Application Notes

A series of interviews conducted by HypOxygen, Don Whitley Scientific Limited's US distributor.

Prof. Margaret Petroff is at the Department of Anatomy and Cell Biology at the University of Kansas Medical Center in Kansas City, Kansas and has recently relocated to Michigan State University in East Lansing, Michigan.

a. *"Minor Histocompatibility Antigens Are Expressed in Syncytiotrophoblast and Trophoblast Debris: Implications for Maternal Alloreactivity to the Fetus" (2012) Olivia Holland, Caitlin Linscheid, Herbert Hodes, Traci Nauser, Melissa Gilliam, Peter Stone, Larry Chamley, and Margaret Petroff; Am J Pathol. 2012 Jan; 180(1): 256–266*

b. *"Maternal CD4$^+$ and CD8$^+$ T Cell Tolerance Towards a Fetal Minor Histocompatibility Antigen in T Cell Receptor Transgenic Mice" (2013) Antoine Perchellet, Susmita Jasti, and Margaret Petroff; Biol Reprod. 2013 Oct; 89(4): 102, 1-12.*

From an immunological perspective, pregnancy is fascinating in that the fetus and its placenta represent a semi-allograft which must normally be tolerated, but sometimes isn't. Women who have borne children possess T cell populations specific for fetal mHAgs, and Hypoxystation users Margaret Petroff and Caitlin Linscheid examined where and how the maternal immune system is exposed to them. As oxygen levels in the placenta are very low at the beginning of pregnancy and increase as vascularization progresses, they use an Hypoxystation for their cell culture, both to mimic the 8% O2 in a normal term placenta and the approx. 2% found in pre-eclampsia. Maternal alloimmunization during pregnancy has implications for organ and cell transplantation, autoimmunity, and recurrent miscarriages.

The lab has been using an Hypoxystation for about two and a half years now. We spoke with **Dr. Caitlin Linscheid** at the lab there; HypOxygen asked how the lab uses the workstation for hypoxic cell culture.

What is the focus of the research at your lab?

We have three different project areas we are looking into: fetal antigens and maternal recognition of the fetus during pregnancy; autoimmune diseases and their impact on fertility; and the relationship between pregnancy and cancer.

I'll be presenting a poster at the Society for Reproductive Investigation conference in San Francisco in March regarding trophoblast-derived exosomes and their effects on primary dentritic cells. My research, broadly speaking, is on fetal antigens expressed in the placenta and maternal recognition of the fetus

during pregnancy. It has been demonstrated that the maternal immune system can form a T cell response to fetal antigens, and those can be coded on the Y chromosome or be polymorphic among individuals. These are expressed in the placenta, in the syncytiotrophoblast, and are shed into the maternal bloodstream. This form of antigen exposure during pregnancy is balanced out by other signals sent out by the placenta that promote tolerance of the essentially foreign fetus. The trophoblasts do not express the classical MHC's, so they cannot actively present their antigens, but they do express some non-classical MHC's like HLAG, which have been shown to be implicated in tolerance. Regulatory T cells also play an important role in maintain tolerance of the fetus. I am characterizing the fetal antigens in the placenta, specifically in the context of pre-eclampsia, which is a disorder occurring in 5-8% of pregnancies and is characterized by high blood pressure, proteinuria and kidney failure. Pre-eclampsia can have very serious effects for both the mother and child.

Can you describe the course of a typical assay with the placenta cells?

We obtain placentas from C-sections at term (37 weeks), from a breech pregnancy or a repeat Cesarian, to preclude any sort of unknown pathologies. The placenta is fetal in origin, not maternal. In the lab, we isolate trophoblast cells from the placenta and culture them in dishes (6 well or 60 mm) in the hypoxia workstation for 72 hours. We use either 8% oxygen, which resembles the normal situation in a term placenta, or 2%, which is hypoxic for a term placenta and is representative of the oxygen parameters in disease states where the placenta is dysfunctional. The Hypoxystation allows us to keep the cells at their physiological oxygen, instead of at the hyperoxia that ambient air would represent. After 72 hours, we collect the supernatant from the trophoblast cultures and separate out the exosomes using differential ultracentrifugation. We use mass spectrometry for proteomics analysis of the exosomes from trophoblasts cultured at normal placental oxygen levels. We have also looked at antigenic proteins that are expressed in trophoblast cells themselves, rather than in exosomes.

Do you ever use the oxygen profiling/cycling option to simulate hypoxia/reperfusion or slow deoxygenation processes?

We intend to use the oxygen profiling ability of the Hypoxystation to simulate ischemia/reperfusion processes in the placenta. This kind of fluctuation, spatially and temporally, approximates the situation in the hypoxic placenta as oxygenation is disturbed in pre-eclampsia. The logical next step is to ramp down or intermittently change the oxygen level in the workstation and examine the effects on gene expression in the trophoblasts.

During the 18-72 hrs culture process, what are typical steps the cultures require, and how many times total would you say are you going in and out for a typical cell culture?

The trophoblast cells are at 21% through the lengthy process of isolation from the placenta, and then we allow them to equilibrate overnight, change the media, and then transfer them to the Hypoxystation. They stay in that atmosphere for 72 hours, and we don't disturb them there at all. The advantage of having them in the workstation, as compared to an incubator, is the uninterrupted, stable hypoxia they experience. We appreciate the total stability of the atmosphere inside the chamber. That stability of the

oxygen level is essential to having those reproducible parameters which induce the pre-eclampsia proteome.

I love the Hypoxystation, I call it my third baby! It's so easy, I love working with it.

What is the typical volume of dishes/flasks in the w/s?

Because we plate at high density, and we isolate mRNA and proteins in the workstation, we have a fairly small volume of dishes in the workstation at any given time, perhaps 3-4 dishes at once. I can reach everywhere I need to get and I have the room to maneuver that I need.

Is there any instrumentation in the w/s?

We would like to place a small microscope inside the chamber soon, to monitor the morphology of our trophoblasts.

How do you clean your workstation?

Once we have removed the cells, we allow the workstation to dry out and then clean it thoroughly using the Labdet solution HypOxygen provide. It's very easy to clean the chamber once the front is removed. We've never had any issues with contamination in the workstation.

See more of what Dr. Petroff and Dr. Linscheid are doing at: http://www.kumc.edu/school-of-medicine/anatomy-and-cell-biology/faculty/peggy-petroff-phd.html