Optimizing cryopreservation of human spermatogonial stem cells: comparing the effectiveness of testicular tissue and single cell suspension cryopreservation

Pamela Yango, B.S.,[a] Eran Altman, M.D.,[a,b] James F. Smith, M.D.,[a,c] Peter C. Klatsky, M.D., M.P.H.,[d] and Nam D. Tran, M.D., Ph.D.[a]

[a] Department of Obstetrics, Gynecology and Reproductive Sciences and [c] Department of Urology, University of California, San Francisco, San Francisco, California; [b] Helen Schneider Hospital for Women, Rabin Medical Center, Petah-Tiqva, Israel; and [d] Department of Obstetrics and Gynecology, Albert Einstein University, Bronx, New York

Objective: To determine whether optimal human spermatogonial stem cell (SSC) cryopreservation is best achieved with testicular tissue or single cell suspension cryopreservation. This study compares the effectiveness between these two approaches by using testicular SSEA-4+ cells, a known population containing SSCs.
Design: In vitro human testicular tissues.
Setting: Academic research unit.
Patient(s): Adult testicular tissues (n = 4) collected from subjects with normal spermatogenesis and normal fetal testicular tissues (n = 3).
Intervention(s): Testicular tissue versus single cell suspension cryopreservation.
Main Outcome Measure(s): Cell viability, total cell recovery per milligram of tissue, as well as viable and SSEA-4+ cell recovery.
Result(s): Single cell suspension cryopreservation yielded higher recovery of SSEA-4+ cells enriched in adult SSCs, whereas fetal SSEA-4+ cell recovery was similar between testicular tissue and single cell suspension cryopreservation.
Conclusion(s): Adult and fetal human SSEA-4+ populations exhibited differential sensitivity to cryopreservation based on whether they were cryopreserved in situ as testicular tissues or as single cells. Thus, optimal preservation of human SSCs depends on the patient's age, type of samples cryopreserved, and end points of therapeutic applications. (Fertil Steril® 2014;102:1491–8. ©2014 by American Society for Reproductive Medicine.)
Key Words: Testicular cell cryopreservation, testicular tissue cryopreservation, spermatogonial stem cells

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/yangop-cryopreservation-spermatogonial-stem-cells/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Advances in oncological medicine have provided definitive cures for many patients allowing them to live healthy productive lives. Therefore, oncologists are encouraged to refer patients to fertility specialists to discuss fertility preservation strategies before gonadotoxic cancer therapies (1). Sperm cryopreservation is an established and proven technique to restore fertility in adolescent and adult males (1). However, this approach requires the presence of mature spermatozoa, which is not possible for prepubertal boys. At present, fertility preservation for prepubertal boys is considered to be experimental because there is a significant lack of scientific knowledge with regard to optimal cryopreservation techniques, isolation of spermatogonial

Received February 19, 2014; revised July 25, 2014; accepted July 28, 2014; published online September 18, 2014.
P.Y. has nothing to disclose. E.A. has nothing to disclose. J.F.S. has nothing to disclose. P.C.K. has nothing to disclose. N.D.T. has nothing to disclose.
P.Y. and E.A. should be considered similar in author order.
Supported by American Society for Reproductive Medicine new investigator award, UCSF Rap Grant, Weston Haven Foundation (NDT).
Reprint requests: Nam D. Tran, M.D., Ph.D., Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, San Francisco, California 94143 (E-mail: trann@obgyn.ucsf.edu).

Fertility and Sterility® Vol. 102, No. 5, November 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.07.1250

stem cells (SSCs), and subsequent transplantation or in vitro differentiation (2–4). Hence, there is a lack of established standard protocol for fertility preservation for this vulnerable patient population.

The discovery of mouse SSCs and their ability to reconstitute spermatogenesis after heterotopic and orthotopic transplantations provide potential novel therapeutic applications of SSC transplantation in humans for fertility preservation and infertility treatment (5–8). Encouraging results from the murine model have garnered support from many fertility centers that view cryopreservation of prepubertal testicular tissues, presumably containing SSCs, as an acceptable strategy for fertility preservation in this patient population (9–11). Heterotopic xenografts of hamster, marmoset, and mouse testicular tissues into castrated immunodeficient nude mice resulted in limited and finite restoration of spermatogenesis (8). Specifically, heterotopic xenografts of marmoset testicular tissues did not result in successful differentiation of spermatogonia beyond the primary spermatocyte stage (8). Furthermore, autologous heterotopic transplant of fresh testicular tissues in marmoset monkeys also resulted in differentiation arrest at the primary spermatocyte stage (12). Whether cryopreserved testicular tissues exhibited similar engraftment potential to fresh testicular tissues with heterotopic transplants remained to be investigated (13, 14).

Alternatively, orthotopic SSC transplantation uses single cell suspensions. This allows SSCs to be positively selected and cancer cells eliminated by fluorescence-activated cell sorting (FACS), which can greatly ameliorate the risk of malignant cell contamination associated with testicular grafting (15–17). Positive selection of SSCs by FACS for transplantation was shown to eliminate the risks of malignant cell contamination (15, 16). Unlike heterotopic transplantation of testicular tissues, orthotopic transplantation of SSCs resulted in long-term reconstitution of spermatogenesis, capable of fertilization, in adult rhesus macaques (18, 19).

The current clinical practice of fertility preservation for prepubertal boys involves obtaining testicular tissues by testicular sperm extraction (TESE) and then subjecting tissues to a controlled slow-freezing standard protocol with either dimethyl sulfoxide (DMSO) or vitrification (9, 10, 20–27). Tissue cryopreservation preserves both options for heterotopic testicular tissue and orthotopic SSC transplantations in the future. Alternatively, testicular tissues can be enzymatically digested and cryopreserved as single cell suspensions (2, 28, 29). Although, single cell cryopreservation eliminates the possibility of heterotopic tissue transplant, it may be more effective in preserving testicular cells and SSCs specifically (30).

Previous studies have investigated the effect of different cryopreservation conditions on overall post-thawed cell survival using single cell suspensions (30). However, cell viability does not provide quantitative information on the efficiency of the total number of viable cells recovered after cryopreservation as freezing injuries often result (31). In addition, fertility preservation and resumption of spermatogenesis critically rely on the survival of SSCs and essential somatic cells after cryopreservation (32). At present, it is unclear whether human SSCs would be best preserved in situ as testicular tissues or as single cell suspensions. We and other investigators have demonstrated that cells expressing either SSEA-4 and THY-1 are enriched in adult human SSCs and somatic cells (Sertoli and stromal cells), essential for in vitro SSC expansion, respectively (11, 32, 33). Using SSEA-4 as a marker for testicular cell population enriched with human SSCs, Pacchiarotti et al. (2) reported similar post-thawed SSEA-4+cell recovery, regardless whether they were cryopreserved as testicular tissues or as single cell suspensions. However, the testicular tissues were collected from patients undergoing sexual reassignment surgery who were on extended high dose estrogen (E) therapy. Because spermatogenesis is inhibited with high dose E therapy (34), it is unknown whether those results are applicable to patients with normal spermatogenesis who are not on E therapy. Important, similar studies on human prepubertal SSC cryopreservation are not currently available mainly due to the scarcity of available tissues and the associated ethical dilemma. However, we have recently demonstrated that human fetal testicular tissues shared significant similarity in seminiferous cord morphology and primitive spermatogonia composition with prepubertal testicular tissues, thus making them viable surrogates for prepubertal tissues (11). In contrast to adult testicular tissues, fetal SSCs coexpressed SSEA-4 and THY-1 (11).

The present study uses testicular tissues collected from adult men with normal spermatogenesis to investigate the effectiveness of human SSC cryopreservation after either testicular tissue or single cell suspension cryopreservation using SSEA-4 as a marker for SSCs. In parallel, human fetal testicular tissues were also used as a surrogate for human prepubertal testicular tissues. We hypothesize that the viability fraction and number of surviving SSEA-4+cells after cryopreservation depend on the approaches of cryopreservation (tissues vs. single cells) and the age of the patients.

MATERIALS AND METHODS

Testicular Tissues

Adult human testicular tissues were collected by TESE from four subjects (34–62 years of age) with normal spermatogenesis. Each patient had normal semen parameters or prior paternity. Subjects 2, 33, and 105 underwent TESE either for failed vasovasotomy or anejaculation as part of their fertility treatments. Subject 123 underwent a spermatocelectomy (Supplemental Table 1, available online). Tissues were transported in sperm wash medium with gentamycin (Vitrolife) and processed within 2 hours. All subjects signed a written informed consent allowing use of their testicular specimens for research purposes as part of the University of California, San Francisco Lifestyle, Infertility, Fertility, and Evaluation Study (IRB approved CHR # 10-04868). Human 22–23 weeks of gestation fetal testes (n = 3) were obtained following elective termination of pregnancy with appropriate consent from subjects prior to procedure (Institutional Review Board-approved CHR 12-08704). None of the terminations were for reasons of fetal abnormality, and all fetuses appeared to be morphologically normal. Gestational age was determined by last menstrual period, confirmed with ultrasound and foot length measurement. Tissues were transported at 4°C in