

## **Defining Fetal Hematopoietic Microenvironments to Understand Human Stem Cell Development**

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### **Background:**

The developmental processes required to generate functional tissue specific stem cells from pluripotent human embryonic stem cells (hES) are poorly understood. Identification of the microenvironmental cues that support the development of tissue specific stem cells during embryogenesis will be a necessary first step in making stem cell therapy efficient and practical. It was recently discovered that in mice, the placenta is one of the first organs to develop self-renewing, multipotential hematopoietic stem cells (HSC). We are now investigating whether the human placenta possesses a similar ability to harbor HSCs. Isolation of fetal HSCs at different developmental stages will initiate further molecular studies to define how “stemness” is established in HSCs and how fetal microenvironments support this process.

### **Objective:**

We propose to investigate whether the human placenta possesses the ability for hematopoiesis and support of HSCs. We will subsequently identify the areas of hematopoiesis within the placenta that may contain supporting niche cells.

### **Material and Methods:**

Placenta and control hematopoietic organs were obtained from discarded material from elective terminations. To assess clonal hematopoietic potential, two methods were used. Hematopoietic tissues were processed into single cell suspension by using enzymatic and mechanical dissociations and transferred to semi-solid methylcellulose colony assays where their differentiation potential was analyzed. In parallel, tissue explants were plated onto OP-9 stroma co-culture, which has been shown to be the most efficient co-culture system for assaying murine and human HSCs. To verify that placental hematopoietic activity is derived from fetal cells, individual colonies were genotyped. Localization of placental hematopoietic cells at different developmental stages was performed by immunohistochemistry.

### **Results:**

- Hematopoiesis occurs in the early gestation human placenta as evidenced by multilineage progenitors seen as early as 4 weeks developmental age. Hematopoietic growth from blighted ovum sample suggests de novo hematopoiesis in the placenta.
- Hematopoiesis continues in the placenta at least throughout the first trimester.
- Hematopoietic progenitors/candidate HSCs are localized in the villous mesenchyme and within the vasculature
- A macrophage population develops separately within the mesenchyme.

### **Conclusion:**

The first trimester human placenta supports multipotential hematopoietic cells, which may represent developing HSCs.