elsewhere.[1–3] Thereafter, injury-related deaths rose significantly, with more than one third of injuries being intentional, a similar proportion to that in a study of 129 maternal deaths in Australia.[4] This study did not capture out-of-hospital births, which are less than 1% of births in Ontario, or maternal deaths occurring antepartum. Although direct and indirect causes of death were not differentiated herein, the striking difference in maternal mortality rates following a stillbirth vs a livebirth deserves further study. Future studies should further classify maternal deaths by injury subtypes, including overdoses, and additionally evaluate antepartum deaths. ■

ACKNOWLEDGMENT

Author contributions included the following: Joel G. Ray and Allison L. Park developed the study concept, analysis and interpretation of the data, drafting of the manuscript, manuscript revision, and approval of final version. Jonathan Zipursky was responsible for the interpretation of the data, manuscript revision, and approval of final version.

Joel G. Ray, MD, MSc
Departments of Medicine
Health Policy Management and Evaluation, and
Obstetrics and Gynecology
St Michael's Hospital
University of Toronto
30 Bond Street
Toronto, Ontario, Canada M5B 1W8
rayj@smh.ca

Jonathan Zipursky, MD, FRCPC
Department of Medicine
Division of Clinical Pharmacology and Toxicology
University of Toronto
Toronto, Ontario, Canada

Alison L. Park, MSc
Institute for Clinical Evaluative Sciences
University of Toronto
Toronto, Ontario, Canada

The views expressed therein are those of the author and do not necessarily reflect those of the Ontario Registrar General or the Ministry of Government Services.

The opinions, results, and conclusions reported in this paper are those of the authors and are independent from the funding sources. No endorsement by the Institute for Clinical Evaluative Sciences or the Ontario Ministry of Health and Long-Term Care is intended or should be inferred.

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. Parts of this report are based on Ontario Registrar General information on deaths, the original source of which is Service Ontario.

This work was supported by a grant from the Canadian Institutes of Health Research and by the Institute for Clinical Evaluative Sciences, which is funded by an annual grant from the Ontario Ministry of Health and Long-Term Care.

The authors report no conflict of interest.

REFERENCES

1. Cristina Rossi A, Mullin P. The etiology of maternal mortality in developed countries: a systematic review of literature. Arch Gynecol Obstet 2012;285:1499–503.
2. MacDorman MF, Declercq E, Thoma ME. Trends in maternal mortality by sociodemographic characteristics and cause of death in 27 states and the District of Columbia. Obstet Gynecol 2017;129:811–8.
3. Schutte JM, Steegers EA, Schuitemaker NW, et al. Rise in maternal mortality in The Netherlands. BJOG 2010;117:399–406.
4. Thornton C, Schmied V, Dennis CL, Barnett B, Dahlen HG. Maternal deaths in NSW (2000–2006) from nonmedical causes (suicide and trauma) in the first year following birth. Biomed Res Int 2013;2013:623743.
5. Chiu M, Lebenbaum M, Lam K, et al. Describing the linkages of the immigration, refugees and citizenship Canada permanent resident data and vital statistics death registry to Ontario's administrative health database. BMC Med Inform Decis Mak 2016;16:135.

 https://doi.org/10.1016/j.ajog.2018.05.025

Human term amniotic fluid: a novel source of stem cells for regenerative medicine

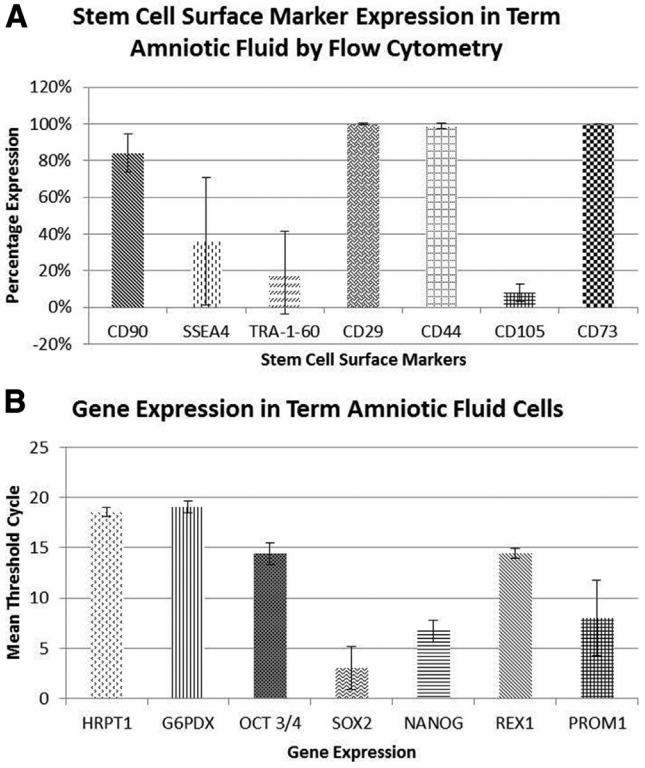
Check for updates

OBJECTIVE: Our objective was to characterize term amniotic fluid stem cells and compare them with previously described mid-trimester amniotic fluid stem cells. Stem cells are undifferentiated cells with the ability to self-replicate without differentiation. Mid-trimester amniotic fluid stem cells have been well-characterized as multipotent, but scant data exist for term amniotic fluid.[1] With the advent of noninvasive prenatal diagnosis, mid-trimester amniotic fluid will become less available from normal patients. In contrast, term amniotic fluid is available in large quantities from normal pregnancies and might be a resource for clinical transplantation.

STUDY DESIGN: Institutional Review Board approval and informed consent were obtained before research began. Amniotic fluid was collected from uncomplicated, scheduled term cesarean deliveries with a small uterine incision and a soft catheter to collect fluid before the attempt to deliver the baby. Immediately after collection, cell viability from each fresh sample was assayed before culture with the use of Trypan Blue staining; cells were counted via hemocytometer. Cells were then cultured for expansion; aliquots were taken and frozen from each subsequent passage. Cells were seeded on gridded culture dishes and counted daily for 4 days to assess proliferation. Flow cytometry for surface markers

and gene expression analysis by real-time polymerase chain reaction for genes that were associated with stem cells were performed at passage 3. Differentiation toward neural lineage was demonstrated by immunofluorescent microscopy with the use of monoclonal antibodies for nestin, B-tubulin III, and glial fibrillary acidic protein. Chondrocyte differentiation similarly was shown with monoclonal antibodies for aggrecan. Differentiation toward osteocyte lineage was assayed quantitatively for alkaline phosphatase activity and verified by alkaline phosphatase and Alizarin Red staining.[2]

FIGURE
Stem cell markers in term human amniotic fluid–derived stem cells



A, The graph shows the results of flow cytometry studies that were performed on term human amniotic fluid cells that were harvested at passage 3 to determine the presence of surface markers associated with stem cells. For all samples, the average percent of cells that expressed each marker is shown. **B,** The graph shows the results of real-time polymerase chain reaction studies to determine the expression of genes that were associated with stem cells (*OCT3/4*, *SOX2*, *NANOG*, *REX1*, and *PROM1*) in term human amniotic fluid. The stem cell genes are compared to housekeeping genes normally expressed by all cells (*HRPT1* and *G6PDX*) to compare gene expression levels.

Dolin. Human term amniotic fluid. Am J Obstet Gynecol 2018.

RESULTS: Amniotic fluid was collected from 19 women with mean age of 33.9±2.7 years and mean gestation age of 39.0 ±0.7 weeks. Median volume of amniotic fluid that was obtained during collection was 61 mL (range, 20−118 mL). Samples from 8 patients were cultured successfully. The others were discarded because of meconium, blood, or slow growth. Mean cell viability was 80±7%, and cell counts were 1.3×10^5 to 1.5×10^6 per mL. The mean doubling time was 27 hours. Samples tolerated 10 passages without senescence and cryostorage for up to 18 months. All samples demonstrated cells with multiple surface markers and transcription genes that were associated with multipotent stem cells (Figure). Multipotency was demonstrated by differentiation into neural, osteocyte, and chondrocyte lineages.

CONCLUSION: Term and mid-trimester amniotic fluid stem cells demonstrate similar characteristics. We showed that term amniotic fluid stem cells were multipotent, differentiated into multiple cell lineages, and withstood cryostorage. Previous studies on term fluid have demonstrated only mesenchymal cells that required induction to pluripotent stem cells to achieve differentiation to neural lineage.[3] Induced pluripotent cells are genomically unstable and tumorigenic; amniotic stem cells are not.[4] Our study demonstrates for the first time that both ectodermal and mesenchymal differentiability are present in term amniotic fluid stem cells. Neural differentiation was achieved without the induction of pluripotency. Term amniotic fluid contains approximately 1 million cells per milliliter, which may represent a readily available, abundant, and noncontroversial source of stem cells with therapeutic potential. ■

Cara D. Dolin, MD
Michael K. Chan, BA
Ross S. Basch, MD
Bruce K. Young, MD
New York University Langone Health
Division of Maternal-Fetal Medicine
Department of Obstetrics and Gynecology
New York, NY

The authors report no conflict of interest.

REFERENCES

1. Loukogeorgakis SP, De Coppi P. Stem cells from amniotic fluid: potential for regenerative medicine. Best Pract Res Clin Obstet Gynaecol 2016;31:45–57.
2. Chen Z, Chan MK, Strelchenko N, et al. Heterogeneity of stem cells in human amniotic fluid. J Regen Med 2014;3.
3. Moraghebi R, Kirkeby A, Chaves P, et al. Term amniotic fluid: an unexploited reserve of mesenchymal stromal cells for reprogramming and potential cell therapy applications. Stem Cell Res Ther 2017;8:190.
4. Rebuzzi P, Zuccotti M, Redi CA, Garagna S. Achilles' heel of pluripotent stem cells: genetic, genomic and epigenetic variations during prolonged culture. Cell Mol Life Sci 2016;73:2453–66.

 https://doi.org/10.1016/j.ajog.2018.05.035