

Quadruplet pregnancy following transfer of two embryos: Case report

David J.Cahill^{1,4}, Julian M.Jenkins¹, Peter W.Soothill¹, Andrew Whitelaw² and Peter G.Wardle³

University of Bristol Divisions of ¹Obstetrics and Gynaecology, St Michael's Hospital, Bristol BS2 8EG, ²Child Health, Southmead Hospital, Bristol BS10 5NB and ³The Cotswold Centre, Southmead Hospital, Bristol BS10 5NB, UK

⁴To whom correspondence should be addressed at: Level D, University of Bristol Division of Obstetrics and Gynaecology, St Michael's Hospital, Bristol BS2 8EG, UK. Email: d.j.cahill@bris.ac.uk

Following a long period of secondary unexplained infertility, a couple had assisted conception treatment with IVF and transfer of two embryos 2 days later. The woman conceived and was found to have a quadruplet pregnancy. Following spontaneous labour onset at 34 weeks gestation, she was delivered by immediate Caesarean section. All four babies (three boys, one girl) were eventually discharged home with the mother. All the boys were found to be of differing zygosity. The quadruplet pregnancy was attributed to natural conception following intercourse and fertilization of oocytes not collected at the time of IVF. The wisdom of intercourse during a cycle of IVF is discussed.

Key words: chorionicity/quadruplet pregnancy/zygosity

Introduction

While success at assisted conception was initially considered solely in terms of pregnancy rates, currently the quality of the outcome is also gaining importance. Achieving a singleton or at most a twin pregnancy provides the best chance of a 'take-home' baby. Considerable efforts are exerted to reduce the likelihood of a multiple pregnancy. Here we report a case where, despite the efforts of the clinicians involved in direct care, a woman still achieved a quadruplet pregnancy.

Case report

A 32 year woman and her husband presented for infertility treatment with a 6 year history of secondary unexplained infertility. She had previously conceived without difficulty and delivered at term. Investigations carried out previously included a laparoscopy that was normal, sperm function tests that were all satisfactory, gonadotrophin levels that were all within normal limits and there was evidence of regular ovulation. In view of the prolonged duration of secondary infertility, it was agreed that the couple would undergo IVF and this was planned. As the couple had already had one previous successful pregnancy and delivery, they agreed to have just two embryos replaced at transfer.

Ovarian stimulation was undertaken using down-regulation with a long protocol luteal phase GnRH agonist (Buserelin; Shire, Basingstoke, Hants, UK), gonadotrophin stimulation, and, following menstruation, a recombinant FSH product (Gonal F; Serono, Feltham, Middlesex, UK). Final oocyte maturation was undertaken with hCG (Profasi; Serono). Two

days later, a vaginal ultrasound-guided oocyte collection was undertaken. At oocyte recovery, of the five mature follicles (≥ 17 mm) on the right, oocytes were collected from two follicles. From the six mature follicles on the left, six oocytes were collected. The embryologists evaluated all eight oocytes as being mature based on the appearance of the surrounding cumulus oophorus and all were inseminated. After 19 h, they were evaluated. Of the eight oocytes, five showed pronucleate development. The remaining three were still unfertilized. These were discarded. The five pronucleate oocytes were maintained in culture. They were further evaluated the following day, 44 h after oocyte recovery. Of the five, two had developed to the 4-cell stage, two to the 2-cell stage, and one was a 6-cell embryo. The two 4-cell embryos were considered most appropriate for embryo transfer and this was undertaken later that day at 49 h post-oocyte recovery. Embryo transfer was undertaken without difficulty using a one piece Frydman catheter (Rochford Medical, Oxford, UK). The remaining three embryos were further evaluated on day 3 at 69 h. As none of them had achieved the criteria for cryopreservation, they were all discarded.

Three weeks later, the patient reported a positive pregnancy test and at 5 weeks and 2 days from oocyte recovery, an ultrasound was performed. At this initial ultrasound scan, a multiple pregnancy was diagnosed and two intrauterine sacs, with fetuses each measuring 11 mm were seen (appropriate for the equivalent menstrual age). A normal heartbeat was noted in both fetuses. One week later, she presented to the local early pregnancy clinic with vaginal bleeding. Here, the pregnancy was noted to be ongoing, but four intrauterine gestational sacs

were noted, each with a fetus, showing a normal heart rate and each appropriately grown for the gestational age. Not surprisingly, the woman was shocked and surprised by this news and she was referred back to the treating IVF unit for further discussion and counselling. This counselling took the form of an outline of the risks of high order multiple pregnancy, the possible treatment options open to her at that time including selective reduction, the supervision and management of the pregnancy should she choose to continue and the likely options for, and the gestational age at which, delivery might occur.

At 11 weeks, she was seen in the fetal medicine unit when the crown–rump length measurements were 30, 25, 24 and 35 mm respectively. The nuchal translucency measurement were 0.8, 0.8, 0.7 and 1 mm respectively and so were all low risk. Chorionicity was examined by looking at the intersac membranes and they appeared to be quatro-chorionic. After extensive counselling about the risks of a quadruplet pregnancy, given the encouraging nuchal translucency and chorionicity results, the woman and her husband chose to continue with the pregnancy without reduction.

At 34 weeks gestation the woman had a transvaginal scan of her cervix which showed a shortened cervical length and a dilated internal cervical os. Delivery was advised and undertaken by semi-elective Caesarean section the following day. At this, she was delivered of four healthy infants, three boys and one girl. Their weights were 2052, 2258, 1144 and 1660 g respectively. The neonatal period was complicated by mild to moderate respiratory distress syndrome in all babies, moderate in the third boy and the girl. In addition, she subsequently required mechanical ventilation because of necrotizing enterocolitis (which did not require surgery) and a patent ductus arteriosus (which did require surgical ligation). In total, they stayed for 154 days in the neonatal intensive care unit.

Because of the clear indication that these quadruplets did not arise from a monozygotic twinning of the embryos replaced, blood was taken from the parents and from the three boys for zygosity testing. Using 11 different probes, examination of parental allele combinations on chromosomes 4, 7 and 19 showed that the three boys originated from independent zygotes. Permission was sought and obtained from the couple to publish this report.

Discussion

The need to explain these four infants of different zygosity remained. The laboratory worksheets from the IVF laboratory clearly detailed the fate of all the oocytes and subsequent developing embryos. It seemed quite clear that there was no possibility that these four infants of different zygosity arose from transfer of more than two embryos. The parents were taken through these laboratory worksheets and this was fully explained to them. In further discussion, it transpired that the couple had had intercourse 2 days prior to hCG injection and 4 days prior to oocyte recovery. As fewer oocytes were collected than expected from the follicles seen by ultrasound on the day of hCG, an hypothesis is proposed: that the additional embryos resulted from natural fertilization of the oocytes which were not collected at the time of oocyte recovery. Given the level of

security expressed in the laboratory sheets, this hypothesis seems the most likely explanation.

The presence of two fetuses and sacs at the first scan and four at the next deserves some comment. The first scan was undertaken 5 weeks and 2 days post-oocyte recovery. Not all the sacs were seen but all were sufficiently grown a week later to be seen. At 11 weeks, the crown–rump lengths were in two distinct groups, but the possibility of spontaneous conception was still not seriously considered. One explanation for the difference in size was that the two putative fetuses which had undergone monozygotic twinning were of different sizes. In retrospect, it is easier to see that the difference in size was related to the difference of several days gestation between the two sets of ‘twins’, one from spontaneous and one from assisted conception.

Despite the intentions of the IVF unit to provide this couple with a minimal risk of a multiple pregnancy, they ended up with a quadruplet pregnancy. It appears that this multiple pregnancy came about because of the natural fertilization of oocytes left behind at oocyte recovery by sperm that were introduced 4 days prior to oocyte collection. The practice of a couple having intercourse during a treatment cycle was not discouraged by the IVF unit at the time and is advocated by some as being safe (Tremellen and Norman, 2001) and conferring an improved implantation rate (Tremellen *et al.*, 2000). However, and this is the main message of this case report, such a practice carries a risk of multiple pregnancy in a couple with patent Fallopian tubes and normal sperm function. There is a well established risk of 1–2% of conception occurring in an IVF cycle prior to oocyte collection in association with the use of GnRH agonists (Cahill *et al.*, 1994; Cahill, 1998). However, reports of spontaneous pregnancies occurring following oocyte collection are rarely reported. Most twin and triplet pregnancies are assumed to result from the transfer of two or three embryos. When two embryos are replaced the normal likelihood of a multiple pregnancy should be far lower (0.5% triplets and 10% twins; unpublished data). When a twin pregnancy occurs after a transfer of two embryos, this is assumed to result from the implantation of both embryos, though embryo studies may refute this. When triplets or higher order pregnancy occurs after the transfer of two embryos, monozygotic twinning is assumed to have occurred in at least one of the embryos replaced. In one published case, a quadruplet pregnancy (and delivery) resulted from a two embryo transfer. In that case, it appears that monozygotic twinning occurred in one transferred embryo, a third gestation sac resulted from a second transferred embryo and a fourth sac arose from the fertilization of a residual oocyte (not collected at oocyte recovery) (Milki *et al.*, 2001). In a second published case, quintuplets resulted from ‘monopaternal superfecundation’ following the transfer of two embryos with subsequent selective reduction to two fetuses at 12 weeks (Amsalem *et al.*, 2001). Despite the outcome, Amsalem *et al.* advised that couples should ‘have intercourse after retrieval to increase their chances for a pregnancy’, although only in selected cases where the likelihood of success is suboptimal. In our report, monozygotic twinning of the two replaced embryos was ruled out by the zygosity testing of the

three male infants. Consequent to the information we gained from this case and the previous case reports, we have changed our policy in the IVF unit to advise couples not to have intercourse without contraception during their stimulation cycle. We suggest that in young couples with no sperm disorder and patent tubes, this policy would prevent further undesired multiple pregnancies following assisted reproductive technology.

References

- Amsalem, H., Tsvieli, R., Zentner, B.S., Yagel, S., Mitrani-Rosenbaum, S. and Hurwitz, A. (2001) Monopaternal superfecundation of quintuplets after transfer of two embryos in an *in vitro* fertilization cycle. *Fertil. Steril.*, **76**, 621–623.
- Cahill, D.J. (1998) Risks of gonadotrophin releasing hormone agonist administration in early pregnancy In Filicori, M. and Buletti, C. (eds) *Ovulation Induction Update 1998*. Parthenon Publishing, Camforth, UK, pp. 97–105.
- Cahill, D.J., Fountain, S.A., Fox, R., Fleming, C.F., Brinsden, P.R. and Hull, M.G. (1994) Outcome of inadvertent administration of a gonadotrophin-releasing hormone agonist (buserelin) in early pregnancy. *Hum. Reprod.*, **9**, 1243–1246.
- Milki, A.A., Hinckley, M.D., Grumet, F.C. and Chitkara, U. (2001) Concurrent IVF and spontaneous conception resulting in a quadruplet pregnancy. *Hum. Reprod.*, **16**, 2324–2326.
- Tremellen, K.P. and Norman, R.J. (2001) The effect of intercourse on pregnancy rates during assisted human reproduction. *Hum. Reprod.*, **16**, 2030–2031.
- Tremellen, K.P., Valbuena, D., Landeras, J., Ballesteros, A., Martinez, J., Mendoza, S., Norman, R.J., Robertson, S.A. and Simon, C. (2000) The effect of intercourse on pregnancy rates during assisted human reproduction. *Hum. Reprod.*, **15**, 2653–2658.

Submitted on August 12, 2002; accepted on October 30, 2002