UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2015/16
NB: This 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:

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Name of supervisor(s):

Michelle Welsh

Project Title: (no more than 220 characters)

Is there a link between excess progesterone in assisted reproduction and placental abnormalities?

Project aims: (no more than 700 words)

Previous literature has established an increased incidence of IUGR (intrauterine growth restriction) in babies born to IVF (in vitro fertilisation). IUGR is defined as the presence of a reduced neonatal size at birth - more specifically having a weight or length below the 10th percentile for gestational age and an abdominal circumference below the 2.5th percentile with a pathologic restriction of foetal growth. This condition is often associated with high rates of perinatal morbidity and mortality. Valsamakis et al. suggest an increase in the prevalence of metabolic syndrome, related to type 2 diabetes, obesity and hypertension, in IUGR babies. Moreover, Manikkam et al. consider IUGR as a marker of various adult disorders some of which include cryptorchidism, male subfertility, PCOS (polycystic ovarian syndrome) and CVD (cardiovascular disease). These findings imply that changes in the prenatal environment leading to growth restriction could have longterm effects on the development and health of the individual, thus understanding the cause and mechanism of IUGR is vital to its prevention and/or the treatment of related disorders.

One of the possible origins of IUGR in IVF babies is the high progesterone level in the maternal environment compared to natural conception. The increased progesterone concentration results from elevated hormone secretion by multiple corpora lutea following oocyte release due to ovarian hyper-stimulation. Previous research using a rat model depicted that MPA (progesterone) treated pregnant rats have smaller placentae and give birth to low birth weight pups compared to controls. Pilot data has illustrated an alteration in placental morphology following progesterone administration. Therefore, this project focused on investigating placental vascularisation and possible alterations in blood vessel supply related to increase in progesterone concentration. Since the placenta plays a vital role in oxygen and nutrient delivery, waste removal, as well as hormone production and metabolism, which collectively control foetal development within the uterus, changes in placental vascularisation could result in abnormal growth and maturation of the foetus. The process by which tissue structure and function are transformed as a result of insults during early development is known as intrauterine programming.

In the current study, three aspects of foetal placental blood vessels were considered: blood vessel density, blood vessel wall thickness and blood vessel diameter. All were measured in control and MPA treated rats and a comparison between the two groups was made. In order to investigate blood vessel density, IHC (immunohistochemistry) for CD34 was performed on placental sections, to visualise blood vessel endothelium. The cycloid test system was in turn used (as by Gambino et al.) to calculate the length density of blood vessels for each placenta and statistical analysis was performed. Both blood vessel wall thickness and diameter were determined using Masson's Trichrome staining.

Although the effects of antenatal hormone treatment have been studied in various rodent models, they are yet to be established in IVF babies. In case treatment is related to low birth weight, this could have a significant impact on future development of IVF procedures.

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

In order to investigate foetal blood vessel density, wall thickness and diameter, IHC and basic staining were performed on pre-cut sections of paraffin embedded placental tissue blocks from control (n=4) and MPA treated (n=5) rats.

Following an optimisation run, 1:5000 dilution of CD34 primary antibody raised in rabbit and goat anti-rabbit IgG secondary antibody were used. The negative control (lacking the primary antibody) remained unstained, while the blood vessel endothelium was localised in the remaining sections. Length density of blood vessels was calculated for each placenta using the cycloid test system. An independent samples t-test was performed, illustrating a significant decrease in blood vessel density in the MPA treated group compared to control ($p<0.05$). This suggests a possible alteration in angiogenesis, normally responsible for maintaining sufficient levels of vascularisation in the growing placenta throughout pregnancy. Therefore, future studies could investigate the distribution of angiogenic factors and their receptors in MPA treated versus control placentae at different stages of gestation, to determine whether progesterone affects blood vessel density by altering angiogenesis and if so at what point in pregnancy the process takes place. Understanding the mechanism behind the reduced density is the first step to preventing and/ or counteracting the effects of increased progesterone levels on placental morphology.

Masson's Trichrome staining was used to highlight the blood vessels to allow measurement of blood vessel wall thickness and diameter. No statistically significant difference was found between the two groups for either of these factors. However, this project focused on examining only foetal blood vessels, therefore, future investigations could explore the diameter of maternal blood vessels instead, since its decrease would lead to reduced foetal blood supply and consequent growth retardation. The morphology of maternal blood vessels in hemochorial placentae (as those of the rat and human), however, renders the determination of wall thickness difficult and possibly non-essential to the issue at hand.

Throughout this project I had the opportunity to experience how research is carried out first hand. I learned various laboratory techniques including microscopy and immunohistochenistry. Moreover, the level of independence I was provided with, gave me the chance to make many decisions regarding how to go about a particular task based on the present literature and previous knowledge. For example, I had to decide which stain was most appropriate for studying blood vessel diameter and thickness or which primary antibody was most suitable for investigating blood vessel density. I also gained a lot of experience in data gathering and analysis. One task, which I found especially interesting and challenging was using the cycloid test system for determining blood vessel density. Lastly, I acquired general knowledge on the topic of assisted reproduction, IUGR and its consequences, placentation and the effects of changes of hormone concentrations on placental vascularisation.

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

Winter 2016

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

It is proposed that we will submit a poster for the Anatomy Society Winter 2016 meeting.

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.

Is there a link between excess progesterone in assisted reproduction and placental abnormalities?

Previous literature has suggested that IVF babies are at greater risk of low birth weight as well as various metabolic conditions (diabetes, cardiovascular disease, obesity) during adulthood. One potential underlying cause is the increased progesterone levels in the maternal environment of hormonally stimulated mothers. A rat model has been developed illustrating that progesterone-treated rats give birth to smaller pups and have smaller placentae compared to controls. The current project focused on investigating histological changes in the placentae following treatment. Blood vessel density was measured using the cycloid test system following immunohistochemistry for CD34, to localise blood vessel endothelium. A significant decrease of density was noted in the progesterone-treated group, suggesting that increased hormone concentration could be responsible for lower blood vessel density, which in turn results in a smaller placenta that restricts foetal growth. Blood vessel wall thickness and diameter were measured following Masson's Trichrome staining but no significant difference was present between the two groups. Future experiments could focus on the mechanisms underlying blood vessel density reduction, such as possible changes in angiogenesis, which will allow to prevent or treat such outcomes. Moreover, further studies are required to determine whether progesterone has the same effects in humans, and if so, this could greatly impact the future of assisted reproduction technologies.

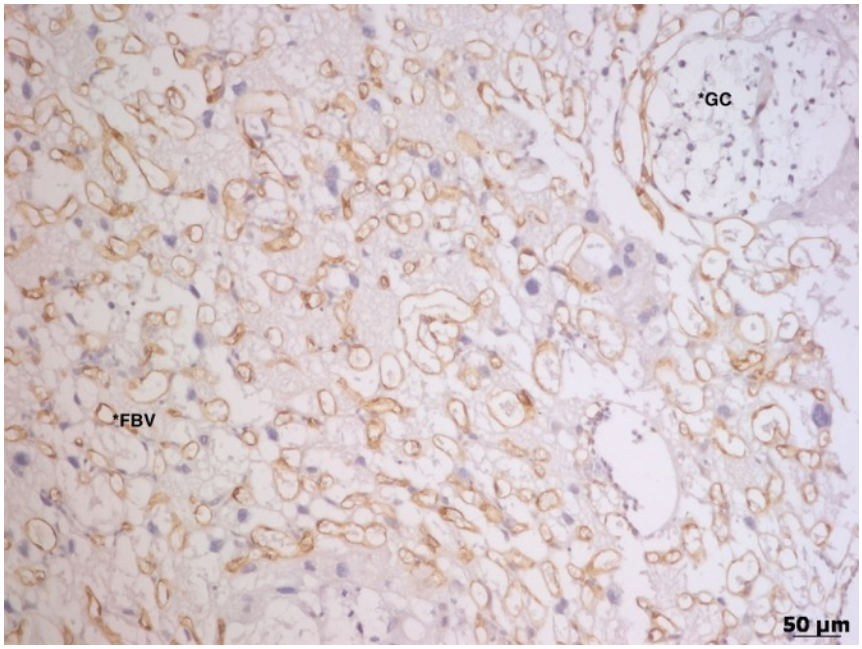

Figure 1. CD34 Immunohistochemistry of Control placenta localising blood vessel endothelium - stained in brown; FBV - foetal blood vessel; GC - glycogen cells

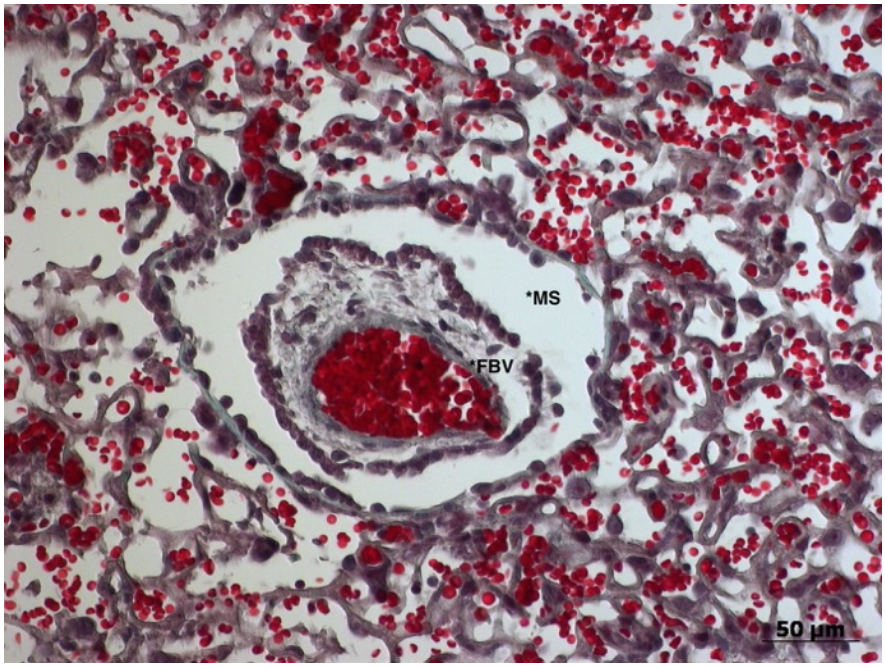

Figure 2. Masson's Trichrome stain of Control placenta; cytoplasm, keratin and muscle fibres in red, collagen in green, and nuclei in black; FBV - foetal blood vessels - filled with red blood cells and visible tunica adventitia in green; MS - maternal space filled with maternal blood (not visible) bathing foetal blood vessels

Other comments: (no more than 300 words)

Signature of student……Date ……

Signature of supervisor…… *……..... Date……*

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