CASE REPORT

Ongoing twin pregnancy after transfer of vitrified oocyte injected with sperm recovered from cryopreserved testicular tissue

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ABSTRACT

Cryopreservation of human gametes and embryos has become an essential part of assisted reproduction. It is now possible to cryopreserve gametes and embryos at their different stages of development. It allows patients undergoing chemotherapy or radiotherapy to preserve their fertility, and helps to attain all benefits from the costly ovarian superovulation therapies prior to assisted reproductive techniques (ART). Owing to the extremely high survival and pregnancy rates, vitrification is now being widely used in ART laboratories in addition to its low cost and simplicity as an optimal cryopreservation procedure for human oocytes and embryos. Here, we would like to report an ongoing twin pregnancy after Vitrification/warming of oocytes which were injected by ICSI with patient’s husband spermatozoa retrieved from cryopreserved testicular tissue and transfer of day three cleavage stage embryos was.

Key words: Vitrification; oocytes; embryos.

INTRODUCTION

Cryopreservation of human oocytes would potentially benefit young women affected by cancer who must undergo fertility threatening chemotherapy or radiation. Moreover the ability to store oocytes successfully could overcome legal and ethical problems concerning the cryopreservation of the embryos. Human oocytes cryopreservation by using the traditional slow-freezing method has been performed; however, the results are still variable and not sufficiently successful to justify routine use (1-3). Vitrification is a novel method of human embryos and gametes cryopreservation and being now widely used in ART laboratories. Kuleshova et al. (1999) reported the first human pregnancy achieved from vitrified-thawed human oocytes. (4) After that, the number of reports concerning pregnancies and deliveries following vitrification has steadily increased.(5,6)

CASE REPORT

An infertile couple, 29 years-old female and 34 years-old male partner with severe oligoatheratozoospermia presented at the outpatient ART clinic of university of Lübeck,
department of gynecology and obstetrics since February 2007. The couple was thoroughly cancelled for in-vitro fertilization program. Ovulation induction was performed by administration of gonadotrophin-releasing hormone (GnRH) antagonist (Cetrotide, Serono, Germany) together with gonadotrophins (Gonal F, Serono; Menopur, Ferring, Germany). Ovulation was triggered by the i.m. administration of human chorionic gonadotrophin (HCG) (10,000 IU/ml, Choragon, Ferring) as soon as three follicles of a diameter of ≥17 mm were observed by transvaginal ultrasonography and with estradiol concentrations corresponding to the number of follicles. Oocyte retrieval was performed 35–36 h after triggering under ultrasonographic guidance. Twenty two retrieved oocytes were prepared for ICSI. Unfortunately, no sperms were found in two consecutive ejaculates of the male partner, in addition to it was a weekend, for this reason the testicular biopsy was not possible.

So we counseled the couple to perform vitrification of the oocytes and to refer the husband to the urology department later to perform a testicular biopsy in order to identify the cause of azoospermia. After having the consent, 19 Metaphase II oocytes were submitted to vitrification. The husband underwent testicular biopsy few days later. Testicular tissue was obtained under local anesthesia using an open multiple-biopsy technique (one cranial and one caudal tissue section in each testis). From each part a sample was fixed for the histopathological examination. Testicular specimens in Ham’s F10 medium were examined where the presence of spermatozoa was confirmed, and were subsequently frozen in fractions as previously described (7, 8), and stored until the time of ICSI.

One month later, the patient was subjected to a programmed cycle for embryo transfer. The endometrium prepared with the induction of proliferative phase by incremental dose of E2 (Progynova 1-6mg/day/Germany weeks, Schering, Germany) and vaginal progesterone (Crinone gel, 7.5 mg/ml, Serono, Germany) that was added on the same day and 48h before embryo transfer. Thereafter 6 mg of E 2 and vaginal progesterone were maintained until pregnancy test. Four oocytes were thawed on 15th day of the cycle; all of them survived and were subjected to ICSI 2h after warming. Sixteen hours later, two oocytes were fertilized. On day 3, two twelve-cell stage embryos with grade I morphology were transferred after 72 h culture in cleavage stage medium (Ham’s F-10 + 20% serum). Unfortunately according to the hormonal profile on day 12 after embryo transfer, the patient was not pregnant. Therefore a subsequent programmed cycle was prepared. So, another four vitrified oocytes were warmed, and all of them survived, and three oocytes were fertilized and one was abnormal. On day 2, two four–cell stage embryos with grade I morphology were transferred.

One month later, the patient was subjected to a programmed cycle for embryo transfer. The endometrium prepared with the induction of proliferative phase by incremental dose of E2 (Progynova 1-6mg/day/Germany weeks, Schering, Germany) and vaginal progesterone (Crinone gel, 7.5 mg/ml, Serono, Germany) that was added on the same day and 48h before embryo transfer. Thereafter 6 mg of E 2 and vaginal progesterone were maintained until pregnancy test. Four oocytes were thawed on 15th day of the cycle; all of them survived and were subjected to ICSI 2h after warming. Sixteen hours later, two oocytes were fertilized. On day 3, two twelve-cell stage embryos with grade I morphology were transferred after 72 h culture in cleavage stage medium (Ham’s F-10 + 20% serum). Unfortunately according to the hormonal profile on day 12 after embryo transfer, the patient was not pregnant. Therefore a subsequent programmed cycle was prepared. So, another four vitrified oocytes were warmed, and all of them survived, and three oocytes were fertilized and one was abnormal. On day 2, two four–cell stage embryos with grade I morphology were transferred.

Protocol for vitrification and thawing procedures

The vitrification/warming protocol was performed according to the method described previously (Kuwayama et al., 2005a, Al-Hasani et al 2007) (9, 10). The oocytes were incubated in equilibration solution comprising 7.5% ethylene glycol (EG) (Sigma-Aldrich, Steinheim, Germany) and 7.5% dimethyl sulphoxide (DMSO) (Sigma-Aldrich) in Ham’s F-10 media supplemented with 20% patient serum for 15 min at room temperature. After an initial shrinkage and recovery, they were then aspirated and placed into the vitrification solution (15% EG, 15% DMSO, 0.5 M sucrose) (Merck, Darmstadt, Germany) in Ham’s F-10 medium supplemented with 20% patient serum for 50–60 s at room temperature. After having observed cellular shrinkage, oocytes were aspirated and placed into the vitrification solution (15% EG, 15% DMSO, 0.5 M sucrose) (Merck, Darmstadt, Germany) in Ham’s F-10 medium supplemented with 20% patient serum for 50–60 s at room temperature. After having observed cellular shrinkage, oocytes were aspirated and placed on the tip of the Cryotop (Kitazato, Japan). No more than two oocytes were placed on each Cryotop. Cooling of the oocytes was done by direct contact with liquid nitrogen. The Cryotops were stored in liquid nitrogen. Warming of oocytes was performed by placing the Cryotop in thawing solution (1 M sucrose) for 50–60 s at room temperature and then into dilution solution (0.5 M sucrose) for 3 min, followed by another dilution solution of 0.25 M sucrose for 3 min, both at room temperature. The warmed oocytes were placed 4–5 times into washing solution (Ham’s F-10 + 20% sucrose) for 3–4 min, followed by another washing solution for 3 min. After washing the oocytes were transferred into culture medium (Ham’s F-10) for 2 h before ICSI.
serum) before incubation. The intact oocytes were cultured in Ham’s F-10. The ICSI procedure was performed 2 h later for the survived oocytes. The embryo quality was scored according to Steer et al. (1992) (11).

CONCLUSION

Storage of female gametes might offer potential benefits, such as: (i) maintenance of fertility options for young women suffering from pathological entities of the reproductive system (premature ovarian failure, endometriosis), for those who wish to delay their reproductive choices or for those about to undergo anticancer therapy (chemotherapy, radiation therapy); (ii) increased flexibility of assisted reproduction programmes in patients whose initial treatment cycle has to be halted because of events such as hyperstimulation or inability of the partner to produce viable spermatooza as in this case report; and (iii) formation of donor ‘egg banks’ to facilitate and lessen the cost of oocyte donation (12,13).

To date, although numerous studies have been conducted utilizing different slow-cooling procedures, results still remain contradictory: These data suggest that the best slow freezing procedure has not yet been established. On the other hand, the results obtained by vitrification with mixtures of Cryoprotectants achieved a 100% survival rate, 93% fertilization rate, 96% cleavage rate. (6, 9) In conclusion, recent results provide optimism for the use of oocyte freezing particularly vitrification as a routine in IVF/ICSI protocols.

To our best knowledge, this is the first case report using vitrified oocyte injected with cryopreserved testicular sperm.

REFERENCES


Received on September 12, 2007; revised and accepted on October 24, 2007