

## Fetal cellular microchimerism in miscarriage and pregnancy termination

Suzanne E Peterson<sup>1,2</sup>, J Lee Nelson<sup>3,4</sup>, Vijayakrishna K Gadi<sup>4,5</sup>, and Hilary S Gammill<sup>1,4,\*</sup>

<sup>1</sup>Department of Obstetrics and Gynecology; University of Washington; Seattle, WA USA; <sup>2</sup>Swedish Medical Center; Seattle, WA USA;

<sup>3</sup>Division of Rheumatology; University of Washington; Seattle, WA USA; <sup>4</sup>Division of Clinical Research; Fred Hutchinson Cancer Research Center; Seattle, WA USA; <sup>5</sup>Division of Medical Oncology; University of Washington; Seattle, WA USA

**F**etal cells transfer to the mother during pregnancy and can persist long-term as microchimerism. Acquisition of microchimerism may also occur during pregnancy loss, either miscarriage or pregnancy termination. Because nearly half of all pregnancies end in loss, we recently investigated the magnitude of fetal cell transfer during pregnancy loss and whether obstetric clinical factors impacted cell transfer. Prospective measurement of fetal cellular microchimerism before and after miscarriage and termination of pregnancy demonstrated a significant transfer of fetal cells in these pregnancies, with higher concentrations of fetal microchimerism in pregnancy termination vs. miscarriage and in those that were managed surgically vs. medically. The frequency of pregnancy loss as a proportion of all pregnancies, and the overrepresentation of fetal genetic abnormalities in pregnancy loss suggest that the resultant acquisition of fetal microchimerism could have a unique and substantial impact on women's health.

The durable persistence of fetal cells (or microchimerism) acquired by a woman through natural fetal-maternal exchange during pregnancy may be considered the biological legacy of pregnancy. Long-term, microchimerism may have both beneficial and detrimental effects on health and has been associated with both risk of and protection from diseases, e.g., systemic sclerosis<sup>1</sup> and breast cancer,<sup>2</sup> respectively. The number and type of cells exchanged likely varies by pregnancy outcome, and transfer

can occur during pregnancy loss resulting from miscarriage or elective pregnancy termination.<sup>3,4</sup> Several factors underscore the need to characterize microchimerism acquisition during pregnancy loss specifically. Nearly half of pregnancies end in miscarriage or pregnancy termination;<sup>5-7</sup> thus, cells acquired during these pregnancies have the potential to substantially impact maternal health. In fact, previous studies demonstrate that fetal microchimerism is most commonly detectable years after pregnancy termination compared with other pregnancy outcomes.<sup>8,9</sup> Additionally, aneuploid or otherwise genetically abnormal fetal cells are overrepresented in miscarriage and pregnancy termination compared with uncomplicated term deliveries, and the implications of acquiring genetically abnormal cells are unknown.

We recently conducted a prospective study to specifically characterize and quantify the number of fetal cells transferred to the maternal circulation during care for miscarriage and pregnancy termination.<sup>10</sup> Because clinical factors could influence fetal cell transfer, we also explored relationships between aspects of obstetric care and fetal microchimerism concentration. Women undergoing treatment for singleton pregnancy loss were enrolled prospectively after informed consent. Peripheral blood samples were collected before and approximately 30 min after treatment (n = 150 samples from 75 women). In this study, male microchimerism was considered a surrogate for fetal microchimerism. From Ficoll-purified peripheral

**Keywords:** microchimerism, miscarriage, pregnancy termination, fetal, aneuploidy

**Abbreviations:** PBMC, peripheral blood mononuclear cells

Submitted: 05/01/13

Accepted: 05/02/13

<http://dx.doi.org/10.4161/chim.24915>

\*Correspondence to: Hilary S Gammill;  
Email: hgammill@u.washington.edu

Article Addendum to: Peterson SE, Nelson JL, Guthrie KA, Gadi VK, Aydelotte TM, Oyer DJ, et al. Prospective assessment of fetal-maternal cell transfer in miscarriage and pregnancy termination. *Hum Reprod* 2012; 27:2607-12; PMID:22752611; <http://dx.doi.org/10.1093/humrep/des244>.

**Table 1.** Fetal microchimerism detection and concentration after miscarriage and pregnancy termination

	Pre-treatment	Post-treatment (30 min)	Post-treatment (2 weeks)
Fetal microchimerism detection, n (%)	4/75 (5%)	18/75 (24%)	2/5 (40%)
Fetal microchimerism concentration,* median (range), mean	0 (0.0–0.7), 0.1	0 (0.0–36.0), 10.6	0 (0.0–0.7), 0.2

\*In microchimerism genome equivalents per 100,000 maternal cells.

blood mononuclear cells (PBMC), DNA was extracted, and quantitative PCR for a male-specific DNA sequence, DYS14, was performed.

Clinical characteristics that were considered included miscarriage vs. pregnancy termination defined by sonographic viability (the presence or absence of cardiac activity by ultrasound), medical vs. surgical treatment, and gestational age. As expected, our population was enriched for fetal abnormalities (both karyotypic and structural) with 33% (10/30) of those karyotyped demonstrating abnormalities (most commonly Trisomy 21 and Trisomy 13) and 70% (28/40) of those for whom tissue examination or formal autopsy was performed demonstrating anomalies (most commonly central nervous system anomalies, cystic hygromas, or complex cardiac defects). The median fetal gestational age was 16.6 weeks (range 5.0–24.0 weeks).

We found that fetal cell transfer was common in both miscarriage and abortion, with a post-treatment range of fetal microchimerism concentrations of 0–36 gEq of fetal microchimerism per 100,000 maternal cells, compared with 0–0.7 gEq per 100,000 before treatment ( $p < 0.001$ ), results summarized in **Table 1**. Exploratory analyses suggested that higher concentrations of fetal cells were associated with abortion compared with miscarriage (rate ratio for detection 24.7, 95% CI 1.6–389.8; among confirmed male fetuses, rate ratio 26.9, 95% CI 1.6–456.4) and with surgical compared with medical management (rate ratio for detection 5.9, 95% CI 0.7–51.8; among confirmed male fetuses, rate ratio 16.7, 95% CI 1.6–173.3). Cellular fetal microchimerism did not vary with gestational age.

Though we did not find an association of gestational age with the quantity

of fetal cells transferred when evaluated immediately after pregnancy loss/termination in our population, gestational age remains a factor that may differentially influence immediate fetal cell transfer vs. durable persistence. It has been hypothesized that possible heightened “stemness” of fetal cells early in gestation may facilitate maternal engraftment of fetal cells.<sup>11</sup> The focus of our recent work was on cellular transfer early in pregnancy, with a study population median gestational age of 16.6 weeks (range 5.0–24.0 weeks). The relationship between gestational age and fetal-maternal transfer also differs considerably for cell-free material compared with intact cells. Studies of whole blood or plasma, which reflect cell-free fetal DNA, show gradually increasing concentrations with increasing gestational age.<sup>3,12,13</sup> In contrast, cellular transfer, though also positively associated with gestational age, appears to follow a different pattern, with the large majority of transfer occurring much later in pregnancy, around the time of delivery in uncomplicated pregnancies.<sup>14</sup> The relationship between gestational age and cellular fetal microchimerism is likely complex, reflecting differential influences on the number of cells transferred, and their engraftment potential. In other words, though we did not see a difference in the quantity of cells transferred immediately after treatment for pregnancy loss in subjects at 5–24 weeks of gestation, it remains unknown: (1) how the number of cells immediately transferred after delivery at gestational ages beyond 24 weeks compares with earlier gestational ages, and (2) whether long-term engraftment varies dependent on acquisition across gestational age.

These differences highlight the importance of investigating both the immediate

and in particular the long-term reproductive consequences of fetal microchimerism. Durable persistence of microchimerism is of particular interest as it has been associated with later-life health status. Although our study was not specifically designed to evaluate fetal microchimerism concentrations long after treatment for pregnancy loss, we were able to evaluate a few samples ( $n = 5$ ) obtained approximately 2 weeks after treatment. Of five samples tested, two were positive for fetal cellular microchimerism (**Table 1**).

Another factor that may uniquely influence later life health is fetal genetic status. Aneuploidy is overrepresented among pregnancy losses, and the potentially important implications of acquisition of aneuploid microchimerism are unexplored. Women who have given birth to a child with Down Syndrome have a higher risk of developing Alzheimer dementia (a common risk in individuals with Down Syndrome themselves).<sup>15–17</sup> Whether this could be related to microchimerism from the child with Down Syndrome is unknown, but it is notable that both murine and human studies have demonstrated localization of microchimeric cells in the brain of the recipient.<sup>18,19</sup> Notably, higher concentrations of cell free fetal DNA have been found in pregnancies complicated by fetal aneuploidy compared with normal fetuses.<sup>20,21</sup> In our recent study, we found no association of fetal cell transfer with aneuploidy status, though we were not powered for this specific comparison, and further studies are needed. Speculatively, could aneuploid fetal cells in maternal brain serve as a nidus for neurofibrillary plaque formation and thus serve as an explanation for the observed epidemiologic association?

It is clear that pregnancy loss (miscarriage or pregnancy termination) can result in acquisition of cellular fetal microchimerism that can persist long-term. Further investigation of the relationship between pregnancy outcomes and both immediate transfer and long-term persistence of fetal microchimerism is needed in order to better understand reproductive influences on disease. Fetal microchimerism has been associated with both beneficial and detrimental effects. Furthering our understanding of the physiology of

maternal-fetal cell transfer and the ways in which obstetric management can influence the process may allow us to shift this balance toward benefit. Many important questions remain in this area, including: How does transfer after delivery (at term or preterm gestational ages) compare with transfer after pregnancy loss? Are there differences in factors that influence immediate fetomaternal cell transfer compared with factors that influence long-term persistence of fetal microchimerism? What are the implications of acquisition of genetically abnormal fetal cells on a woman's health? At a societal level, increasing maternal age at reproduction and its attendant increase in failed pregnancy completion would imply that more women harbor aneuploid fetal microchimerism now than in any time in history. Could an increase in aneuploid fetal microchimerism in the population impact the societal burden of certain diseases (e.g., Alzheimer disease)? Additional studies are warranted to investigate this new scientific frontier.

#### Disclosure of Potential Conflicts of Interest

All authors confirm that they have no conflicts of interest in relation to this work.

#### Acknowledgments

We gratefully acknowledge the women who agreed to participate in the original study, as well as the study coordination efforts of Evangelyn Nkwopara. The original study was supported by NIH grant HD01264.

#### References

- Nelson JL, Furst DE, Maloney S, Gooley T, Evans PC, Smith A, et al. Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. *Lancet* 1998; 351:559-62; PMID:9492775; [http://dx.doi.org/10.1016/S0140-6736\(97\)08357-8](http://dx.doi.org/10.1016/S0140-6736(97)08357-8).
- Gadi VK, Nelson JL. Fetal microchimerism in women with breast cancer. *Cancer Res* 2007; 67:9035-8; PMID:17909006; <http://dx.doi.org/10.1158/0008-5472.CAN-06-4209>.
- Bianchi DW, Farina A, Weber W, Delli-Bovi LC, Deriso M, Williams JM, et al. Significant fetal-maternal hemorrhage after termination of pregnancy: implications for development of fetal cell microchimerism. *Am J Obstet Gynecol* 2001; 184:703-6; PMID:11262475; <http://dx.doi.org/10.1067/mob.2001.111072>.
- Sato T, Fujimori K, Sato A, Ohto H. Microchimerism after induced or spontaneous abortion. *Obstet Gynecol* 2008; 112:593-7; PMID:18757657; <http://dx.doi.org/10.1097/AOG.0b013e31818345da>.
- Jones RK, Kooistra K. Abortion incidence and access to services in the United States, 2008. *Perspect Sex Reprod Health* 2011; 43:41-50; PMID:21388504; <http://dx.doi.org/10.1363/4304111>.
- Boklage CE. Survival probability of human conceptions from fertilization to term. *Int J Fertil* 1990; 35:75-59, 79-80, 81-94; PMID:1970983.
- Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, et al. Incidence of early loss of pregnancy. *N Engl J Med* 1988; 319:189-94; PMID:3393170; <http://dx.doi.org/10.1056/NEJM198807283190401>.
- Yan Z, Lambert NC, Guthrie KA, Porter AJ, Loubiere LS, Madeleine MM, et al. Male microchimerism in women without sons: quantitative assessment and correlation with pregnancy history. *Am J Med* 2005; 118:899-906; PMID:16084184; <http://dx.doi.org/10.1016/j.amjmed.2005.03.037>.
- Khosrotehrani K, Johnson KL, Lau J, Dupuy A, Cha DH, Bianchi DW. The influence of fetal loss on the presence of fetal cell microchimerism: a systematic review. *Arthritis Rheum* 2003; 48:3237-41; PMID:14613289; <http://dx.doi.org/10.1002/art.11324>.
- Peterson SE, Nelson JL, Guthrie KA, Gadi VK, Aydelotte TM, Oyer DJ, et al. Prospective assessment of fetal-maternal cell transfer in miscarriage and pregnancy termination. *Hum Reprod* 2012; 27:2607-12; PMID:22752611; <http://dx.doi.org/10.1093/humrep/des244>.
- McGrath H Jr. Elective pregnancy termination and microchimerism: comment on the article by Khosrotehrani et al. *Arthritis Rheum* 2004; 50:3058-9, author reply 3059; PMID:15457487; <http://dx.doi.org/10.1002/art.20650>.
- Watanagana T, Chen AY, LeShane ES, Sullivan LM, Borgatta L, Bianchi DW, et al. Cell-free fetal DNA levels in maternal plasma after elective first-trimester termination of pregnancy. *Fertil Steril* 2004; 81:638-44; PMID:15037414; <http://dx.doi.org/10.1016/j.fertnstert.2003.07.028>.
- Lo YM, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *Am J Hum Genet* 1998; 62:768-75; PMID:9529358; <http://dx.doi.org/10.1086/301800>.
- Adams Waldorf KM, Gammill HS, Lucas J, Aydelotte TM, Leisenring WM, Lambert NC, et al. Dynamic changes in fetal microchimerism in maternal peripheral blood mononuclear cells, CD4+ and CD8+ cells in normal pregnancy. *Placenta* 2010; 31:589-94; PMID:20569981; <http://dx.doi.org/10.1016/j.placenta.2010.04.013>.
- Cutler NR, Heston LL, Davies P, Haxby JV, Schapiro MB. NIH Conference. Alzheimer's disease and Down's syndrome: new insights. *Ann Intern Med* 1985; 103:566-78; PMID:2864010; <http://dx.doi.org/10.7326/0003-4819-103-4-566>.
- Schupf N, Kapell D, Lee JH, Ottman R, Mayeux R. Increased risk of Alzheimer's disease in mothers of adults with Down's syndrome. *Lancet* 1994; 344:353-6; PMID:7914304; [http://dx.doi.org/10.1016/S0140-6736\(94\)91398-6](http://dx.doi.org/10.1016/S0140-6736(94)91398-6).
- Schupf N, Kapell D, Nightingale B, Lee JH, Mohlenhoff J, Bewley S, et al. Specificity of the fivefold increase in AD in mothers of adults with Down syndrome. *Neurology* 2001; 57:979-84; PMID:11571320; <http://dx.doi.org/10.1212/WNL.57.6.979>.
- Chan WFN, Gurnot C, Montine TJ, Sonnen JA, Guthrie KA, Nelson JL. Male microchimerism in the human female brain. *PLoS One* 2012; 7:e45592; PMID:23049819; <http://dx.doi.org/10.1371/journal.pone.0045592>.
- Zeng XX, Tan KH, Yeo A, Sasajala P, Tan X, Xiao ZC, et al. Pregnancy-associated progenitor cells differentiate and mature into neurons in the maternal brain. *Stem Cells Dev* 2010; 19:1819-30; PMID:20707697; <http://dx.doi.org/10.1089/scd.2010.0046>.
- Bianchi DW, Williams JM, Sullivan LM, Hanson FW, Klinger KW, Shuber AP. PCR quantitation of fetal cells in maternal blood in normal and aneuploid pregnancies. *Am J Hum Genet* 1997; 61:822-9; PMID:9382092; <http://dx.doi.org/10.1086/514885>.
- Lo YM, Lau TK, Zhang J, Leung TN, Chang AM, Hjelm NM, et al. Increased fetal DNA concentrations in the plasma of pregnant women carrying fetuses with trisomy 21. *Clin Chem* 1999; 45:1747-51; PMID:10508120.