Analysis of Placental Steroidogenesis as a Cause of Recurrent Miscarriage

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Background

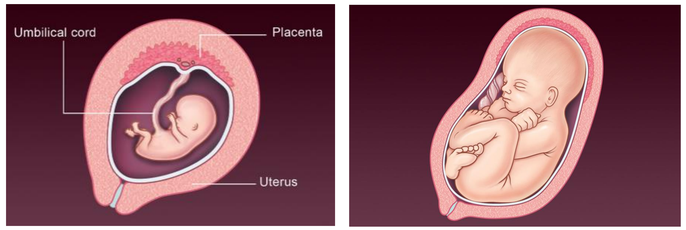

Figure 1: Illustration of a fetus during 10-12 weeks and at 30-31 weeks gestation [1]

- Recurrent miscarriage (RM) is a condition, traditionally defined as the loss of three or more consecutive clinically recognised pregnancies before 20 weeks of gestation
- RM affects 1-3% of couples trying to conceive; factors such as uterine abnormalities, maternal hypothyroidism and parental balanced translocations are associated with this condition
- In approximately 40-50% of these couples, the underlying causes are currently unexplained, but many do go on to have successful pregnancies
- These conditions have a huge psychological and clinical impact on families and couples trying to conceive, especially as the underlying cause is often unknown
- The coexistence of RM and live births in many families suggests a potential association with an underlying recessive, X-linked or imprinted condition
- The main steroid needed to maintain pregnancy is progesterone (P_4). In humans, progesterone synthesis occurs from the maternal corpus luteum in the first 6-8 weeks of gestation at which point the fetally-derived placenta takes over progesterone production ("luteal-placental shift")
- The progesterone receptor (PGR) is the only classic liganded nuclear receptor without an associated disorder in humans

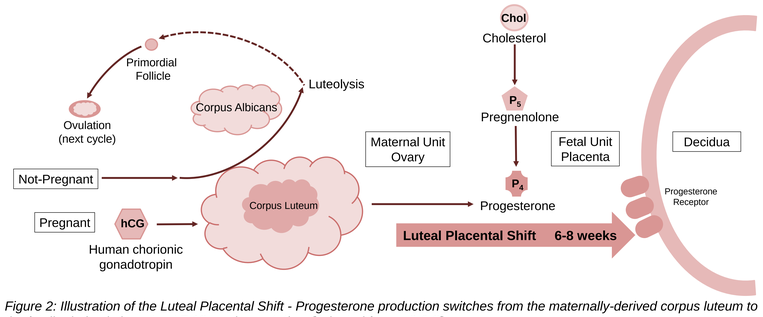

Figure 2: Illustration of the Luteal Placental Shift - Progesterone production switches from the maternally-derived corpus luteum to the fetally-derived placenta at 7-10 weeks gestation [Adapted from – 2 & 3]

Hypothesis

Are There Genetic Mechanisms Disrupting Placental Steroidogenesis That Cause Recurrent Miscarriage?

- Progesterone is a key hormone involved in the maintenance of pregnancy. We hypothesise that defects in progesterone pathway could be a cause of RM where the current aetiology is unknown

Methods

Targeted Sequencing of RM Cohorts and Placental Single Cell Data

- A custom targeted array capture (Agilent HaloPlex) was designed that included up to 257 genes potentially involved in placental steroidogenesis, growth and fetal survival
- Capture libraries were prepared from the following cohorts and underwent next generation sequencing (Illumina NextSeq)
- Using online placental single cell RNA-sequencing (scRNA-seq) data repositories, we short-listed genes expressed in the placenta (in particular, the syncytiotrophoblast cluster) and genes involved in placental steroidogenesis for analysis

Table 1: Recurrent miscarriage cohort characteristics and number of samples used in the study

Recurrent Miscarriage Cohort	Number
Women and their Products of Conception (POC)	19
Couples and their POC	3
Couples	110

Results

Placental Single Cell Data

- Our review of data focused on genes involved in placental steroidogenesis; in particular, the progesterone (P_4) synthesis pathway
- Key P_4 pathway genes were found to be localised to a cell cluster in the syncytiotrophoblast
- The progesterone receptor (PGR) was localised to the maternal decidua, as expected

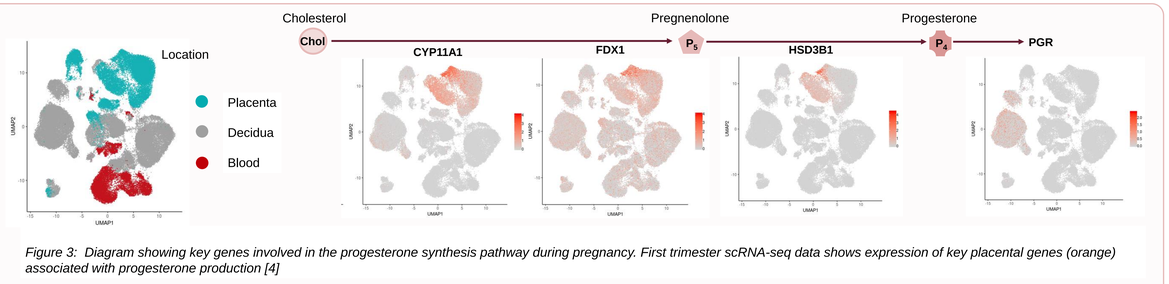

Figure 3: Diagram showing key genes involved in the progesterone synthesis pathway during pregnancy. First trimester scRNA-seq data shows expression of key placental genes (orange) associated with progesterone production [4]

Targeted Sequencing of Couples With Recurrent Miscarriage

- Targeted sequencing data from 110 couples with a history of recurrent miscarriage were analysed for mutations *shared between partners* at variant level, focussing on rare variants ranging from 20% to 0.5% Minor Allele Frequency (MAF) within key P_4 pathway genes
- No likely pathogenic variants were found in key components of the P_4 synthesis pathway

Table 2: Showing the number of couples within the recurrent miscarriage (RM) cohort sharing variants in key progesterone pathway genes, at different minor allele frequencies (MAF)

Gene	RM Couples present at 20% MAF	RM Couples present at 10% MAF	RM Couples present at 5% MAF	RM Couples present at 2% MAF	RM Couples present at 1% MAF	RM Couples present at 0.5% MAF
CYP11A1	1	1	1	1	0	0
FDXR	2	2	2	2	0	0
HSD3B1	2	2	1	0	0	0

Summary

- **The luteal-placental shift is a key event in progesterone synthesis and the maintenance of pregnancy past the first trimester**
- **Defects in core components of placental progesterone production are not likely to be common causes of pregnancy loss in couples with a history of recurrent miscarriage**

References:
[1] NHS England (2018) Your pregnancy week by week - NHS. Available at: https://www.nhs.uk/conditions/pregnancy-and-baby/pregnancy-week-by-week/ (Accessed: 3 October 2019)
[2] Han, K. H. et al. (2013) 'Protective Effect of Progesterone during Pregnancy against Ovarian Cancer', Journal of Cancer Prevention, 18(2), pp. 113–122. doi: 10.15430/JCP.2013.18.2.113
[3] Hennebold JD. Corpus luteum. In: Encyclopedia of Reproduction. Elsevier; 2018:99-105. doi:10.1016/B978-0-12-801238-3.64396-9
[4] Vento-Tormo, R. et al. (2018) 'Single-cell reconstruction of the early maternal–fetal interface in humans', Nature. Nature Publishing Group, 563(7731), pp. 347–353. doi: 10.1038/s41586-018-0698-6

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