Randomized controlled trial of cervical cap with intracervical reservoir versus standard intracervical injection to inseminate cryopreserved donor semen

C.Coulson, E.A.McLaughlin1, S.Harris, W.C.L.Ford and M.G.R.Hull

University of Bristol Department of Obstetrics and Gynaecology, St Michael's Hospital, Bristol BS2 8EG, UK

1To whom correspondence should be addressed

Introduction
Male factors account for at least one quarter of all infertility (Hull, 1992), and even with the introduction of advanced techniques such as intracytoplasmic injection (ICSI) to achieve fertilization in vitro, donor insemination still provides the only practical means for most of these couples to have children. Approximately 3000 couples seek donor insemination treatment in the UK every year and there were more than 16 000 cycles of treatment in 1992 [Human Fertilisation and Embryology Authority (HFEA), 1994], the last year for which figures are currently available.

The use of quarantined cryopreserved semen (Department of Health, 1991) is mandatory to minimize the risk of infection with HIV (Stewart et al., 1985) but reduces the chance of success (Richter et al., 1984; Di Marzo et al., 1990) and in the UK in 1992 the pregnancy rate per cycle was only 6.4% per cycle (HFEA, 1994). That is similar to other findings with cryopreserved in contrast to fresh semen (Richter et al., 1984; Di Marzo et al., 1990).

By comparison with those rates using cryopreserved semen, separation of spermatozoa from semen for intrauterine insemination (IUI) has been reported to double the chance of pregnancy (e.g. Byrd et al., 1994). However, that requires several times more semen and monitoring of the luteinizing hormone (LH) surge for precise timing of insemination. Furthermore, benefit of IUI treatment has not been a consistent finding (Byrd et al., 1990; Patton et al., 1990, 1992; Hurd et al., 1993; Depypere et al., 1994), nor has it been compared with simple insemination by any controlled study. Combination with ovarian stimulation has been reported to increase success rates of IUI with donor spermatozoa (Depypere et al., 1994), although this has also not been evaluated by controlled study and incurs substantially greater cost of drugs, requires ovarian monitoring and involves the risk of multiple pregnancy. For all those reasons intracervical injection of unprepared semen remains the standard primary method of donor insemination in the UK, because of simplicity, safety and low cost, before considering IUI or in-vitro fertilization (IVF).

Studies with cryopreserved bovine semen have shown that the conception rate is related to the number of motile spermatozoa inseminated (see Watson, 1990). With frozen/thawed human semen the chance of conception has been found to decline when $<2\times10^6$ motile spermatozoa were inseminated (Fédération CECOS et al., 1989). The British Andrology Society (BAS) has recommended in recent guidelines (BAS, 1993) that individual inseminates should contain a minimum of $3\times10^6$ progressively motile spermatozoa. However, the conception rate cannot be improved by simply increasing the inseminated volume. Doubling the volume of the inseminate in a prospective controlled trial from 0.25 to 0.5 ml, corresponding to an average of 3 or $6\times10^6$ motile spermatozoa after thawing respectively, failed to improve the pregnancy rate (Corrygan et al., 1994). It was observed that most of the extra inseminate leaked out of the cervical canal and may have been wasted in the vagina.

The conventional method of ensuring that the semen and cervical mucus remain in close contact is the use of a cervical cap acting as an ectocervical reservoir, typically holding 2 ml of semen (Kossoy et al., 1989). However, such caps have not been evaluated by any controlled studies, and now the restricted availability of quarantined cryopreserved semen makes it impractical to use such large volumes of semen unless highly effective. Therefore the Belaisch insemination cap or ‘dome’ (Prodimed: Neuilly en Thelle, France), designed to hold only 0.5 ml semen mostly in an intracervical reservoir, seemed
Materials and methods

Given approval by the local Medical Ethics Committee, all patients requiring donor insemination treatment between February and September 1994 in the University of Bristol Department of Obstetrics and Gynaecology were recruited into the study with their consent. The main indications for treatment were azoospermia (64%), severe oligozoospermia and/or severe asthenozoospermia (25%), and seminal antisperm antibodies (9%); ejaculatory dysfunction and a history of genetic disease accounted for the remainder (2%). The women underwent basic screening assessment of their fertility potential by history, chlamydia serology and measurement of early follicular phase serum LH and follicle stimulating hormone (FSH). Ovulation was confirmed by mid-luteal serum progesterone measurement (≥30 nmol/l). Women with suspected tubal damage or any who failed to conceive after 6–12 cycles of treatment underwent hysterosalpingography or laparoscopy. Each patient was inseminated using a standard intracervical injection method (with a Rocket ‘gun’: Rocket of London Ltd, Watford, UK) or intracervical reservoir in alternate cycles, the initial choice by year of birth: odd for the reservoir (85 patients) and even for injection (113 patients).

Semen was donated mainly by young university students screened according to BAS guidelines (BAS, 1993). After cryopreservation (McLaughlin et al., 1990) in 0.25 ml straws the semen was quarantined for at least 6 months. When required the straws were removed from liquid nitrogen and the semen allowed to thaw on the bench top at room temperature. Only semen meeting the minimum standard of 3×10⁶ progressively motile spermatozoa per 0.25 ml straw after thawing was used, thus each patient received a minimum of 6×10⁶ progressively motile spermatozoa per insemination. Standard intracervical insemination by injection was done as described previously (Corrigan et al., 1994) with two 0.25 ml semen straws used per insemination (total volume 0.5 ml). Figures 1 and 2 show the cap with intracervical reservoir, which is a 26 mm wide saucer shaped polyethylene device with a strengthened ring base for grasping with forceps and a 12 mm long hollow stem (blind at the base) to fit into the cervical canal and act as a reservoir of slightly less than 0.5 ml. A nylon string is attached for removal of the device. Insemination was carried out according to the manufacturer’s instructions. After thawing as described above, semen from two straws was loaded into the reservoir and any excess semen allowed to overflow into the hollow of the cap. After exposing the cervix with a bivalve speculum, the device was gripped by the base ring using sponge forceps, and the central reservoir was inserted into the cervical os. The device was held in position with the forceps until the speculum had been removed allowing the vagina to close onto the cervix. Two to 6 h after insemination the device was removed by the patient pulling on the string.

The timing of insemination was based either on previous cycle length and recognition of pre-ovulatory cervical mucus, the first insemination being scheduled for 2 days prior to the expected day of ovulation, or on urinary detection of the LH surge (Ovuquick: Medimarketing Ltd, Oxford, UK), in which case inseminations were scheduled for the first day of detection of the surge and the next day. One or two inseminations were performed each cycle at the patient’s request.

Clinical pregnancy was defined by detection of urinary human chorionic gonadotrophin (HCG) after delayed menstruation. The results were expressed as pregnancy rates per cycle and as cumulative conception rates calculated by the Kaplan–Meir life table method, compared statistically using the log-rank test. Standard errors were calculated from Peto’s formula (Peto et al., 1977).

Results

A total of 201 patients were recruited but three patients were excluded because of protocol violations. The mean age of the female partner was 30.4 years (range 21–40) and the median duration of infertility at initial counselling was 48 months (range 3–192).

The 198 patients remaining in the study received a total of 635 treatment cycles (median three per patient: range one to seven) including 326 with the standard injection method and 309 with the reservoir (range zero to four cycles with either one insemination method). The number of inseminations per cycle was mostly two and the range was one to three. There were 56 pregnancies, giving an overall pregnancy rate per cycle of 8.8%; 32 with the standard injection method (9.8% per cycle) and 24 with the reservoir (7.8%). The difference was not significant by the χ² test. Comparison of the women who conceived after insemination with either method and those who failed to conceive revealed no significant differences in their mean age, duration of infertility, proportion nulligravid, cycle length or the concentrations of follicular phase LH or FSH or mid-luteal progesterone; nor in the distribution of causes of their partners’ infertility.

The cumulative conception rates for the first five cycles are
Figure 3. Cumulative conception rates by intracervical insemination of 0.5 ml cryopreserved donor semen using a cervical cap and reservoir compared with a standard injection technique. The total cycles of treatment appear on the horizontal axis and because treatment alternated from cycle to cycle the group of women used to calculate the conception rates also alternates from cycle to cycle. Vertical bars show standard errors.

Discussion
The results of this randomized controlled study of intracervical insemination of cryopreserved donor semen in unstimulated cycles shows no improvement in the chance of pregnancy using a cervical cap combined with an intracervical reservoir holding 0.5 ml semen, compared with a standard method to simply inject a similar volume. The average clinical pregnancy rate per cycle was 8.8% and the six-cycle cumulative rate was 37%.

It has been suggested that improvement in fecundability by donor insemination can be achieved simply by increasing the volume and therefore number of spermatozoa inseminated (Behrman, 1979; David et al., 1980; Shapiro, 1991; Marshburn et al., 1992). Our own randomized controlled study of low volumes showed no benefit of 0.5 ml compared with 0.25 ml (Corrigan et al., 1994), possibly due to wastage in the vagina. The only effective way of increasing the numbers of spermatozoa delivered may be by separation and concentration in culture medium for intrauterine insemination. Cryopreserved semen appears less fertile than fresh semen in vivo (Richter et al., 1984) but fertilization in vitro and implantation rates appear similar to fresh spermatozoa (Mahadevan et al., 1982; Williams et al., 1992). Cryopreserved spermatozoa exhibit both a reduced capacity to penetrate cervical mucus (Keel et al., 1987) and reduced motility characteristics in vitro (McLaughlin et al., 1992; Marshburn et al., 1992) which may account for this discrepancy.

Other authors have attempted to improve donor insemination conception rates by closely controlling the timing of insemination by the LH surge, detected by urinary (Robinson et al., 1992) or serum (Brook et al., 1994) testing, or by vaginal ovarian ultrasound scanning (Irons and Singh, 1994) but neither method gave conception rates greater than those achieved by Fédération CECOS of France using cervical mucus scoring and temperature charting (Le Lannou, 1987; Fédération CECOS et al., 1989). Our own experience using urinary LH testing to time inseminations showed no improvement in conception rates (McLaughlin and Ford, unpublished observations). Comparative studies of urinary or serum LH testing have shown no better pregnancy rates than by mucus testing or prediction from previous temperature patterns (Brook et al., 1994).

In one recent retrospective study (Ahmed Ebbiary et al., 1994) significantly higher pregnancy rates occurred with increasing number of intracervical inseminations. Whether this was due to more effective timing of one of the inseminations, which may differ between individuals, or to increase in the total number of effectively delivered spermatozoa, remains speculative. An increased chance of pregnancy was also associated with a higher Insler cervical mucus score and a larger follicle diameter at the time of first insemination (Ahmed Ebbiary et al., 1994). This confirms that intracervical insemination with cryopreserved semen is most successful when performed before ovulation (Fédération CECOS et al., 1993).

We encourage patients to attend for two inseminations on consecutive days just prior to the expected day of ovulation. Historically, conception rates per cycle of 9-10% have been achieved with this protocol using either 0.25 ml or 0.5 ml semen (Corrigan et al., 1994). Because we felt that the failure of the larger volume of semen may have been due to wastage in the vagina, we were initially attracted by the design of the new cap with its intracervical reservoir. On reflection, the failure of this cap may have been due to the narrow reservoir allowing only a small area of semen–mucus contact (see Figures 1 and 2). It is possible that a modified reservoir might be more successful and this is an attractive possibility in view of the costs incurred by more advanced reproductive technologies which employ large volumes of valuable donor semen and often involve ovarian stimulation as with IUI, IVF or gamete intrafallopian transfer (GIFT) (Khalifa et al., 1995; Williams et al., 1995).

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References


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