APOPTOSIS IN REGRESSING FOLLICLES FROM *Solea solea* AND *Gadus morhua*

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Introduction

In order to measure individual egg production (realised fecundity) in wild fish we need to determine the fate of vitellogenic follicles as they regress into post ovulatory or atretic follicles. The cellular process underlying follicular regression is thought to start with the production of apoptotic nuclei and this was investigated using the TUNEL reaction and by measuring short chain oligonucleotides (180-200 base pairs).

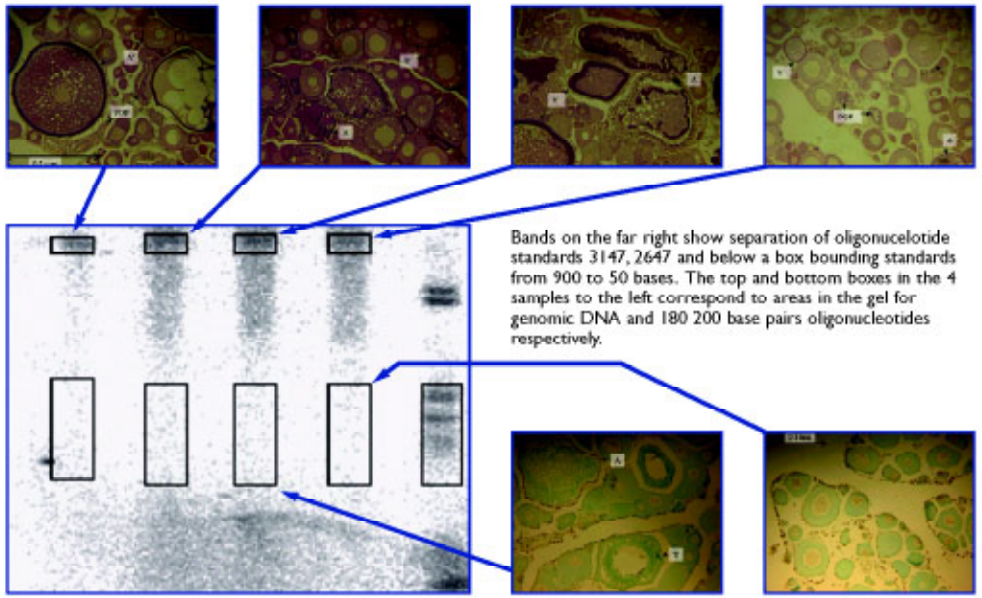
Experiment 1

Method

Ovary samples from wild *Solea solea* caught towards the end of the spawning season were processed to prepare histological sections stained by PAS Mallory and TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling). The latter was performed using an *In Situ* Cell Death Detection Kit. (Roche Biochemicals) in accordance with the manufacturer's instructions and counter-stained with 1% methyl green. The effective suppression of endogenous peroxidase was investigated by a negative control (no reaction enzyme) after incubating with 3% hydrogen peroxidase in methanol. DNA was also extracted from the ovaries followed by electorphoretic size fractionation of 3'-end-labelled oligonucleotides on agarose gels.

Results

Three examples below (from left) show sections of ovaries where 0, 14 and 26 % respectively of vitellogenic (V) follicles were in various stages of atretic (A) or post ovulatory follicle (POF) regression. The section on the right was from a spent fish. Arrows point to results of the electrophoresis for each ovary and below for TUNEL.



Bands on the far right show separation of oligonucelotide standards 3147, 2647 and below a box bounding standards from 900 to 50 bases. The top and bottom boxes in the 4 samples to the left correspond to areas in the gel for genomic DNA and 180 200 base pairs oligonucleotides respectively.

Only the spent ovary contained detectable quantities of short chain oligonucleotides by electrophoresis even though there were many regressing follicles in different stages of breakdown. The TUNEL showed abundant apparently apoptotic nuclei (dark brown spots) but they were not associated particularly with atretic follicles.

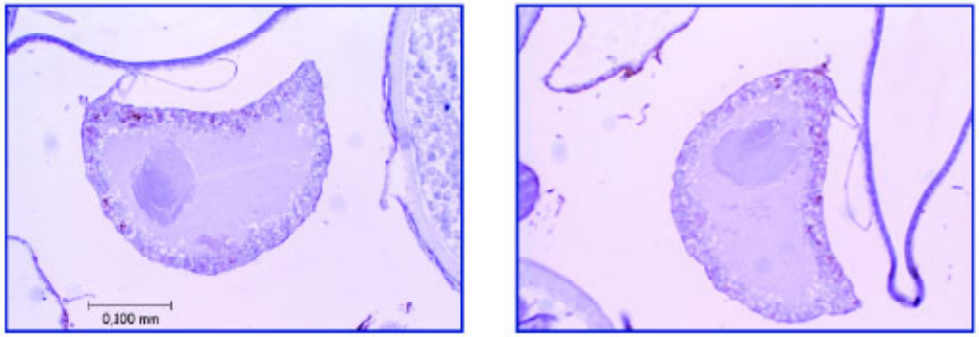
Experiment 2

Method

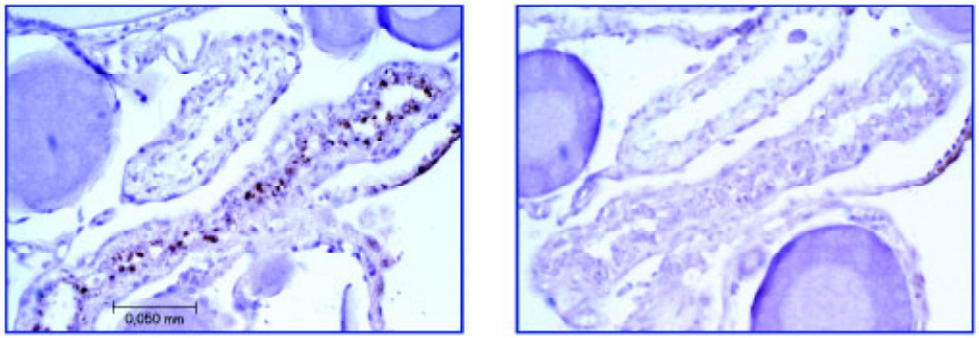
Ovary samples were removed immediately from humanely killed captive cod at intervals during the batch production cycle to study the production of apoptotic nuclei in atretic and post ovulatory follicles. After 1 minute microwave antigen retrieval in 0.01M citrate buffer wax sections of these samples were stained by TUNEL using the same procedure as in Experiment 1 and counter-stained with Haematoxylin.

Results

Atretic follicles were stained by TUNEL both in the normal and negative control stained section (right).



Post ovulatory follicles were stained by TUNEL in the inner granulosa layer of the POF and this was mostly absent in the negative control stained section (right).



Conclusions

Apoptosis occurs in the teleost ovary as part of the cell turnover process and in regressing post ovulatory follicles but the situation in atretic follicles is less clear. Endogenous peroxidase confounded the results and needs to be excluded by optimising the assay conditions to suppress their activity.

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