



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Chromosome manipulation

Dr. Amrutha Gopan
Assistant Professor
School of Fisheries
Centurion University of Technology and Management
Odisha

Centurion University of Technology and Management
Odisha



Centurion
UNIVERSITY

Shaping the Future
Empowering Communities...

The changes in the number of chromosome sets is brought out by destruction of one set, as in egg or sperm cells or by the disruption of the metaphase spindle during karyokinesis in either somatic or germinal cells.



METHODS OF CHROMOSOME MANIPULATION

Centurion
UNIVERSITY

*Empowering Lives
Empowering Communities*

1) Inactivation of gametes

Irradiation of spermatozoa with gamma radiation, X-ray or U-V light or dimethylsulphate destroys the genetic material without inactivating the spermatozoa.

UV irradiation form pyrimidine dimers in DNA leading to its genetic inactivation.

The optimum dose of UV varies with spermatozoa concentration.



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

De-chromosomed spermatozoa activates the egg followed by shock treatment (to prevent second polar body release), and establishes XX condition.

To induce androgenesis, the maternal (egg) genome is inactivated by irradiation and fertilized with normal sperm.



Centurion
UNIVERSITY
Shaping Lives..
Empowering Communities...

Fish eggs - difficult to manipulate - large size.

Fish spermatozoa - easy to manipulate - small size.

Gynogenetic, androgenetic individuals - haploids with low survival rate.

Diploidization by shock treatment improves the survival rate.



2) Shock treatment

Ploidy induction - multiplication of the chromosome set during embryonic development, by insemination, release of polar body and first-cell division.

Manipulation is done by application of thermal or temperature (cold and heat), pressure or chemical shock.

Shocking of inseminated fish egg causes depolymerization of tubulin polymers that form microtubules essential for formation of spindle apparatus.



Centurion

UNIVERSITY

Shaping Lives...

Empowering Communities...

- ❑ Shock treatment results in the inhibition of spindle formation and aster movement.
- ❑ When heat shock is applied shortly before first cleavage, cytokinesis is inhibited and cause zygotes to undergo two genomic replications with only one cytoplasmic division.
- ❑ This is necessary for diploidizing gynogenetic and androgenetic offspring and in inducing triploidy and tetraploidy.



Centurion
UNIVERSITY
Thrive & Live...
Empowering Communities...

THERMAL SHOCK

Cold shocks for cold water species (salmonids) - 0°C

Cold shocks for warm water species (Common carp, Tilapia and Indian major carps), 8-12°C.

Heat shock for cold water fishes around 26-28°C.

Heat shock for warm water fishes 39-42°C.



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

PRESSURE SHOCK

- Simple to administer.
- Pressure range varies between 7000 to 9000 pascals (Psi).
- The hydrostatic pressure is applied by French Cell Press designed by mechanical engineering method.
- Less side effect than the thermal shock.



Centurion
UNIVERSITY

*Slapting Live...
E... ..*

CHEMICAL SHOCK

Colchicine and cytochalasin-B disrupt cell division and induce ploidy induction.

Anaesthetics such as nitrous oxide and Freon 22 induce triploidy. But the results are inconsistent and unsatisfactory.



DETECTION OF POLYPLOID INDIVIDUALS

Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities.*

1) Chromosome analysis

- Simplest, appropriate method for determining the ploidy level.
- Very much labour intensive , time consuming.



Jai Group of
INSTITUTIONS
UNIVERSITY
Shaping Lives
Empowering the Future

ii) The ploidy level can be distinguished by measuring and comparing the nuclear volume and cell volume of the erythrocytes.

-This can be done with the help of light microscopy.

iii) Flow cytometry is used to estimate the DNA concentration of diploid and triploids.



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

1. ANDROGENESIS

Results in all-paternal inheritance.

Involves genetic inactivation of the egg's genome and fertilization with haploid sperm (followed by diploidization) or diploid sperm.

Method requires the suppression of the first mitotic cleavage.



Centurion
UNIVERSITY
*Shaping Lives...
Empowering Communities...*

Survival of the androgenotes is very low due to,
irradiation damage suffered by eggs.

homozygous expression of lethal gene.

-damage inflicted by thermal shock treatment to suppress the first mitotic cleavage.

Induced in fish by fertilizing irradiated eggs (gamma or X ray or U-V ray) with normal spermatozoa.



Centurion
UNIVERSITY
*Shaping Lives...
Empowering Communities...*

U-V (254 nm) irradiation is easy, inexpensive, can be easily set up under laboratory condition.

Diploid androgenetic individuals can then be produced by various treatments (thermal, pressure or chemical shock) to suppress the first cleavage division to yield a homozygous diploid.



Centurion
UNIVERSITY
*Shaping Lives
Empowering Communities...*

It can be used to generate clonal lines.

It has the advantage of storing and regenerating lines from cryopreserved sperm.

Androgenetic fishes were successfully produced only in about a dozen economically important food fishes (e.g., common carp, tilapia, rainbow trout and Siberian sturgeon).

Androgenetic tilapia can be useful for monosex fish culture.



Centurion
UNIVERSITY
Shaping Lives...
Empowering Communities...

2.GYNOGENESIS

It is a reproductive manipulation resulting in all-maternal inheritance.

It involves egg activation by genetically inactivated homologous or heterologous sperm and diploidization by retention of second polar body (*meiotic gynogenesis*), or suppression of the first mitotic cleavage (*mitotic gynogenesis*).



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

The shocks destroy the aster formation or the microtubules of the spindle and inhibit nuclear division. Thus, a diploid embryo containing maternal genetic material alone can be produced.

Gynogenesis is a natural form of reproduction in the teleost, *Mollienesia formosa*.



IRRADIATION OF SPERMATOZOA

- Gamma, UV radiation are used to inactivate sperm.
- Gamma radiation has greater penetration and helpful in the treatment of large quantities of sperm at a time.
- Residual chromosome fragments found in gynogenetic offspring after fertilization with gamma – irradiated sperm, reduce survival or cause abnormalities therefore, in the gynogenetic offspring, UV is a preferred method for sperm chromosome inactivation.

Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...



Centurion
UNIVERSITY
*Shaping Lives...
Empowering Communities...*

II) DIPLOIDIZATION

- The haploid embryo generated by gynogenesis dies unless some special treatment is conducted, so apparently it is necessary for the embryo to become diploid by doubling its chromosomes.
- Polyploidization treatment can be performed by inhibiting meiotic phase II after insemination with sperm that has received a genetic inactivation treatment.



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities*

MEIOGYNogenesis

- Achieved by inhibiting the extrusion of the second polar body.
- The resulting offspring are homozygous at a locus only if no recombination occurred.
- Determination of the percentage of heterozygous offspring, helps to calculate the recombination frequency.



Centurion
UNIVERSITY
Shaping Lives...
Empowering Communities...

MITOTIC GYNOGENESIS

- Polyploidization can be caused by the inhibition of cell division during the first cleavage.
- The original haploid set of chromosomes during meiotic phase I and II will be duplicated before the first cleavage.
- Pairs of chromosomes after the first cleavage inhibition are homologous to each other irrespective of crossing over.



Centenary
UNIVERSITY

Shaping Lives...
Empowering Communities...

Mitotic gynogenesis results in fully homozygous offspring, since it is achieved by inhibiting the first mitotic cleavage after duplication of the haploid genome.

It has been achieved in zebra fish (*Danio rerio*), medaka (*Oryzias latipes*), common carp (*Cyprinus carpio*) Nile tilapia (*Oreochromis niloticus*) and Indian catfish (*Heteropneustes fossilis*).



Centurion

UNIVERSITY

Shaping Lives...

Empowering Communities...

APPLICATION OF GYNOGENESIS

In female homogametic species all female population is produced.

50 to 100% inbred individuals can be produced in a single generation.

Inbred lines of fish can be crossed to produce hybrid vigour or heterosis.

It has the ability to map genes relative to their centromeres in fish after retention of the second polar body.



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

Generates homozygous lines by applying a second cycle of gynogenesis to the homozygous fish produced initially by gynogenesis.

This is useful for the production of female or monosex exotic species for release into the natural environment without risk of reproduction.

Combining gynogenesis and sex reversal it is possible to produce males with female genotype.



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

POLYPLOIDY

Polyploidy is the induction of individuals with extra (triploids, tetraploids) sets of chromosomes.



Centurion
UNIVERSITY

*Shaping Lives
Empowering Communities...*

TRIPLOIDY

- They are sterile and therefore useful for stocking natural bodies of water where population control is desirable.
- They grow faster at and after sexual maturity (in triploids 20-30% of the energy utilized for normal gonadal development is diverted to somatic growth).



Centurion

UNIVERSITY

Shaping Lives...

Empowering Communities...

HETEROGENEOUS POLYPLOID

Polyploidization treatment after hybridization yields heterogeneous polyploids.

When triploidization treatment is conducted, heterogeneous triploids that contains 2 genomes from the mother and one genome from the father is produced.



Centurion
UNIVERSITY

*Shaping Lives,
Empowering Communities.*

TETRAPLOIDS

- Induction of tetraploidy involves applying shock treatment soon after zygote formation.
- This is to prevent the first mitotic cleavage thereby inducing the tetraploid condition.
- Unlike triploids, tetraploids reach sexual maturity.



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

THANK YOU