SRF Summer studentship report:

The effect of endocrine disrupting chemicals on bovine luteal function.

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Back ground:

The current decline in fertility exhibited by the majority of dairy herds within the UK poses a significant economic problem (Hudson et al., 2010). The decline in fertility can be attributed to a variety of causes. However, one important potential cause for the decline in fertility could be the exposure of cows to endocrine disrupting chemicals (EDCs). EDCs can be of organic or inorganic origin; however those of greatest concern have been produced artificially by man. DEHP and PCB are two EDCs of particular interest due to their effects on reproduction. DEHP is a plastic softener found in PVC, however it is unable to form strong chemical bonds so readily leaches from any products made of PVC (Herreros et al., 2013). A large number of PCB isoforms exist, all of which are known to be very stable in the environment, giving a long time period for exposure to take place (Tavolari et al., 2006). Both of these chemicals have been found in the environment at low levels (Rhind, 2005) and it has been demonstrated that DEHP has the ability accumulate in the soft tissues of many farmed species (Herreros et al., 2013). One of the main routes of exposure for farmed species is through the application of human sewage sludge to pastures for use as a fertiliser.

The bovine corpus luteum (CL) is integral to the establishment and maintenance of pregnancy, being the main structure responsible for the secretion of progesterone following ovulation (Niswender et al., 2000). The rapid growth of this structure from an ovulated follicle is critical to its function (Fraser and Lunn, 2001). This fast rate of growth could not occur without angiogenesis, the development of new blood vessels from existing vasculature (Robinson et al., 2009). Angiogenesis is a tightly regulated process which is mediated by a variety of pro and inhibitory factors.

Project aims:

Due to the importance of angiogenesis in luteal growth and subsequent progesterone secretion we tested for the presence of DEHP and several PCB isoforms in bovine ovaries. We then investigated the effect DEHP could have on angiogenesis and luteal function by utilising a physiologically relevant luteal cell co-culture angiogenesis system *in vitro*.

We hypothesise that dairy cows are exposed to EDCs which are detrimental to luteal function.

DEHP and PCB whole ovary analysis:

Bovine ovaries were collected from a local abattoir and then sent for DEHP and PCB content analysis (James Hutton Institute) as described by Rhind *et al.* 2010. The results of this are illustrated in Table. 1 and Table. 2. Samples had DEHP and a range of PCBs present. DEHP was present in considerably larger quantities than all PCBs, with the mean content of the 4 samples equating to 1.51µg/g dry material of DEHP. PCB 153 was the PCB found in greatest abundance in all the samples (mean 0.04µg/kg), whilst other PCBs were detectable, but at much lower levels. These findings demonstrate that DEHP and PCB have the capacity to accumulate in ovarian tissue following host exposure to the chemicals in the environment. Although, DEHP does this to a much greater extent than PCB. These results are similar; if not slightly lower, than previously reported DEHP concentrations observed in the muscles and livers of ewes grazed on pastures treated with EDC contaminated sewage sludge (Rhind et al., 2005).

Table 1. Results from DEHP content analysis of bovine ovary samples (dry material).

Sample ID.	DEHP µg/g dry material
1186665	1.04
1186666	1.25
1186667	2.41
1186668	1.34

Table 2. PCB content of entire ovarian samples (dry material).

	µg/kg dry material						
Sample ID.	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180
1186666	<0.02	<0.02	0.03	0.02	0.04	0.06	0.04
1186667	ND	<0.02	<0.02	<0.02	<0.02	0.03	<0.02
1186668	<0.02	0.02	0.07	<0.02	0.03	0.03	<0.02

The effect of DEHP on luteal angiogenesis *in vitro:*

The CL was dissected from the ovary and enzymatically dispersed. Luteal cells were washed and isolated using centrifugation. Cells numbers were estimated and then cells were diluted to a final plating concentration of 1x10^5 cells/ml before being added to fibronectin coated glass coverslips in 12 well plates. Cells were treated with DEHP 24 hours after plating, new treatments were administered along with new media every 48 hours, until the end of culture on day 9. DEHP was administered at 1x, 2x and 5x doses, whilst controls were treated with ethanol (Fig.1.). Immunohistochemistry for von Willebrand factor was carried out in order to show areas of endothelial cell (EC) growth. Due to the time constraints of a 6 week project, the area of EC growth was not quantified. However, a generalised increase in EC network size and number was observed following microscopic examination of cultures. This may suggest that DEHP affects angiogenesis by promoting EC growth and proliferation *in vitro*. A

similar increase in luteal angiogenesis *in vivo* would lead to the rapid growth of a mature CL causing an improvement in reproductive performance.

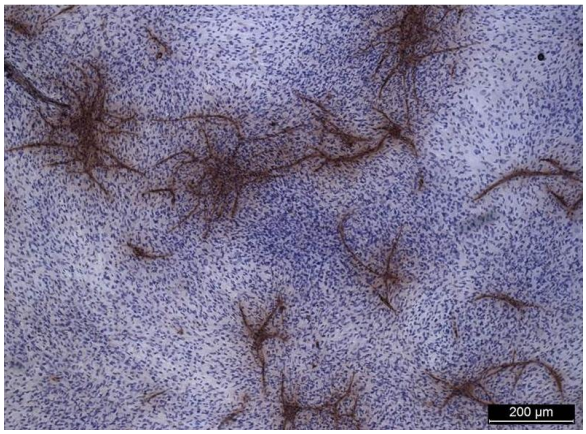

Fig.1. Endothelial cell network formation shown by immunostaining for von Willebrands factor (brown) in a control well after 9 days of culture.

Future work:

Quantification of the effect of DEHP on EC growth will be performed. In addition, progesterone analysis of spent media will be performed to demonstrate the effect of this chemical upon luteal steroidogenic function. Another important focus of future work would be to establish the effects of the different isoforms of PCBs upon luteal angiogenesis.

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