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 **UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2018/19**

***NB: This whole report will be posted on the Society’s website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.***

**Name of student:**

Amy Wilkinson

**Name of supervisor(s):**

Professor Graham Burton & Dr Tereza Cindrova-Davies

**Project Title: (no more than 220 characters)**

Signalling pathways involved in the functional response of human endometrial gland organoids to early pregnancy hormones

**Project aims: (no more than 700 words)**

Contrary to existing dogma, maternal blood flow to the placenta is not fully established until the end of the first trimester of pregnancy. Instead, the conceptus is supported by secretions from the endometrial glands during this critical period of organ development. All mammalian uteri contain endometrial glands that secrete a complex array of molecules including glucose, proteins, enzymes, growth factors, cytokines, hormones, transport proteins, lipids and other substances, collectively termed histotroph or uterine milk. The importance of the glandular secretions for the survival and normal development of the conceptus during early pregnancy is well established in domestic species. Paracrine signalling between the conceptus and the endometrium increases the secretion of uterine milk proteins in early pregnancy, constituting a servomechanism by which the nutritional demands of the embryo are met. Thus, in the sheep, horse, rabbit and pig, expression and secretion of growth factors, such as EGF, and uterine milk proteins, such as glycodelin, from the glands increases in early pregnancy. Recently, human endometrial gland organoids have been established and characterised. The organoids are self-organising, genetically stable, 3D culture systems containing both progenitor and differentiated cells that resemble the tissue of origin. The aim of this project was to use these organoids to determine whether a similar servomechanism operates in humans, and if so, what signalling pathways mediate it. There were three specific points this studentship aimed to address:

1) What is the functional response of human endometrial gland organoids to pregnancy hormones?

This aim addresses the functional response of glands to hormonal stimulation in terms of their uterine milk production, specifically the production of glycodelin and EGF, in order to establish if they are indeed upregulated by early pregnancy hormones as hypothesised.

2) What are the signalling pathways mediating the endometrial response to pregnancy hormones in humans?

In the sheep, the glands undergo hyperplasia between days 15 and 50 post-fertilisation. Exposure to interferon-τ, placental lactogens and placental growth hormone activates the JAK/STAT pathways, specifically involving STAT5. In the human, we speculate that hCG plays the role of interferon-τ, as the molecule of recognition of pregnancy. As well as the pathways described in the domestic species, additional stimulation is likely to come from the decidual cells, which secrete high levels of prolactin. The aims were to:

i. Analyse changes in the STAT, MAPK, and cJun signalling pathways in response to pregnancy hormones using phosphorylation-sensitive antibodies.

ii. Test the functional relevance of any changes observed by culturing organoids in inhibitors and analysing their effect on uterine milk production.

3) How do pregnancy hormones affect the gland signature in terms of their receptor profile?

In the sheep, downregulation of the progesterone receptor is necessary to increase uterine milk production. The aim was to see if this receptor is also downregulated in the human organoids in response to the hormones, and to analyse any changes in levels of additional receptors, such as the prolactin receptor and EGF receptor.

**Project Outcomes and Experience Gained by the Student (no more than 700 words)**

Human endometrial gland organoids were grown for 3 days, treated with estrogen for 3 days, and then subsequently cultured in either estrogen alone (E2), estrogen and progesterone (EP), or estrogen, progesterone, hCG, human placental lactogen and prolactin (All) for varying amounts of time between 6-48 hours. I was involved in culturing and processing the organoids for the experiments. This allowed me to learn aseptic technique, passaging, and different methods of extracting samples – skills that are transferable to other types of tissue and cell culture.

Samples were subject to Western blotting, immunohistochemistry and RT-qPCR. Throughout the project I became proficient in these techniques and gained a good knowledge of the theory behind them. This will be useful to me in any future laboratory work I will undertake. I was also involved in analysing the data obtained, giving me experience in using software packages like ImageJ and Graphpad prism.

We found from our initial investigation that levels of glycodelin mRNA and protein were increased by the All treatment. In addition, we also found that EGF mRNA levels were higher in the All treatment compared to E2. This confirmed that early pregnancy hormones do indeed lead to increased uterine milk production in human endometrial gland organoids.

A stand-out candidate for further investigation was STAT3. Using phosphorylation sensitive antibodies and Western blotting, phospho-STAT3 levels were found to be higher in the All treatment. To assess the functional relevance of this, we cultured organoids in a phospho-STAT3 inhibitor along with the original hormone treatments for 24 and 48 hours, and then analysed glycodelin levels by Western blot and RT-qPCR. Inhibiting phospho-STAT3 prevented the increase in glycodelin production seen with the All treatment. This suggests that at least one of the hormones in the All treatment activates STAT3 which then mediates an increase in glycodelin synthesis. This was an exciting finding given that the STAT pathway has previously been implicated in the sheep servomechanism.

In terms of the receptor profile of the organoids, we found that the progesterone receptor was at lower levels in the EP and All treatments, probably due to the effect of progesterone itself, as in the sheep. In addition, the prolactin receptor was also at higher levels in the All treatment.

Overall, this project has contributed to the evidence in favour of there being a servomechanism for uterine milk production in humans and has additionally provided pilot mechanistic insights. It seems that the action of early pregnancy hormones leads to STAT3 activation which mediates an increase in glycodelin, and hence uterine milk production. Although this is only preliminary work, it has provided potential avenues for further research into this area. Furthermore, on a personal level, this project has given me an insight into what a career in research would be like and has allowed me to develop a wide range of skills.

Please state which Society Winter or Summer Meeting the student is intending to present her poster at:

Winter meeting

**Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)**

Poster abstract:

Maternal blood supply to the placenta is not fully established until the end of the first trimester of pregnancy. During the critical period of organogenesis, the conceptus is supported by endometrial gland secretions, referred to as histotroph or uterine milk. The secretions contain an array of molecules including glycodelin, a glycoprotein with immunomodulatory effects at the maternal-fetal interface, and epidermal growth factor (EGF) that is known to stimulate trophoblast proliferation. In domestic species, paracrine signalling between the placenta and uterus constitutes a servomechanism whereby secretions from the endometrial gland increase to meet the needs of the conceptus. The aim of this project was to determine if a similar mechanism operates during human pregnancy, and to identify the signalling pathways involved.

Organoid cultures were derived from endometrial scratch biopsies of 5 patients undergoing IVF treatment at the Bourn Hall Clinic and grown with ethical permission (NHRA 17/EE/0151). Organoids were grown for 3 days, stimulated with 10nM estrogen for 3 days, and subsequently treated with either i) estrogen (E2), ii) estrogen and 1μM progesterone (EP), or iii) EP and other hormones (20ng/ml prolactin, 20ng/ml human placental lactogen and 100ng/ml hCG (All)) for 6-48 hours. Protein and mRNA expression of glycodelin, EGF, and phopho-STAT3 were examined using Western blotting, immunohistochemistry or RT-qPCR analysis. The treatment with all hormones significantly increased glycodelin and EGF mRNA and protein levels, and phosphorylation of STAT3, compared to E2 and EP treatments. Addition of a P-STAT3 inhibitor, S31-201 (50μM), significantly reduced glycodelin mRNA and protein levels in hormone-treated samples. Organoid receptor profile was also altered with different treatments. Addition of progesterone (EP and All treatments) downregulated the progesterone receptor, compared to E2 alone, whilst the prolactin receptor expression was upregulated by the hormonal treatment (All).

Together these data provide evidence in support of a servomechanism operating in the human, and provide pilot mechanistic insights. The results suggest that early pregnancy hormones lead to the activation of STAT3, which mediates an increase in glycodelin production. These findings may be translatable to pregnancy complications, such as miscarriage, where endometrial function is compromised and placental development post-implantation is deficient.

**Brief Resume of your Project’s outcomes**: **(no more than 200-250 words)**.

*The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.*

Signalling pathways involved in the functional response of human endometrial gland organoids to early pregnancy hormones

Maternal blood supply to the placenta is not fully established until the end of the first trimester of pregnancy. During this critical period of organogenesis, the conceptus is supported by endometrial gland secretions, referred to as uterine milk, containing an array of molecules including glycodelin and EGF. In domestic species, paracrine signalling between the placenta and uterus constitutes a servomechanism whereby secretions increase to meet the needs of the conceptus. The aim of this project was to determine if a similar mechanism operates during human pregnancy and to identify the signalling pathways involved.

Treatment of human endometrial gland organoids with early pregnancy hormones resulted in increased glycodelin and EGF at both RNA and protein levels compared to estrogen alone. Additionally, levels of phospho-STAT3 were significantly higher with the hormone treatment. Treatment of organoids with a phospho-STAT3 inhibitor prevented this increase in glycodelin. The receptor complement was also altered, whereby the progesterone receptor was downregulated by progesterone and the prolactin receptor was upregulated by treatment with hormones.

(B)

(A)



*Fig. 1 – (A) Western blot of organoid samples cultured in the hormones stated detecting phospho-STAT3. Levels are highest in the treatment with all hormones. (B) RT-qPCR data showing glycodelin (PAEP) mRNA levels of samples cultured with or without phospho-STAT3 inhibitor. There is reduced glycodelin mRNA in the treatment with all hormones plus the inhibitor.*

Together this provides evidence in support of a servomechanism operating in humans, and pilot mechanistic insights. The results suggest that early pregnancy hormones lead to STAT3 activation, which mediates an increase in glycodelin production, and hence uterine milk production. These findings may be translatable to pregnancy complications, such as miscarriage, where endometrial function is compromised and placental development is deficient.

**Other comments: (no more than 300 words)**

I would like to thank Professor Graham J Burton and Dr Tereza Cindrova-Davies for giving me the opportunity to work in their lab over the summer, and for teaching me a wide range of skills that will be invaluable to me in the future. I thoroughly enjoyed the experience! I would also like to thank the Anatomical Society for funding this project, making it possible for me to gain experience and to get to know what it is like to work in a lab.

 *Signature of student.......Amy Wilkinson.....................................Date 29/10/19.*

 *Signature of supervisor…Graham Burton………………………………... Date 31/10/19*

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