Pregnancies after activated oocyte transfer: a new option for infertility treatment

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Introduction

The development of in-vitro fertilization (IVF) procedures have revolutionized the treatment of infertility (Steptoe and Edwards, 1978). Consistent fertilization and pregnancy rates can now be obtained in couples with a wide spectrum of reproductive disorders. However, the treatment of infertility remains stressful and time consuming for the patient. A typical IVF treatment cycle requires repeated visits to the centre for follicular monitoring, hormone therapy and finally oocyte retrieval and embryo transfer. Furthermore, IVF centres are usually located in city centres, causing considerable disadvantages to patients living in rural areas.

Same-day low technology procedures such as intrauterine insemination (IUI) are advantageous, but offer low pregnancy rates with severe or moderate oligozoospermic patients (see Hughes, 1997). Several years ago a higher technology procedure called transcervical oocyte and sperm transfer was reported where same day transfer of oocytes and spermatozoa to the uterus resulted in pregnancy (Veersema et al., 1989). Again this latter procedure is not suitable for oligozoospermic patients. In this report we describe a new same-day procedure, called activated oocyte transfer (AOT), where oocytes are transferred to the uterus 1 h following intracytoplasmatic sperm injection (ICSI).

Materials and methods

Patients

Following informed consent, a total of 19 patients with the following characteristics was included in this preliminary trial: previously failed IUI or IVF attempt, male factor infertility, <40 years of age and normo-ovulatory.

Ovarian stimulation, oocyte retrieval and preparation

An ultrashort protocol was used with gonadotrophin releasing hormone (GnRH) analogue and a step-down ovarian stimulation regime with 150 IU/l urinary human follicle stimulating hormone (FSH; Metrodin®; Serono, Geneva, Switzerland) stepped down to 75 IU/l FSH. Transvaginal oocyte retrieval was performed 36 h after the administration of 10 000 IU human chorionic gonadotrophin (HCG) when ultrasound imaging indicated the development of at least two leading follicles of 18 mm mean diameter. Oocyte retrieval was performed in an office setting with local anaesthesia.

Oocytes were washed in pre-equilibrated in-vitro fertilization medium (IVF; Medicult, Copenhagen, Denmark), and then incubated for 2 h at 37°C and 5% CO2 in medium drops under oil before further treatment. Cumulus complexes were then removed from oocytes with 80 IU hyaluronidase (Medicult) and metaphase II oocytes selected for injection.

Sperm preparation

Semen samples were classified using World Health Organization criteria (WHO, 1992) and morphology was assessed according to (Kruger et al., 1987). Semen samples were collected immediately after oocyte retrieval. The sample was treated using standard swim-up techniques. Motile spermatozoa for injection were isolated following 30 min of incubation.

Injection and activated oocyte transfer

Following micro-injection, oocytes were incubated at 37°C and 5% CO2 for 1 h. The number of oocytes selected for transfer to the uterus was based on the assumption that between 50–80% would be successfully activated and 25–50% of these develop into potentially viable embryos (Van Steirteghem et al., 1993). Oocytes were transferred in IVF medium using Edwards–Wallace catheters (SIMS Portex, Hythe, UK). Supernumerary injected oocytes were left in culture and checked 16–18 h later for fertilization, and resulting zygotes were cryopreserved. The luteal phase was supported with progesterone supplementation (50 mg i.m./day) starting the day after HCG administration. Implantation was confirmed 14–16 days after oocyte transfer by the assessment of plasma βHCG. Clinical pregnancies confirmed only by ultrasound indicating gestational sac and fetal heartbeat were considered for analysis.
Clinical outcome of activated oocyte transfer

| No. patients included in study | 19 |
| Age (mean ± SD, years)         | 32.2 ± 6.4 |
| No. FSH ampoules               | 16.0 ± 7.5 |
| Age (mean ± SD)                | 5.3 ± 4.8 |
| No. metaphase II oocytes injected (mean ± SD) | 76 (4.0 ± 2.6) |
| No. activated oocytes transferred (mean ± SD) | 64 (3.6 ± 1.5) |
| No. patients transferred       | 18 |
| Clinical pregnancy rate (%)    | 5/18 (28) |
| Implantation rate (%)          | 7/64 (11) |

*Range 1–5. FSH = follicle stimulating hormone.

Results

Nineteen couples were included in this preliminary study. The mean age of females selected for AOT was 32.2 ± 6.4 years of age. A total of 101 oocytes was exposed to hyaluronidase, of which 75% were at the metaphase II stage. Following injection, 64 activated oocytes were selected for transfer. The number of oocytes transferred to the uterus ranged from 1–5 with a mean of 3.6 ± 1.5. In the 18 patients undergoing transfer, five ongoing pregnancies were obtained. The implantation rate per activated oocyte transferred was 11%. Pregnancies were obtained in all spectra of male factor infertility, from moderate to severe oligoasthenoteratozoospermic males. The results are shown in Table I.

Discussion

In this short communication, we describe the successful use of activated oocyte transfer using a combination of oocyte retrieval, ICSI and the transfer of activated oocytes in a 1 day, out-patient procedure. This technique, assuming a fertilization rate of 80% in ICSI with ejaculated spermatozoa (Van Steirteghem et al., 1993), has the advantage over the previous 1 day technique, transcervical oocyte and sperm transfer (Veersema et al., 1989), in that it may be used with oligozoospermic patients and allows an estimation, albeit rough, of the number of potentially viable embryos transferred to the uterus. Previous authors have applied this criterion in transferring zygotes to the uterus with considerable success (Scott and Smith, 1998).

It is not possible to determine human oocyte or embryo viability. Overall, 50–75% will arrest before the blastocyst stage. However, batches of embryos vary from patient to patient, with some having a high percentage of viable embryos, others a lower percentage. Extended culture from day 2 to day 3 improved implantation rates (Dawson et al., 1995), and more recently the use of sequential media and day 5 transfers has improved this even further (Jones et al., 1998). Blastocyst culture is a method of selecting viable embryos and therefore reducing the risk of multiple pregnancies; however, considering the advantages of AOT or zygote transfer, efforts should be made to identify cytoplasmic markers of viability.

It is feasible that the lower implantation rate of 11% per activated oocyte, compared to 25% per blastocyst (Choteau et al., 1998), is in part due to the oocyte’s incapacity to regulate the acid conditions (Dale et al., 1998) encountered in the uterus, or simply that at least 50% of early embryos are destined to block in development (Gardner and Schoolcraft, 1998). This lower implantation rate necessitates the transfer of 3–5 activated oocytes to achieve the same pregnancy rate achieved when transferring two blastocysts (Ménézo et al., 1998). Of the five ongoing clinical pregnancies, two are twin pregnancies, giving a multiple pregnancy risk of 40%. It is interesting that the full range of developmental events, from release of intracellular calcium through syngamy to early cleavage, is able to proceed in the potentially hostile uterine cavity as shown in other species (Marston et al., 1977).

The new technique yields a pregnancy rate of 28%, comparable to that in our laboratory over the same time period with analogous patients after standard ICSI and culture of embryos (33%). This new procedure may be highly advantageous for some groups of patients. AOT could emerge as the treatment of choice for patients with moderate to severe male factor infertility requiring a cost-effective, low stress approach to assisted reproductive transfer.

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References


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