GENE EXPRESSION DURING OOGENESIS IN THE MOZAMBIQUE TILAPIA (*O. MOSSAMBICUS)*

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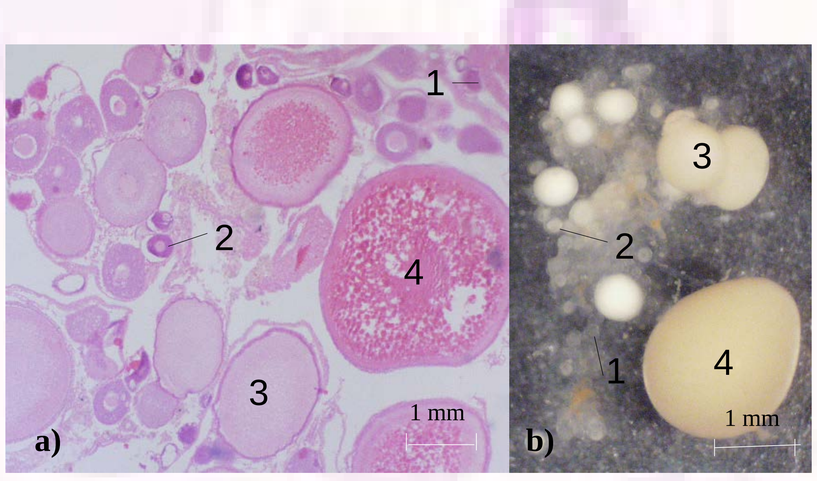
Introduction

Tilapias are teleost fish with asynchronous ovaries, exhibiting variable interspawning intervals (ISI), with average cycles of about two weeks but which vary from a few days to more than one month [3]. Throughout oogenesis a multitude of factors regulate the physiological processes that lead to the fully developed oocytes. Here we present the activity during this process of 3 genes involved in oogenesis:

- *FoxL2*, which is a member of the forkhead family of transcription factors, with functions in both developmental processes and cellular differentiation, and has as known target genes *CYP19* in brain and ovary, *GnRH* in the hypothalamus and *StAR* in the adult ovary. Moreover, it exhibits expression in ovary but not in testes, being involved in ovarian development and function [1,2,5];
- *CYP19a* encodes ovarian aromatase cytochrome P450, the key and terminal enzyme involved in estrogen production from androgen [2];
- *Vas* encodes a putative RNA helicase expressed only in germ cells, as a possible indicator of oocyte quantity [4].

Methods

Ovarian follicles were dissected individually under a microscope and total RNA was extracted from pools of 80-100 of 4 main stages of oocyte development: oogonia, primary oocytes, early vitellogenenic and late vitellogenic oocytes. cDNA synthesis was then carried out. Quantification of gene expression was done by semi-quantitative RT-PCR, having *18S* as reference.



1- Oogonia (SS)
2- Primary oocytes (S)
3- Early vitellogenic oocytes (M)
4- Late vitellogenic oocytes (L)

Fig.1 – (a) Hematoxylin-Eosin coloration of ovary tissue and (b) fresh oocytes under a light microscope. The oocytes were measured in order to create a coherent collection of oocytes in the same stage.

Results

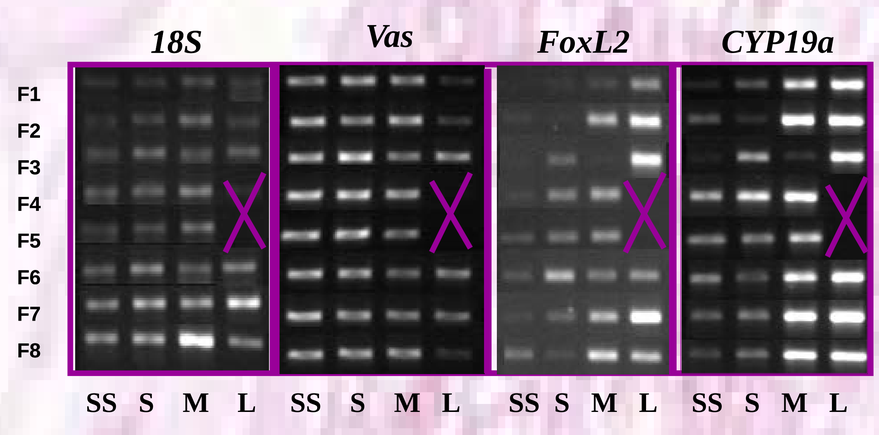


Fig. 2 – Gel electrophoresis of the RT-PCR products for *18S*, *FoxL2*, *CYP19a* *(P450arom)* and *Vas*. Quantification of the bands was done using *BIORAD*'s Quantity One. *FoxL2* and *CYP19a* bands are more intense for vitellogenic stages while *Vas* bands are stronger for the pre-vitellogenic stages.

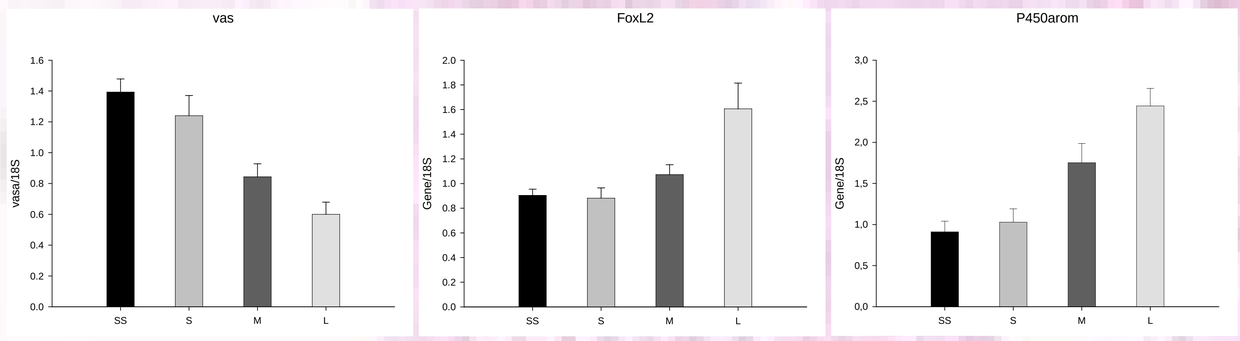


Fig.3 – Expression patterns for *Vas*, *FoxL2* and *CYP19a* (*P450arom*) using as reference *18S* (arbitrary units). The bars indicate the intensity of the bands obtained for each gene in each stage of oocyte development / expression of 18S in those same cells.

Conclusions

Using *18S* gene expression as a reference, the results show a progressive decrease in *Vas* expression (Fig 3). Simlar results have been shown in the closely related *O. niloticus* [4] and this has been attributed to RNA dispersion through the greater volume of the growing oocytes. However, it could also be related to accumulation of maternal *18S* rRNA in the oocytes, or to a *de facto* decrease in *Vas* gene expression. If the former hypothesis is confirmed *18S* may no be appropriate as reference in RT-PCR. If the second, *Vas* cannot be used to standardize germ cell number. In any case it is clear that *FoxL2* and *CYP19a* have increased mRNA expression during vitellogenesis. This is consistent with previous work showing *FoxL2* and *CYP19a* expression to increase with follicle development [1,2], in particular with the increased levels of 17β-estradiol produced in vitellogenic follicles of various teleosts. Further work is required to know whether *Vas* or *FoxL2* are regulated by hormones and what is their specific role in the ovary. The question of what reference RNA should be used for standardization of RT-PCR needs to be addressed.

Bibliographic references

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