QRT-PCR analysis of the effect of *in utero* exposure to sewage sludge on steroidogenic gene expression in the ovine fetal adrenal gland

Erin A.M. Cooper[1], Sreedath Reddy[2], Abbie Z. Allenson[1], Duncan P. Cooper[1], Paul A. Fowler[3], Michael T. Rae[2] & Steven D. Morley[1,4]

[1]University of Edinburgh Medical School, UK; [2]School of Life, Sport & Social Sciences, Edinburgh Napier University, UK;
[3]Institute of Medical Sciences, University of Aberdeen, UK; [4]Division of Health Sciences, University of Edinburgh, UK.

Why are endocrine disruptors important?

- Both animal livestock & humans are routinely exposed to a cocktail of environmental chemical pollutants. These include endocrine disrupting chemicals (EDCs) which can disrupt hormone synthesis or signaling[1].
- EDCs can enter the fetal compartment[2], potentially affecting development of fetal endocrine organs[3] & programming of adult disease[4].
- Steroid hormones made in the fetal adrenal gland regulate the oestrogenic milieu of pregnancy, maturation of other fetal organs & onset of parturition, so that altered steroid hormone levels could disrupt these proceses[5].
- EDCs are present in dried sewage sludge pellets, a biosolid by-product of soil water purification, commonly used as a fertiliser on livestock pasture[6].
- Texel sheep were chosen as a large animal model of EDC exposure due to their similar fetal development & gestation period to humans[2,3,4].
- The study aim was to compare steroidogenic gene expression in e110 ovine fetal adrenals from mothers exposed before or during pregnancy to pasture treated with sewage sludge or an organic fertilizer control.

Methods

- *PTCHD1, SHH, STAR, HSD3β2, HSD11β1, HSD11β2, CYP11A1, CYP17A1 & MR* expression in e110 ovine fetal adrenals was determined by QRT-PCR, based on a literature search for key regulatory steps. Normally-distributed data was analysed by one-way ANOVA & Tukey's post-hoc tests to assess significance.

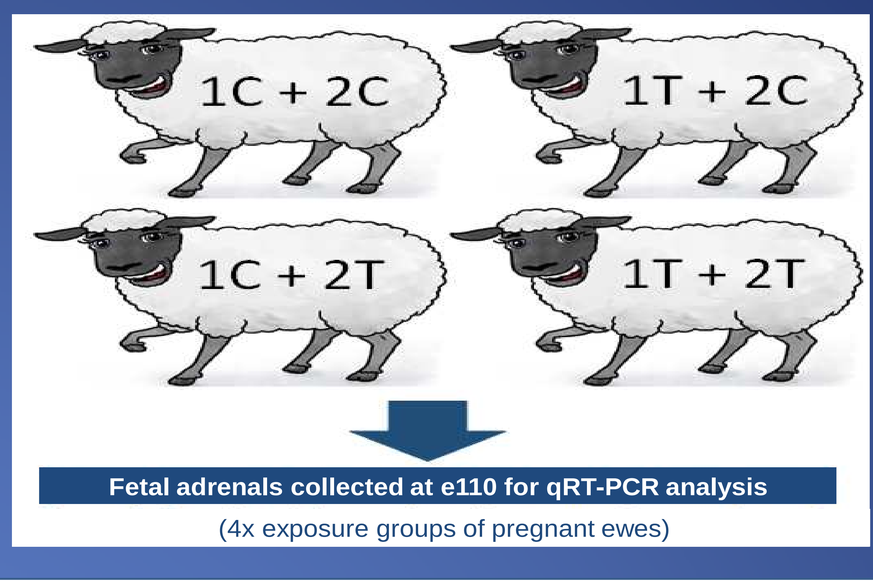
Key:

1. Pre-pregnancy exposure

2. Mid-pregnancy exposure

C. Control (organic fertiliser)

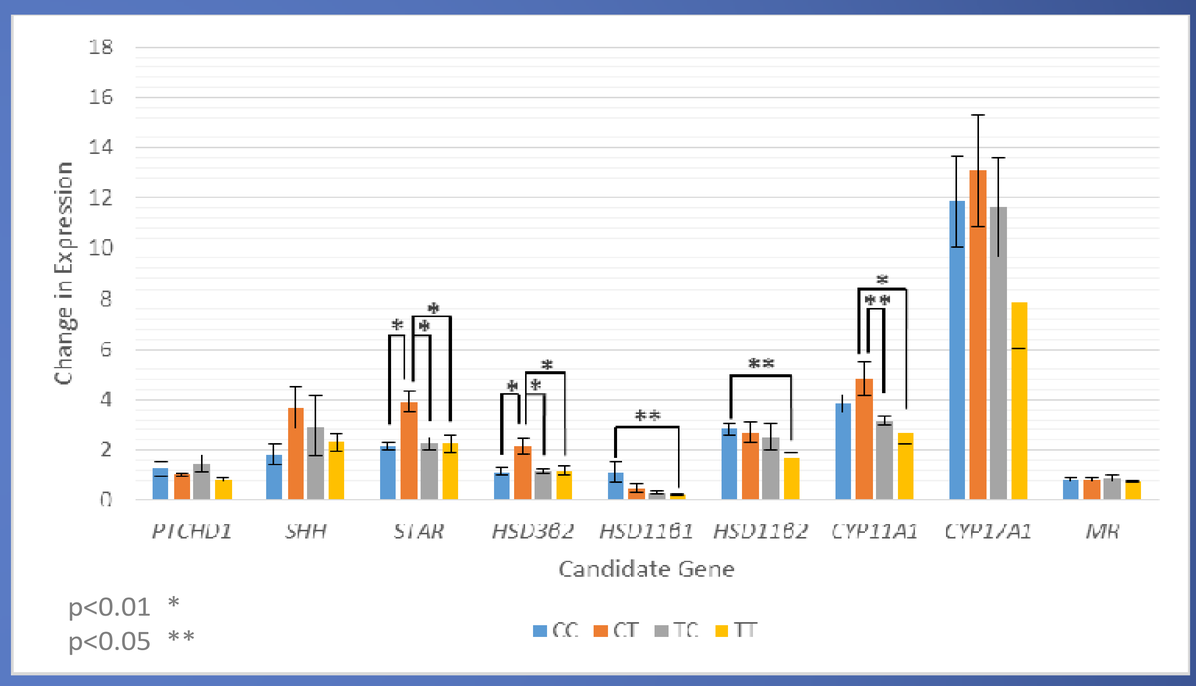
T. Treatment (sewage sludge)



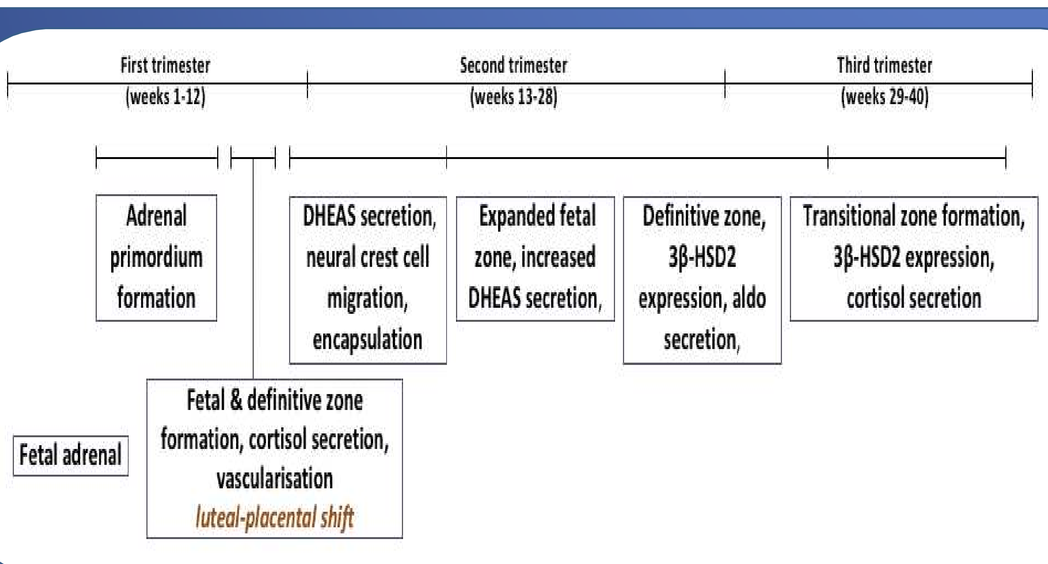
Candidate steroidogenic genes analysed

Candidate genes	Function of encoded protein	Changes in gene expression
PTCHD-1	Receptor for Shh Receptor	No significant change
SHH	Role in development and maintenance of the adrenal gland.	No significant change
STAR	Rate-limiting transport of cholesterol from outer to inner mitochondrial membrane.	Up-regulation in expression (CT vs all other groups $p<0.01$)
HSD3β2	Conversion of: pregnenolone to progesterone 17OH-pregnenolone to 17OH-progesterone DHEA to androstenedione	Up-regulation in expression (CT vs all other groups $p<0.01$)
HSD11β1	Conversion of cortisone to cortisol 11-dehydrocorticosterone to corticosterone	Down-regulation in expression (TT vs CC group $p<0.05$)
HSD11β2	Oxidation of cortisol to cortisone.	Down-regulation in expression (TT vs CC group $p<0.05$)
CYP11A1	Conversion of cholesterol to pregnenolone.	Up-regulation in expression (CT vs TT group $p<0.01$)
CYP17A1	Conversion of: pregnenolone to 17OH-pregnenolone, progesterone to 17OH-progesterone, 17OH-pregnenolone to DHEA (human), 17OH-progesterone to androstenedione (human)	No significant change
MR	Receptor for mineralocorticoids & glucocorticoids.	No significant change

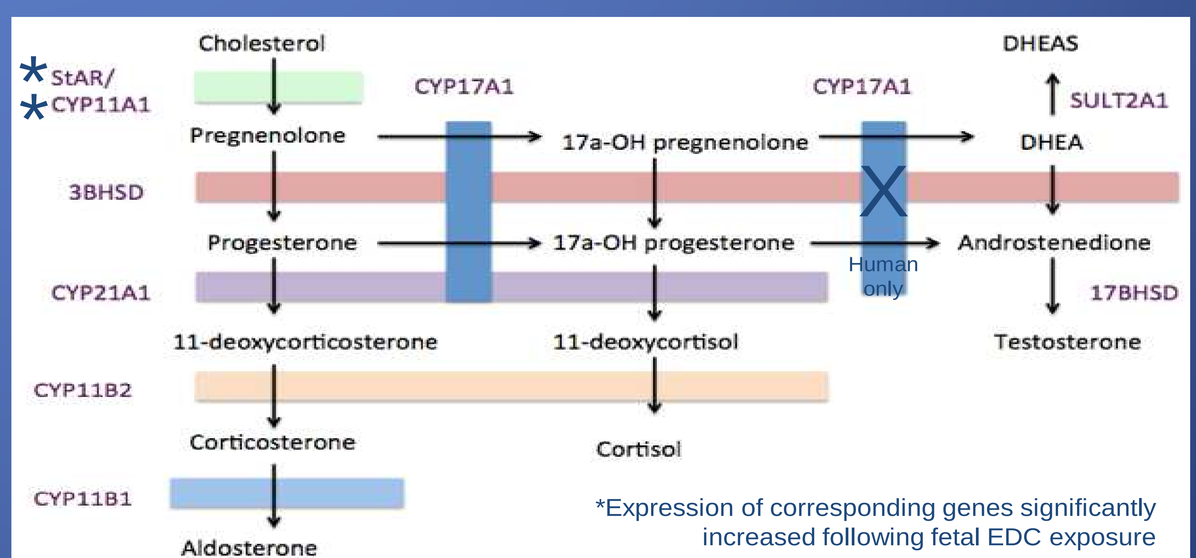
Sewage sludge exposure affects some steroidogenic genes



Human fetal adrenal development



Pathways potentially affected by increased STAR & Cyp11A1 activity



*Expression of corresponding genes significantly increased following fetal EDC exposure

Discussion & conclusions

- *STAR & HSD3β2* are significantly increased in CT vs other groups while *CYP11A1* is also elevated, consistent with SSC-positive cell numbers[7].
- Both *HSD11β1 & HSD11β2* are decreased in TT exposure versus CC control groups, while *PTCHD1, SHH & MR* are unchanged, suggesting they are unaffected by maternal or fetal EDC exposure.
- *STAR, CYP11A1 & HSD3β2 c*atalyse key regulatory or rate-limiting steps in the steroidogenic pathway. EDC-mediated upregulation may lead to increased progesterone & elevated cortisol production in sheep and cortisol & adrenal androgens in humans. Decreased *HSD11β1* may reduce oxidation of cortisol to cortisone, maintaining high cortisol.
- Samples were collected at e110 (ovine term = e144-151) when the fetal adrenal is regulated by pituitary ACTH, but before the normal *prepartum* surge in fetal steroid concentrations.

Discussion & conclusions

- Fetal adrenal steroids (cortisol in sheep, DHEAS in higher primates) play key roles in parturition, driving increases in maternal plasma oestrogen & other factors accompanying the onset of normal spontaneous term labour. Potentially, precocious EDC-mediated elevation of steroids could perturb fetal organ maturation & predispose to preterm birth.

Acknowledgements:
We thank Cathal Grace for technical assistance, the Society for Endocrinology for awards of BES Registration Grants to EC & DC, Wellcome Trust grant 080388 to PAF, Neil P. Evans & Stewart M Rhind for sheep experimentation & tissue collection & the Charlie Walters Bequest Fund to SDM.

References:
(1) Sweeney T. Domest. Anim. Endocrin. 23, 203-209 (2002)
(2) Rhind SM, Kyle CE, Mackie C, *et al*. J. Enviro. Monitor., 12, 1582-1593 (2010)
(3) Bellingham M, Amezaga MR, Mandon-Pepin B, et al., Mol Cell Endocrinol. 376, 156-172 (2013).
(4) Harris A, Seckl J. J. Horm Behav. 59, 279-89 (2011)
(5) Ishimoto H, Jaffe RB. Endocr Rev. 32, 317-55 (2011)
(6) EU Council directive on the protection of the environment, 86/278/EEC (1986)
(7) Buckley CB, Danga M, Hogg K,et al. Endo Abstracts 28, P312 (2012)

BES2018 38--P
Adrenal and steroids
Steven Morley
Poster presented at: SfE BES 2018 19-21 November GLASGOW
PosterSessionOnline.com