

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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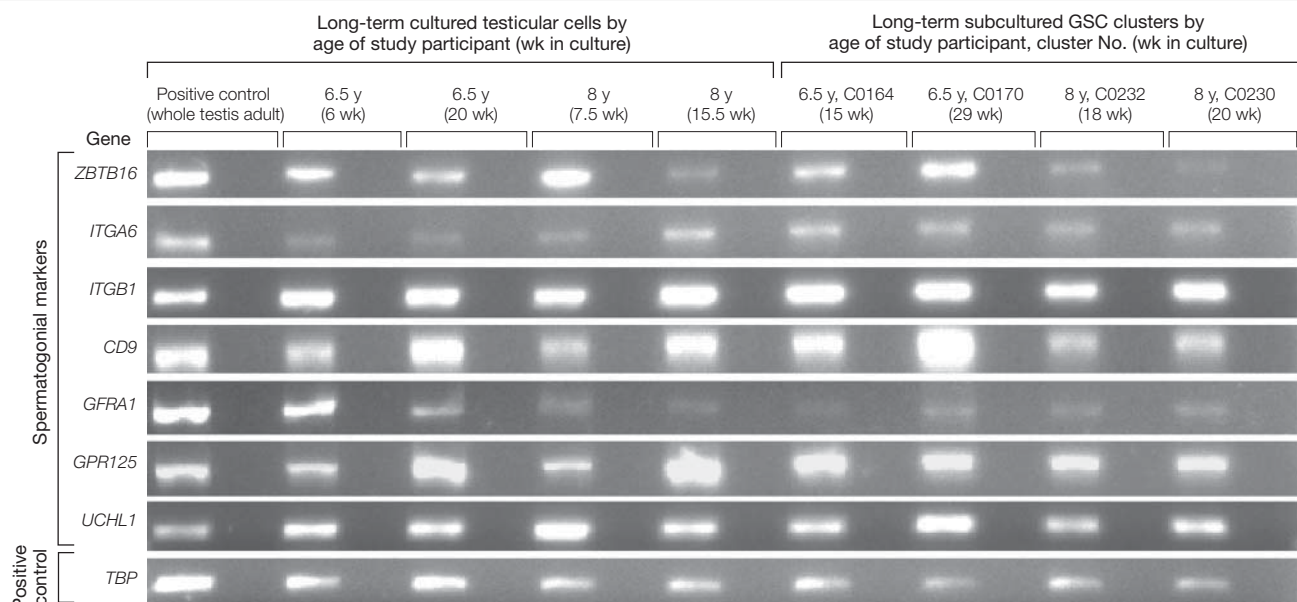
## RESEARCH LETTER

### 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.<sup>1</sup> We describe in vitro propagation of human prepubertal SSCs using a culture system for adult human SSCs.<sup>2</sup>

**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-

**Figure 1.** Culture Characterization



Reverse transcriptase–polymerase chain reaction showing expression of *ZBTB16* (PLZF), *ITGA6*, *ITGB1*, *CD9*, *GFRA1*, *GPR125*, and *UCHL1* (spermatogonial markers) in human adult testis (control), long-term cultured testicular cells, and several independent subcultured germline stem cell (GSC) clusters of the 6.5-year-old (clusters C0164 and C0170) and 8-year-old boys (clusters C0232 and C0230). The *TBP* gene was used as a positive control, and the second lane for each sample tested shows the negative control (without reverse transcriptase).

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.<sup>3</sup> 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.<sup>2</sup> All visible testicular-derived, embryonic stem cell–like colonies<sup>4</sup> were removed from the culture.

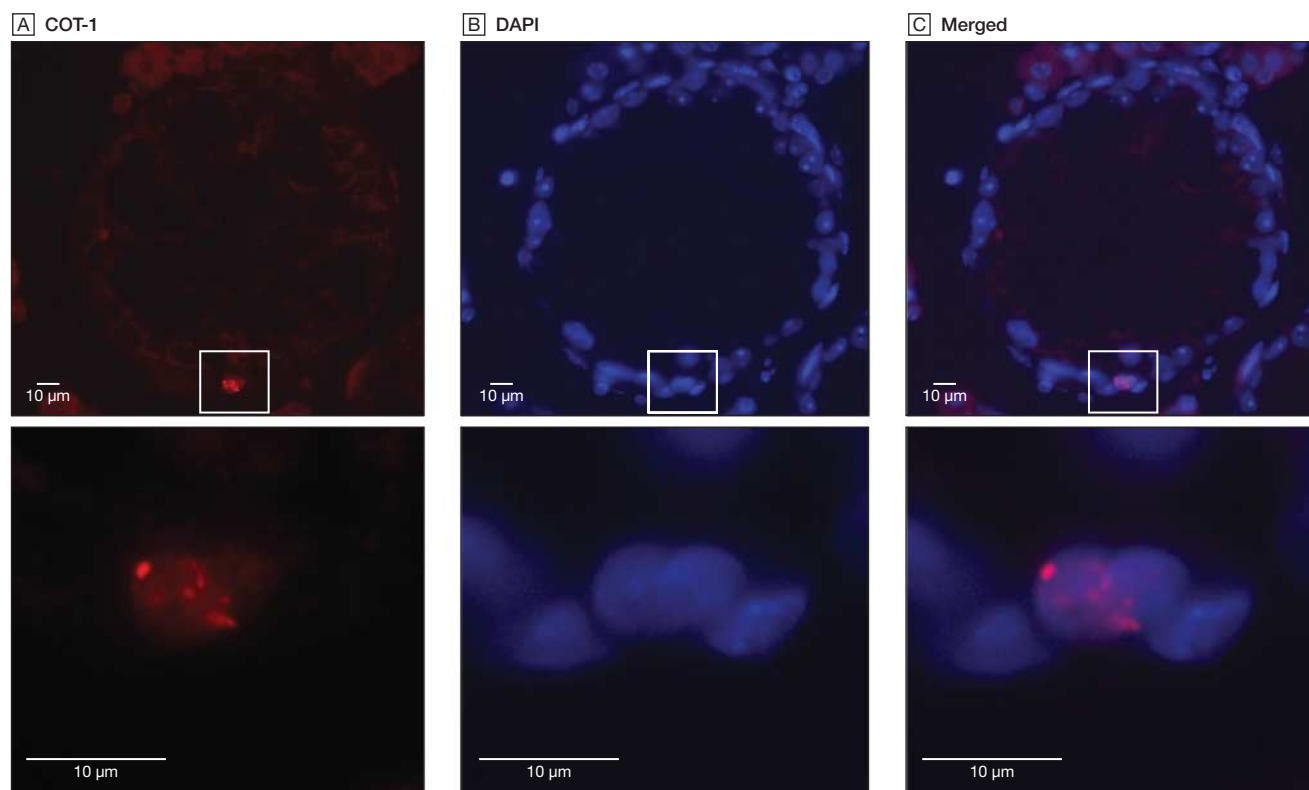
To determine the presence of spermatogonia during culture, the expression of spermatogonial markers<sup>5,6</sup> was studied by reverse transcriptase–polymerase chain reaction, immunohistochemistry, or both. To confirm the presence and

propagation of SSCs during culture, cells of early and later passages were transplanted into testes of busulfan-treated immunodeficient mice.<sup>2</sup>

**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

**Figure 2.** Detection of Human Prepubertal Spermatogonial Stem Cells After Transplantation to Immunodeficient Mouse Testis



Migration of human spermatogonial stem cells (cultured cells from testicular cell culture of the 8-year-old boy, passage 6 at 9 weeks) to the basal membrane of the seminiferous epithelium of immunodeficient mouse testis 8 weeks after transplantation. Cells were detected using a (A) human COT-1 fluorescence in situ hybridization probe, and detection with Cy3 (red) and cells were visualized using (B) DAPI (blue) staining. The merged image (C) indicates COT-1 staining in the nucleus of a migrated human spermatogonial stem cell.

would be necessary to achieve the 1300-fold increase in SSC number that we previously estimated as necessary for repopulation of adult human testes after autotransplantation.<sup>2</sup> 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.

**Study concept and design:** Sadri-Ardekani, Akhondi, van der Veen, Repping, van Pelt.

**Acquisition of data:** Sadri-Ardekani, Repping, van Pelt.

**Analysis and interpretation of data:** Sadri-Ardekani, Repping, van Pelt.

**Drafting of the manuscript:** Sadri-Ardekani, Repping, van Pelt.

**Critical revision of the manuscript for important intellectual content:** Sadri-Ardekani, Akhondi, van der Veen, Repping, van Pelt.

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## 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.