BTY 303 ANIMAL & AQUACULTURE BT

UNIT-I

In Vitro Fertilization (Ivf)

The term in vitro refers to outside of the body, and the term fertilization states to the condition where sperm has attached to and entered the egg.

- Overall, **in vitro fertilization** is defined as the condition where the fertilization takes place in a laboratory dish (i.e. outside the body).
- IVF is one of the techniques of the assisted reproductive technology (ART).
- The IVF is mainly employed to assist with fertility and avoid genetic problems.
- While performing IVF at lab, firstly mature eggs are retrieved from the ovaries and are fertilized by sperm.
- Then, the fertilized eggs are placed to a uterus.
- One complete cycle of IVF may take about 3 weeks.
- The first successful test tube baby was Louise Brown, born on the year 1978 performed by Dr. Robert G.

Steps For In Vitro Fertilization

The IVF comprises of basic 5 steps. They are enlisted as follows:

- Stimulation
- Egg retrieval
- Insemination and fertilization
- Embryo culture
- Embryo selection and embryo transfer

Step I: Stimulation

- It is also termed as super ovulation.
- In a normal condition, a woman produce one egg during each menstrual cycle.
- However, IVF has requirement of multiple eggs because use of multiple eggs increase the probability of developing a viable embryos.
- Fertility drugs are prescribed to the woman, so that the egg production boosts up.
- The fertility drugs consist of exogenous gonadotropins and similar substances, that causes hormonal stimulation of the ovary causing the production of large number of eggs per cycle.

- On the third day of menstruation, treatment cycles are generally started, following the use of fertility medicines to trigger the development of multiple follicles of the ovaries.
- During this step, females are required to undergo regular transvaginal ultrasounds and blood tests to check hormone levels.
- The stimulation of ovary can be performed by **2 major protocols:**
- 1. **Lengthy protocol** is the one where the suppression (down regulation) of the pituitary ovarian axis is performed by the prolonged use of a gonadotropin-releasing hormone (GnRH) agonist.
- Once the process of down regulation is accomplished, usually after 10-14 days, subsequent ovary hyperstimulation generally using follicle stimulating hormone (FSH) starts.
- 2. **Short protocol** is the one where down regulation part is neglected and consist of prescription of injectable gonadotropins under regular monitoring inorder to trigger the development of multiple follicles of the ovaries.
- The frequent monitoring checks the level of estradiol, and the follicular growth is checked via gynecologic ultrasonography.
- Usually ten days of injection is required.
- During the last days of stimulation, the use of GnRH antagonists usually prevents the spontaneous ovulation during the cycle.
- It blocks the natural surge of luteinizing hormones (LH) facilitating the start of the ovulation process by use of injectable human chorionic gonadotropins.

Step II: Egg Retrieval

- It is also termed as follicular aspiration.
- It is a minor surgery performed for the removal of eggs from the woman's body.
- After the ovarian follicles reach a certain level of degree of development, final maturation is induced by an injection of human chorionic gonadotropin (hCG).
- hCG hormone plays a role as that of luteinizing hormone(LH).
- After a single hCG injection, ovulation would take place between 38 and 40 hours.
- However, the eggs are retrieved between 34 and 36 hours after hCG injection, which is, just before the rupture of follicles.
- This assists for scheduling the process of egg retrieval at a time when the eggs are completely matured.
- A technique called transvaginal oocyte retrieval is used to retrieve eggs.
- In this process, the woman are given anesthesia, prior to surgery.

- The health care provider by using ultrasound images as a guide, inserts a thin needle through the vagina into the ovary and sacs (follicles).
- Then, the needle is connected to a suction device, that pulls the egg and fluid out of each follicle, one at a time.
- The same process is repeated for other ovary.
- Generally, 10-30 eggs are removed.

Step Iii: Insemination And Fertilization

- The best quality of embryos that are potent for successful pregnancy are selected.
- It is also termed as oocyte selection.
- Along with it, the process called as sperm washing is also conducted.
- In this process, the inactive cells and seminal fluids are removed from semen in order to prepare it for fertilization.
- In the case where semen is supplied by a sperm donor, the preparation for treatment takes place before being frozen and quarantined, then it will be thawed ready for use.
- For about 18hrs, the incubation of sperms and egg (at the ratio of about 75000:1) is done in the culture media.
- In majority of the cases, the egg will be fertilized by that time and the fertilized egg shows two pronuclei.
- In specific cases such as low sperm count or motility, intracytoplasmic sperm injection(ISCI) can be used to inject a single sperm directly into the egg.
- Now, the fertilized egg is transferred to a special growth medium and left for about 48hrs until the egg reaches the 6-8 cell stage.

Step Iv: Embryo Culture

- After, the fertilized egg reaches 6-8 celled stage, embryos are cultured usually 3 days after retrieval.
- Embryo culture can be performed either in an artificial culture medium or in an autologous endometrial co-culture.

Embryo culture in artificial culture medium:

- In this type of culture, there can be either the same culture medium throughout the process or embryo can be sequentially placed in different media by use of sequential system. Ex. One medium can be used for culture to day 3, and second medium is employed for culture after it, when culturing to blastocyst stage.

- For the culture of human embryos to the blastocyst stage, both the single and sequential medium are equally effective.
- The media for artificial culture usually contain glucose, pyruvate, and energy supplying components.
- However, the addition of the nucleotides, amino acids, vitamins, and cholesterol enhances the performance of embryonic growth and development.
- The techniques that allow dynamic embryo culture along with fluid flow and embryo movement are also present.
- A new technique in development where the embryos are encapsulated in permeable intrauterine vessel.

Step V: Embryo Selection And Embryo Transfer

1. Embryo selection:

- On the basis of the number of cells, evenness of growth and degree of fragmentation, embryos are graded by embryologists.
- For the selection of embryos, morphological scoring system is considered as best strategy that optimizes pregnancy rates as well.
- If it is to be choosed between embryos of morphologically equal quality, presence of soluble human leukocyte antigen-G (HLA-G) is regarded as a second parameter.
- Embryos that have reached 6-8 celled stage are then transferred 3 days after retrieval.
- It has been seen that blastocyst stage transfer results in higher pregnancy rates.

2. Embryo transfer:

- Thenumber of embryos that are to be transferred depends on the number available, the age of the woman and other health and diagnostic factors.
- Most of the clinics and country regulatory bodies tend to reduce the risk of pregnancies carrying multiples.
- The best embryos are transferred to the patient's uterus by means of a thin plastic catheter that goes through the cervix.

Summaries Of Steps Of Ivf:

- The follicle maturation along with ovulation is promoted by ovarian hormonal stimulation.
- To achieve fertilization by assisted reproductive technology (ART) several fertilization methods are used.
- Under the cultured conditions, the re-implantation embryo spends certain time that will influence its further development.

- Pre implantation embryo biopsies can be used during this period of time.
- Finally, the embryo is transferred to a recipient female.

Cryopreservation

The word Cryo comes from the Greek word "kayos" meaning "frost". It means preservation in a "frozen state". It is the process of cooling and storing cells, tissues, or organs at very low temperatures to maintain their viability. Cryopreservation is a technique in which low temperature is used to preserve the living cells and tissue. In this technique, tissues can be preserved for a very long time. The science that deals with cryopreservation is known as “cryo biology”. It can be done over the following temperature :

- Solid carbon dioxide (at -79^{o}C)
- Low-temperature deep freezer (at -80^{o}C)
- In vapor phase nitrogen (at -150^{o}C)
- In liquid nitrogen (at -196^{o}C)

Organelles, cells, tissues, extracellular matrix, organs, and other biological structures that are vulnerable to harm from uncontrolled chemical kinetics are maintained by cooling to extremely low temperatures. Cryo-preservation or cryo-conservation is the term for this process.

The temperature which is normally used is:

Using solid carbon dioxide −80 ^{o}C or Using liquid nitrogen −196 ^{o}C

The main aim of the Cryopreservation technique is to achieve low temperatures without incurring further harm due to ice crystal formation during freezing.

In the past, Cryopreservation was based on coating the material to be frozen with cryoprotectants. Due to the intrinsic toxicity of many cryoprotectants, new techniques are being studied and worked upon.

Steps of Cryopreservation

The technique followed by the regeneration of plants involves the following steps.

Selection of Material:

For Cryopreservation, the selection of proper plant material is important. Two important factors depend on it such as nature and density. Any tissue can be selected for this purpose, for example embryo, meristem, ovules seeds, etc. The density should be high.

- **Addition of Cryoprotectant:** The chemical material is important as it prevents cryo destruction. Some examples of cryoprotectants are alcohol, some amino acids like proline, and dimethyl sulfoxide. Mainly two cryoprotectants should be used together instead of a single one as they are considered to be more effective.

- **Freezing:** Different species of plants show different types of sensitivity to low temperatures. They are different types of methods:

  - **Slow Freezing Method**- In this process, the tissue or plant material is slowly frozen at a slow cooling rate. The major advantage is that the plant cells are partially hydrated and serve in a better manner.

  - **Rapid Freezing Method** - The vials are plunged in liquid nitrogen. In this process, the temperature decreases from -300 to - 1000 degrees rapidly.

  - **Dry Freezing Method** - In this method hydrated cells and seeds are stored.

- **Storage in Liquid Nitrogen:** It is also important for the maintenance of the sale or material at a specific temperature. In general, the temperature is kept - 70 to - 196°C. Prolonged storage is done at the temperature of -196 °C in liquid nitrogen. A continuous supply of nitrogen is needed to prevent damage.

- **Thawing:** The thawing process is usually carried out by plunging the vials into a warm water bath with vigorous swirling. It also causes the vials to get transferred or move to another bath at 0 °C

- **Washing & Reculturing:** The preserved material is washed to remove the cryoprotectant. Furthermore, the material is recultured in a fresh medium.

- **Measurement of Viability:** Due to storage stress, there is a possibility of cell death. The presence of viability can be seen in most cases.

It is calculated by the formula:

(no of cells growing/no of cells thawed)×100

- **Regeneration of Plants:** After that, the viable seeds are cultured on a non-specific growth medium. Suitable environmental conditions are maintained.

Steps, Applications and Advantages

Steps

The major steps in Cryopreservation are

- The process of combining CPAs with cells or tissues before cooling
- The freezing of cells or tissues at a low temperature, followed by their storage
- The process in which cells or tissues are being warmed up
- After freezing, the process of removal of CPAs from cells or tissues

Applications

➢ In Medical sciences

- Cryopreservation gained prominence in human medicine after its use in infertility treatment. Since then, gamete cryopreservation has been developed to combat infertility.
- Sperm was the first successfully frozen reproductive cell and remains the easiest to freeze due to its tiny cytoplasm and thus low water content. Also, sperm nuclear material is compressed and protected from damage. For these reasons, cryopreservation of sperm cells is frequently used in human medicine today.
- Live births from assisted reproductive cycles employing frozen semen or embryos have been observed in recent years. Human oocytes and ovarian tissues have also been cryopreserved. Studies and research on immunological memory lymphoid cells, aortic root allografts, and osteoblasts for bone banking are still going on.
- Human medicine is also now commonly performing cryopreservation of cornea, umbilical cord, and hematopoietic cells, as well as sperm banking.

- Cryopreservation of bull semen has been used to reproduce rare and threatened species. Every year, more than 25 million bovine calves are artificially impregnated with frozen-thawed bull sperm. Tissues, cell lines, DNA, and serum samples can also now be kept in cryogenic banks.

➢ In Biological sciences

- Cryopreservation is one of the most reliable strategies for preserving plant genetic resources for the long term.
- In agriculture, germplasm cryopreservation is used to improve domestic varieties' genetics and adaptability to environmental changes. While the practice of preserving plant germplasm in cryogenic temperatures is relatively new, scientists have been developing cryopreservation procedures for plant cells and tissues for over 40 years now.
- These strategies can now be used for plant genotypes also. New cryogenic methods utilizing cryoplates (V and D) have recently been developed. These technologies have advantages such as ease of application and excellent regeneration rates after cryopreservation.
- Aquatic biotechnologies rely on cryopreservation of gametes, embryos, and embryonic cells to propagate economically significant species, safeguard endangered species, and maintain genetic variety.
- The results of studies show that marine fish sperm cryopreservation is more successful than freshwater fish cryopreservation and that fertilization rates are similar to mammalian species.

Advantages

- Cryopreservation boosts the efficiency of assisted reproductive treatments by allowing all extracted and/or fertilized cells to be kept for future use.
- By freezing embryos between cycles, ovarian stimulation is not required each time, and if the woman's ovaries are overstimulated, implantation can be postponed without squandering retrieved oocytes.
- Cryopreservation allows couples who conceive in their first treatment cycle to contribute their unused frozen embryos to research.
- It is currently common to implant only one or two embryos, with any remaining embryos being cryopreserved for future treatment cycles.

- Cryopreservation allows people who are losing their fertility to keep their reproductive cells and maybe conceive via aided methods in the future. Women who want to delay childbearing or have a family history of early menopause may use it.
- Cryopreservation is a powerful tool for preserving endangered species' germplasm. It can also help to maintain plant fertility.

Applications of Cryopreservation

- It is an ideal method for long term conservation of material.
- Disease-free plants can be conserved and propagated and recalcitrant seed can be maintained for a long time.
- Endangered species can be maintained.
- Pollen can be maintained to increase longevity.

Advantages of Cryopreservation

- Once the material is successfully conserved at a particular temperature, it can be preserved identifiably.
- No change or contamination of fungus or bacteria takes place after the storage process is completed and material is preserved.
- Minimal space is required for the purpose of cryopreservation.
- Minimal labor is required for the purpose of cryopreservation.

Cryopreservation of Animal Cells

The development of animal cell lines is expensive, time-consuming, and labor-intensive.

The continuous cell line has several advantages of over fertilizers cell lines such as:

- They survive indefinitely.
- They grow more rapidly.
- They can clone more easily.

Cryopreservation of Plant Cell

Due to the gradual disappearance of economic and rare species the necessity for storage of genetic resources increases. The convent journal method of the storage fails to prevent losses caused by:

- Attack of pathogen and pest
- Climatic disorders
- Natural disorder
- Political and economic causes
- The material to be preserved is stored at low temperatures due to which growth rate of cells retards. Consequently, biological activities are reserved for a long period of time.

Pest Management

A pest species can be any species that humans consider undesirable. Any organism that reduces the availability, quality, or value of a human resource can be classified as a pest. This designation in no way reflects the organism's role in the natural ecosystem but is more an indicator that they are in conflict with humans. Plant pests, also referred to as weeds, are included in the discussion of Non-native Invasive Plant Removal.

A pest in one area may not be considered a pest elsewhere. Often organisms rise to pest status because they escape normal control by natural regulating agents. This is achieved through direct or indirect importation to a new region or by human activities which reduce or eliminate the efficiency of their natural enemies. Without controls on population growth, organisms can rapidly achieve levels at which damage is caused thus becoming pests (e.g., locust swarms stripping landscapes bare). However, organisms do not need to exist in large numbers to be a pest. For example, the codling moth (Cydia pomonella) does not lay many eggs compared to many insects and often produces only one generation each year (Begon et al. 1996). However, because it blemishes apples, making them commercially undesirable, the codling moth is considered an important agricultural pest.

Pest management is therefore a means to reduce pest numbers to an acceptable threshold. An acceptable threshold, in most cases, refers to an economically justifiable threshold where application of pest control measures reduces pest numbers to a level below which additional applications would not be profitable (i.e.,

where additional costs of control exceed additional benefits). Pest eradication (i.e., complete removal) is usually not a viable option.

Methods of control can be categorized as chemical, biological, cultural, physical/mechanical, or genetic, and are discussed in further detail below.

➢ Chemical Methods:

Chemicals (e.g., insecticides, herbicides, rodenticides) can be broad-spectrum (non-selective) or narrow-spectrum (selective), and can be organic or inorganic. Chemicals used to regulate pest abundance can act as nerve toxins (for insects and mammals) and growth regulators/inhibitors. Chemicals can also be used to affect pest abundance through more indirect means, such as releasing pheromones to disrupt breeding behavior and interfere with mating. Chemical pesticides are often toxic to non-target organisms including the pest's natural enemies, can persist in the environment affecting water supply, soil productivity, and air quality, and can be biomagnified in the food chain. Inappropriate use of pesticides can result in target pest resurgence from killing off natural enemies, secondary pest outbreaks by removing natural enemies of other organisms and allowing them to rise to pest status, and evolved resistance to the pesticide.

➢ Biological Methods:

Due to any number of reasons, including those mentioned in the Chemical and Cultural sections, compromising the effectiveness of natural enemies often allows potential pest organisms to experience virtually unregulated population growth and enables them to reach pest status. Biological control involves the use of a pest's natural enemies (e.g., predators, pathogens, parasites and parasitoids), to control pest abundance. Measures to conserve or enhance the impact of natural enemies should be attempted first. Perhaps biological control is most known for importation of natural enemies, often from the pest's area of origin, to control non-native pests (e.g., importing vedalia bettles to control cottony cushion scales which were attacking California citrus orchards). A number of safeguards are necessary before implementing importation actions to ensure imported organisms will not pose additional threats to non-target organisms. A third approach to biological control involves augmenting natural enemies through rearing and periodic releases and can be inoculative (natural enemies are released early in the season) or innundative (natural enemies are released as a biological pesticide).

➢ Cultural Methods:

The effectiveness of natural enemies can be compromised by human practices. Application of broad-spectrum pesticides which kill off natural enemies in addition to target pest species, the type of crop plant, the crop environment, and cropping practices. Modern crop varieties often inadvertently create conditions which favor pest species (e.g., pest species which have bored deeper into larger fruit making them inaccessible to natural enemies). Crops are often monocultures, consisting of a single crop species, which

creates a homogenous habitat often lacking key requirements of natural enemies, thus favoring pest species. Moreover, many harvesting practices prevent natural enemies from persisting in annual crops. Examples of cultural practices that encourage natural enemies and discourage pest persistence include intercropping (multiple crops in the same field) to make it more difficult for pests to find a host plant, planting trap crops which attract pests away from harvest crops and which can later be treated with select application of pesticides, and delaying planting times to coincide with times where pests have emerged and died off for the season.

➢ Physical Methods:

Manual or mechanical removal, or installation of physical barriers can be used to exclude pest species. Removal methods include use of animal traps, sticky cards for insects, manual removal of insects from plants (e.g., hand picking or spraying with a hose), removing diseased or infected materials (e.g., pruning branches or removing diseased litter). Physical barriers such as fences, nets, mulch, and tree trunk guards can exclude pests and reduce the damage they inflict.

➢ Genetic Methods:

Genetic alteration to reduce pest impacts is not as widely known or publicly available as other control options. Autocide is one type of genetic control and involves using the pest itself to induce increased mortality rates. Sterile males are introduced into the population, which, after mating with females, creates infertile eggs. This is an expensive option with many limitations including potential for reduced competitive viability of the introduced sterile males versus naturally occurring fertile males. Straightforward genetic manipulation to create pest resistant plant strains is another form of controlling pest impacts. However, genetic manipulation research and development is costly, and introduces a whole other series of ethical and environmental issues that are not easily addressed. Genetic manipulation is not a viable control option for the general public.

- Integrated Pest Management (IPM) is an increasingly popular process for controlling pests. IPM considers the ecosystem as a whole and takes into consideration a balanced mix of the aforementioned control methods to produce the most effective and least damaging plan. All the methods are mutually augmentative with chemical control means as the last resort in the plan. Ideally, an IPM plan would result in a sustainable system without need for much costly follow-up maintenance.

- A number of insects and pathogens have been identified as pests particularly for their impacts to vineyards, orchards, and other agricultural industries important to Napa County's economy. The following table presents a number of important pest species in Napa County as well as their deleterious effects.

Mosquito management

Mosquito control manages the population of mosquitoes to reduce their damage to human health, economies, and enjoyment. Mosquito control is a vital public-health practice throughout the world and especially in the tropics because mosquitoes spread many diseases, such as malaria and the Zika virus.

Mosquito-control operations are targeted to multiple problems:

- Nuisance mosquitoes bother people around homes or in parks and recreational areas;
- Economically important mosquitoes reduce real estate values, adversely affect tourism and related business interests, or negatively impact livestock or poultry production;
- Public health is the focus when mosquitoes are vectors, or transmitters, of infectious disease.
- Mosquito-born diseases can threaten endangered species.

Depending on the situation, source reduction, biocontrol, larviciding (killing of larvae), or adulticiding (killing of adults) may be used to manage mosquito populations. These techniques are accomplished using habitat modification, pesticide, biological-control agents, and trapping. The advantage of non-toxic methods of control is they can be used in Conservation Areas.

Integrated pest management (IPM) is the use of the most environmentally appropriate method or combination of methods to control pest populations. Typical mosquito-control programs using IPM first conduct surveys, in order to determine the species composition, relative abundance and seasonal distribution of adult and larval mosquitoes, and only then is a control strategy defined.

physical control

physical control makes an environment uninviting for mosquitoes to breed in. Physical controls include barriers like screens, bed nets and long sleeves and pants. Another form of physical control is source reduction or habitat modification such as draining or removing debris from a body of water to filling in tree holes.Physical control is usually the most effective of the mosquito control techniques available and is accomplished by eliminating, or significantly reducing, mosquito breeding sites. The primary operational objective of physical control is to reduce the mosquito carrying capacity of a source to preclude the use of control methods that would adversely impact the environment and wildlife. This can be as simple as properly discarding old containers which hold water or as complex as developing a regional drain system for storm water. Physical control can virtually eliminate the need for pesticide use in and adjacent to the

affected habitat. There are many types of mosquito breeding sources capable of being reduced by physical control techniques.

Biological Control

Biological mosquito control methods protect the public from mosquitoes and the transmission of mosquito-borne diseases. Mosquito biological control agents include a wide variety of pathogens, parasites and predators. A biological control agent used by the District is **Gambusia affinis**, the mosquitofish. Mosquitofish are small live-bearing minnows closely related to the common guppy. Used in mosquito control in California since 1922, these fish consume mosquito larvae and pupae and can survive in varying water conditions. Because mosquitofish are surface feeders, they are extremely efficient mosquito predators. Mosquitofish have been said to consume upwards of 80-100 mosquito larvae per day, and are capable of quickly populating a source if conditions are favorable. The fish are placed in a variety of permanent and semi-permanent fresh water habitats, including dirty swimming pools, water troughs, and ponds.

Chemical Control

Chemical control of mosquitoes is the application of natural or manmade compounds (insecticides) to reduce mosquito populations to tolerable levels. Chemical control methods are applied to obtain immediate control when physical and biological control methods fail to maintain mosquito numbers below a tolerable level or during an epidemic of mosquito-borne disease when emergency control measures are needed.

Larvicides may be applied to water in which larvae or pupae are developing. Pastures, irrigation ditches, dairy waste ponds, sloughs, catch basins, storm drains and roadside ditches are examples of areas the District regularly inspects to reduce mosquito populations.

Adulticides may be applied as space sprays, mists, or fogs to kill adult mosquitoes and as a residual insecticide on surfaces likely to be contacted by adult mosquitoes. Adulticides are applied by hand held equipment as well as, truck mount, and aerial mounted equipment.

- ### Larviciding

Control of larvae can be accomplished through use of contact poisons, growth regulators, surface films, stomach poisons (including bacterial agents), and biological agents such as fungi, nematodes, copepods, and fish. A chemical commonly used in the United States is methoprene, considered slightly toxic to larger animals, which mimics and interferes with natural growth hormones in mosquito larvae, preventing development. Methoprene is frequently distributed in time-release briquette form in breeding areas. Another

chemical is Temefos or temephos, a sand granular insecticide is used to treat water infected with disease carrying insects.

It is believed by some researchers that the larvae of *Anopheles gambiae* (important vectors of malaria) can survive for several days on moist mud, and that treatments should therefore include mud and soil several meters from puddles

UNIT-II

Transgenic fish production

Transgenic are organisms into which transgene has been artificially introduced and the transgene stably integrated into their genomes. Transfer of transgene into the nucleus of a target cell where integration into the host genome takes place.

Interestingly, fish offer easier genetic manipulation compared to mammalian counterpart, as they bred easily and large number of eggs is laid by female. The primary goals for genetic manipulation of fish species used in aquaculture are to:

a) Intensify the growth and efficiency of food conservation,
b) Increase tolerance to environmental variables, such as temperature and salinity,
c) Bring out new colour variants of ornamental fishes, and
d) Develop disease resistant fishes.

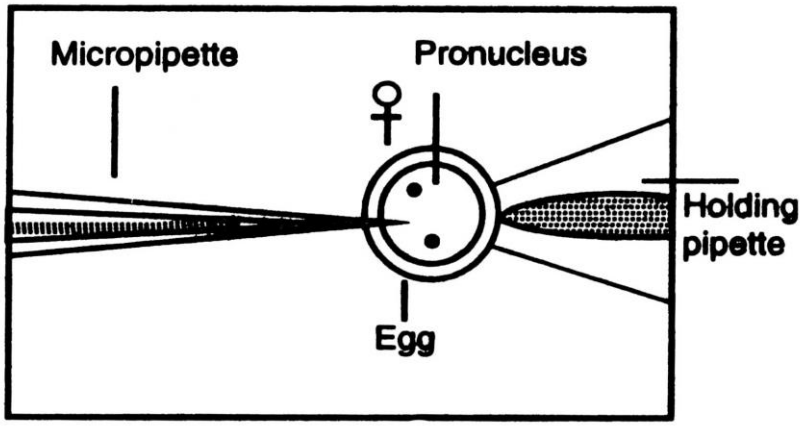
The first report of the generation of transgenic fish in the aquaculture field was in goldfish (Zhu et al. 1985) and rainbow trout (Onchorhycus mykiss; (Maclean et al. 1992).

- Over the period, a growth hormone gene has been targeted as a transgene aiming to increase the growth rate in more than 20 teleost species.
- A number of fish species are in focus for gene transfer experiments and can be divided into two main groups: animals used in aquaculture and model fish used in basic research.
- Among the major food fish species are carp (Cyprinus sp.), tilapia (Oreochromis sp.), salmon (Salmo sp., Oncorhynchus sp.) and channel catfish (Ictalurus punctatus) while zebrafish (Danio rerio), medaka (Oryzias latipes) and goldfish (Carassius auratus) are used in basic research.
- Transgenic fish show better gross food conversion, the increase in fish weight per unit of food fed.
- The global scenario of transgenic food/ ornamental fishes Over the past four decades, the various transgenic fishes have been developed consisting of transgenic lines expressing growth hormone for aquaculture, industrial, and pharmaceutical applications.
- Most of the transgenic fishes were developed by using a growth hormone construct because of its importance and highly conserved gene sequence.
- Those genetically modified fishes depicted dramatic improvement in growth rate, thus showing the useful application of the transgenic in aquaculture.
- Besides the growth trait other traits of disease resistance; cold or hypoxia tolerance and FCR (Feed conversion ratio) improvement were also considered for transgenesis.

- The first FDA approved transgenic food fish is "AquAdvantage Salmon" with augmented growth rate and size compared to wildtype.
- Apart from food fishes, ornamental transgenic fishes have been developed using different color genes.
- The value-added aquarium fishes have already been commercialized, such as „GloFish" with six attractive fluorescent color combinations, including Starfire pink. Methods in the production of transgenic fish

➢ **Microinjection method**:

Microinjection method has been used successfully in the production of transgenic fish and is a commonly used technique due to its simplicity and reliability. The use of the microinjection method results in higher survival rates for manipulated fish embryos than the electroporation method. The most established method for gene transfer in fish is microinjection. Microinjection that allows delivery of the transgene directly into the nucleus, Transgene is directly microinjected into the male pronuclei of fertilized eggs.



Disadvantages :

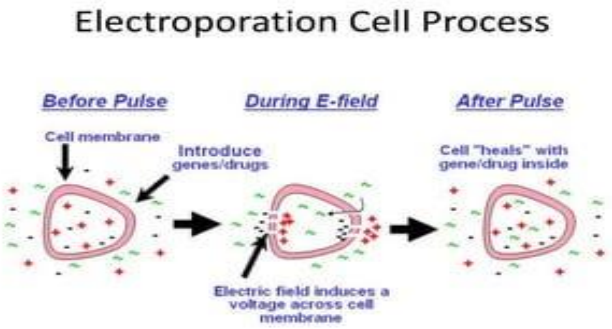
Time-consuming and labour intensive .The nuclei of fish eggs are small and difficult to visualize The chorion, hardens soon after fertilization and in many fish species the pronuclei of fertilized eggs is not visible and transgenes are usually injected into the egg cytoplasm. Low efficiency of generation of transgenics.

To improve the efficiency of selection of transgenics, genetic markers are coinjected with the transgene to monitor for transformed zygotes. The Green Fluorescent Protein (GFP) from Jellyfish (Aequorea victoria) has been used for this purpose in zebrafish.

2.Electroporation method:

Electroporation has been shown to be the most effective means of gene transfer in fish since a large number of fertilized eggs can be treated in a short time by this method. Electroporation utilizes a series of short electric pulses to permeate the cell membrane that make possible the formation of temporary pores on the

surface of the target cells through which the transgene is introduced into the cytoplasm where it is then delivered to the nucleus by the cellular machinery. Electroporation has been preferred in many laboratories for gene transfer in fish systems because of its efficiency, speed and simplicity.

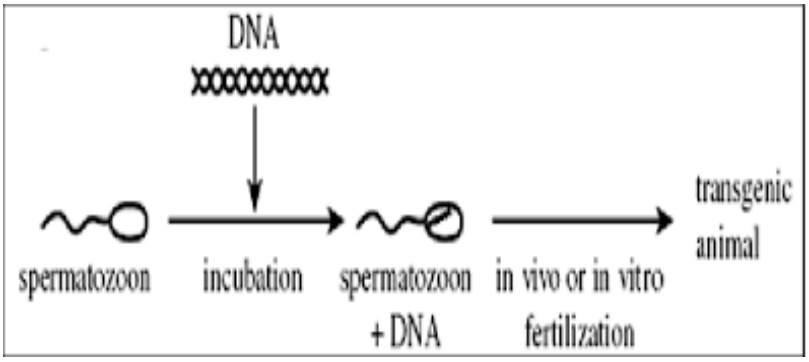


Disadvantages:

The presence of a tough chorion layer around the fish eggs reduces efficiency, removal of the chorion is a tedious procedure and introduces additional stress on newly fertilized eggs. The efficiency of the electroporation reduced because of voltage, number and frequency of pulses.

3. Sperm mediated gene transfer method:

SMGT is a transgenic technique that transfers genes based on the ability of sperm cells to spontaneously bind to exogenous DNA and transport it into an oocyte during fertilization to produce genetically modified animals. Exogenous DNA refers to DNA that originates outside of the organism. SMGT have low efficiency and it is mainly due to low uptake of exogenous DNA by the spermatozoa.



Mechanism of SMGT:

The exogenous DNA molecules bind to the cell membrane of the head of the sperm cell. This binding and internalization of the DNA is not a random event. The exogenous DNA interacts with the DNA-binding proteins (DBPs) that are present on the surface of the sperm cell. Spermatozoa are naturally protected

against the intrusion of exogenous DNA molecules by an inhibitory factor present in mammals‟ seminal fluid. This factor blocks the binding of sperm cells and exogenous DNA because in the presence of the inhibitory factor, DBPs lose their ability to bind to exogenous DNA. In the absence of this inhibitory factor, DBPs on sperm cells are able to interact with DNA and can then translocate the DNA into the cell. Therefore, the seminal fluid must be removed from the sperm samples by extensive washing immediately after ejaculation. After the DNA is internalized, the exogenous DNA must be integrated into the genome.

4. Retro viral infection method:

Although transgenic techniques have been adopted successfully by microinjection and electroporation techniques with great efficiency, mosaicism is the major concern in resulting individuals. Therefore, a retroviral vector, such as long terminal repeat (LTR) sequences of Moloney murine leukemia virus (MoMLV) and pantropic retroviral vector, has been developed for improving

efficiency of integration of the gene. Because of its higher efficiency, it has been used to produce transgenic organisms, including fishes. Regrettably, these viral vectors are inclined to unstable expression or even complete silencing of transgene expression and integration rates may be increased because of active infection. The successfully used retroviral vector or constructs reported to produce transgenic crayfish and top minnows, Poeciliposis lucida. In a recent study, pantropic retroviral vector has been used as a tool for transducing sea urchin embryos. This is a valuable gene transfer technique for fishes, in general, but, introduction of viral sequences into food fish may not be accepted by the public, but it could be used for ornamental fish development. **Disadvantages:**

- The preparation of retroviral particles including the transgene of interest is a very laborious process, increases costs and requires technology.

Advantages of transgenic fish:

- The growth rate of Transgenic fish can be increased by 400% to 600% Can reduce feed input by up to 25% per unit of output, thereby improving food conversion ratios.
- Transgenic fish have been developed for various applications such as the experimental models for biological research, environmental monitoring, ornamental fish and aquaculture production.
- Two to three fold increases relative to non-transgenic fish have been reported for tilapia and Atlantic salmon and up to two fold increases in common carp.
- Use of transgenic fish as bioreactors for the large-scale production of rare human therapeutic proteins or novel foods for specific dietary requirements.
- Transgenic lines of tilapia engineered to produce human clotting factor VII, which is used in liver transplants and in treating injuries.

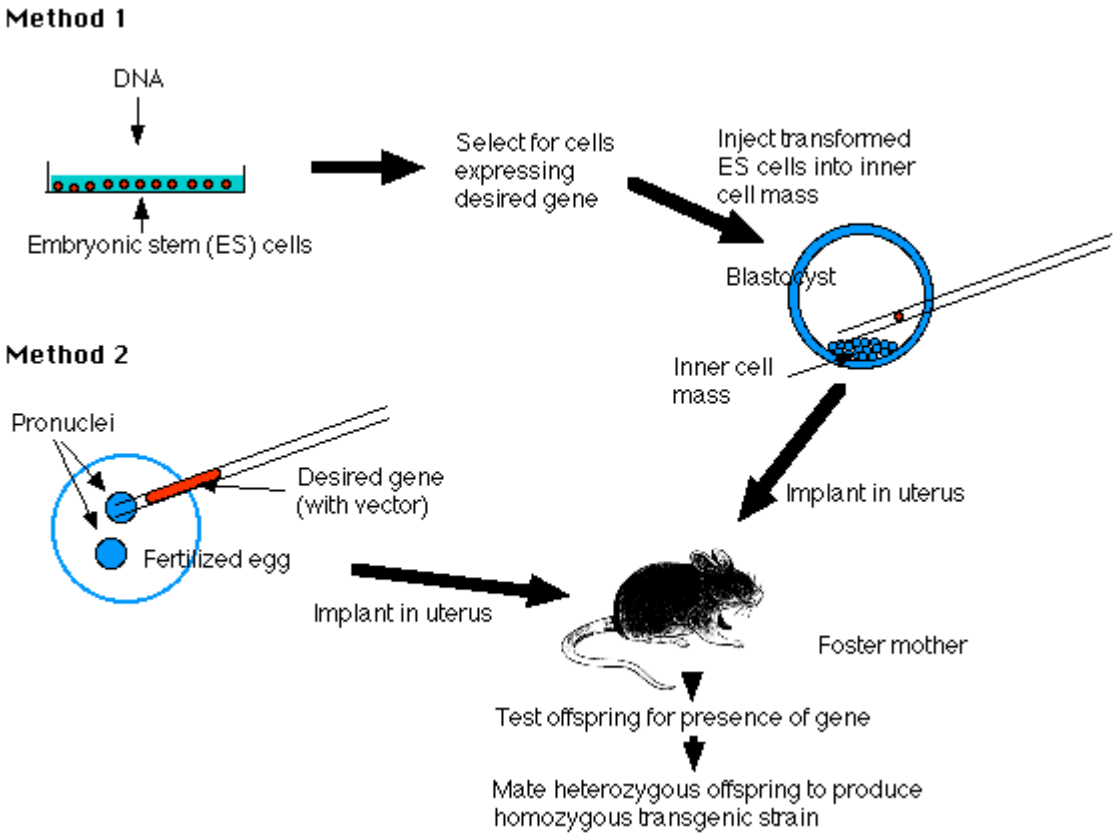
- Development of transgenic animal models represents a revolutionary advance in the study of a variety of disease processes.
- Fish as cost-effective and important animal models in genetics, developmental biology and toxicology.
- Development of transgenic fish as a model in reducing or replacing selected mammals used in toxicity testing.

Transgenic MiceProduction

A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. The foreign gene is constructed using recombinant DNA methodology. In addition to the gene itself, the DNA usually includes other sequences to enable it to be incorporated into the DNA of the host and to be expressed correctly by the cells of the host. Transgenic sheep and goats have been produced that express foreign proteins in their milk. Transgenic chickens are now able to synthesize human proteins in the "white" of their eggs. These animals should eventually prove to be valuable sources of proteins for human therapy.

Two methods of producing transgenic mice are widely used:

- transforming **embryonic stem cells** (**ES** cells) growing in tissue culture with the desired DNA
- injecting the desired gene into the **pronucleus** of a fertilized mouse egg



The Embryonic Stem Cell Method - Method 1

Embryonic stem cells (**ES** cells) are harvested from the **inner cell mass** (ICM) of mouse blastocysts. They can be grown in culture and retain their full potential to produce all the cells of the mature animal, **including its gametes**.

1. Make your DNA

Using recombinant DNA methods, build molecules of DNA containing

- the gene you desire (e.g., the insulin gene)
- **vector** DNA to enable the molecules to be inserted into host DNA molecules
- **promoter and enhancer sequences** to enable the gene to be expressed by host cells

2. Transform ES cells in culture

Expose the cultured cells to the DNA so that some will incorporate it.

3. Select for successfully transformed cells

4. Inject these cells into the inner cell mass (ICM) of mouse blastocysts.

5. Embryo transfer

- Prepare a **pseudopregnant** mouse (by mating a female mouse with a vasectomized male). The stimulus of mating elicits the hormonal changes needed to make her uterus receptive.
- Transfer the embryos into her uterus.
- Hope that they **implant** successfully and develop into healthy pups (no more than one-third will).

6. Test her offspring

- Remove a small piece of tissue from the tail and examine its DNA for the desired gene. No more than 10–20% will have it, and they will be heterozygous for the gene.

7. Establish a transgenic strain

- Mate two heterozygous mice and screen their offspring for the 1 in 4 that will be **homozygous** for the transgene.
- Mating these will found the transgenic strain.

The Pronucleus Method - Method 2

1. Prepare your DNA as in Method 1

2. Transform fertilized eggs

- Harvest freshly fertilized eggs before the sperm head has become a pronucleus.
- Inject the male pro-nucleus with your DNA.
- When the pro-nuclei have fused to form the diploid zygote nucleus, allow the zygote to divide by mitosis to form a 2-cell embryo.

3. Implant embryos in a pseudo pregnant foster mother and proceed as in method 1.

ANIMAL CLONING

Modern biotechnology revolutionized the world of science and medicine. One biotechnological technique in particular which remains a topic of controversy is **animal cloning**. Cloning refers to the process of creating an identical genetic copy of one organism. Cloning can be done on genes, single-celled organisms, and even entire animals. Clones also occur naturally, as is the case for identical twins; identical twins originate from the same embryo and thus contain the same genes. Other instances of natural cloning include bacteria that reproduce by making exact copies of themselves. Scientists also clone single genes by inserting them into single-celled organisms such as bacteria and yeast. Each time these organisms reproduce, the gene is cloned and replicated.

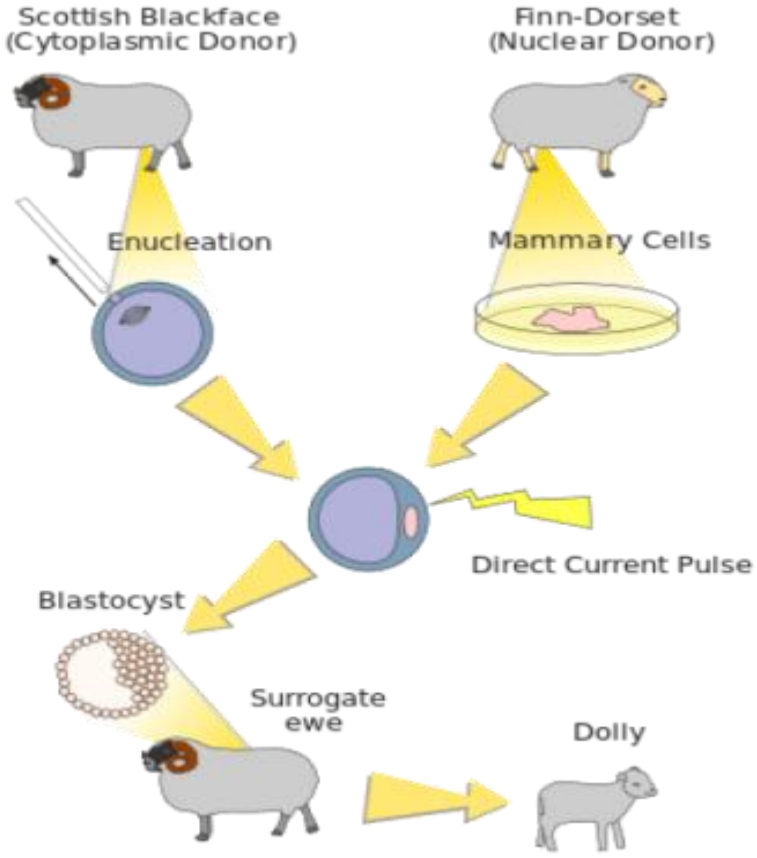
The first recorded successful attempt at animal cloning gave birth to Dolly the Sheep in 1996. Dolly was cloned from another sheep and lived for six years. Dolly was genetically identical to its mother sheep, which would not have been the case if it was as a result of sexual reproduction. Sexual reproduction involves the fusion of haploid gametes from the father and the mother to result in a genetically different and unique offspring; on the other hand, cloning would result in an identical genetic copy of the parent.

The animal cloning process can occur via two separate methods: **artificial twinning** or **somatic cell nuclear transfer**. The process of artificial twinning is done in many labs in order to induce the birth of identical animal twins. The embryo to be transferred into the mother's womb is split into two, and each developing embryo is allowed to develop to form its own organisms. The resulting offspring would then form identical

twins. Twinning can be used to increase livestock and produce more offspring than normal. The other process, somatic cell nuclear transfer, is more intricate and involves several steps that include:

- Enucleation: The host egg is enucleated. Its DNA is removed, and its genetic information is lost.
- Nucleus transfer: The nucleus of the animal to be cloned is removed.
- Insertion: The nucleus of the animal to be cloned is inserted into the enucleated host egg to produce a zygote.
- Stimulation: The zygote is stimulated to divide via electric current.
- Embryo transfer: The zygote is then transplanted into the surrogate mother's uterus.

The Process of Animal Cloning



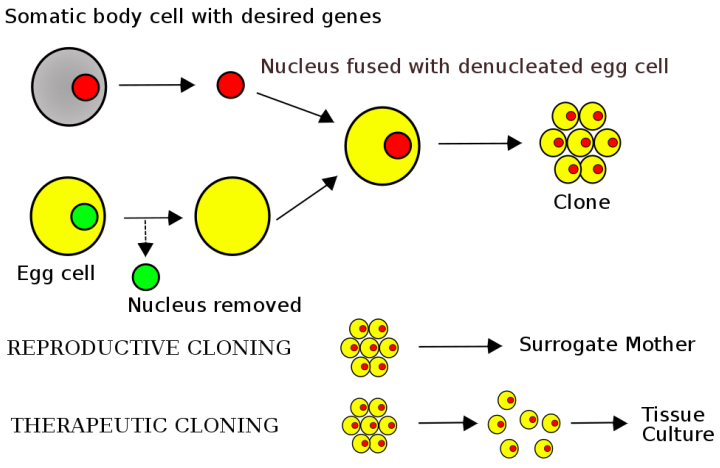
Application of cloning:

1. Proteins, vaccines, and antibiotics are all produced by the process of cloning.
2. This can include DNA copies, cells, organs, or even the complete animal aid using cloning.
3. In agriculture, cloning is employed to create pest-resistant plants.

4. Cloning is also utilized to create transgenic animals and for gene therapy.
5. Cloning livestock is a way to duplicate a desirable combination of features, like as rapid development and abundant milk production, without the genetic "lottery" and mixing that takes place during sexual reproduction.
6. It enables faster reproduction of an animal with a particular genetic change than does normal mating, such as the capacity to generate a medicine in milk.
7. Additionally, it is simpler to alter a gene in cultured cells than it is in an entire animal, and the changed cell nucleus may be added to an enucleated egg to create a clone of the desired genotype.
8. Cloning is used in scientific study to better understand the basic biological processes of animals used in tests, such as mice.

Somatic cell nuclear transfer (SCNT),

- It is a technique in which the nucleus of a somatic (body) cell is transferred to the cytoplasm of an enucleated egg (an egg that has had its own nucleus removed).
-]Once inside the egg, the somatic nucleus is reprogrammed by egg cytoplasmic factors to become a zygote (fertilized egg) nucleus.
- The egg is allowed to develop to the blastocyst stage, at which point a culture of embryonic stem cells (ESCs) can be created from the inner cell mass of the blastocyst.
- Mouse, monkey, and human ESCs have been made using SCNT; human ESCs have potential applications in both medicine and research.



Dolly

- In 1996 Dolly made history as the first successfully cloned mammal. She is pictured standing in her pen at the Roslin Institute, near Edinburgh.
- The most practical application of SCNT is in the reproductive cloning of farm animals that have exceptional qualities, such as the ability to produce large quantities of milk.
- Reproductive cloning is accomplished by implanting an SCNT-derived blastocyst into the uterus of a surrogate mother, in which the embryo develops into a fetus carried to term.
- Dolly the sheep, born in 1996, was the first mammal cloned using SCNT. The technique also could be used to resurrect extinct species.
- For example, cells collected from a frozen woolly mammoth could be used as nuclear donors for enucleated elephant eggs.
- Proof of principle for such "resurrection" was provided by an experiment in which mice were cloned using somatic cell nuclei derived from a mouse that was frozen for more than 15 years.

Applications

The main areas of application of SCNT are:

- Reproductive cloning,
- therapeutic cloning and basic research.
- A great application potential of SCNT based cloning is the production of genetically modified (transgenic) animals.

UNIT-III

Role of Biotechnology in Aquaculture

1. Nutrition:

Indian aquaculture industry lags behind in the science of nutrition. A good approach would be to improve the quality and utilisation of various agricultural and industrial by-products for efficiency.

Various foodstuffs having potential values as fish feed ingredients have not been nutritionally utilised as they contain toxic or anti-nutritional factors. The need of biotechnology is to develop new varieties of foodstuff with totally free or low content of such intoxicants.

(1) Development of food additives, such as anabolic steroids and thyroid hormones, have resulted in increased growth of salmonid fishes.

(2) Development of new artificial diets, such as micro-encapsulations for filter-feeders (oysters and mussels).

(3) Improved culture of single-celled feed organisms (algae, yeast, bacteria).

2. Feeding Stimuli and Chemical Signal:

To obtain the full sequence of feeding behaviour from initial recognition of feeding 'strike' to ingestion of food particles, it is necessary to identify the feeding stimuli or chemical signal. It is thus required to genetically design or engineer the bacterial strain with characteristics of having better cellulose degradation capacity.

3. Reduce the In-Pond Chemical Oxygen Demand:

Microbial processing of livestock manure through anaerobic digestion in biogas plants reduces the in-pond COD.

It also improves the fertilisation quality of the inputs, Biotechnology has attained great significance in aquaculture development by increasing the nitrogen fixation levels in ponds through bio-fertilisation with blue-green algae (azolla) and green-manuring and through genetic manipulation of bacteria to improve their nitrogen fixing capacity. This has definitely increased fish production.

4. Administration of Mammalian Hormones:

Mammalian gonadotropic hormones have been used extensively for spawning of carps and catfishes. Hormonal manipulation of sex is being practised to control unwanted reproduction in prolific breeders such as common carp and Tilapia. Administration of mammalian growth hormones through feed or injection has enhanced fish growth. Manipulated monosex population improves nutritive value of fish flesh and growth.

5. Cryopreservation:

Cryopreservation refers to preservation of gametes and embryo generally at below freezing temperature for ready availability of genetic material for breeding and research purposes. Cryopreservation of milt has been successfully developed for several fishes.

It is essential since the males of some fish species mature earlier than the females and there would be dearth of oozing males when fully mature female fishes would be available. Cryopreservation of zygotes and embryos have facilitated large scale fish seed production programme.

6. Use of Allomones and Pheromones:

In large scale induced breeding, use of allomones and pheromones is of great significance. Under biotechnological programme it is necessary to study the role of pheromones in alarm and social behaviour, species and sex recognition, sex behaviour and territorial and space recognition.

7. Genetic Manipulation:

Change in genetic make-up of any organism by application of improved bio-techniques is referred to as genetic manipulation. Fishes, generally, can tolerate a wide range of genetic manipulation than terrestrial farm animals.

(a) Chromosome Manipulation:

Through chromosome manipulation fish stock can be improved for breeding and culture by way of gynogenesis, androgenesis and polyploidy.

(i) Gynogenesis (all Maternal Inheritance):

Gynogenesis in fishes can be induced by stimulating parthenogenetic development of fish eggs by artificially inactivated spermatozoa. Through X-ray or UN-rays, the genetic contents (DNA) in spermatozoa can be destroyed.

With such spermatozoon fertilisation would occur without any contribution from them. The diploid parthenogenetic individuals would give rise to offsprins that would be females having maternal inheritance only Ex. Grass carp, Salmon, Rainbow trout, etc.

(ii) Androgenesis (all Paternal Inheritence):

Similarly with X-rays or UN-rays the genome of ovums can be destroyed and fertilisation with normal spermatozoon would result in homogametic males having paternal inheritance only. Ex. Common carp.

(iii) Polyploidy:

Polyploidy (individuals having extra sets of chromosomes) can be achieved by subjecting fertilised eggs to heat, cold, pressure or chemical shocks. Polyploidy produces

(1) Sterile individuals,

(2) Enhanced growth and survival,

(3) Improved quality of flesh in some fishes,

(4) More viable and ideal for producing new hybrids.

(b) Gene Transfer or Transgenesis:

Transgene- sis results in formation of 'Transgennic fish'. The techniques involved is through micro-injection and electroporation, genes are introduced into one-celled embryo or oocyte.

The potentialities of transgenesis are:

(1) Tolerance to physical factors. Ex. tolerance to cold using antifreeze gene.

(2) To accelerate growth by using growth hormone genes.

(3) Efficient use of food by fishes through manipulation of biochemical pathways.

(4) To increase disease resistance by using specific disease resistance genes (Ex. T-cell receptor, immunoglobulin, lymphokines, etc.).

(5) Behavioural modification (maturation, reproduction, sex control, etc.) by regulation of endocrine function.

(c) Application of Cell Culture Biotechnology to Marine Macro-Algae:

Seaweeds can be easily genetically engineered. In many maritime countries, tons of seaweeds are produced through mariculture, which are used as human food (green seaweeds) and for commercial production of phyco-colloids (agar, algins, carrageenas, etc.) for industrial purposes.

8. Hormonal Manipulation for Sex Control:

Sex hormones (steroids) are used to produce monosex for culture of tilapia in particular. Tilapia being a prolific breeder, creates problem in pond culture as it results in overcrowding with small size fishes and thus, in decreased production. Therefore, monosex culture controls reproduction and also helps in increased production.

To obtain only males by sex reversal method, the early fry of tilapia are fed with androgen 17-a methyl testosterone for induction of sex reversal from genetic females to phenotypic males. Similarly, in species where females are bigger and grow faster than males, only females can be produced for monosex culture by feeding fry with estrogen 17b-Estradiol benzoate or diethyl stibestrol.

9. Fish Disease Management:

Fish diseases can be controlled through development of biotechnologically produced vaccines. Vaccines against vibriosis and furunculosis have been developed and are available in the market. However, they are for limited fish species. There is an increased demand to use biotechnology for developing vaccines against bacterial, viral and other diseases affecting several commercially important culturable fish species.

10. Current Breakthrough and Essentials for Future Research:

Technological advancement has led to freshwater and marine pearl culture product development such as chitin, chitosan, etc. Further studies for even more efficient and large scale production technologies is essential.

Attention should be drawn towards genetic improvement of fish stock through selection and genetic engineering, ex-situ conservation methodologies, gene banking both with live and gamete level approaches, which would subsequently lead to future research. Social impact of fisheries and aquaculture development has to be studied and the need is to develop parameters to undertake Social Impact Assessment (SIA).

Thus, for the development of biotechnology it is urgently required to upgrade laboratory facilities and attract young blood for research and training. It should be noted that water resources in the country are

not exclusively available for aquaculture as there is excessive pressure on their use from several other sectors.

Therefore, any further growth in aquaculture for quantum jump would have to be vertical and technology based. The transfer of technology from the laboratory to the field has had its own causalities.

Today only about 30% of the proven technology available in various fields of aquaculture has been manifested in the field. There is thus, urgent need to narrow the gap between the results of research and those in the farmers field.

Pearl Culture

Introduction to Pearl Culture:

Pearls have always been a source of attraction for ladies and gents. People of all age group and all the profession have the same attraction for pearls. Pearls have been a rare and one of the most valuable objects for people and they are supposed to be done of the best and most loving thing for people of all ranks either rich, poor or of medium economic status.

The proverb “Soft and flawless like a pearl” itself is sufficient to explain the character and quality of a pearl as well as its rank in a society. They have always attracted businessmen, doctors and kings for their price medicinal value and luster. It ranks high amongst the animal products.

Pearl is produced by a special kind of Mollusca commonly known as “Pearl oyster”. It is a small bivalve shelled animal living in seawater, they are found in undisturbed areas of the sea, away from the coasts at a depth of 10-15 fathoms.

All the pearl oysters are not able to form pearl; its rare occurrence is due to its mode of formation. It is known to Chinese since 2300 B.C. but remained in closed-door practice. For Indian Vaidhyas it was known for its medicinal value and is termed as “Mukta” or “Moti”.

It is a small about 100 mm diameter, rounded, flawlessly white, coloured, pinkish, yellowish or bluish coloured calcareous product. In most of the cases the outer structure may be rounded but in general reshaped, oblong and distorted pearls are also founded.

Rounded pearls are supposed to be the best. The nucleus of the pearl may contain a foreign object of different nature otherwise it is a simple deposition of calcium carbonate. The iridescent luster its shooting effect makes it precious. The quality of pearl and its cost depends upon the nucleus of the pearl and its luster along with the shape and size.

Pearl Formation Process:

Pearl formation is a rare and accidental phenomenon, which takes about 2-6 years for attaining a good diameter. The longer time the bigger would be the size of the pearl but the pearl which develops within span of 3-4 years are very good in luster where as those which develop for 6 years or more loose their luster. This is due to the excess deposition of calcium carbonate, which is deposited in concentric rings. The pearls cannot be produced in all the oceans.

They are found in the warmer parts of the world and inhibit the warm water oceans. They are more common in Indian, ocean, coast of China, Japan and Pakistan. Japan is the most advanced country as far as pearl production is concerned.

But India too is one of the first few nations which are pioneer in production of pearl. In India it is found in Gulf of Kutch, Mannar, Bay of Bengal, Kanyakumari, Rameshwaram and at various other centres. Pak bay, Cyclone, Bangladesh and other coastal countries to produce pearl on small scale.

Pearl is produced by bivalve mollusc belonging to class Bivalvia, Family- Pteriidae and genus Pinctada. Some of the selected species are able to produce pearl and they are P. vulgaris, P.chemnitzi, P-margaritifera, P. anomiodes and P. atropurpurea. These are commonly known as Pearl-oysters. Besides this few other marine mollucans are able to produce pearl but their product is of inferior grade and does not have economic value.

These species are Haliotes (Linn.) mytelus, Placuna blanceta and Margratifera erythroseusis. Not only the marine bivalves but some of the fresh water bivalves too are able to produce pearl e.g., Unio and anodonta species but hey are of low grade and have no luster and economic value. Species of P. maxima and P. margaritifera are bigger in size and much healthier so they produce pearl of bigger size and best luster.

Size of a pearl depends upon the size of foreign particle, health and age of the oyster and condition of the seawater. A smaller nucleus requires more deposition as compared with a bigger nucleus. In the same way the shape of the foreign particle also is responsible for the shape of the pearl.

A healthy oyster will produce more calcium carbonate as compared with the weak and young oyster. At the same time the temperature of the water current activates the oyster. In oceans where temperature of water varies periodically the development of pearl oyster is affected and this badly affects the formation of pearl.

Formation of pearl is an interesting phenomenon. It is a protective device against the foreign invaders. It is just a chance that some of the unwanted material likes sand particle, seaweeds, crustacean larvae or any other parasite or object reaches in between the shell and the wall of the maritle.

This area is without any extra device and is unable to expel the object. In general practice the outer margin of the maritle is always attached along all sides with the nacre of the shell and no particle is allowed to invade it. But in case an object reaches this area it starts developing as a pearl.

The composition of a pearl and shell is almost the same. The wall of the maritle on its outer peripheral zone secretes a substance, which is deposited on the margin of the shell, and the shell grows in size but the inner margin of the shell is always attached with the epithelial layer of maritle.

This layer is a secretary layer and produces calcium carbonate and conchyolin spreaded uniformly alternating with each other. This gives a white, soothing, luster to the inner layer of the shell. This is termed as "mother of the pearl" also.

The same technique is applied in the formation of pearl also. The moment an object reaches in between the above said area it creates a pressure on the maritle wall and causes irritation.

This activates the nacre secreting cells of the maritle and the secretion is deposited around the object which is termed now as nucleus. The continuous growth of the nucleus causes more and more irritation and higher glandular discharge. This causes regular growth of the nucleus and thus a pearl is formed in nature.

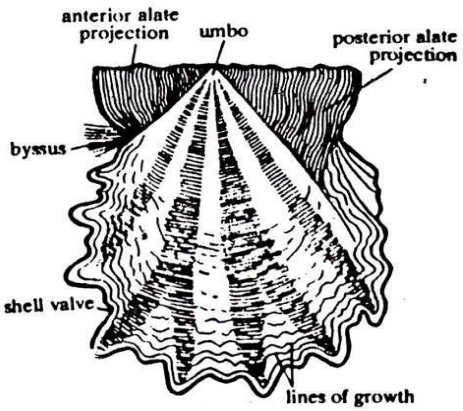
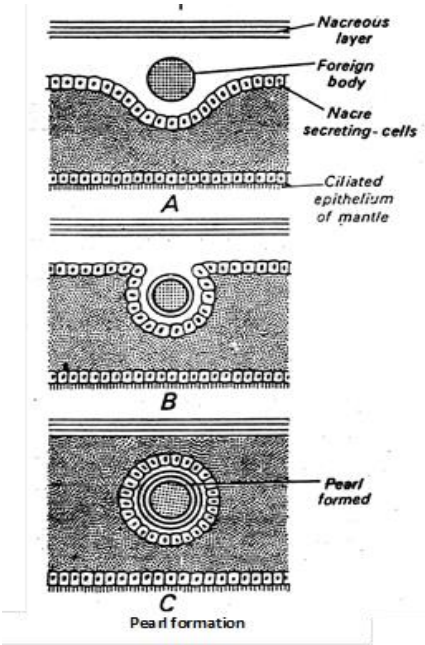


Fig. 47.9. Pinctada margaritifera



Pearl formation

Composition and Quality of Pearl:

Pearl comprises of water, organic matter, calcium carbonate and the residue:

(1) Water- 2.4%

(2) Organic matter- 3.5-5.9%

(3) Calcium carbonate- 90%

(4) Residue- 0.1-0.8%

Quality of Pearls:

The pearls obtained are of variable shapes and sizes. They may be white or cream red or pink red in colour. The spherical pearls of rainbow colour are rarely found. The best quality of pearl is known as 'Lingha pearl' and obtained from marine oysters. Pearl obtained from freshwater bivalves are not as valuable as those obtained from the marine oysters.

Artificial Pearls:

Keeping in mind the high demand and the cost of the pearl, European people have developed a new technology to form the pearl. This pearl is an artificial structure, which is formed by using the by-products of fishing industry. The scales of a cyprinid fish Alburnus species which are commonly known as Bleaks are scraped and the silvery white substance is collected and polished on the inner side of specially prepared hollow glass bead.

This produces a silvery white luster to the glass bead and this artificially coloured bead is sold as pearl. The central hollow space is filled with wax which provides strength and weight to the bead. The fish is confined to the north of Pyrenees and Alps in Europe so, such artificial pearls are common in European countries.

4. Pearl Production Process and Its Industry:

Although pearl industry may be established only on natural basis of pearl formed by oysters in the natural conditions but an artificial device to insert the nucleus as foreign particle in the shell of oyster has proved useful for the production of pearls in greater number.

This whole process is very much complicated, technical and time taking and can be managed as given below:

Collection of Oysters:

The oysters are collected from the bottom sea by the divers particularly women divers in Japan who are called as 'AMA' which in Japan means 'the girls of sea.' The divers usually have got training for proper diving into the seawater since their childhood for the search of seashells or seaweeds. The well-protected suits of cotton with cap are used at the time of diving. Each diver has a small hand net at the time of diving when she goes upto 5 metre depth. The net helps in the collection of oysters from the bottom.

The oysters collected by nets are stocked in the wooden bucket attached to the diver's left wrist by a cord and the diver with bucket comes up on the surface of water. An experienced diver can remain under water up to about one and half minute and can collect 2 to 10 oysters per dive. The best time for diving is form the early morning to mid-day. The best period for the collection of the oysters is of two months in the summer season when the water is nearer and the sea is calm.

During diving in deep seawater the divers operate directly from the side of boat and a rope remains tightly fitted to the diver's wrist through which the operating boatman pulls the diver out with force up to the surface after receiving any signal from the partner. Thus, the whole collected oysters are stored and stored out.

The oysters of same age group are segregated and two years old are kept in shallow water for future. Three years old oysters are sent to shallow water and in the months of April and May they are taken out. For pearl industry and proper supply of oysters, its eggs are incubated artificially which solves the problem of obtaining oysters for pearl culture.

Oysters are also caught by special type of cages (84 × 54 × 20 cm) by covering a heavy wire frame with two centimeter wire mesh. This cage is dipped into hot coltar as a measure against corrosion. Now this cage is dipped into the sand-cement mixture providing rough surface to the cages are suspend at a depth of 6 month from July to November where spots are easily available. These collected oysters are now transferred to rearing cages.

Rearing of Oysters:

The collected oysters are stocked and reared in special type of cages called as rearing cage. These cages are almost similar to those of collection cages except that they are further divided into 4 to 6 smaller

chamber and lack the diagonal sub-divisions. They are also covered with metal mesh and with netting of cotton.

These cages are well protected from natural enemies of oysters like Octopus, Eel, and Devilfishes etc. The collected oysters are first cleaned and then placed into the culture cages for a period of about 10 to 20 days to recover the strain due to excessive handling and for the physiological adjustment to the shallow water conditions.

Insertion of Nucleus:

The insertion of nucleus as foreign particle is very much technical process and is of great important for pearl industry. A number of methods are devised but most practicable and efficient method is one adopted by Nishikows. In this method a piece of mantle of living oyster is cut off and inserted together with a suitable nucleus inside the living tissue of another oyster.

Following steps are taken for the insertion of nucleus:

(a) Fitness of Oyster for Operation:

The selected oysters for the insertion of nucleus should be healthy and strong enough to over-come the shocks during operation. It is suggested that if the ovary and testis of oysters are got rid of they would be more resistant to the shocks of operations for this purpose oysters are dipped into cold and warm current of water alternately which initiates them to eject their sperms and eggs in case of males and females respectively.

Before operation, oysters are kept under stress of suffocation as a result they start to open their shells and at once a bamboo peg(piece)is inserted between the gap of two shells due to which shells may not be closed again.

(b) Preparation of Graft Tissue:

The piece of tissue which is inserted inside the mantle is called as 'Graft' tissue. A strip of about 7 × 0.75 cm is cut from the edge of mantle of healthy oysters by sharp knife.

This piece is smoothed cleaned and washed off the adhering mucus and again wiped off by wet sponge. The border of gill piece is removed by sharp scalpel. Now this tissue is trimmed to 2 to 3 cms long narrow strip and again cut transversely into small squares according to the size of the nucleus for insertion.

These squares are kept in sea water at 22°C where they can survive for about 48 hours. The outer edges of these graft squares must be known because nacre secreting cells are found only on the outer surface of the mantle so it is essential to keep the outer surface in contact with the inserted nucleus.

(c) Preparation of Nucleus:

Although any small particle may function as nucleus to initiate the pearl formation but it is reported that calcareous nucleus is the best because the deposition of nacre was found to be more satisfactory on calcarious nucleus as compared to any other particle.

Best nucleus is formed by the shell of molluscs with heavy deposition of calcareous shells. Such type of molluscs are easily available in India but Japan depends on U.S.A. for good quality of calcarious shells. It is also not able that spherical nucleus is best for the formation of good quality of spherical pearl.

(d) Insertion of Nucleus:

For the insertion of nucleus, oysters are fixed in a desk clamp in the position of right valve facing upward. Mantle folds are smoothly touched to expose the foot and the main body mass, followed by an incision into the epithelium of the foot and a slender channel in to the main mass. Suddenly one graft tissue piece is placed into the channel and the nucleus is placed over the graft tissue which functions as a bed for the nucleus.

Now the bamboo peg is quickly removed and oyster shells are closed automatically. For the insertion of the second nucleus similar operation is performed from the left side in the gonadial tissue and third insertion should never be tried. In Japan one trained girl can operate 25 to 40 oysters per hour and these girls are called as 'Tomarine son' means 'Miss Nucleus Pusher'. The operation period should not increase beyond 30 minutes and the oysters cannot survive beyond one hour of the operation period. So operation and insertion of nucleus should be performed by experienced persons.

(e) Post Operational Care:

Nucleated oysters are placed into cages and suspended into seawater and attached with floating rafts to a depth of 2 to 3 metres for about 6 to 7 days to recover from the shocks due to operation. This period of 6 to 7 days is known as 'Recovery period. Now oysters are examined properly and dead individuals are removed from cages. Sometimes, few oysters expel out the nucleus from the body due to heavy shock.

Now-a-days it is examined by X-rays whether oysters are having inverted nucleus or not. About 3000 to 3600 nucleated oysters are kept in different cages suspended in sea water at 2 to 3 meters depth for 3 to

6 years and undisturbed except at the time of clearing and inspection. The pearl oysters grow best in warm shallow waters generally not more than 14 meter deep.

Harvesting of Pearl:

Pearl are harvested in the month of December to February which may slightly vary according to the climatic conditions of the industrial area. After the completion of 3 years of the insertion of nucleus, pearl oysters are harvested from the sea and the pearls are taken out from the shell.

Clearing of Pearls:

After taking out the pearls from the oysters shell they are washed properly, cleared with the soap solution, but pearls should not be rubbed much.

Fresh Water Aquaculture

Aquaculture has been defined in many ways. It has been called as the rearing of aquatic organisms under controlled or semi controlled condition – thus it is underwater agriculture. The other definition of aquaculture is the art of cultivating the natural product of water, the raising or fattening of fish in enclosed ponds. Another one is simply the large-scale husbandry or rearing of aquatic organisms for commercial purposes. Aquaculture can be a potential means of reducing over need to import fishery products, it can mean an increased number of jobs, enhanced sport and commercial fishing and a reliable source of protein for the future.

Fish is a rich source of animal protein and its culture is an efficient protein food production system from aquatic environment. The main role of fish culture is its contribution in improving the nutritional standards of the people. Fish culture also helps in utilising water and land resources. It provides inducement to establish other subsidiary industries in the country.

- The basic principle of composite fish culture system is the stocking of various fast-growing, compatible species of fish with complementary feeding habits to utilize efficiently the natural food present at different ecological niches in the pond for maximizing fish production.
- Composite fish culture technology in brief involves, the eradication of aquatic weeds and predatory fishes, liming: application of fertilizers on the basis of pond soil and water quality, stocking with 100 mm size fingerlings of Indian major carps-catla, rohu, mrigal, exotic carps, silver carp, grass carp and common carp in judicious combination and density; regular supplementary feeding and harvesting of fish at a suitable time.

- Composite fish culture system is conducted by adopting three types of combinations viz., culture of Indian major caps alone, culture of exotic carps alone, and culture of Indian and exotic carps together.
- Fish production ranging between 3,000 to 6,000 Kg. per hectare per year is obtained normally through composite fish culture system.
- Development of intensive pond management measures have led to increase the fish yield further. Integrated fish and animal husbandry systems evolved recently are the fish-cum-duck culture, fish-cum-poultry culture, fish-cum-pig culture, utilization of cattle farm yard wastes and recycling of biogas plant slurry for fish production.
- Advantages of the combined culture systems, number of birds/animals, quantity of manure required and fish production potentiality of the recycling systems are described.
- Fish culture in paddy fields is an important integrated fish cum agriculture system.
- Essential requirements of paddy fields to conduct fish culture, characteristic features suitable for culture in rice fields, constraints to culture fish in paddy fields due to recent agrarian practices, and improved fish-paddy farming methodologies are discussed.
- Freshwater prawn culture is a recent practice. Giant freshwater prawn Macrobrachium rosenbergii and Indian riverine prawn M. malcolmsonii are the two most favoured species for farming purposes in India.
- Breeding, hatchery management, seed production, culture systems and production potentialities of the freshwater prawns are presented.
- Commercially important air-breathing fishes of India are the murrels, climbing perch, singhi and magur. Techniques of their seed production and culture systems are described.

Fresh Water Culture Systems

- Cultivable organisms are cultured in different types of culture systems.
- Many culture systems are based on traditional ideas that have been used for years, but some encompass new and some times radical concepts that make them unique.
- There are three major culture systems – open, semi-closed and closed culture systems.
- Each has its special characteristics, advantages and disadvantages.
- The choice of system is largely dependent on the function of the organisms to be grown and the resources and ideas of the farmer.

Open culture systems

- Open systems are the oldest and its farming is the use of the environment as the fish farm.
- Natural resources can be used as culture systems and organisms to be cultured are stocked in the water body.
- Capital expenses are low for the open culture systems. There is less management than in the other systems.
- The conditions are more natural and uncrowded in the culture environment, less time is required in monitoring the condition of the culture organisms in open systems.
- The disadvantages like predation and poaching are common. The growth rate and the uniformity of the product are variable compared to other systems.
- Cages, long lines, floats, rafts, trays and clam beds are examples of open system techniques.

Cage culture:

- It is the culture of fish or other organisms in a river, lake or bays by holding them in cages.
- Cages are built of metal rods, bamboo mesh or PVC pipes and covered by mosquito cloth or nylon net.
- Cage culture, in recent years, has been considered as a highly specialized and sophisticated modern aquaculture technique, receiving attention for intensive exploitation of water bodies, especially larger in nature, all over the world.
- In India, cage culture was attempted for the first time in case of air breathing fishes tikeH.fossilis and A. testudineus in swamps.

Pen culture:

Pens are the specially designed nylon or bamboo made enclosures constructed in a water body into which fish are released for culture. Such type of culture is referred to as pen culture.

Raft culture:

- Rafts are generally made of bamboo poles or metal rods with buoys at the top for floating in the water.
- These are used in the culture of oysters, mussels and seaweeds in open seas.

Rack culture:

- Racks are constructed in brackish water areas and inshore areas for rearing oysters, mussels, seaweeds, etc.

Semi-Closed Culture Systems

- In semi-closed culture systems, water is taken from natural sources or ground water and is directed into specially designed ponds and race ways.
- These systems offer an advantage over open systems in that they allow greater control over the growing conditions.
- A greater production per unit area is possible in addition to crop being more uniform.
- Water can be filtered to remove predators, diseases can be observed and treated more easily in semi-closed systems.
- The main disadvantages are more expensive and require more complex management. Ex:- ponds and raceways.

Pond Culture:

- The majority of aquaculture throughout the world is conducted in ponds.
- Earthen ponds or reinforced concrete ponds are used for culturing the fish, shrimp, prawn, etc. in both freshwater and brackish waters.

Raceway culture:

- A series of earthen or cement tanks are constructed along the course of a river or stream and are used for fish culture.
- Raceway is a culture chamber that is generally long and narrow. Water enters at one end and leaves through the other end in most cases.

Closed Culture System

- In closed culture systems, no water is exchanged and the water is subjected to extensive treatment.
- Extremely high densities of organisms may be raised under these conditions. Farmer has complete control over growing conditions in closed systems.
- The temperature is regulated, parasites or predators are not found and harvesting is simple.
- Food and drugs can be added efficiently into the system to grow quickly and uniformly.
- Fish or prawn culture in water re-circulation systems is good example for closed systems.

Water re-circulation systems:

- Here the water is conserved throughout most or all of the growing season by circulating in the culture tanks after purifying it through biological filters.

- Closed recirculating water systems are being used primarily for experimental work and for the rearing of larval organisms in commercial or research facilities.
- Closed systems are generally comprised of four components; the culture chambers, a primary settling chamber, a biological filter (bio-filter) and a final clarifier or secondary settling chamber for purification of water for reuse.

Induced Breeding

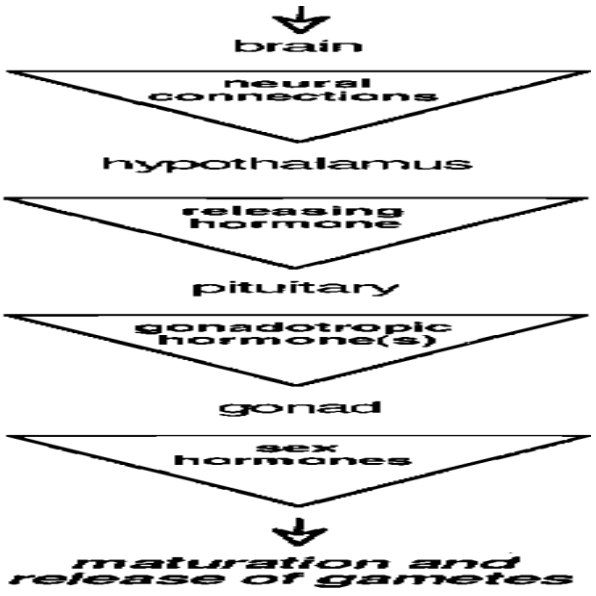
Major carps are most important species from the point of view of their high food and nutritive values. Hence they have kept attention of scientists and aqua farmers. They have peculiar habit of breeding in running waters of rivers and streams where they have large space for movement.

During breeding season, water is sufficiently provided with minerals, O2 and food contents. This friendly aquatic environment provides stimulus for spawning. Carps do not breed in confined water of captivity even if their gonads are matured and ovulation might have taken place in natural environment

For the increasing production of carps it is necessary that they should be made to breed in confined water so that increasing demand of good quality fish and their seed could be available.

This can be done by adopting induced breeding technique by which ripe or mature fishes breed in confined water when stimulated by injection of pituitary hormone. The pituitary hormone is an important gonadotropin, which is extracted from the hypophysis of a mammal or a mature fish.

Induced breeding of carps in captivity by the use of pituitary hormone injection has been successfully done. Many scientists are involved in doing research to improve induced breeding technique. Several attempts are being made to establish pituitary banks to meet out the need of pituitary hormones throughout the year.



Steps of Induced Breeding Technique:

Induced breeding technique has following steps:

1. Collection of Pituitary Extract:

Pituitary gland is collected from a mature fish, which is called as a donor fish. Most widely used donor fish is the common carp, Cyprinus carpio. The best time for preparation of pituitary extract is May and June. The Indian major carps like Catla catla , Labeo rohita, Labeo kalbasu, Labeo gonius and Cirrhinus mrigala do not breed in confined water and have need to be subjected to induced breeding.

To remove pituitary gland, the head of the fish is dissected and brain is exposed. The gland is immediately removed from the brain and stored in a refrigerator. It may be preserved in absolute alcohol at room temperature.

The gland then is homogenized in distilled water. The homogenate is centrifuged and clear supernatant is used as source of hormone to which 0.3% sodium chloride solution is added to it. This extract is ready for the immediate use. If pituitary extract is to be stored for a longer period the glycerin or trichloroacetate acid may be used instead of sodium chloride.

2. Selection of Breeders:

Medium sized fully ripe and healthy fish of around 2 to 4 years of age is preferred for induced breeding. The weight should be 1 to 5 kg. Healthy male and female breeders should be identified and netted out before the breeding season and should be kept in spawning pools. They should be provided with supplementary food.

3. Injection of Pituitary Extract:

To ensure higher success rate of fertilization it is important to coincide time of ovulation with the release of milt of male fish. For this purpose usually ratio of female and male 2:1 is maintained in every set. Dose of pituitary extract to be given is decided according to age, sex, weight size and state of maturity of both donor and recipient. A dose 2 to 3 mg of gland per kg body weight is given to female breeder.

There is no need of injecting dose to the male breeder if it in a state of milt oozing. After 6 hours of the first dose of injection another dose of 5-8 mg of gland per kg of body weight may be given to female if needed. However, a dose of 2-3 mg per kg body weight is recommended for the male breeder. More than 2 injections should not be given.

The injection given may be intramuscular at caudal peduncle or shoulder or intra-peritoneal at the bases of paired fins. The first injection should be given at the early hours of the day, while the second one in the evening. Weather should be rainy or cloudy for easy and early spawning. The fishes should be transferred to the breeding hapa after injecting the pituitary hormone.

4. Spawning in Breeding Hapa:

A pair of breeder is released into the breeding hapa for spawning after injection of pituitary extract. The breeding hapa is a rectangular case of fine netting. For larger fishes its size is 8′ x 3′ x 3′, but for the smaller fishes it is 5'x 3'x 3′. It is held on four bamboo poles, one at each corner of the rectangular case. The roof of the hapa may be open or closed.

The hapa is made of mosquito net cloth through which laid eggs and milt cannot escape out. Three-fourth part of hapa is summarized in water whereas upper one-fourth part remains in air. After 3 to 6 hours of injection of pituitary extract spawning takes place.

The fertilized eggs are white and opaque whereas unfertilized eggs are transparent and bead-like. A hatching hapa is also rectangular and made of muslin or malmal cloth and is open from above. The mosquito net hapa is present inside the hatching hapa.

5. Precautions for Induce Breeding:

(1) To avoid diseases and parasitic infections, breeders should be properly washed with $KMnO_4$ solution (0.5 g in 100 litres of water) for a few minutes. After this they should be kept in formalin (200 mg/ It of water) for one hour.

(2) Breeder should be protected from mechanical injuries during handling.

(3) Water condition should be favourable having temperature about 24 to 31°C and turbidity about 100 to 1000 ppm. Flowing water with higher O_2 content is of great use. The intensity and duration of light also affect the induced breeding and spawning. Pituitary glands taken from the same or related species as the recipient species are said to be more effective.

Advantages of Induced Breeding:

1. The seed spawn is timely available, its availability from natural sources is quite uncertain.

2. A pure spawn of a desired species is made available. The spawn obtained from the rivers are not pure. They are mixed with the spawns of other species and sorting of pure seed from the mixed spawn is not possible.

3. Any quantity of pure spawn can be made available.

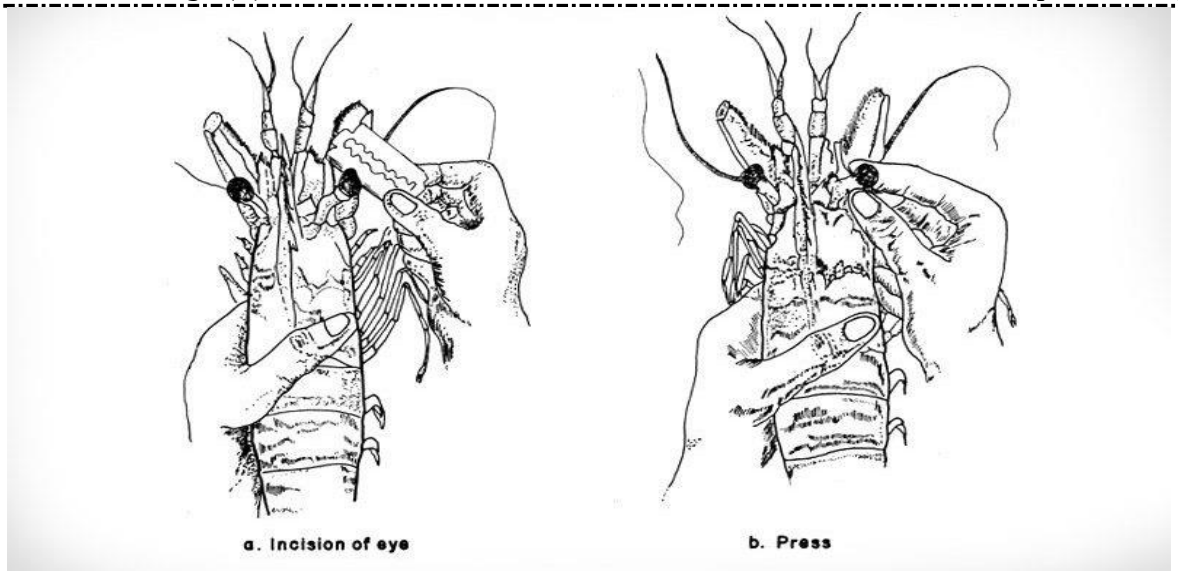
4. Several carps attain sexual maturity in ponds but they do not breed in confined water. Such fish can be subjected to induced breeding and spawn can be collected.

5. It is economical to obtain a spawn from induced breeding experiments in comparison to its collection from the riverine sources.

6. The induced breeding technique is very simple and can be learnt even by a layman.

Eye stalkAblation

- Eye stalk ablation is the removal of one (unilateral) or both (bilateral) eyestalks from a crustacean.
- It is routinely practiced on female shrimps (or prawns) in almost every marine shrimp maturation or reproduction facility in the world, both research and commercial.
- The aim of ablation under these circumstances is to stimulate the female shrimp to develop mature ovaries and spawn.
- Most captive conditions for shrimp cause inhibitions in females that prevent them from developing mature ovaries.
- Even in conditions where a given species will develop ovaries and spawn in captivity, use of eyestalk ablation increases total egg production and increases the percentage of females in a given population that will participate in reproduction.
- Once females have been subjected to eyestalk ablation, complete ovarian development often ensues within as little as 3 to 10 days.
- The most commonly accepted theory of why eye ablation reduces this inhibition is that a gonad inhibitory hormone (GIH) is produced in the neurosecretory complexes in the eyestalk.
- This hormone occurs in nature in the nonbreeding season and is absent or present only in low concentrations during the breeding season.
- It has been reported that in the tiger prawn (Penaeus monodon), the eyestalks fully regenerate in less than 6 months



Techniques :

Techniques for eyestalk ablation include:

- Pinching the eyestalk, usually half to two-thirds down the eyestalk. This method may leave an open wound.
- Slitting one eye with a razor blade, then crushing the eyestalk, with thumb and index fingernail, beginning one-half to two-thirds down the eyestalk and moving distally until the contents of eyes have been removed. This method, sometimes called enucleation, leaves behind the transparent exoskeleton so that clotting of haemolymph, and closure of the wound, may occur more rapidly
- Cauterizing through the eyestalk with either an electrocautery device or an instrument such as a red-hot wire or forceps. If performed correctly, this method closes the wound and allows scar tissue to form more readily. A variation of this technique is to use scissors or a sharp blade to sever the eyestalk, and then to cauterize the wound.
- Ligation by tying off the eyestalk tightly with surgical or other thread. This method also has the advantage of immediate wound closure.

Bio active Compounds From Corals

Bio active compounds derived from corals are of significant interest due to their potential pharmaceutical and biomedical applications. Here are some key points about them:

Diversity of Coral Species:Corals are marine invertebrates that exist in a variety of species, each potentially producing unique bioactive compounds.

Types of Bioactive Compounds:

- **Antibiotics and Antimicrobial**: Some corals produce substances that inhibit the growth of bacteria and other microorganisms. These can be useful in developing new antibiotics.
- **Anti-Cancer Compounds**: Several coral-derived compounds have shown promise in fighting cancer cells or preventing their proliferation.
- **Anti-Inflammatory Agents**: Compounds with anti-inflammatory properties can be derived from corals, which are valuable for treating inflammatory diseases.
- **Neuroprotective Compounds**: Research suggests that some coral-derived compounds may protect nerve cells and have potential applications in treating neurodegenerative diseases.
- **Antioxidants**: Many coral species produce antioxidants that can help protect cells from damage caused by free radicals.
- **Biomedical Applications**: These bio active compounds are being studied for their potential use in medicine, including drug development, wound healing, and as therapeutic agents.

Challenges and Conservation: Research into coral-derived compounds faces challenges such as sustainable sourcing without damaging coral reefs, as many coral species are threatened by climate change and other environmental factors.

Research and Discoveries: Ongoing research continues to discover new bio active compounds from corals and understand their mechanisms of action.

Future Directions:

Future studies may focus on sustainable methods for harvesting coral compounds, as well as exploring their full potential in various medical and biotechnological applications.

Overall, coral-derived bio active compounds represent a promising area of research with potential benefits for human health, though conservation efforts are crucial to ensure their sustainability.