A quick method for an easy identification of ovarian structures: the use of autofluorescence

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INTRODUCTION

Estimation of fish stock reproductive potential has been of increasing importance in recent years. It involves the estimation of several key parameters in a routine basis, such as potential and/or batch fecundity, spawning fraction, atresia… To achieve this routinely it is highly recommended the use of methodologies that reduce time for estimation, such like stereology.

Some of these parameters need a prior identification of the presence of POF structures within the ovary in order to estimate fecundity. However, it requires important skills as very often it is hard to be distinguished from late alfa- or beta-atresia.

In this context it is very important to develop quick diagnosis techniques that reduce the chance of error on identifying both types of structures. In routine histological procedures is common the use of autofluorescence stains, such as eosin. Previous studies have shown the use of fluorescence with eosin allows distinguishing better these structures.

Masson´s trichrome, Voff III, Metanil yellow, Arteta´s trichrorme,pas are specific stain. They have to solutions autofluorescence.

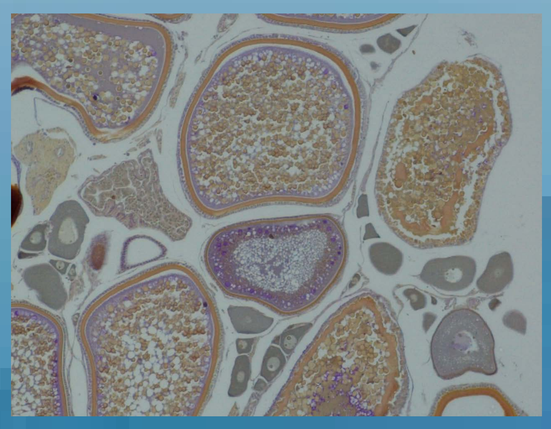
MATERIAL AND METHODS

Ovaries of differentes spices fishs (cod, hake, sebastes, gallo) were sectioned (3m) and stained with Vof III, Metanil Yelow, Arteta´s trichrome, Masson´s trichrome, H&E, eosin, pas and more stain

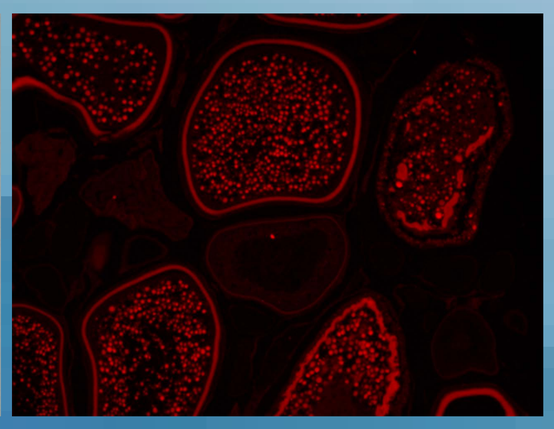
Images of sections were taken in a Nikon Eclipse 90i using brigth field ilumitation and direct fluorecence microscopy, using aB-2 A filter set 450-490nm and aG-2 A filter set 545-590?nm

RESULTS

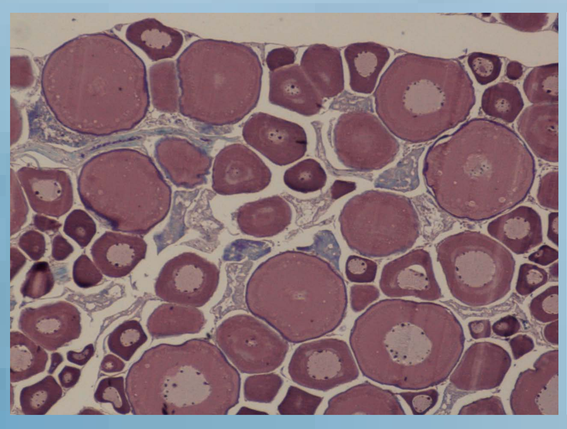
Differentation between POF and atresia



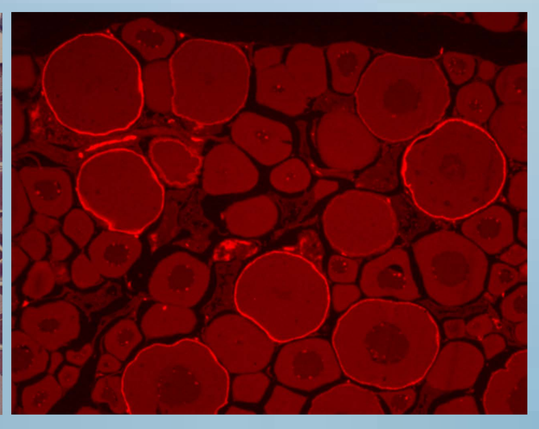
F1.Metanil yellow (hake)



F2.



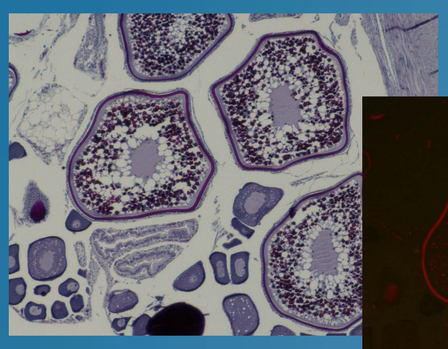
F3.Vof III. Cod



F4.

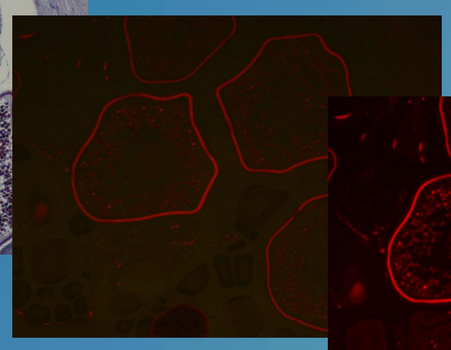
The old yolk drops there are to difficultd recongnize using bright field ilumination with rutin stain H&E. The samples are staining with Metanil Yelow (F.1) and vof (F3) We can see to better the identification structure. When the sample is estudied under the fluorescence microscopy with green filter they were clearly recongnize .B- atresia (flecha roja) and a pof (flecha azul).

Detection of oocyte edges

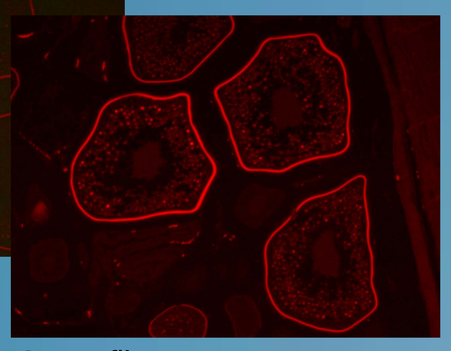


F5. Masson´s Tricromic

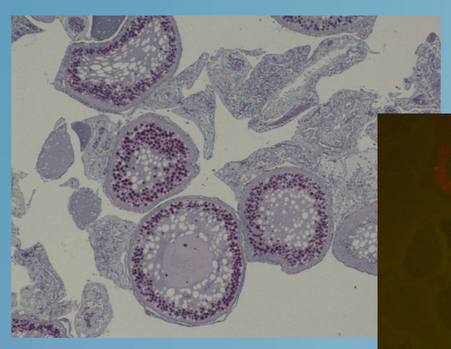
hake



Blue filter

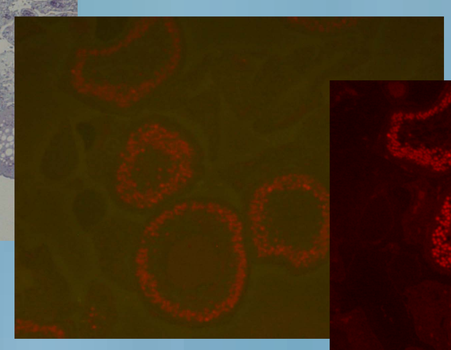


Green filter

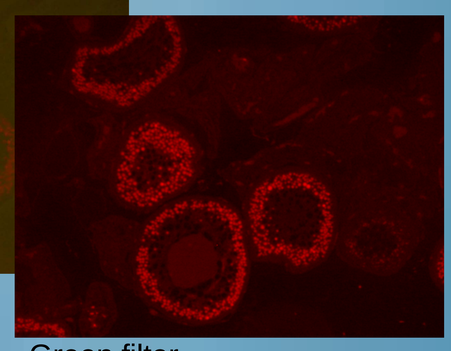


F6.Masson´s Trichormic.

Sebastes



Blue filter



Green filter

Masson´s tricromic in hake stain very well the oocyte membrana and vitellgenic (F5) .Under fluorescence microscopy with green filter the samples are clearly recognize while the blue filter don´t work as well as green.The green filter has to more intensity than blue. The picture Masson´s tricromic with green filter have to contrast and they hep us to automatic deteccion. While Masson´s tricromic in sebastes don´t work as well as in hake.

CONCLUSSIONS

-Are there ***stain*** to better the structures identificationin using bright field ilumintationn (b-atresia- pof)

-The ***fluorescence*** help us to better recognize the structures with the differents filters

-In every ***spice*** there to play with the staining and fluorescence ***filters***

- In the fluorescence have a lot of contrast in the sample. This hep us to ***automatic deteccion*** in the oocytes

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4th Workshop on Gonadal Histology of Fishes

16-19 June 2009