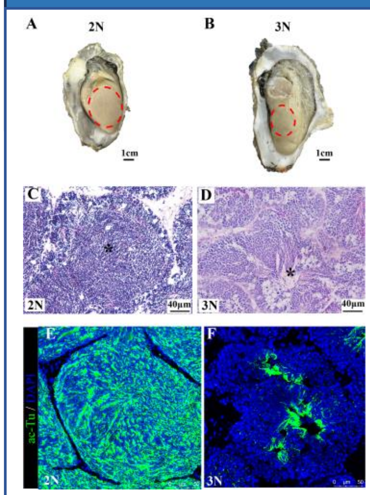
# Studies on the mechanism of spermatogenesis arrest in triploid Pacific oysters

Chen Chen
Fisheries College, Ocean University of China

## Abstract

Spermatogenesis is a complex development process. Meiosis in diploid animals is already an intricate process. The challenges intensify in triploid cells. In this study, histological analysis and immunostaining revealed a markedly low mount of mature spermatozoa in triploids, indicating a severe blockade in spermatogenesis. Ultrastructural analysis and apoptosis analysis demonstrated that triploid males underwent normal spermatogonial mitosis. Analysis of phosphorylated histone H2AX (γ-H2AX) displayed numerous γ-H2AX staining in developed triploid gonads, with a significantly higher number of apoptotic cells confirmed by the TUNEL assay. These findings suggest a potential arrest at the zygotene stage, leading to cell apoptosis. Flow cytometry confirmed spermatozoa from triploids containing approximately 1.5 times the DNA content of spermatozoa from diploids. The sperm head size and flagella length in triploids surpassed those in diploids. This study provides novel insights into the blocked spermatogenesis in triploid oysters, emphasizing the importance of sterility in aquaculture practices.

## Result 1 - Impaired spermatogenesis resulting from triploidization



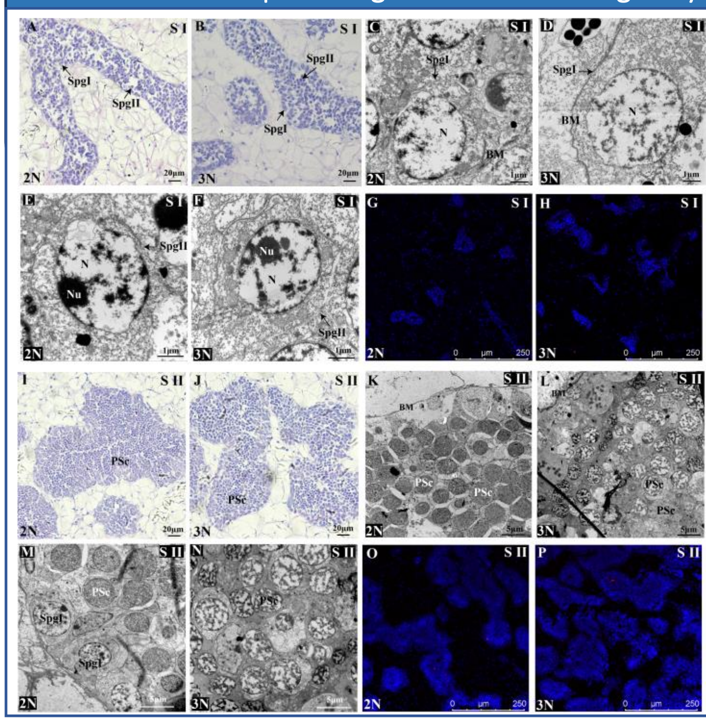
(A-B) Anatomical examination of fully developed gonads suggests that triploids exhibited a scarce presence of gonadal tubules on the gonadal surface.

(C-D) H&E staining shows triploid males display very few mature spermatozoa.

(E-F) Confocal images show sperm flagella staining in mature diploid and triploid males, visualized with an acetylated alpha-tubulin (ac-Tu) antibody.

These findings collectively establish a robust blockade in spermatogenesis in triploid oysters.

## Result 2 - Normal spermatogonial mitosis during early spermatogenesis in triploids

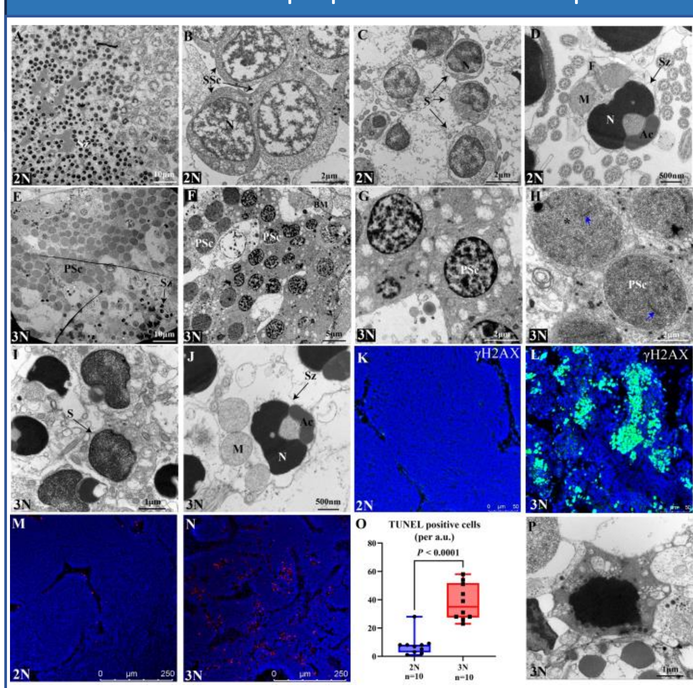


(A-F) Light microscope and electron microscope allow us to distinguish two types of spermatogonia and reveal no morphological difference between diploid and triploids.

(I-N) The gonadal tubules enlarge due to mitotic divisions of type II spermatogonia, giving rise to numerous primary spermatocytes.

(G, H, O, and P) The TUNEL assay results further support the normalcy of early spermatogenesis, with almost no abnormal apoptotic cells observed at the early spermatogenesis stages.

## Result 3 - Meiotic prophase I arrest and spermatocyte apoptosis in triploids



(A-D) At the mature reproductive stage, diploids exhibit gonadal tubules primarily composed of spermatozoa. Minimal secondary spermatocytes and spermatids are observed.

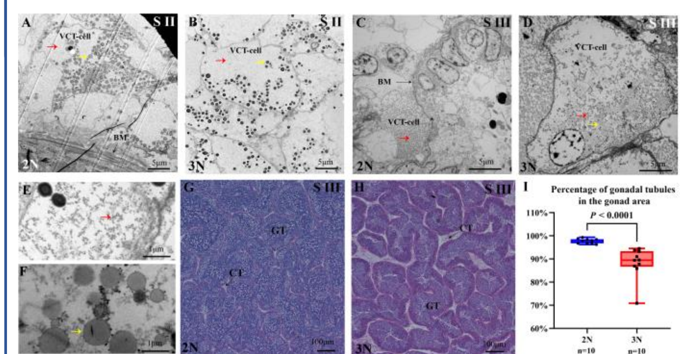
(E-J) Triploids display a distinctive composition, featuring numerous primary spermatocytes and a limited number of spermatozoa.

(K-L) Analysis of phosphorylated histone H2AX (γ-H2AX) reveals minimal positive signals in the follicular edge of mature diploid gonads. In contrast, triploids exhibit numerous γ-H2AX signals, indicating an inability to complete the zygotene-pachytene transition.

(M-P) Meiotic arrest commonly leads to germ cell apoptosis, a programmed cell death process. TUNEL assay results confirmed a significantly higher number of apoptotic cells in the gonads of mature male triploids compared to their diploid counterparts.

## Result 4 - Limited gonadal tubule dilatation in triploid male gonads

In oysters, the gonad is a diffuse organ comprised of numerous gonadal tubules enveloped by connective tissue characterized by vesicular-connective tissue cells (VCT-cells).

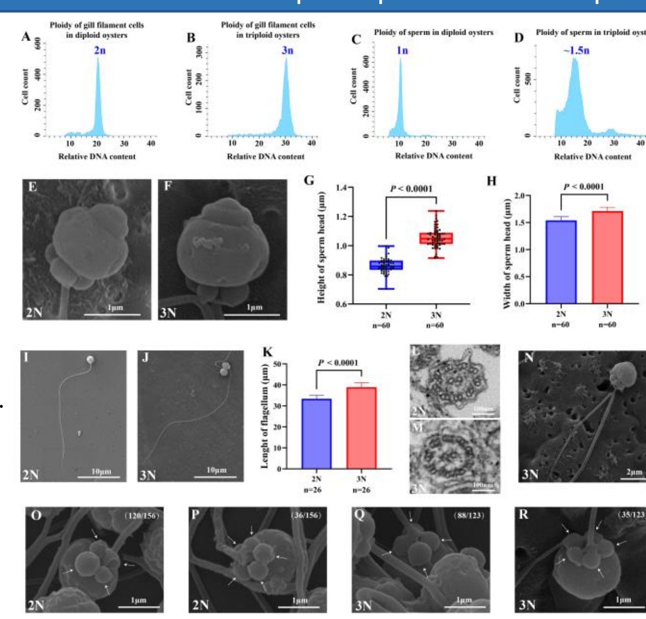


(A-D) TEM micrograph of VCT-cells.

(E-F) The cytoplasm of VCT-cells exhibits varying numbers of glycogen particles (red arrow) and lipid droplets (yellow arrow).

(G-I) The percentage of gonadal tubules in the gonadal area in triploid males was significantly lower than in diploid males.

## Result 5 - Production of abnormal aneuploid spermatozoa in triploids



(A-D) Flow cytometry analysis indicates an approximate DNA content of 1.5n in triploid spermatozoa.

(E-H) The size of the sperm head in triploid oysters is significantly larger than that in diploids.

(I-K) The flagella length in triploid oysters exceeds that in diploids.

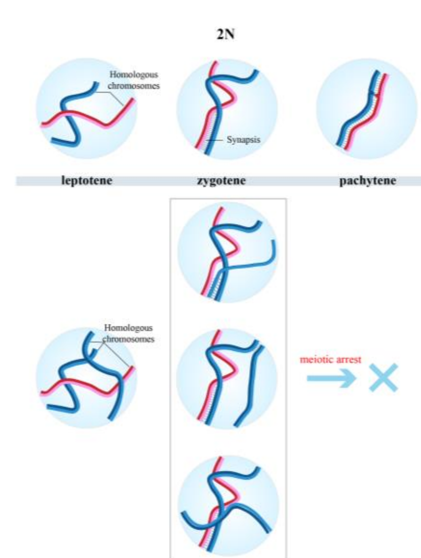
(L-M) The flagellum structure of sperm exhibits the typical 9 + 2 microtubule arrangement.

(N) Two abnormal, two-tailed spermatozoa are observed in all triploid samples.

(O-R) The number of spermatozoa mitochondria in both diploid and triploid oysters ranges from four to five.

## Discussion

We propose a possible explanation for meiotic arrest in triploid male oysters.




(1) Multiple SC may be possible, i.e., synapsis involves three lateral elements and one central element lying side by side.

(2) Another proposed meiotic abnormality is the occurrence of complex synaptic interactions, where three chromosomes pairwise associate at different points along their lengths during zygotene. These synaptic multivalents are called partner switches.

(3) There is a possibility that synaptic multivalents do not occur at zygotene. In this scenario, the two sets of homologous chromosomes form bivalents during meiosis, while the third set of homologous chromosomes remains unpaired.

chenchen920@stu.ouc.edu.cn