a third party to obtain consent, lessens the potential for coercion. But the potential remains. And even though Freischlag, the chair of a department of surgery, notes that none of her patients object to student shadowing, surgeons have enormous influence over their patients. Might some patients be intimidated and find it hard to say no?

Even if patients consent, their response to the presence of a college student during the visit cannot be predicted. For example, what if a patient was planning to inform his physician that he is bisexual, has participated in high-risk behaviors, and now wants a test for human immunodeficiency virus? Will he hesitate to share this information because a student is present? A patient trusts the physician to provide a safe environment in which to share private concerns. What could be more important than maintaining that trust?

A social obligation to educate future physicians exists. This obligation begins when students enter medical school because, at that time, they have both formally committed to and been accepted by the profession. Until then, a line must be drawn regarding who is allowed into the examination room. In the absence of data proving otherwise, shadowing by college students crosses that line.

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Conflict of Interest Disclosures: The author has completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.


RESEARCH LETTER

In Vitro Propagation of Human Prepubertal Spermatogonial Stem Cells

To the Editor: Treatment of pediatric cancer has continuously improved over the past decades, but fertility is often compromised in survivors of childhood cancer. Fertility preservation in prepubertal boys with cancer could theoretically be achieved by cryopreserving testicular tissue before cancer treatment, and then propagating and autotransplanting spermatogonial stem cells (SSCs) from this tissue. We describe in vitro propagation of human prepubertal SSCs using a culture system for adult human SSCs.

Methods. This study was conducted from July 2009 to September 2010. Open testicular biopsies were performed on 2 boys aged 6.5 and 8 years, diagnosed with Hodgkin lym-
phoma, who were referred to the Avicenna Research Institute (Tehran, Iran) for fertility preservation. The biopsy specimens were cryopreserved in 5% dimethyl sulfoxide and 5% human serum albumin. The main part of each biopsy specimen was stored for possible future clinical use, and with written informed consent from both parents, a small part was donated for research and transferred in liquid nitrogen to the Academic Medical Centre (Amsterdam, the Netherlands). Approval for using the material for research was obtained from the ethical committee of the Avicenna Research Institute.

After rapid thawing and washing, testicular tissues were subjected to 2-step enzymatic digestion, and single cells were cultured in supplemented StemPro medium (Invitrogen, Carlsbad, California). The medium was refreshed every 3 to 4 days; cells were passaged every 7 to 10 days; and depending on the ratio of somatic vs germ cells, differential plating was applied. All visible testicular-derived, embryonic stem cell–like colonies were removed from the culture.

To determine the presence of spermatogonia during culture, the expression of spermatogonial markers was studied by reverse transcriptase–polymerase chain reaction, immunohistochemistry, or both. To confirm the presence and propagation of SSCs during culture, cells of early and later passages were transplanted into testes of busulfan-treated immunodeficient mice.

**Results.** Two and a half weeks after initiation of the testicular cell culture, the first germline stem cell (GSC) clusters appeared. Testicular cells were cultured for 20 and 15.5 weeks from the 6.5- and 8-year-old boys, respectively. GSC clusters were subcultured on laminin for a total of 29 and 20 weeks from the 6.5- and 8-year-old boys, respectively. Expression of spermatogonial markers was detected throughout the entire culture period at the RNA (FIGURE 1) and protein levels (ZBTB16 and UCHL1). Eight weeks after xenotransplantation, human SSCs were detected on the basal membrane of seminiferous tubules of recipient mouse testes (FIGURE 2). Xenotransplantation of cultured cells from early and later passages from the 8-year-old boy showed a 9.6-fold increase in the number of SSCs in 11 days of culture. Similarly, subcultured GSCs from the 6.5-year-old boy showed a 6.2-fold increase in SSCs within 21 days and a 5.6-fold increase within 14 days from the 8-year-old boy.

Assuming SSCs grow in an exponential way, 35 days of testicular cell culture or 58 to 83 days of GSC subculture...
would be necessary to achieve the 1300-fold increase in SSC number that we previously estimated as necessary for re-population of adult human testes after autotransplantation.2 No intratesticular tumors were observed in any of the 11 recipient mice after xenotransplantation.

Comment. We have demonstrated in vitro propagation of human prepubertal SSCs. Although these results are preliminary and need to be confirmed, they support the potential for autotransplantation of SSCs in infertile survivors of childhood cancer. Given the time between preservation of testicular tissue during childhood and potential SSC autotransplantation later in adult life, it is important to counsel prepubertal boys with cancer on the possibility of cryopreserving testicular tissue before undergoing gonadotoxic cancer treatment.

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Author Contributions: Dr Repping had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Obtained funding: Repping, van Pelt.

Administrative, technical, or material support: Sadri-Ardekani, Akhondi, van der Veen, Repping, van Pelt.

Study supervision: van der Veen, Repping, van Pelt.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Funding/Support: This study was supported by a grant from the Dutch Children Cancer Free Foundation (Foundation KiKA).

Role of the Sponsor: Foundation KiKA had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

Additional Contributions: We express gratitude to the following staff of the Infertility Clinic at the Avicenna Research Institute: Naser Amirjannati, MD, and Hamed Akhavizadegan, MD, for providing patient samples; Mohammad-Reza Sadeghi, PhD, for cryopreservation of testicular tissue; and Haleh Sohtanghoraei, MD, for the evaluation of testicular histology. Furthermore, we thank Saskia K. van Daalen, BSc; Cindy M. Korver, BSc; and Herman L. Roepers-Gajadin, BSc (Center for Reproductive Medicine, Academic Medical Centre), for their technical assistance. We also thank Dirk de Rooij, PhD (Center for Reproductive Medicine, Academic Medical Centre), for critically reviewing the manuscript. None of these persons received compensation for their contributions.


CORRECTIONS

Omitted Disclosure: In the Editorial entitled “Short-term Use of Unopposed Estrogen: A Balance of Inferred Risks and Benefits,” published in the April 6, 2011, issue of JAMA (2011;305[13]:1354-1355), in the Conflict of Interest Disclosures section, it should read, “Dr Colditz reported that money was paid to his institution (Washington University School of Medicine) by Littlepage Booth for his expert testimony regarding plaintiff-attorney group interpretation of epidemiological data on hormones and breast cancer.” Dr Colditz reported this information to JAMA on his completed ICMJE Disclosure Form, but this disclosure was erroneously omitted from the published editorial. This editorial has been corrected online.

Error in Figure. In the Original Contribution entitled “Patterns and Intensity of Medical Therapy in Patients Undergoing Percutaneous Coronary Intervention,” published in the May 11, 2011, issue of JAMA (2011;305[18]:1882-1889), the x-axis was shifted in both plots in the Figure. This article was corrected online.