Knock-out of vasotocin reduces reproductive success in female zebrafish, Danio rerio

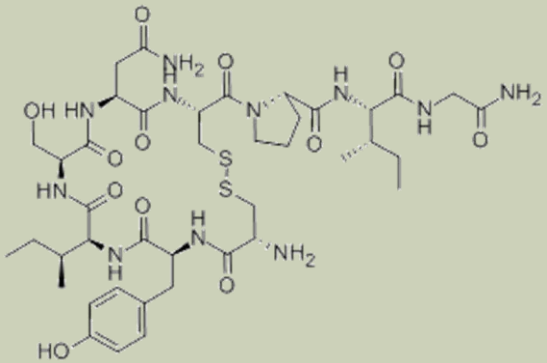
| Kusum Sharma | Divya Ramachandran | Jan Mennigen | Vishal Saxena |

| Department of Biology | University of Ottawa |

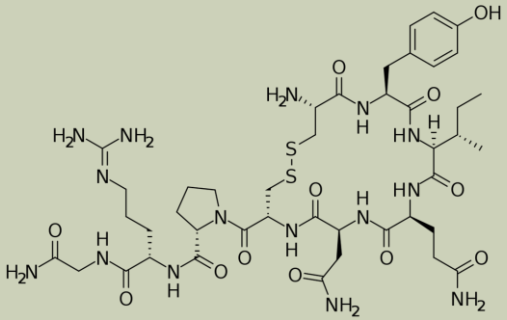
MENNIGEN LAB

uOttawa

BACKGROUND

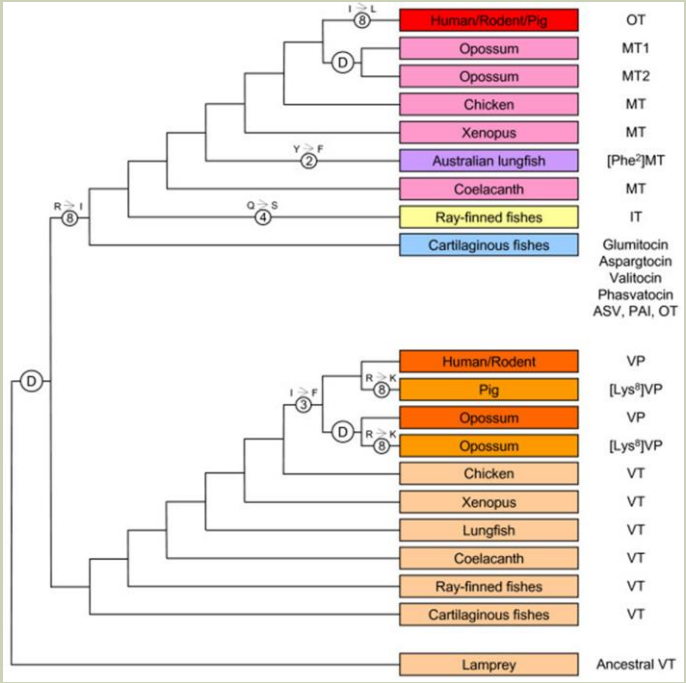


Isotocin



Arginine Vasotocin

Oxytocin and Vasotocin are evolutionarily conserved nonapeptides.[1]



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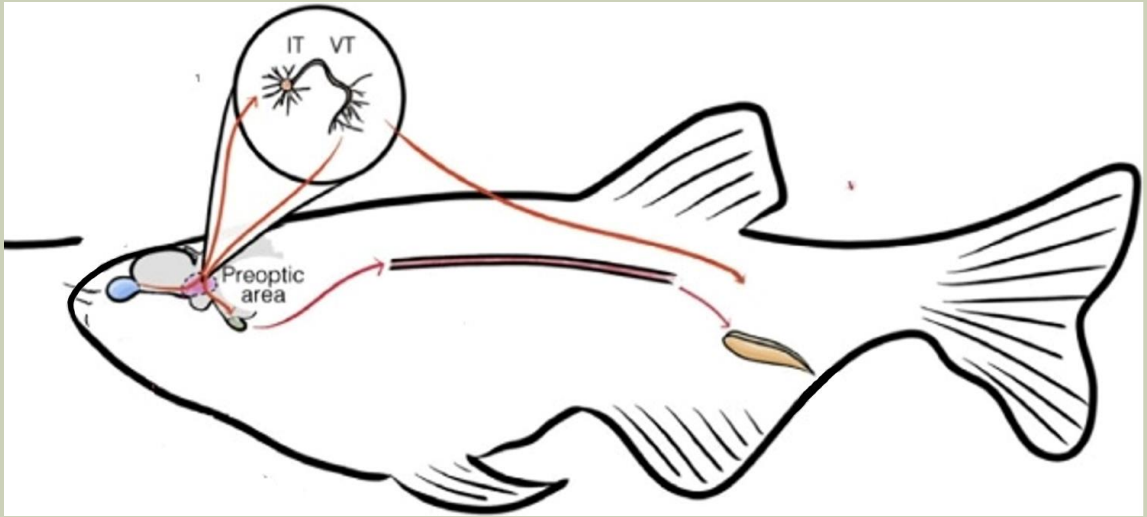
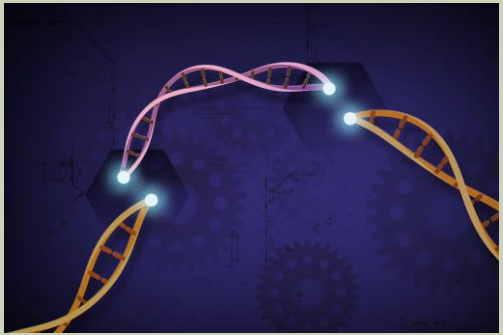


Nonapeptide roles in the Hypothalamus-Pituitary-Gonadal axis.[2]



(avt -/-)

(oxt -/-)

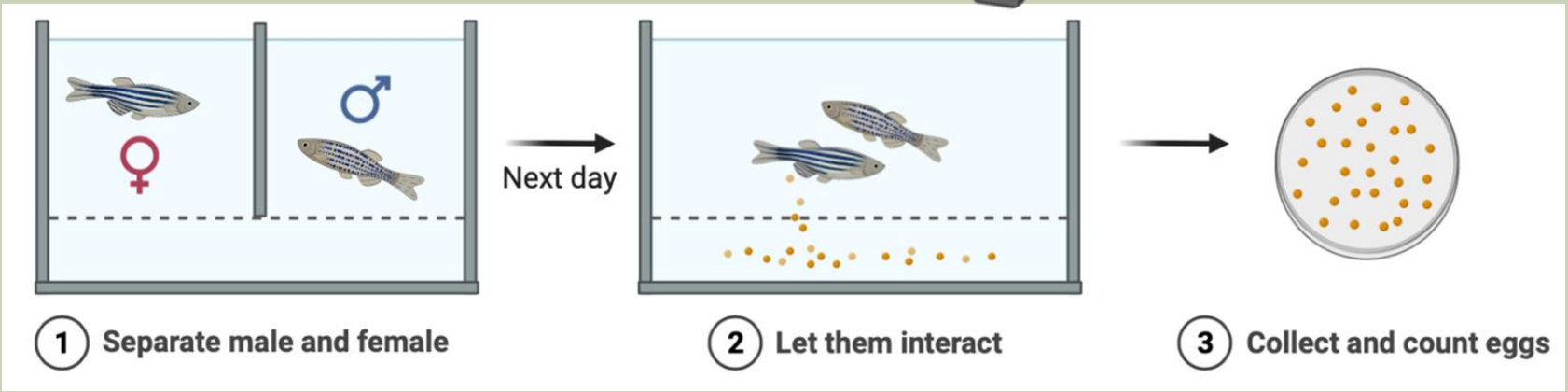
Knockout lines using CRISPR Cas9.

HYPOTHESIS

Arginine vasotocin (avt -/-) but not Isotocin (oxt-/-) affects reproductive success in a female-specific manner.

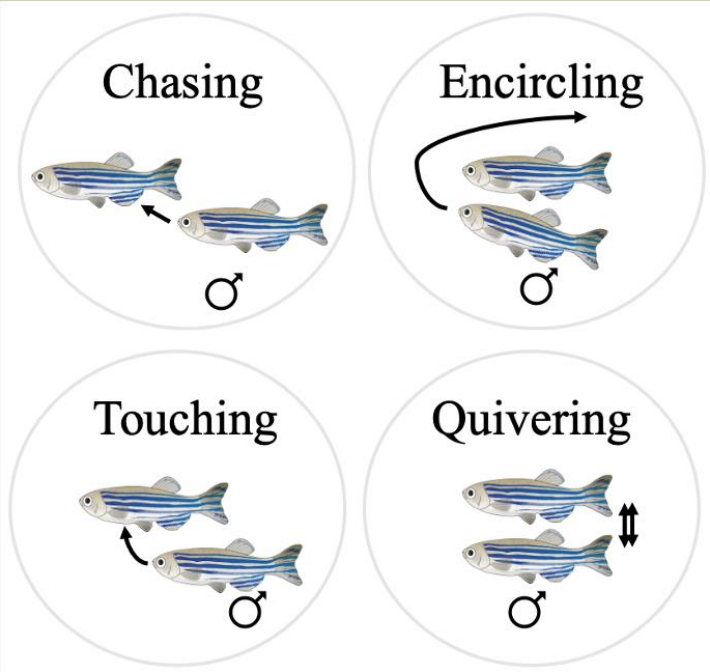
METHODS

A) Mating assays

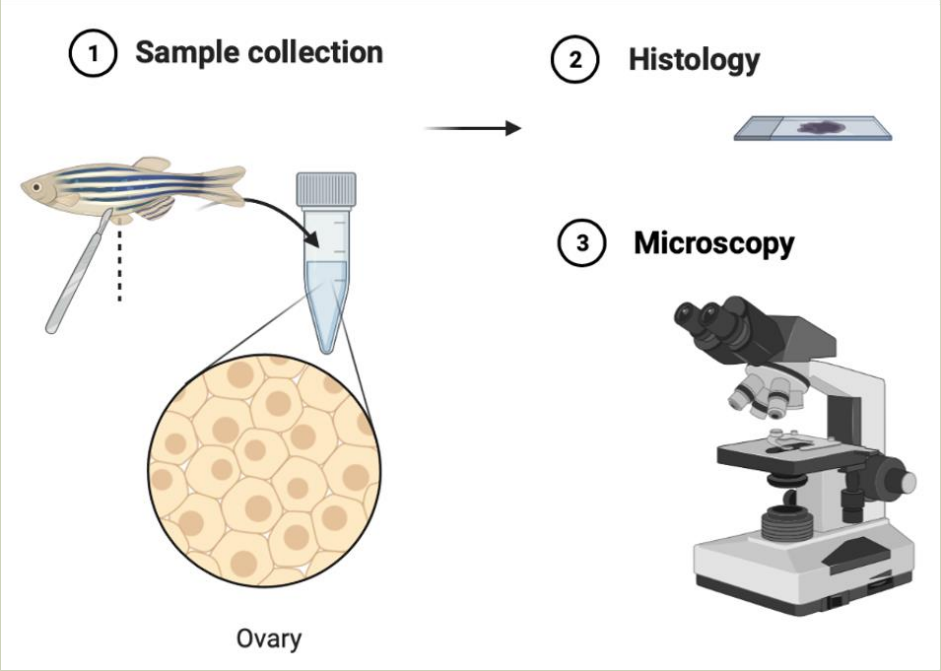


METHODS

B) Zebrafish courtship behaviour

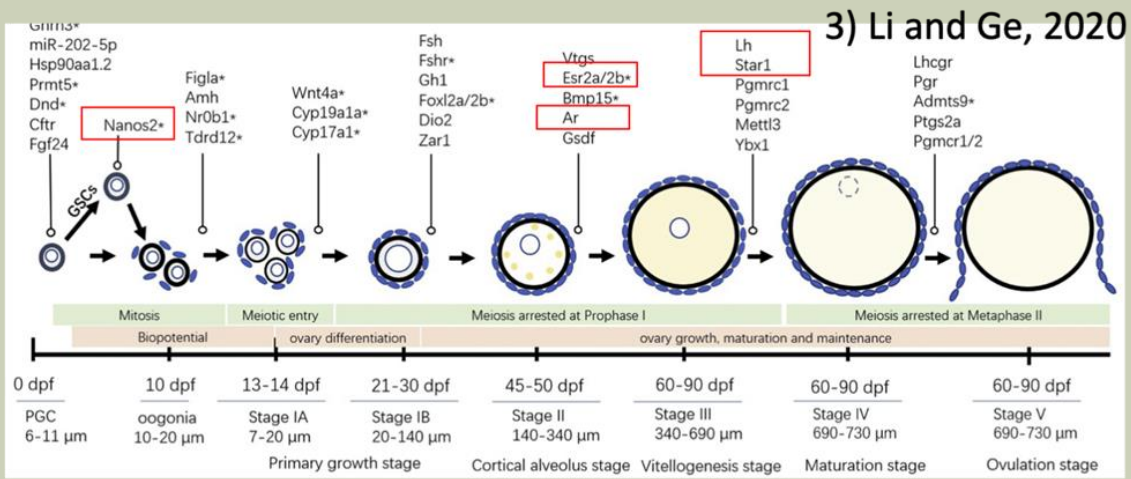


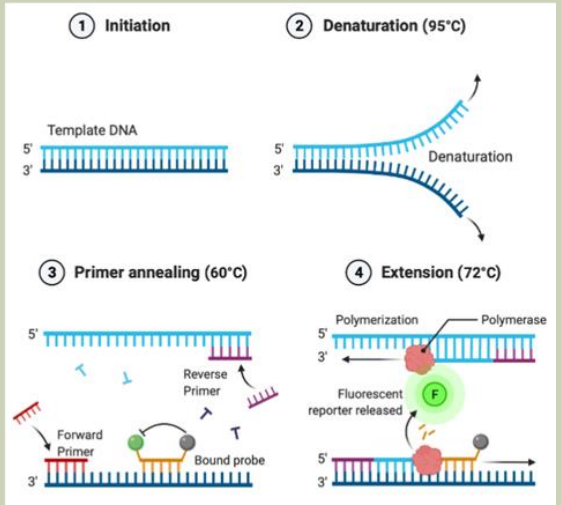
C) Histology



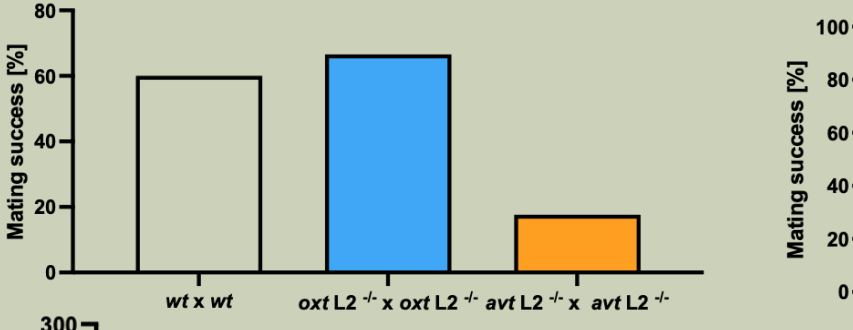
METHODS

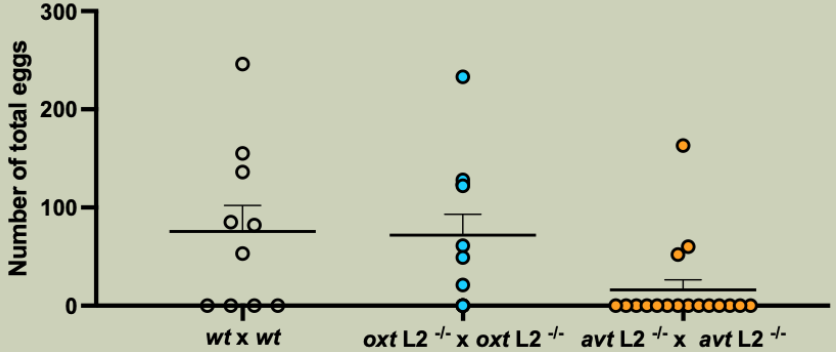
D) Gene expression profiling: qPCR

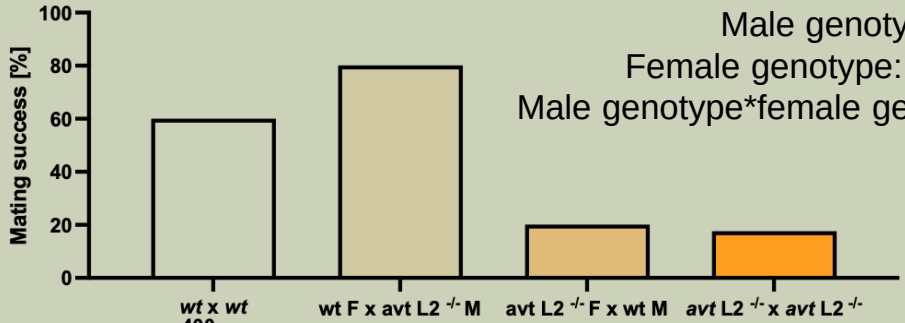




RESULTS







Male genotype: n.s.
Female genotype: P<0.01
Male genotype*female genotype: n.s.

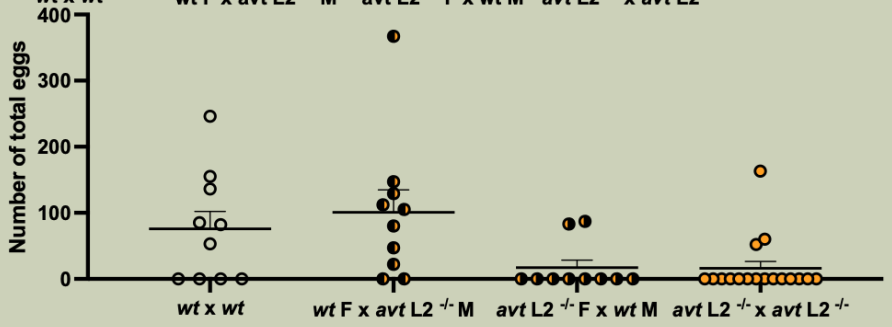
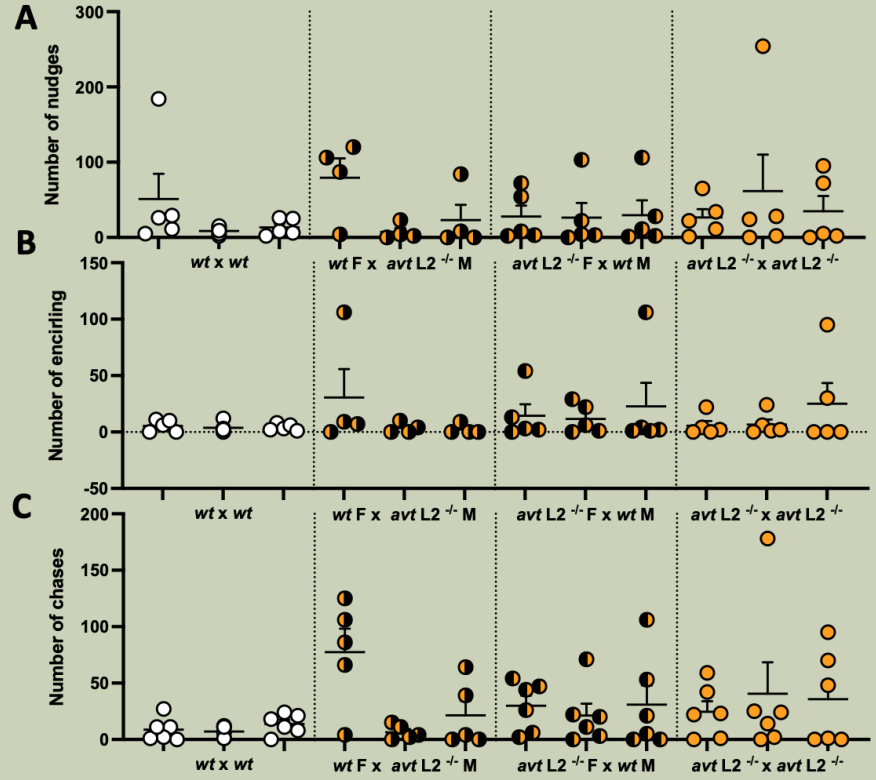


Figure 1. Successful mating trials (A, C) and median number of spawned eggs (B, D) in WT (n= 10), *oxt* L2$^{-/-}$ (n=12) and *avt* L2 $^{-/-}$ (n=17), and backcrosses (n=10) and average values ± S.E.M. indicated. Letters indicate significant differences between groups as determined by Dunn's post-hoc test following significant Kruskal-Wallis omnibus tests.

RESULTS



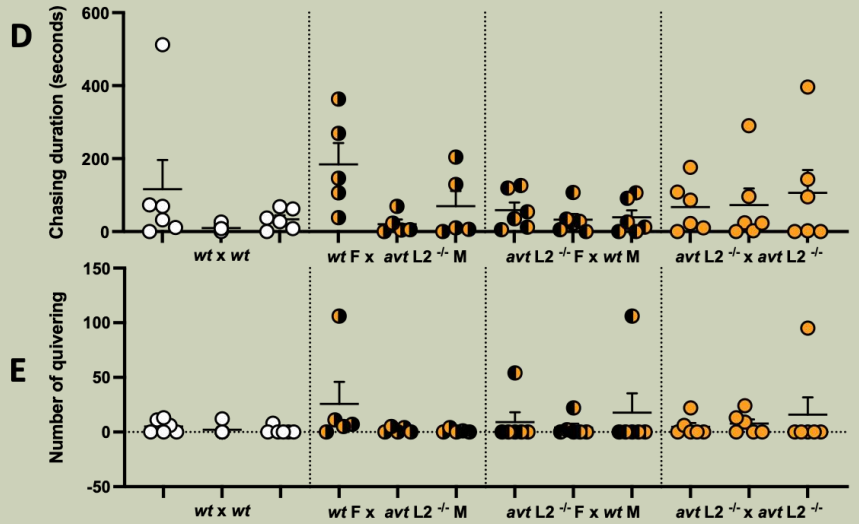
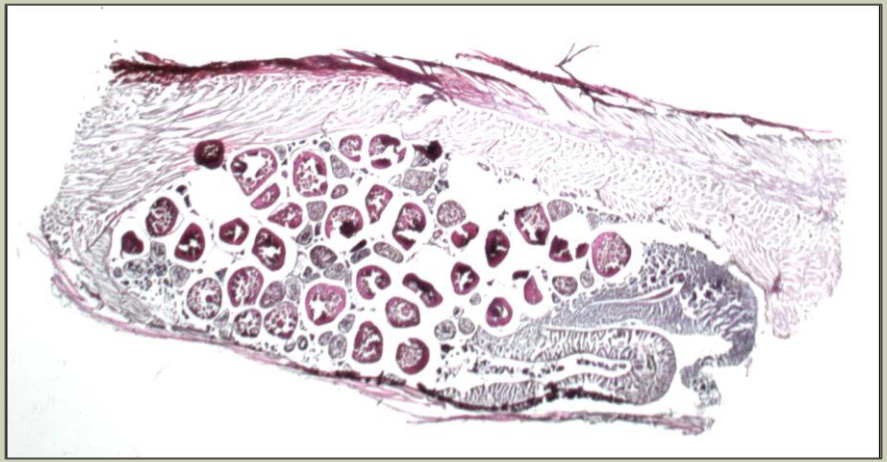


Figure 2. Indices of male courtship behaviours from n = 4–6 trials, of which the first 10 minutes, middle 10 minutes, and final 10 minutes were analyzed. Average values ± S.E.M. indicated

RESULTS



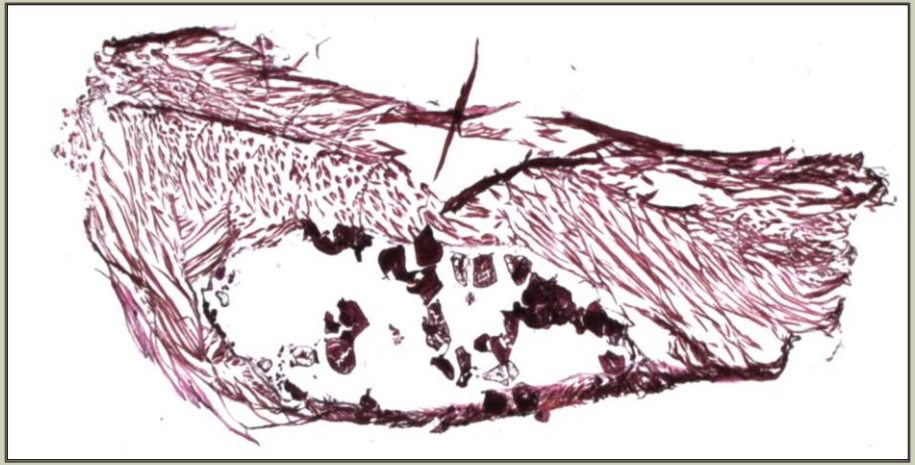


Figure 3. (A) wt and (B) avt -/- ovary sections imaged using a dissection microscope using a 0.67x magnification.

RESULTS

Figure 4. Number of eggs per section in wt and avt-/- (n= 6) as a function of egg maturation stages.

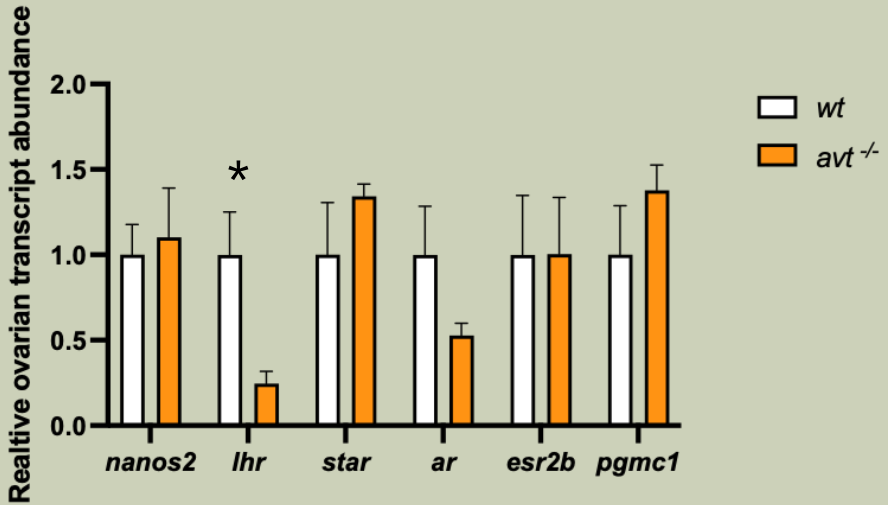


Figure 5. Normalized mean relative ovarian gene expression of oocyte development markers +/- S.E.M. in WT (n=4) and AVT-/- (n=4).

CONCLUSION

- Role of AVT in the female reproduction which can be translated to other fish species.
- Studied behavior, release, and the developmental phenotype - the evidence supports that female avt knockout drives the reduction in phenotype, likely through reduced development of eggs.
- Implications for aquaculture and ecotoxicological research

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2) Altmieme, Z., Jubouri, M., Touma, K., Coté, G., Fonseca, M., Julian, T., & Mennigen, J. A. (2019). A reproductive role for the nonapeptides vasotocin and isotocin in male zebrafish (Danio rerio). *Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology*, *238*, 110333. https://doi.org/10.1016/j.cbpb.2019.110333

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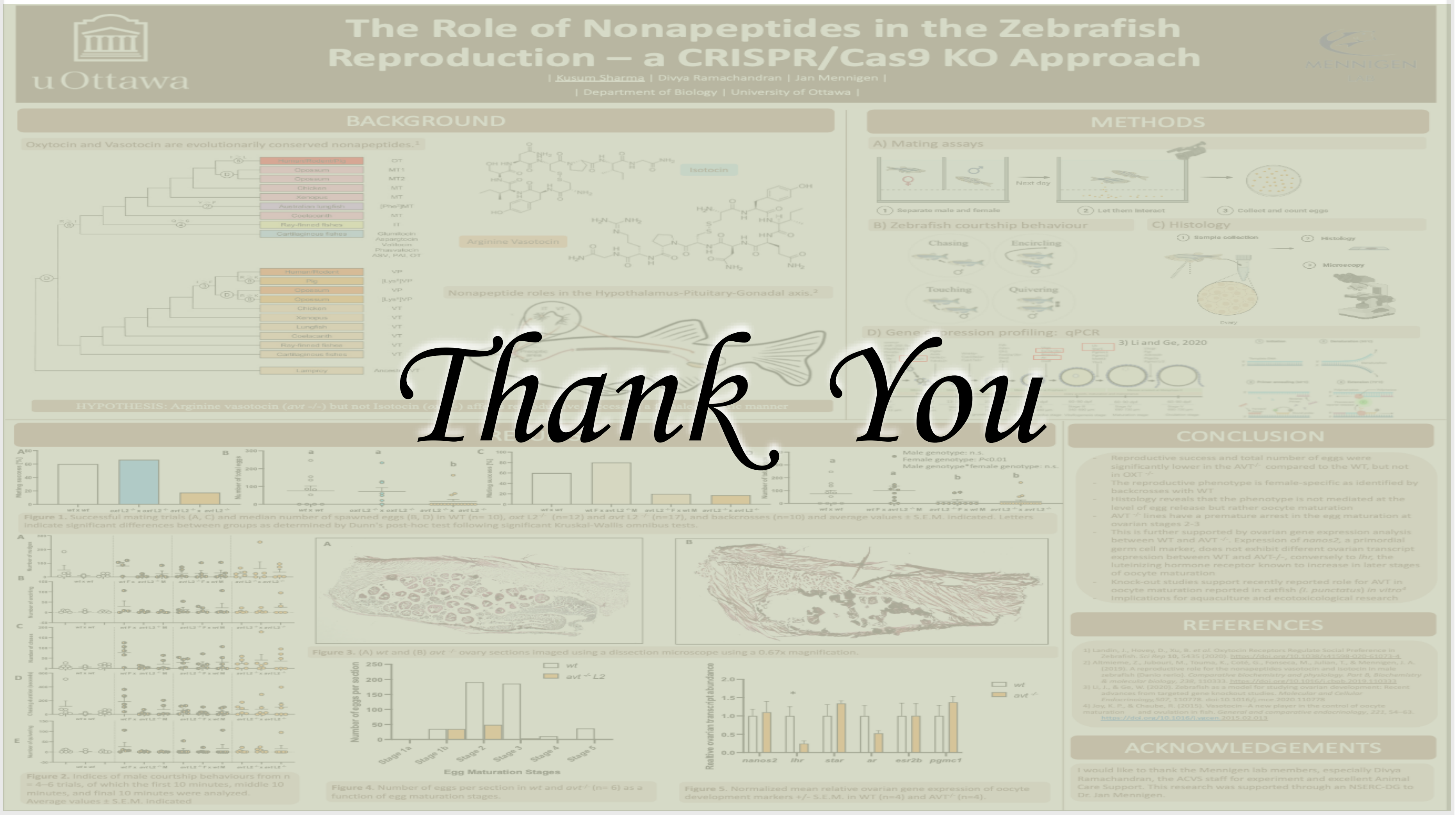
The Role of Nonapeptides in the Zebrafish Reproduction – a CRISPR/Cas9 KO Approach
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B) Zebrafish courtship behaviour
C) Histology
D) Gene expression profiling: qPCR
CONCLUSION
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Egg Maturation Stages

Thank You