Case report

Healthy girl born after cryopreservation of gametes and ICSI in a patient with seminoma

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Abstract

This study reports birth in a case of intracytoplasmic sperm injection with cryopreserved oocytes and spermatozoa banked after radiotherapy and prior to chemotherapy due the occurrence of two non-synchronous seminomas. A 30-year-old male with a diagnosis of seminoma cryopreserved six vials of spermatozoa. After oncological treatment was completed, his partner, a 24-year-old woman, underwent ovarian stimulation. Seventeen oocytes were retrieved; one was at the germinal vesicle stage and two were injected, resulting in two embryos. Fourteen metaphase II oocytes were frozen. The woman presented moderated ovarian hyperstimulation syndrome, and embryo cryopreservation was indicated. After endometrial preparation, two embryos were transferred and a pregnancy was achieved. The woman suffered eclampsia during week 28 of gestation. Caesarean section was performed and a preterm girl weighing 1000 g was born, but died 2 weeks after delivery. A year later, a second procedure was begun. Frozen oocytes and one vial of semen were thawed. Eight of the 14 oocytes survived and were microinjected; two became fertilized and one good quality cleaved embryo was transferred. Pregnancy was achieved and a healthy girl was born with a birth weight of 2800 g. Oocyte cryopreservation associated with sperm banking in cancer patients is a useful tool for couples seeking deferred parenthood.

Keywords: *fertility, intracytoplasmatic sperm injection, oocyte cryopreservation, seminoma, sperm banking, testicular cancer*

Introduction

A seminoma is a malignant tumour that usually affects men of reproductive age. This pathology affects sperm quality, which is also modified by chemo- and radiotherapy; both situations are a significant threat to the potential future fertility of the patient.

Advances in reproductive medicine, especially in gamete cryopreservation, allow patients to preserve the possibility of parenthood by using sperm and oocyte banking (Carson *et al*., 1991; Chen *et al*., 1996; Polak de Fried *et al*., 1998).

Sperm cryopreservation is indicated prior to radiotherapy or chemotherapy treatment. Taking into account the impairment that freezing may produce in sperm samples, even when intrauterine insemination has been proposed (Scammell *et al*., 1985; Redman *et al*., 1987), fertilization in these circumstances may be highly affected, so the thawed semen is usually combined with assisted reproductive techniques such as IVF or more commonly intracytoplasmic sperm injection (ICSI).

Oocyte freezing has the potential to be an important adjunct to assisted reproduction techniques in many indications. Recently, there has been a growing interest in oocyte banking among women who wish to postpone motherhood for professional or social reasons. Oocyte cryopreservation techniques continue to improve. New methods, including the use of choline-based media and vitrification, have proven useful in increasing the survival rate (Stachecki and Cohen, 2004), and many pregnancies have been achieved (Porcu *et al*., 1997; Polak de Fried *et al*., 1998; Tucker *et al*., 1998).

There are very few publications on the use of thawed spermatozoa from patients who have undergone chemo/radiotherapy and then proceeded to undergo assisted reproduction with cryopreserved oocytes. The objective of this study is to report the birth of a healthy baby after the application of this technique.

Case report

A 30-year-old male patient underwent a right orchidectomy and then radiotherapy because of a seminoma. Two years later, he consulted the Reproductive Medicine Department because of an ultrasonographic image in the left testicle compatible with a new seminoma.

Considering the need for more surgery and coadjuvant chemotherapy treatment, semen samples were cryopreserved. The semen count ranged from 1.8 to 6 × 10^6/ml, with a general motility of 50%. The semen was diluted (1:1) with freezing medium test yolk buffer (Irvine Scientific, Santa Ana, CA, USA), cooled and stored in liquid nitrogen at –196°C.

The patient's partner was a 24-year-old woman in good health, normogonadotrophic with regular menses, and displaying a normal infertility work-up, with no history of gynaecological pathology.

Once the male partner's treatment was finished, an ICSI procedure with his frozen spermatozoa was programmed. Ovarian stimulation was carried out as usual (Polak de Fried *et al*., 1993) with gonadotrophin-releasing hormone antagonist (GnRHa; Lupron 2.8 ml; Abbot, Buenos Aires, Argentina) in a long protocol to achieve desensitization, with rFSH (200 IU/day; Puregon; Organon, Buenos Aires, Argentina) for 5 days followed by rFSH 100 IU/day until human chorionic gonadotrophin (HCG) criteria were achieved. Seventeen oocytes were retrieved by vaginal ultrasound-guided follicle aspiration on day 13. Taking into account that the couple had decided to cryopreserve oocytes instead of embryos due to ethical concerns, only two oocytes were inseminated, and all the remaining oocytes were frozen on the day of oocyte recovery.

Of the 17 oocytes, one was at the germinal vesicle stage. Sixteen oocytes were denuded, exposing them for 30 s to a culture medium containing 80 IU/ml of hyaluronidase type VIII (Sigma H-3757; Sigma-Chemical Co., St Louis, MO, USA). Fourteen oocytes were cryopreserved using the 1,2-propanediol slow freeze–rapid thaw method (Polak de Fried *et al*., 1998), following pre-equilibration of the oocytes for 10 min in 1.5 mol/l 1,2-propanediol and transfer to 1.5 mol/l 1,2-propanediol containing 0.1 mol/l sucrose. The oocytes were immediately loaded into plastic straws and placed in a Planer Kryo 10, series II biological freezer (Planer Biomed, Sudbury, Middlesex, UK). They were then cooled from 16°C to –8°C. Ice nucleation was induced manually by seeding each straw with precooled forceps. Gradual temperature reduction was continued to –30°C followed by rapid reduction to –150°C. Oocytes were stored in liquid nitrogen at –196°C. The remaining two oocytes were inseminated using ICSI and two embryos were obtained. These embryos were also cryopreserved 48 h after oocyte recovery using the same technique because the patient showed moderate ovarian hyperstimulation syndrome (OHSS). Thus, even though it had not been the initial intention, deferred embryo transfer was indicated.

Two months later, the woman underwent pituitary desensitization with leuprolide acetate and endometrial stimulation with 17β-oestradiol until a suitable endometrial thickness was achieved and the embryos were thawed. Embryo transfer was performed with a soft Wallace catheter (Cooper Surgical, Trumbull, Connecticut, USA) and a single pregnancy was achieved. At week 28 of gestation the patient suffered from eclampsia, and Caesarean section was performed; a female of 1000 g was born, but died 2 weeks after the delivery.

One and a half years later, the patient was placed under hormonal replacement therapy, following the same protocol as in the previous embryo transfer. Fourteen oocytes were thawed. The straws were removed from liquid nitrogen, maintained at room temperature for 30 s, and transferred into a 30°C water bath for 40 s. The cryoprotectant was removed by transferring the oocytes through decreasing concentrations of propanediol solution (1.5–0.5 mol/l in each) containing 0.2 mol/l sucrose, followed by a final step dilution of 0.2 mol/l sucrose alone. Oocytes were then transferred into culture medium HTF plus 15% serum substitute supplement (Irvine Scientific).

After 1 h incubation, eight survived (survival rate: 57%). A semen vial was immediately thawed at room temperature; the post-thaw count of this vial was lower, 0.5 × 10^6/ml, the motility was 50% and ICSI was performed as usual. Two eggs were fertilized (fertilization rate: 25%) and one 8-cell embryo was transferred on day 3. Serum β-HCG 14 days after the transfer was 135 mIU/ml.

Pregnancy was achieved and delivery was by Caesarean surgery at 36 weeks of gestation; a healthy girl was born with a birth weight of 2800 g.

Discussion

There are many studies published in the literature reporting the use of cryopreserved semen and/or oocytes that had been frozen, for different reasons, in assisted reproduction procedures that led to normal pregnancies and healthy babies being born (e.g. Porcu *et al*., 1997, 2001).

In 1996, Chen *et al*. reported the case of a man with a diagnosis of seminoma in the right testicle; a semen sample was cryopreserved prior to orchidectomy and radiotherapy. Intrauterine inseminations were performed and after three failed cycles with no pregnancy achieved, ICSI was indicated. This case led to a viable pregnancy after the same diagnosis of the man as in the present case, but the thawed spermatozoa were injected into a fresh, rather than a cryopreserved oocyte (Chen *et al*., 1996).

Chia *et al*. (2000) reported a case of a triploid pregnancy using frozen testicular spermatozoa from a man with obstructive azoospermia and thawed oocytes that were cryopreserved 12 h after retrieval because of insufficient number of motile spermatozoa. This procedure led to an abnormal chromosomal pregnancy that ended in a spontaneous abortion after week 9.

The authors suggested many explanations for the triploidy, such as the fertilization of a digynic egg, the ageing of the eggs, the cryopreservation technique and suboptimal oocyte quality following ICSI with human oocytes; they put more emphasis on the abnormality of the male patient gamete quality (Chia *et al*., 2000).

Four births of healthy babies from cryopreserved oocytes fertilized by cryopreserved spermatozoa were reported by Porcu *et al*. in cases where oocytes were frozen because the men were unable to produce a semen sample. In these cases, the semen samples had normal parameters and there was no male pathology (Porcu *et al*., 2002).

In the present report, the semen was cryopreserved due to a neoplasic pathology with high risk of the loss of future fertility. The decision to freeze the oocytes arose from ethical concerns, as previously reported by Borini *et al*., describing their experience with patients with specific demands (Borini *et al*., 2003). The couple had no intention of freezing embryos and preferred to cryopreserve oocytes. However, 24 h after fertilization the patient developed OHSS, and the embryos had to be frozen.

In this case, the expected oocyte post-thaw survival rate was achieved. Embryo quality was good and a normal pregnancy resulted in the birth of a healthy female baby, who has so far shown normal psychometric and neurological development.

Oocyte freezing is a useful tool when there is no intention of storing supernumerary embryos. This procedure offers a solution because gametes can be used or not, so no legal or ethical conflicts arise, especially in cases when the woman is young and has a good chance of pregnancy.

Oocyte cryopreservation still requires further work. Ethical concerns about embryo freezing and the disposal of surplus embryos can be greatly reduced by oocyte freezing.

The feasibility of the oocyte cryopreservation method will provide an option to those women who postpone maternity for social and professional reasons as well as patients who are scheduled to undergo radio/chemotherapy.

This technique combined with banked spermatozoa from a cancer patient is an alternative procedure for couples in need of deferred parenthood.

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Paper based on contribution presented at the 59th Annual Meeting of the American Society for Reproductive Medicine, San Antonio, Texas, USA, 11–15 October 2003.

Received 27 July 2004; refereed 10 August 2004; accepted 10 September 2004.