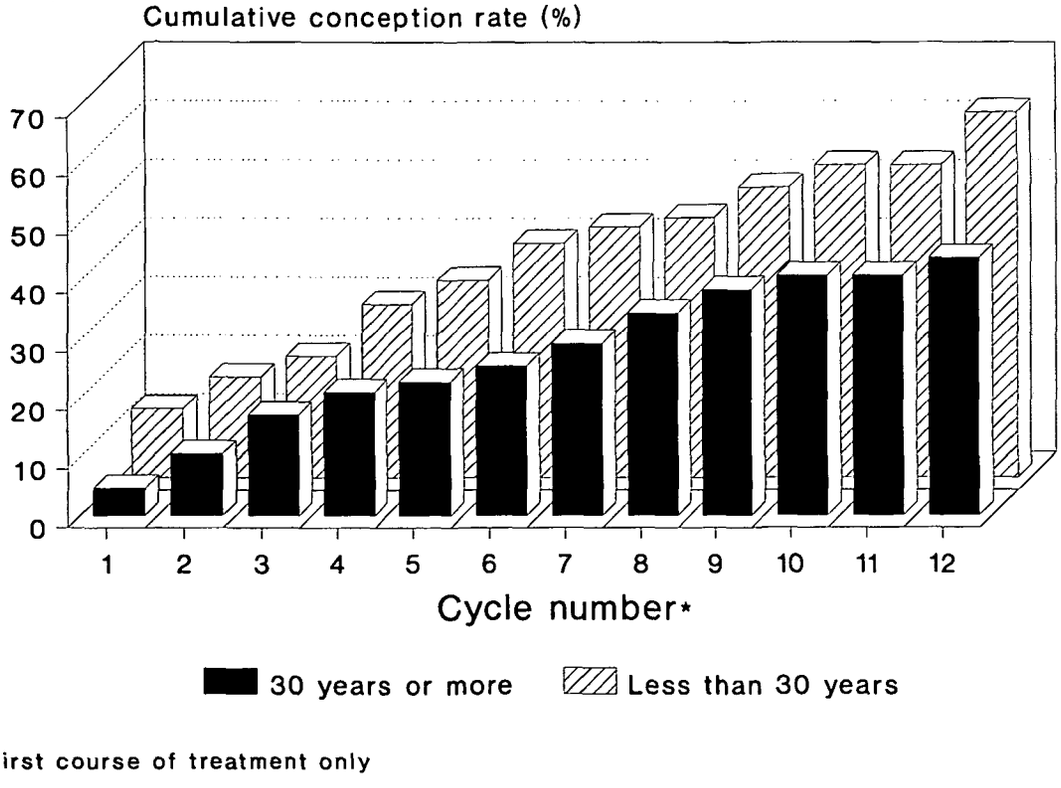

Fig. 2. Cumulative conception rates by age of women at start of first course of treatment.

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motility <20%). There was no significant difference in the cumulative conception rates in the two groups (35 and 27% after six cycles and 55 and 52% respectively after twelve cycles in the first course of treatment) (Table II).

The results of the first course of treatment were analysed according to the age of the recipient. It was found that the cumulative conception rates after 3, 6 and 12 cycles were 21, 40 and 62% for patients <30 years compared with 17, 26 and 44% for those ≥30 years of age (Table II, Figure 2). Cox survival analysis of the influence of age on the cumulative conception rate showed age to be a statistically significant factor ($P = 0.008$).

Table II also shows the results of the first treatment course analysed by gravidity of the recipient at the beginning of treatment. Previous pregnancy did not significantly affect the probability of conception. The cumulative conception rate was 54% after 12 cycles in the nulligravid women compared with 53% in the multigravid women.

Discussion

Variability in the quoted success of any infertility therapy may be due to differences in patient populations, management techniques or methods of analysis. Suboptimal methods of analysing data are a major source of variation, which is avoidable. With regard to donor insemination, for example, success rates based on the proportion of women treated who conceive would not take into account the number of treatment courses undertaken by each woman or the number of women who did not complete treatment because they dropped out of treatment, or were still under therapy when the data were analysed. Cumulative conception rates based on life-table analysis takes into consideration the experience of the entire cohort of women presenting for donor insemination and, therefore, provides a true measure of prognosis. The homogeneity of the present series should also be emphasized. Only one doctor was involved in all the donor insemination procedures in the entire series.

Since age is a major determinant of success of any infertility therapy (Tan *et al.*, 1990, 1992a), we have looked at the effect of donor insemination. Previous studies on this subject have shown conflicting results. While the majority of studies have suggested that success rates are significantly reduced after 30 to 35 years of age (Federation CECOS *et al.*, 1982, 1989; Kovacs, 1988), other studies have reported no significant differences in results based on age (Albrecht *et al.*, 1982; Bordson *et al.*, 1986). Our results support the notion that the success rate declines after 30 years of age. A number of different mechanisms have been suggested to account for the decline of fertility with age (Gosden, 1985) including progressive follicular depletion, decline in granulosa cell function (Hughes *et al.*, 1990), poor oocyte quality (Navot *et al.*, 1991) and reduced endometrial receptivity. It is known that serum inhibin levels reflect granulosa cell function. In a recent study comparing the time courses of serum inhibin responses to ovarian hyperstimulation in patients undergoing in-vitro fertilization (IVF), inhibin responses were found to be significantly lower in women over the age of 35 years (Hughes *et al.*, 1990).

Some previous reports have suggested that pregnancy rates following donor insemination in women whose partners are azoospermic are significantly higher than those whose partners are merely oligozoospermic (Albrecht *et al.*, 1982; Emperaire *et al.*, 1982). The explanation postulated is that women married to azoospermic men have normal fecundability, while women married to oligozoospermic men and who come to a fertility clinic are a selected population of women with subnormal fertility unable to compensate for the deficient sperm concentrations of their partners (Emperaire *et al.*, 1980). However, in the present study we found no significant difference in results between women whose partners were azoospermic and those whose partners were oligozoospermic. This finding is consistent with the experience of Kovacs *et al.* (1988). The reason for this disparity in results may be due to the differing severity of oligozoospermia in the various studies. The patients in our study had sperm concentrations $<5 \times 10^6$/ml, motility <20% and normal morphology <20%. These criteria were somewhat more strict than those in some of the other studies where patients with motility <30% and normal morphology <40% were accepted for donor insemination (Emperaire *et al.*, 1982), and may have accounted for the difference in results.

Interestingly, but perhaps not surprisingly, we found that the small subgroup of patients, who returned for treatment after having previously conceived through donor insemination, had a very high pregnancy rate and the majority of them achieved pregnancy within a few cycles of treatment.

As patients who require donor insemination may themselves be subfertile, investigation of female causes of infertility should be instituted before the patients embark on the treatment (Chauhan *et al.*, 1989). Correction of female infertility factors prior to commencement would improve the prognosis of these patients. The exception would be investigation of tubal infertility, especially by laparoscopy, in the absence of any clinical suspicion of pelvic pathology. Although pelvic pathology cannot be ruled out without performing a laparoscopy, in view of the invasive nature of the investigation, it appears prudent to defer the operation until the patient has failed to conceive after six cycles of donor insemination. Such an approach would save a significant proportion of women from requiring the investigation.

With the advent of the new reproductive technologies such as IVF, gamete intra-Fallopian transfer (Asch *et al.*, 1986), transvaginal peritoneal oocyte and sperm transfer (Tan *et al.*, 1992b) and superovulation and intrauterine insemination (Serhal *et al.*, 1988), consideration should be given to referring patients for one of these methods of assisted conception if pregnancy is not achieved with repeated treatment cycles of donor insemination. There is a progressive decline in the success rate of donor insemination with repeated treatment cycles, especially after twelve cycles (Kovacs *et al.*, 1988). The precise timing of referral depends on individual circumstances and earlier referral may have to be considered in patients over the age of 30 years.

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