Tau protein function in pregnancy

Dr Michael Roberts discusses his ongoing research into the biology of mammalian reproduction, the relevance it has for human placental diseases such as pre-eclampsia and the importance of collaboration

Can you begin by explaining what a trophoblast is?

Trophoblasts are essentially placental cells derived from the first recognisable cell lineage (trophectoderm) that emerges in the early embryo when the transition from a ball of cells (the morula) to a hollow sphere (the blastocyst) is made. The outer layer of the embryo at this stage is known as trophectoderm, which gives rise to the specialised cells that make up much of the mature, foetal placenta.

How did you reveal tau protein function?

The work for which I am best known is the discovery of the interferon tau proteins, which cause maternal recognition of pregnancy in ruminants. What facilitated this breakthrough was my realisation that the best way to isolate this factor would be to culture whole embryos in absence of serum, but initially in the presence of radioactive amino acids to 'tag' the secreted proteins. We then utilised 2D electrophoresis to follow the radioactive protein as we purified it from the culture medium.

What are the translational applications of your research, in biomedicine and beyond?

Our hypothesis asserts that the placenta triggers pre-eclampsia in the mother and that a paternal component contributes to the early onset form of the disease. If so, it may be possible to understand features of the pathophysiology of pre-eclampsia from the cellular phenotypes (eg. gene expression, invasiveness and production of hormones) of the trophoblast cells created *in vitro*. Should this be the case, the opportunity presents itself for studying the effects of drugs and other compounds that might reverse the *in vitro* phenotype. At the very least, we may be able to discard some theories (of which there is a multitude) that have been proposed to account for pre-eclampsia.

How has the reproductive biology field changed since you began your career?

When I entered the field I was a biochemist working primarily amongst physiologists and developmental biologists who had little experience of purifying and characterising proteins. This presented myself and my students with opportunities to perform novel tasks; I'm a great believer in bringing new technologies to the field. Like most disciplines, reproductive biology has gone 'molecular', which, in turn, has been made so much easier with the advent of kits. As something of a pioneer, the laboratory often had to manage without such tools initially and to carry out research from scratch. Also bear in mind that reproductive biology of 1972 (when I began to switch from plant sciences to re-focus my efforts on pig reproduction) was before human *in vitro* fertilisation (IVF) and preceded most of the now universal technologies of assisted reproduction and stem cells.

Are you collaborating with other projects in the course of your investigations?

My research in reproduction has always depended upon collaboration and team work. At Florida, my colleagues in the area all knew more about reproductive physiology than I could possibly hope to learn. They also had the resources (farms, surgery suites) that were inaccessible to me as a member of the Medical School faculty. The same situation occurred when I arrived in Missouri. The present project on pre-eclampsia would be impossible without the involvement of Drs Danny Schust and Laura Schulz in the department of ObGyn. Danny, in particular, provides access to patients and clinical insight into the disease, and Laura bring expertise in the study of trophoblast invasion, the process thought to be defective in pre-eclampsia. Furthermore, Toshihiko Ezashi has been an associate since the mid-1990s and essentially runs the laboratory, initiating most of the forays into new technologies.

Porcine potential in pregnancy

Scientists at the University of Missouri are exploiting stem cells to understand the developmental processes involved in the formation of the human placenta and the emerging risks of pre-eclampsia

HUMAN REPRODUCTION IS incredibly complex. The process of fertilisation, if completed successfully, produces a genetically viable zygote, one that must differentiate and grow and eventually embed in the wall of the maternal endometrium. This process is modulated and controlled by a complex interplay of genetics and hormones, leading to the implanted embryo successfully developing into a foetus, dependent on the nutrition and oxygen of its mother – all of which is provided via the placenta. Unfortunately, the innate complexity of this process means that errors can occur. One common condition thought to be associated with flawed placental development is pre-eclampsia.

Pre-eclampsia is estimated to affect around 3 per cent of births globally, leading to 50,000 deaths annually. The suggested cause of pre-eclampsia is the failure of arteries to properly remodel in response to the presence of a developing embryo. Further complicating the issue is the typically late presentation of pre-eclampsia, meaning sufferers may not be aware of the risk until late in their pregnancy.

With a clear and pressing need for improved diagnostic techniques, research into pre-eclampsia amongst other maternal conditions is ongoing. Led by Dr Michael Roberts, a research team at the University of Missouri is focusing on the biochemical and physiological processes in pregnancy, attempting to replicate these processes with a stem-cell model. This approach has the potential to enable a new methodological framework; a paradigm invoking a new wave of knowledge which will underpin clinical advances.

A stem-cell model for placental diseases is extremely valuable. Pregnancy can be difficult to study and invasive or non-invasive methodologies involving pregnant women are open to ethical accountability and justification. Even if methodologies can be designed and suitably approved, persuading pregnant women to participate in such studies remains a challenge. For these reasons, *in vivo* analysis of placental diseases is not practical. In this context, the value of a stem cell *in vitro* methodology is apparent: "These models should help define the pathogenesis of placental disease and possibly provide novel diagnostic and treatment strategies," expands Roberts.

A TWO-CELLED APPROACH TO MODELLING

Roberts and his team have successfully produced models examining the developmental behaviour of two cell types found in the placenta: the extravillous trophoblasts (EVT) and the syncytiotrophoblasts (ST). The former is a placental cell that invades the wall of the womb: "Failure to invade properly can lead to serious consequences for mother and child, including the onset of pre-eclampsia," explains Roberts. The second of the two cell types, ST, is responsible for the uptake of dissolved nutrients and oxygen from the mother's blood supply and, in the process, the production of hormones regulating maternal physiology to favour the baby.

In order to produce both of these cell lines, the researchers had to formulate and alternative method of differentiating human embryonic stem cells (hESC) to form ST and EVT cells, as Roberts elaborates: "We accomplish this by treating the cells with bone morphogenic protein-4 (BMP4) and/or inhibitors of activin/nodal signalling". This now well-established process allows the group to follow the development of hESC into EVT and ST, and attempt to elucidate key biochemical and physiological patterns distinguishing abnormal development from healthy development. It is hoped this new understanding will provide the key to opening the door for pioneering therapeutics research, reducing the risk of pre-eclampsia and other placental diseases.

PROBING PORCINE POTENTIAL

A second goal of Roberts and his colleagues at Missouri – notably Drs Randall Prather and Kevin Wells – is the genetic manipulation and cloning of pigs. Although not immediately apparent, this work has mutualistic benefits for the group's work within placental diseases. Being large mammals, porcine models are a useful tool for early stage stem-cell research: "Pigs continue to be widely used large animal models to study various aspects of human disease, including metabolic conditions, heart disease, gut disorders, skin development, cancer and respiratory disorders," highlights Roberts. Today, Roberts, along with many like-minded researchers, exploit porcine models to test early stage stem-cell therapies prior to testing on human patients.

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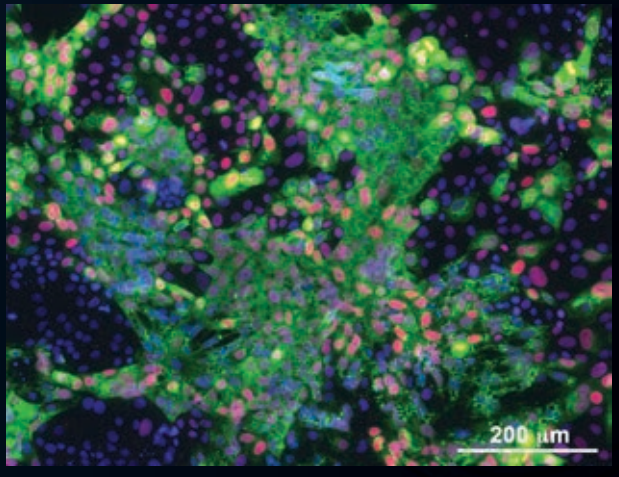
Roberts's early career focused on the study of reproduction in livestock, most notably ruminants. Through his early work in this field, he discovered the role of a family of proteins called interferon tau, which is important in maternal recognition of pregnancy in the early stages of ruminant reproduction. This research has proved both economically and socially important, as Roberts explains: "Early pregnancy loss is the most important cause of lost productivity and income in livestock farming".

Roberts' experience in large mammal biology and research has afforded him a unique window into the changing face of biological research. Contemporary laboratory work



The image illustrates regions of differentiating syncytial trophoblast cells in a colony of an induced pluripotent stem cell line. The green coloured cells indicate hCGα (one of two polypeptides of hCG), while the red colour shows the syncytial transcription factor GATA2. Blue coloured organelle exemplifies nuclei (DAPI staining). The scale bar is 200µm. iPSCs were treated with BMP4 and inhibitors of FGF2 and ACTIVIN signalling and fixed on day six for staining.

From **left** to **right**: Toshihiko E, DVM, PhD; Danny Schust, MD; Laura Schulz, PhD; and Michael Roberts at the University of Missouri-Columbia campus.

is largely dependent on mouse models, an animal with a short lifespan, and one that is easy to genetically manipulate and cheap to house. Despite these advantages, Roberts feels too much focus is placed on an animal fundamentally very different from humans: "Many of us believed, as we still do, that you can learn as much, or more in some cases, about the human by using a farm animal model as you can from a rodent, but we couldn't compete with the genetic manipulation, short generation times and relative low cost of the mouse". Whilst Roberts is concerned with this heavy dependence on mice, the situation may now be changing again, placing significant importance on larger mammals with a biology more analogous to humans.

From his early career in ruminant and large mammal biology, to his current interest in human placental diseases, Roberts has always been fascinated by the complexity of reproductive processes in mammals and the formation of the placenta. This biological complexity has unfortunately led to natural setbacks and human tragedy, causing the loss of both animal and human life.

Only by comprehensively understanding the mechanisms underpinning these afflictions can medicine advance; identifying compounds and appropriate treatments can help to reduce, and even eliminate, their incidence. In this context, it is clear the work being conducted at the University of Missouri is socially and medically valuable. By reproducing the natural processes of placentation with stem cells in a laboratory setting, the team hopes to contribute to our knowledge of placental diseases. If nothing else, they will prove, or disprove, whether pre-eclampsia and other placental diseases are caused by errors in the formation of the placenta in addition to unexpected responses of the mother to early pregnancy.

INTELLIGENCE

PLURIPOTENT STEM CELLS

OBJECTIVES

- To study the formation of the early placenta through a process whereby pluripotent stem cells are directed and then committed to the placental cell lineage in the culture dish by the use of specific growth factors and pharmaceutical agents
- To study the pathophysiology of pre-eclampsia by generating induced pluripotent stem cells from cells derived from babies born to mothers who suffered from a severe, early onset form of the disease

KEY COLLABORATORS

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Laura Schulz, **PhD**

Toshihiko Ezashi, **PhD**

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The Society for the Study of Reproduction

FUNDING

National Institutes of Health – award nos. 1R01HD069979, 1R01HD067759 and 1R01HD077108

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MICHAEL ROBERTS obtained his DPhil in Botany from the University of Oxford in 1965 and went on to complete postdoctoral work in plant biochemistry at the State University of New York at Buffalo. He was Assistant (1970-73), Associate (1973-76) and full Professor (1976-85) in the Department of Biochemistry, University of Florida. Since 1985 he has resided the University of Missouri as Professor of Animal Sciences and Biochemistry. He was elected to the US National Academy of Sciences in 1996 and awarded the Wolf Prize for Agriculture in 2003.