Expression of oxytocin in mouse vaginal cells over the estrus cycle.

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Objective

- Oxytocin is a neuropeptide which is heavily involved in social behavior and bonding[5]
- Some studies have suggested that maternal oxytocin expression may impact offspring behavior[1]
- Oxytocin may also be expressed in keratinocytes[2]
- Oxytocin also acts as a protective molecule during birth, and is heavily involved in multiple other aspects of birth and parenting[6]
- For these reasons, our lab is investigating the expression of oxytocin (OXT) and the oxytocin receptor (OXTR) in murine vaginal cells

Materials and Methods

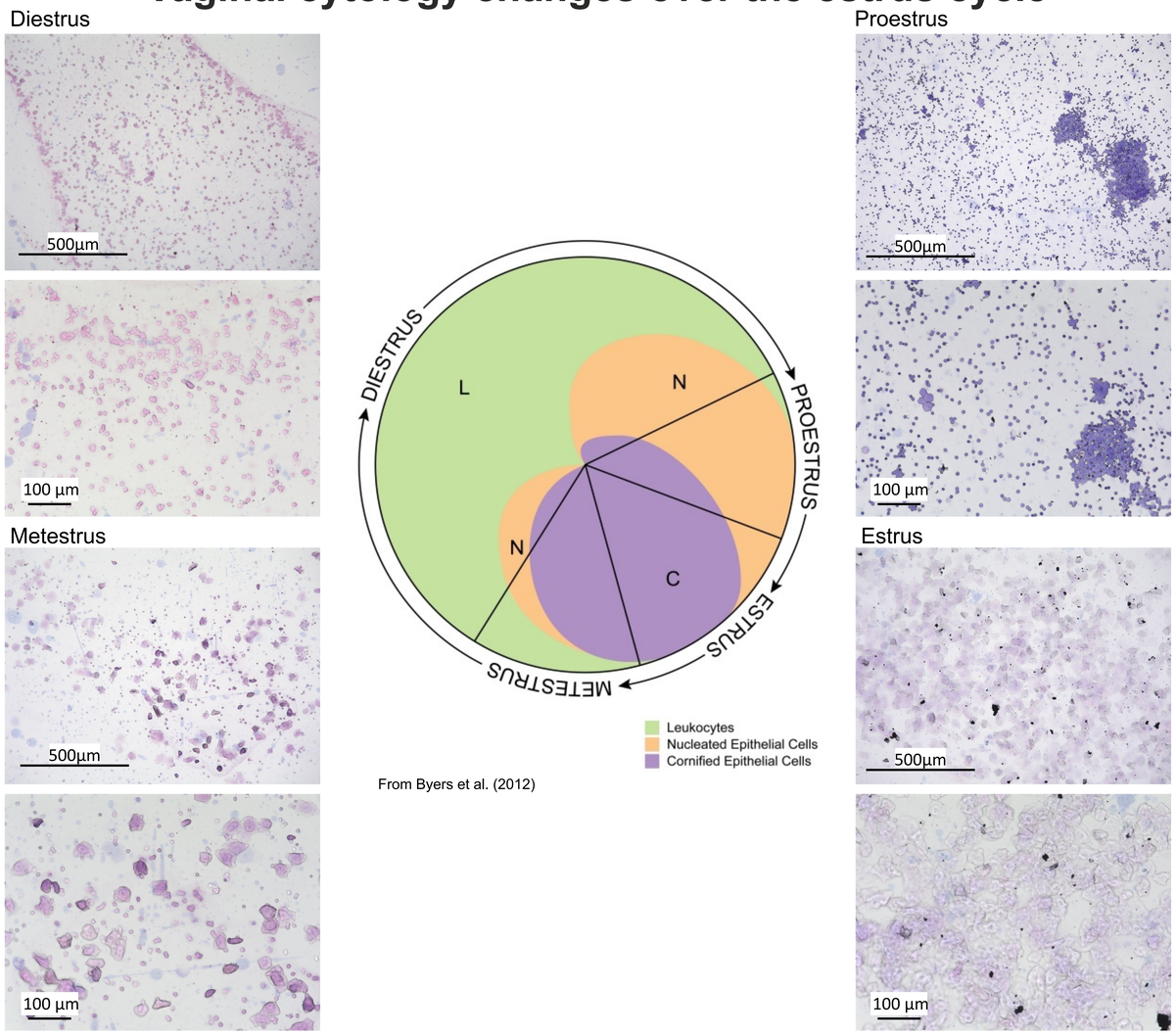
Postmortem vaginal lavage with 1x phosphate-buffered saline (PBS) was used for sample collection. A small aliquot was spread on slides for imaging, and remaining cells were pelleted and frozen for downstream processing.

Cytology was used to asses stage in estrus cycle. Cells from vaginal lavage were spread on a slide, and stained with Giemsa stain. Estrus stage was determined according to the guidelines in Cora et al[1].

RNA extraction was performed following sample collection and cell pelleting using the TRIzol reagent protocol, and **cDNA synthesis** was performed according to manufacturer's instructions.

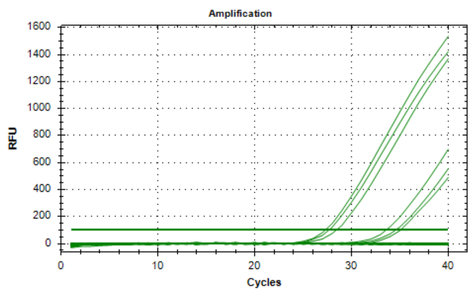
Quantitative RT-PCR was used to assess the expression of OXT and OXTR. Expression of these transcripts were evaluated relative to GAPDH expression.

Vaginal cytology changes over the estrus cycle



From Byers et al. (2012)

OXT and OXTR are not expressed in superficial layers of the non-pregnant vaginal epithelium



RT-qPCR amplification curve for GAPDH, OXT, and OXTR. GAPDH, a housekeeping (control) gene, was the only gene expressed in samples, as shown by the two sets of curves present.

Multiple factors seem to affect overall gene expression

- *Cellularity of samples*: samples with more cells have higher expression of housekeeping genes
- *Stage in estrus cycle*: expression of housekeeping gene is greatest in metestrus, and very low over rest of cycle

Oxytocin does not seem to be expressed in superficial vaginal cells

- RT-qPCR showed only expression of the housekeeping gene, GAPDH
- Neither OXT nor OXTR were expressed at any stage in the estrus cycle

Summary

- Oxytocin does not seem to be expressed in murine vaginal cells
- Oxytocin receptor does not seem to be expressed in murine vaginal cells
- Estrus cycle does not affect the expression of oxytocin in vaginal cells

Implications

- OXT does not seem to be expressed in the outer layer of vaginal cells
- Maternal oxytocin genotype likely affects offspring behavior in another way

Future directions

- The outer layer of the vagina primarily consists of cells which have undergone cornification, and therefore have little functional DNA or RNA; the sample collection method used here would collect mostly cells from this layer
- Future research may benefit from an alternative collection method which would allow for analysis of deeper layers of the vaginal epithelium
- Future research may investigate oxytocin expression during pregnancy and birth, as well as the accumulation of oxytocin peptide before cornification

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