

Can progesterone supplementation in early pregnancy affect normal male fetal development?

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Background: It is well known that some adult diseases get “programmed” in utero by fetal exposure to abnormal concentrations of steroids. Threatened miscarriage in early pregnancy is treated by progesterone in many countries around the world even though there does not seem to be any high quality studies on the efficacy and safety of this treatment. Data on what effects progesterone might have on the fetal development is lacking, and since Dr Duncan’s team has previously shown, that exogenous maternal androgens have negative effects on both the fetal pituitary as well as the testes (1), they hypothesized that progesterone, being a steroid hormone, could also alter fetal development and its administration to pregnant women should therefore be researched in more detail.

Methods: This was a pilot project on a small number of pregnant sheep to provide data to inform a larger application for funding. A sheep model of early pregnancy progesterone supplementation was produced (200mg progesterone given by intramuscular injection twice a week from d20 of gestation) and at d75 of gestation the sheep were sacrificed and fetal pituitaries and testes (n=3 for progesterone treated group; n=6 for controls) fixed and frozen for further analysis. Blood sera were also collected and frozen. Samples were analysed by immunohistochemistry, qRT-PCR and ELISA.

Results: *Immunohistochemical analysis* looked at the expression of progesterone receptor (PR), LH receptor (LHR), oestrogen α receptor (ER α), oestrogen β receptor (ER β), androgen receptor (AR) and WT1 in the fetal testes. In the fetal pituitary we looked at LH β and PR expression. PR and LHR could be detected in fetal testis but we did not see any differences in the testes of progesterone treated and control fetuses. ER α and ER β were not detected by our immunohistochemical analysis and WT1 immunostaining was not successful for technical reasons. Interestingly, blinded analysis showed that AR staining was consistently different in progesterone treated animals compared to controls (Fig. 1), with AR being expressed more strongly in the Sertoli cells. In the pituitaries both PR and LH β were detected but we did not observe any difference in staining between the control and progesterone treated animals.

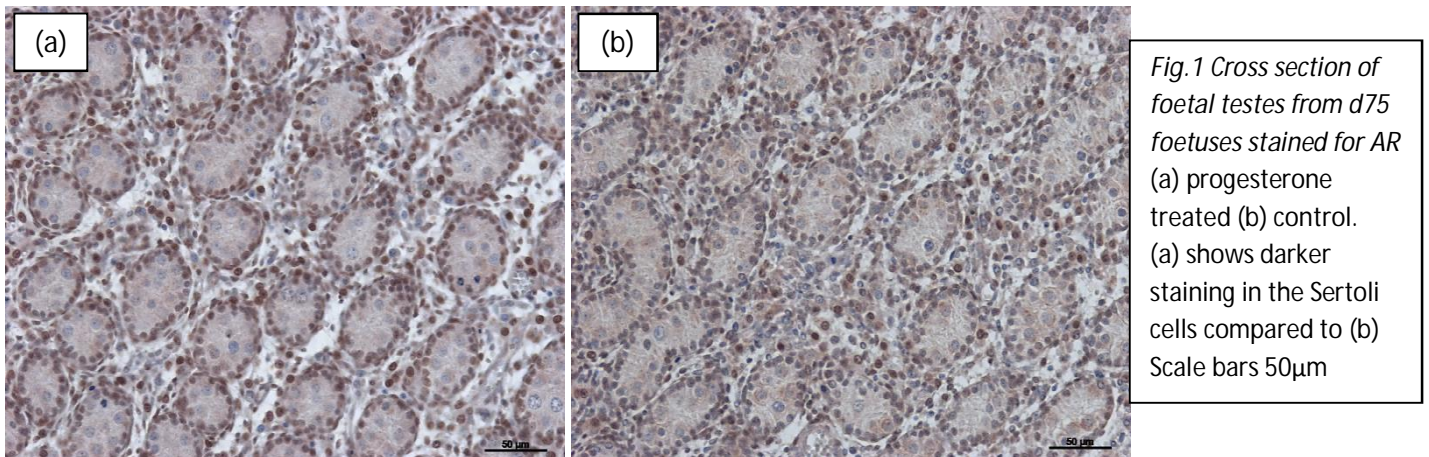


Fig. 1 Cross section of foetal testes from d75 fetuses stained for AR (a) progesterone treated (b) control. (a) shows darker staining in the Sertoli cells compared to (b) Scale bars 50µm

We next used *qRT-PCR* to look at the expression of AMH (Anti Müllerian Hormone), AR, ER β , ER α , LHR, STAR, INSL3 and WT1 in the fetal testes. Unfortunately, we were not able to carry out a statistical analysis on the data obtained, because one of the samples from the progesterone treated group yielded very little RNA compared to the other samples, and thus the PCR results were abnormal. That left only 2 animals in the progesterone treated group, making any statistical analysis impossible to carry out. However, the data gave us a small insight into what might be happening at the testes level. We saw an overall trend of increased expression of AMH, INSL3, ER β , LHR and STAR in the progesterone treated group compared to the controls, suggesting that progesterone might have some effects on the development of the testes. This can be used to determine the potential magnitude of effect to inform power calculations for future studies.

ELISA analysis of the blood sera of the mothers as well as the fetuses (both males and females together) showed, that fetuses from mothers that were supplemented by progesterone had higher blood progesterone levels compared to controls ($p=0.04$, $n=25$, two-tailed t-test), see Fig. 2. This suggests that progesterone must be able to cross the placenta and enter the fetal blood circulation, which is a finding of great importance, proposing that progesterone could directly act on the fetal development by

binding the progesterone receptors. The increase in maternal progesterone blood levels of progesterone treated animals did not reach statistical significance ($p=0.05$), which to some extent would have been expected, since progesterone was given to the mothers only twice a week and could have been therefore possibly metabolised in the meantime.

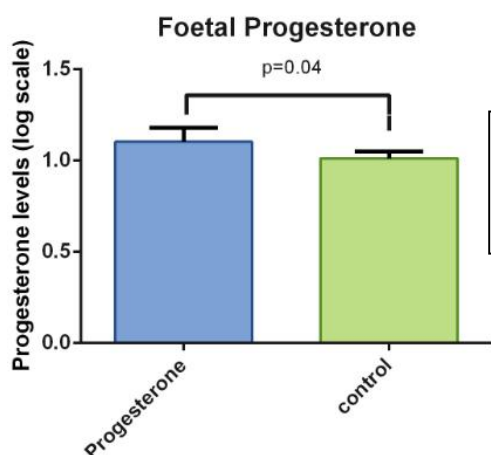


Fig.2 shows foetal progesterone blood levels measured in both male and female fetuses. (N=12 controls and 13 progesterone treated animals)

Conclusions and future work: The main finding of this 8 weeks long project was the discovery of increased fetal blood progesterone levels in fetuses, whose mothers were given the progesterone supplementation from d20 of gestation. This suggests that the progesterone given to mothers somehow crosses the placenta and enters the fetal circulation and can thus possibly exhibit direct effects on the fetuses via progesterone receptors. These were found to be present in small numbers in the fetal testes and also the pituitary; however, it is necessary to confirm this by PCR analysis. Other fetal tissues, such as the brain, adrenal glands, etc. could also be expressing receptors for progesterone and thus progesterone could also affect their function directly. Importantly, progesterone could also possess indirect effects on many different organs and tissues. It would be very interesting to look at the placentas of progesterone treated animals, in order to determine how progesterone crosses it and whether there are any changes to the specific transporters. Since progesterone also plays a crucial role in the production of many other steroids, such as oestrogens, testosterone, cortisol and also the mineralocorticoid aldosterone, it seems important to look at the steroid and mineralocorticoid profiles of both the mothers and fetuses to look at what might happen with the excess progesterone inside the animals.

We discovered some evidence for progesterone altering fetal testicular function. We saw slightly but consistently stronger expression of AR in the Sertoli cells of progesterone treated fetuses and this is something that should be looked at in more detail and using a large number of animals as it might have consequences in further reproductive development of the fetuses. Moreover, looking at the female fetuses would also be intriguing, since they might be more sensitive to the effects of higher progesterone levels due to its normal presence in adult females. Progesterone levels are also tightly linked with the thyroid function of adult females and thyroid hormone is known to cross the placenta and affect fetal development, so it would be a good idea to also look at the levels of thyroid in both the mothers and fetuses to see whether progesterone could influence the fetal development indirectly in this way.

There are many other questions that this research has brought up and it indeed opens a large field of future research that should be carried out, if we are to use progesterone supplementation to prevent threatened miscarriage and want to be sure that this method is safe, efficient and does not have any long-term effects on the health of both the offspring and the mothers.

References:

(1) Connolly F, Rae MT, Bittner L, Hogg K, McNeilly AS, Duncan WC (2013) Excess androgens in utero alters fetal testis development. *Endocrinology* 154:1921-1933.