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Freshwater Pearl Culture



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ABSTRACT

Pearls are considered as "Queen of Gems" and multi-billion dollar industries with the world's largest aquaculture activities in terms of value. Nowadays, cultured pearls are formed in a remarkable collision between nature and science. Pearl is a Gem produced within the mantle of a living shelled mollusk. It is a mineral and is composed of calcium carbonate. Pearls were the first Gem discovered by human beings thousands of years ago. Pearls are the only organic Gem and it requires no processing. The ideal pearl is perfectly round and smooth. Pearl is a composition of 85% of calcium carbonate, 12% organic matrix and water. Pearl has 3.5 to 4.5 hardness with a specific gravity of 2.7. A pearl is formed when a foreign particle *i.e.* piece of sand, insects, etc. by chance enters into the body of mussels and cannot reject that out and instead makes a shine coating on the particle layer by layer. This simple phenomenon is being applied in pearl culture practices. The pearl is similar to the inner shining layer of shell called "mother of pearl layer" or nacre constituted by calcium carbonate, organic matrix and water. Pearl is produced by oysters in marine and mussels in the freshwater environment. Pearls which are farmed and created by using freshwater mussels are known as cultured pearls.

1. INTRODUCTION

According to the Indian system of gemology of nine "Maha-Ratnas," the pearl is only next to diamond. Pearls are viewed as a symbol of purity, love and source of wisdom and power. While the demand for pearls in India and other countries is increasing, their supply from nature is reduced due to over-exploitation and pollution. India is importing a large number of cultured pearls every year from the international market to meet the domestic demand. In nature, a pearl is formed when a foreign particle *i.e.* piece of sand, insects, etc. by chance enters into the body of mussels and cannot reject that out and instead makes a shine coating on the particle layer by layer. The pearl is similar to the inner shining layer of shell called "mother of pearl layer" or nacre constituted by calcium carbonate, organic matrix and water. The pearls available in the market could be artificial. Imitated pearls are not pearls but pearl-like material that simply contains a rigid, round core or base and outer pearly coating^{5, 8}.

2. History:

Long known as the Queen of Gems pearls were once the exclusive property of the rich and powerful. No one can say who discovered pearls probably they were first found by ancient people searching the shores for food. The Romans and Egyptians prized and used them as decorative items as far back as the fifth century BC. Chinese records mention them earlier still. Pearls used often to symbolize purity and perfection^{2, 9}. The techniques for introducing an irritant into the oyster to stimulate the secretion of nacre which forms the pearl. The era of cultured pearls had begun though it would take decades before the original techniques were perfected^{1, 6}.

The original Japanese cultured pearls, known as akoya pearls, are produced by using a species of small pearl oyster, *Pinctada fucata*, which is no bigger than 6 to 8 cm in size; hence, akoya pearls larger than 10 mm in diameter are extremely rare and highly priced. Today, a hybrid mollusk is used in the production of akoya pearls. Other species of oyster used for producing cultured pearls are the penguin's wing oyster *Pteria penguin* and the specific wing-oyster *Pteria sterna*. India recognized the pearls about thousands of years ago and has a long and glorious history of appreciating pearls. Hyderabad is known as the Pearl City from the time of Royal Kings of Nizam. In natural pearls the core or nucleus in minutes with thick pearl nacre. Generally, a natural pearl is small in size and irregular in shape. A cultured pearl is also natural pearl, the only different, being the human intervention in

surgical implantation of a live mantle graft and nucleus for hastening pearl formation to the desired^{7, 13, 15}.

3. Pearls:

It is a Gem having deep lustre with wide ranges of colours and is perfectly round in shape. It is a composition of 85% of calcium carbonate, 12% organic matrix and water. Pearl has 3.5 to 4.5 hardness with a specific gravity of 2.7^{16, 20}. Any shell mollusk can produce a pearl of some sort, but those mollusks who gave a pearl lining or pearl nacre in the interior of the shell surface can produce lustrous pearls. The mollusks, the univalve gastropods and the two bivalves, with a nacreous layer, can produce pearls. Pearl is produce by oysters in marine and mussels in the freshwater environment. Pearls are classified into three types: Natural Pearls, Cultured Pearls, and Artificial or Imitation Pearls. A pearl is a natural Gem created by a living organism. When a foreign object is introduced into a mussel or oyster, the animal coats the irritant with a substance called nacre, the same material with which it makes its shell. Layers of the nacre gets built up to form pearls.

3.1. Types of Pearls:

3.1.1. Natural Pearls:

Natural pearl is small in size and irregular in shape. The surfaces of natural pearls are rough. The nucleus of natural pearl is small with thick crystalline nacre. When a foreign particle such as piece of sand or parasite enters into any species of mollusks that cannot be expelled, then natural pearls are formed. Mollusk secretes nacre to coat the foreign particle to avoid irritation due to it. As a result of which many layer by layer around the irritant of natural pearl is formed. There are very less natural pearls left due to the over-exploitation of the natural stocks^{10, 14, 20}.

3.1.2. Cultured Pearls:

Cultured pearls are also natural pearl, the only difference being the human intervention in surgical implantation of live nucleus of desired shape into the body of mollusk where it cannot be expelled. Cultured pearls are generally larger and are of a more consistent size and colour than that of natural pearl. Cultured pearls are produced in both marine and freshwater environment with desired size, shape, colour and lustre. There are various steps and methods

involved in pearl culture. Most wild, natural pearl producing oyster beds have vanished due to over-fishing, oil drilling and pollution. Today, the world's most beautiful pearls are cultured. Cultured pearls share the same properties as wild pearls. The difference is that a technician opens the shell and inserts the irritant which stimulates nacre production. Seawater oyster have a round shell bead grafted in as the irritant. This is called Nucleating. Oysters are suspended in water from rafts and at risk from typhoons, parasites, predators and algae. Freshwater mussels have a small piece of mantle tissue (nacre producing from another mussel) introduced as irritant. This tissue desiccates leaving a solid pearl. Mussels are farmed in inland lakes and rivers^{15, 22}.

3.1.3: Designed Cultured Pearls:

When images designed by man is intentionally placed between the mantle and outer cover of all shell, to avoid unbearable pressure nacre cells will start growing around the foreign matter. Gradually the foreign matter will transform into pearl. In one way it is a natural pearl only. Only thing is that it is not accidental but due to human intervention. This designed cultured pearl will stay as the symbol of beauty forever. Only upper part of the bead will have pearl covering and when it covers completely the remaining part is cut from the outer shell. The foreign matter inside the part is removed and special type of cement is filled in place. This type of pearl is used in necklace and earrings and will certainly increase the beauty of the wearer. In Bristol pearl one side is flat. If the pearl have any other design instead of hemisphere it can be called as designed pearl. The colour of the pearl will depend on the food given and the type of mussel. Usually, pearl made from freshwater shells are seen in pure white, cream, pink, green, steel grey, gold rose and peacock blue^{21, 24}.

3.2: Pearl Colours:

Pearls come in a variety of colours. Natural colours are mainly down to the breed of mollusks. Other influences include diet, water temperature and pollutants. No-one can predict or control what colour pearls will be produced in any hatchery. Black but blue, green, silver, grey, aborigine, copper, peacock and many more. Green is the predominant colour. Another classification of pearls is based on freshwater pearls or saltwater pearls. Farmed pearls are mainly two types: tissue nucleated and bead nucleated. Nucleation is the process of growing cultured pearls and starts with inserting a foreign object the nucleus into mollusk. That

triggers the mollusks to cover the nucleus with nacre until a pearl is formed. The nucleus can either be a bead or a piece of mantle tissue from another shell¹.

3.3: Quality of Pearls:

In order to stimulate more rapid secretion of nacre, the mussels should be suspended 20-30 cm below the water surface. The culture period of freshwater pearl is 2-3 years. About 6 months before harvest, they are lowered to 80-100 cm below the surface. Although during these last 6 months, the nacre will be secreted more slowly, the pearl will become denser and its colour lustre will improve. Culturing for more than 3 years, the pearls tend to become duller. Pearls cultured for just one year are small in number and size, asymmetrical and of bad quality^{23, 24}.

4: The Mussels:

There are two genera of pearl mussels can be used: *Lamellidens* and *perreysia*. The one used for pearl culture should be *Lamellidens marginalis*, for two reasons. First, due to its size (the mature ones range from 7 to 10 cm, measured from anterior to posterior end) as compared to *perreysia*, it is suitable to operate on. Second, they are common in most inland bodies of water. The varieties of freshwater mollusks are called triangular oyster^{15, 19, 22}. They are well suited for cultured pearl production. They are tissue nucleated and left to grow and develop in the freshwater pond. During this time, water quality and management is very important. After 2-3 years pearls are harvested. After tissue and pearls are removed from the triangle oysters, the meat is separated and the pearls are rinsed. After the pearls are harvested, various techniques are used to polish and treat the pearls including tumbling, polishing and heating. The purpose is to maintain the integrity of the pearl while optimizing its colour and lustre. Pearls are then drilled and sorted according to colour, size and quality.

4.1: Feeding Habit:

Freshwater pearl mussels are filter feeders, removing phytoplankton and other suspended particulate matter from the water.

4.2: Life Cycle:

Mussels have a very unique life cycle. In order to maintain, mussels larvae need to attach to a fish and use some of fish energy to develop internal organs. They do parasitize the gills, fins

or other external structures of fish, but usually, don't hurt it. After a few weeks, the mussels drop off the fish and are now juveniles. Blue gill sunfish, *Lepomis macrochirus*, juveniles of salmonids and *Oreochromis niloticus* are the important host fishes of glochidia larvae. The juveniles can live by themselves on the substrate, eating algae and bacteria and eventually grow until becomes adults.

4.3: Culture of Juvenile Mussels:

The juvenile mussels are cultured in a separate large tanks/smaller size ponds until they reach the adult stage. The juveniles are provided with adequate amount of phytoplankton for its accelerated growth. The matured mussels are used for pearl production through implantation.

4.4: Reproduction in Mussels:

The reproductive biology of freshwater mussels is unique among all mollusks. The larvae (glochidia) are obligating parasites on the gills or fins of fish and exhibit various degree of host fish specificity. Females are fertilized internally by siphoning in the sperm released by males, and eggs are fertilized in the suprabranchial cavity. Fertilized eggs are retained in the water tubes of the gills and develop to the glochidium stage. Females can contain between 50,000 and 5 million glochidia, depending on the species and size of females. When glochidia are mature, they are released as free-floating individuals or in packets called conglutinates. These glochidia must come in contact with or be ingested by a suitable host fish for attachment and metamorphosis to the juvenile stage. Mussel species vary in host fish specificity, but most are restricted to a limited number of suitable hosts. After metamorphosis, juveniles drop from the host fish and begin their benthic life².

4.5: Pearl Sac:

The process of pearl-sac formation starts with the proliferation of the outer mantle epithelial cells to make a continuous sheet in the mesodermal tissue, where the mantle allograft (transplantation) can be implanted. Subsequent secretion of shell materials (*i.e.* periostracum, prismatic and nacreous) causes the accumulation of pearl substance inside the pearl sac itself. The functions of each type of epithelial cells are different. Tall, stratified, columnar epithelium secreting substances which makes periostracum, while simple cuboidal and squamous epithelium without cilia produced pearl substances for the nacreous layer. Mucous cells infiltrating between epithelial cells are responsible for mucosubstances controlling the

production of organic matter and at the same time absorbing the calcium from the surrounding water into mussels. These mucous substances are eventually combined with calcium and become a part of the pearl layer³.

5: Implantation Procedure:

The mussels from which the epithelial implants will be taken are collected and put in buckets of clear water to flush them. First, the adductor muscle is cut so that the shell may be easily opened. A pair of scissors is then used to cut strips of epithelial lining between the inhalant siphon and the adductor muscle. The epithelial strips are placed on the hard wooden board and the connective tissue is cleaned off the back of the strips. They are then cut into 3-4 mm long and 0.5-1 mm thickness of pieces. Healthy mussels are selected and put into assembly that will force the valves open 1-1.5 cm apart, are made in the epithelium of the marginal zone. The previously cut epithelial pieces are then inserted into the holes, about 0.5 cm deep, making sure they are firmly in place. About 25-30 slices can be transplanted in mussel of 10 cm in length.

6: Care and Culture of Operated Mussels:

Operated mussel can be held in net bags, net cages and folders. Operated mussels should be reared in the water bodies free from pollution and disease. The water should contain adequate phytoplankton for its growth. The ideal temperature range for culturing mussel is 20-30°C. Temperature <10°C will prevent the mussel from secreting nacre^{23, 24}. If oxygen level is too low (<30 mg/l) mussels will easily die. Within 2-3 weeks after transplantation would have healed, but the mussels will still be weak. It is therefore easy for bacteria and parasites to infect the mussels. Dead mussels should be quickly removed. In the presence of any dead mussels in pond, high mortality is observed through spreading of infection.

6.1: Nutrient Enrichment:

Use of artificial fertilizers, spreading of slurry, untreated sewage and industrial waste with a high biological oxygen demand, even when at low levels as a point source of enrichment, combine to increase nutrient levels within a river system to an extent which can in time change the river from oligotrophic to mesotrophic to eutrophic. Initial negative effects may be stress on the filter feeding mechanism within adult mussels, adapted for systems where nutrients are low. Excessive nutrients encourage filamentous algal growth, which then coats

the bed of the river or pond which is culture site for freshwater mussels, leading to oxygen deprivation below the substrate surface, making it uninhabitable for juvenile mussels. As the alga dies, it decomposes into fine organic silt which reaches deeper into the apertures of the gravel, causing long term clogging and less chance of fresh oxygenated water reaching the substrate. Calcium is the most important component of both the mussels shell and pearl. Mussels and pearl production depend on assimilation of calcium. It is most important that calcium content of water should be over 10 mg/l for better mussel growth and pearl production. If calcium is more than 10 mg/l of the water, it is also good for mussels and pearl production rate⁴.

6.2: Pollution Incidents:

Severe pollution events such as slurry, sheep dip, silage and industrial spills are well known for causing large fish kills. In the mussel culture site when such accidents occur, salmonids fish which are essential for mussel life cycle can be lost. That's why long term stress and death of young and adult mussels from oxygen deprivation, to immediate death of the entire mussel population from toxic poisoning¹³.

7: Freshwater Pearl Culture Integrated with Ornamental Fishes:

Integrated farming may be defined as a sequential linkage between two or more farming activities. The basic principles involved in integrated farming are the utilization of the synergic effect of inter-related farming activities and conservation, including the full utilization of farm waste. It is based on the concept that "There is no Waste" and "Waste is only a Misplaced Resource" which can become a valuable material for another product. It is not only an efficient way of recycling farm waste but also produces high economic returns from ornamental fishes as well as from freshwater pearls. Fish excreta and other organic suspended materials are efficiently utilized by freshwater mussels. Hence, reduce water pollution and waste becomes pearls. Freshwater pearl mussels are live in lakes, rivers, fish ponds and irrigation canals, it occurs in flowing waters with slightly firm and mud or mud bottom. It can live in areas that have slow currents.

7.1: Limitations:

Even though freshwater pearl production along with ornamental fish culture has much of benefits, which has some limitations too. While the mussels integrated with ornamental

fishes, if it reproduces, the glochidia cause severe problems to ornamental fishes. To overcome these problems, the possible ways are: 1) Only one sex either male or female mussel used for pearl culture in the integration with ornamental fishes. 2) Triploid mussels can be used for this integration. Triploid mussels being a sterile animal; they could not reproduce in their life.

7.2: CONCLUSION:

The culture of freshwater pearl mussels and ornamental fishes in the same pond improve the productivity and reduce the space requirement. It also minimizes water requirements. The freshwater pearl mussels consume the excess amount of algae available in the fish pond and thereby protect the pond from algal bloom formation. It reduces the feed cost of the freshwater mussels. And water exchange from ornamental fish farming is also minimized by this integration which ultimately lowers the cost for power utilization. Freshwater pearls produced through this implantation method utilize the pest (F/W pearl mussel) to make profit by recycling the organic waste. If the seeds collected from the primary feeder canal through the screen, it is advisable to go for scattering method of culture where no contact between fish and mussel. The scattering method is possible if the farm yard with adequate land facility. The net suspension method is advisable for monosex or triploid mussel which produced through captive breeding not by natural collection^{5, 11, 12}.

8: Production Technology:

In order to undertake the culture of pearls, there are four necessary requirements; a culture area, mussels, instruments and irritant or nuclei.

8.1: The Culture Site:

The site, where the mussels will culture in a pond, lakes, rivers etc.

8.2: The Mussels:

Known species of mussels for freshwater pearl culture will be used.

8.3: The Instruments:

8.3.1: Equipments for Operation:

To operate on the mussels for culturing pearls, the following special instruments are needed:

- 1. Shell Opener:** Made of stainless steel. When the handle is pressed, the end opens. This is used to pry open the shells of mussels and prevent them from closing to the prior to the introduction of nuclei and grafts.
- 2. Graft Cutter/Knife:** Made of flat stainless steel and used in the preparation of graft tissues and made from the mantle of the mussel.
- 3. Incision Knife:** Made of stainless steel with a plastic handle. It has a rounded, flat sharp tip which is used to make slits on the gonad, through which the nucleus and graft tissues are inserted.
- 4. Nucleus Carrier/Lifter:** Similar to the incision knife except that the tip is cup-shaped. It is used to carry a piece of nucleus/irritant for insertion into the gonad. In doing so, the tip should first be wet with water; this due to surface tension will enable the instrument to lift the nucleus.
- 5. Graft Carrier/Lifter:** Similar to that of the incision knife and nucleus lifter except that the tip is pointed. By pricking and lifting a piece of the mantle tissue, used to insert the tissue inside the gonad.
- 6. Spatula with a Hook:** Made of stainless steel with a flat end. It is used to lift the gills of a mussel to allow an unobstructed view of the body prior to the operation. The hook is used to hold the foot when making an incision during the operation.
- 7. Mussel Holder:** A wooden block with a large ordinary paper clip fitted at the end, holds the mussel to be operated on in place.
- 8. Pinchers:** An ordinary pincher which is used in the preparation of graft tissue, as in picking up the cut/detached mantle from the mussel.

9. Wooden Peg: Made of wood 0.7 cm in diameter and 3.5 cm long. It is placed between the shells of the mussel, after it opened by the shell opener, to prevent the shells from closing during the operation.

10. Graft Cutting Board: A wooden block used in cutting the mantle to a desired size and shape.

11. Miscellaneous Equipment: Other items needed during the operation include basins, trays, rubber sponges, dishes and bowls.

8.3.2: Fixing Equipments:

For fixing operated mussels, the following materials are use. Bamboo or wood stakes for sustaining the plastic ropes. Plastic ropes for hanging net bags, net cages, net folders and floats. Foam, glass ball, bamboo section and plastic bottle as floats for supporting the mussels culture bags, cages and folders.

8.3.3: Equipments for Holding Operated Mussels:

Operated mussels can be held in the net bag, net cage and net folder then they can be hanged with plastic ropes.

8.3.3.1: Net Bag:

One net bag can hold 2-3 mussels, material of which may be polyvinyl chloride. The bag should be fixed with the plastic rope, which is the popular system in pearl culture.

8.3.3.2: Net Cage:

Net cage may be different shaped and can be made of net and bamboo ring in which 2-3 mussels can be held. Then the cage should be fixed with the plastic rope for operated mussel culture.

8.3.3.3: Net Folder:

It can be made of net and wooden or iron frame, with one row or several rows of pocket. One mussel can be kept in one pocket, and then the folder should be fixed with the plastic rope. This system is suitable for nuclei pearl culture and image pearl culture because it can keep

mussels ventral margin upward, promote wound recovery and prevent the nuclei out of the wounds.

8.4: Nuclei or Irritants:

With the use of nuclei or irritants, the pearls can be produced in a shorter culture period as they start from larger material than is naturally found. The best material for nuclei would be made of shell as it has the same composition as that of pearls. For the culture of pearls, substitute material can be used. The size should range from 2 to 4 mm for the average local mussel and not any larger. Ceramic is a good substitute nuclei as it has almost the same weight as that of a pearl. Other material such as sand can also be used. The smooth type of sand should be selected. Round object for nuclei can also be formed out of either ordinary or white cement. When using these they should first be thoroughly dried for several days.

8.4.1: Preparation Procedure:

The first type of nucleus (stelon beads), is prepared by using commercially available self-cure acrylic repair material (powder and solvent) mostly required in dentistry. The second type of nuclear material (shell bead) is made from the dead shell piece of the freshwater mussel *Lamellidens marginalis*. For the preparation of stelon nuclear material, required amount of acrylic powder is taken in a glass container and slowly the solvent is added to it, (preferably through a syringe) which is then mixed thoroughly to prepare dough. Immediately, nuclear material of desired shape and size are prepared and allowed to air dry. Later, they are stored in dust free close containers. The nuclear material is boiled in water, air dried and cooled few hours prior to implantation. For the preparation of the shell bead nucleus, dead shell of *Lamellidens marginalis* are collected and subjected to through washing in water to remove dirt and sand particles. The dry flesh materials, if present are scraped out. The shells are then dipped in 5000 ppm of chlorine solution (50 gm of bleaching powder containing 10% chlorine in 0.1 liter of water) for 24 hours or 48 hours required. The completely lye-peeled shells are sorted out and are then continuously washed in tap water. They are then kept in an oven maintained at 60°C, for more than 2 hours or can even be sun dried, for a longer duration to ensure the complete removal of chlorine from the treated shells. The dried shells are made into small pieces by using a mortar and pestle and are then finely powdered by means of an electric grinder. The powdered shells are then processed through a sieve of 0.01-0.05 mm mesh size. The commercial glue Araldite hardener and resin (which acts as a

binder) are mixed in a ratio of 1:1 to prepare a paste. To this paste the sieved shell powder is added gradually to prepare dough of this consistency. The ratio of the shells powder to the paste should be 5:1. Immediately, nuclei of desired shape and size are prepared and then are air dried till they become hard. Prior to implantation, the nuclei are boiled in water and cooled¹⁰.

8.5: Supplies and Sources of Raw Materials:

Pearl oysters are collected from a species of freshwater mussels which are found in abundance in inland bodies of water such as lakes, rivers, ponds and dams.

9: Operation of Mussels:

There are two methods of operating on mussels; operations using the gonad for round pearls and then use of the mantle for seed pearls.

9.1: Basic Steps Involved in Freshwater Pearl Culture Operations:

In India, common species which is used for pearl culture are *Lamellidens marginalis*, *L. corrianus* and *P. corrugata*. Farming practice of the freshwater pearl culture operation involves six major steps sequentially.

9.1.1: Collection of Mussels:

The healthy mussels are collected from the freshwater bodies like pond, river etc. they are collected manually and kept in buckets or container with water. The ideal mussel size used for pearl culture is over 8 cm in anterior-posterior length.

9.1.2: Pre-Operative Conditioning:

The collected mussels are kept for pre-operative conditioning for 2-3 days by keeping them in crowded condition in captivity with aged tap water at a stocking density of 1 mussel/l. Pre-operative conditioning helps in weakening of adductor muscles which helps in easy handling during surgery.

9.1.3: Implantation of Grafts and Nuclei:

Mussel surgery is depending on the place of surgery, the implantation is of three types. Mantle cavity, mantle tissue and gonadal implantations. The key materials required during

the surgical implantations are beads or nuclei, which are usually made from mollusks shell or other calcareous materials.

9.1.3.1: Mantle Cavity Implantation:

In this procedure round (4-6 mm diameter) or designed beads are inserted into the mantle cavity region of mussel after opening the two valves (without causing injury to mussels at both ends) of animal and spreading carefully the mantle of anterior sides from the shell with the help of surgical set. Implantation could be done in mantle cavities of both the valves. In case of implantation of designed beads care is taken such a way that the design portion faces the mantle. After placing the beads in desired place the gaps created during implantation are closed just by pushing the mantle onto the shell.

9.1.3.2: Mantle Tissue Implantation:

The mussels are divided into two groups; the donor and the recipient mussels. The first step in this procedure is preparation of graft (small piece of mantle tissue). This is done by preparing a mantle ribbon (a strip of mantle along the ventral side of the mussel) from a donor mussel, which is sacrificed and cutting that into small pieces. The implantation is done on recipient mussels, which is of two types *i.e.* non-nucleated and nucleated. In the former only the graft pieces are introduced into the pockets created at the inner side of posterior pallial mantle present at the ventral region of the mussel. In the nucleated method, a graft piece followed by a small nucleus (2 mm diameter) is introduced in the pockets. In both the producers care is taken so that graft or nucleus does not come out of the pocket. Implantations could be done at mantle ribbons of both valves. This method of implantation is adopted for production of mode pearls per mussels and also the stress on the animal is minimum.

9.1.3.3: Gonadal Implantation:

This procedure also involves preparation of grafts as mantle tissue method. First a cut is made at the edge of the gonad of the mussel. Then a graft is inserted into the gonad followed by nucleus (2-4 mm diameter) so that the nucleus and graft should be in close contact. Care is taken such a way that nucleus touches the outer epithelial layer of the graft and the intestine is not cut during the surgery^{13, 14}.

9.1.4: Post-Operative Care of Mussels:

Implanted mussels are kept in post-operative care unit in nylon bags for 10 days with broad spectra-antibiotic treatment of Chloramphenicol at the rate of 1-2 ppm as a prophylactic measure is beneficial for the survival and wound healing of the implanted mussels and supply of natural food with aged tap water. It is desirable to add green water (algae enriched) into these units after 3 to 4 days of post operation care. The units are examined daily with removal of dead mussels and the ones that reject the nucleus.

9.1.5: Pond Culture of Implanted Mussels:

After post-operative care, the implanted mussels are stocked in the ponds. The mussels are kept in nylon bags (1 cm mesh-12 sq.cm) at the rate of 2 mussels per bag and are hung from bamboo or PVC pipes and placed in ponds at 1 m depth. The mussels can be placed at deeper zones (up to 2.0 m) during hot summer months to avoid surface heating. The mussels are cultured at stocking density of 20,000-30,000/ha. The implantation in pearl culture operations are carried out throughout the year, except during hot summer months for minimizing post-operative mussel mortality and rejection rate of implanted graft and nuclei. Ponds (2.5 m deep) with clay soil base and slightly alkaline waters are suitable for pearl culture operations. A rectangular shaped pond with proper in-lets and out-lets is ideal for implanted pearl mussel rearing. Ponds without aquatic macrophytes and algal blooms such as *Microcystis* sp. and *Euglena* sp. are suitable for pearl culture operations. The ponds are provided with PVC tubing (2 diameter) platforms as rafts for hanging pearl mussel culture units. The implanted mussels at a density of 50,000 to 75,000/ha are placed in nylon bags. The area of the pockets and mesh size in these simple culture units are sufficient for the mussel to open and close their shell valves for feeding and operation. These bags are then tied to the PVC floating platform units or bamboo rafts maintained in the culture environments. The ideal hanging depth of the pearl mussel culture units is observed to be 1.5 to 2.0 m deep in the ponds for good survival and growth of mussels.

Alternatively, the mussels can also be placed in plastic crates 20 to 25 mussels per crate. The physicochemical parameters and water level of the ponds are to be monitored throughout the culture period of the mussels. The optimum temperature regime lies between 25°C to 30°C. The freshwater pearl culture pond environment is generally the same as employed for the aquaculture of the carps. The freshwater mussels constitute the benthic invertebrates in the

pond ecosystem and hence free dispersal on pond bottom should be ideal in terms of growth and survival. It has been observed that mussels, when maintained in bottom-set culture units, recorded poor survival (27%) as against surface and column-set units (66%). The reason for poor survival in bottom units may be due to the reduced level of primary production and siltation aggravated by the restricted space in the units. Other important parameters are the extent of macrophytes infestation and movement of water in the culture environment. However, pond bottom distribution of the operated mussels has certain problems in sampling and harvesting of mussels for pearls. The ponds are fertilized with organic and inorganic fertilizer periodically to sustain the plankton productivity. Addition of green water (*Chlorococcum* sp. and *Scenedesmus* sp.) at regular intervals into the pearl culture ponds as direct mussel feed is observed to be an ideal practice for proper keep-up of the pearl bearing mussel standing crop. The green feed is developed by "open culture method" in a series of Ferro-cement tanks (200 litres) arranged all along the pond bundh. The water in the tanks is fertilized as given below¹⁵.

Cowdung: 1000kg/ha/yr.

Urea: 100kg/ha/yr.

Single super phosphate: 100kg/ha/yr.



Once the fertilizer degrades (7 to 10 days), the green water develops. The algal enriched water is lead to the pearl culture ponds. The mussels under being mucoid filter feeders can accept a variety of particular organic material feed. The pearl mussels in captive culture conditions can be maintained on a diet of powdered rice and groundnut oil cake (1:1 ratio) at 1% of the weight of the mussels provided on an alternate day basis. Periodical checking of mussels with the removal of dead ones and cleaning of bags is required throughout the culture period of 12-18 months.

9.1.6: Food and Feeding:

Algae being the predominant component of the first trophic level in the aquatic food chain have got much importance in aquaculture systems. Some species of algae belonging to *Chlorophyta* (green algae), *Bacillariophyta* (Diatoms) and *Cyanophyta* (blue-green algae) are normally used as feed by the freshwater mussels. The commonly preferred algal species by the freshwater mussel *Lamellidens marginalis* are diatoms green algae (*Chlorella*

chlorococcum, *Scenedesmus* etc.) and blue-green algae (*Spirulina*). Culture vessels and tanks of desired capacities are to be selected before algal culture. The suitable medium should be prepared well in advance for different species to be cultured¹⁶. The pond culture of operated mussels varies from six months or more depending upon the size and number of nuclei implanted, the health of the mussels and the condition of the pond environment. The culture units require periodic cleaning of accumulated silt and other fouling fauna, finding entry into the units. The maintenance of the shells after dumping in the pond is to check the mussels weekly and remove the dead mussels as they will harm the healthy mussels in the nets. And add fertilizers or lime if required¹⁷.

9.1.7: Harvest of Mussels and Pearls:

India being a tropical country, the culture period of pearl is narrow as compared to other temperate countries. The pond culture of operated mussels varies from 12 months or more depending upon the size and number of nuclei implanted, the health of mussels and the condition of the pond environment. At the end of the culture period, the mussels are harvested. The mussels are either crushed following by sieving to extract pearl or the mussel is individually sacrificed, or individually pearls are taken out from the pearl sac of the live mussels without sacrificing. The latter method, though difficult, is desirable to prevent depletion stocks of mussels in the natural environment. In the mantle cavity insertion method, the culture period is generally about 6 to 12 months, depending on the size and number of nuclei implanted. In this method, the mussels at the end of the culture period are sacrificed. The mussels are opened one by one and a half round of design, shell attached pearls are cut out of the shell valves. Two to five attached pearls are cut out of the method, depending on the number of nuclei inserted. As on the attached side of the pearl, nacre cannot be deposited, it is ground off and cemented with a piece of mother of pearl layer obtained from the shell interior. The success rate is about 60-70% of the mussels implanted. The culture period in mantle tissue implanted mussel is generally from 12 months to 18 months, in this method the mussels after the culture period are carefully and the pearls are removed one by one from the pearl sacs. Four to eight pearls are obtained per mussels depending on the size of the mussel and the number of correct implantation done¹⁸.

The same mussel can again be used for the next operation. Alive graft piece or even a small nucleus (less than 2 mm diameter) if implanted into the same pearl sac may result in a pearl in less time. In this method, non-nucleated, solid, unattached and irregular to oval pearls (2 to

3 mm size) or round, unattached cultured pearls are obtained. The success rate in this procedure is about 60-70% of the mussels implanted. The culture period of the gonadal implanted mussel is generally 12 months. The mussel after the culture period is opened carefully and the position of the pearl is felt by touching they are close to the incision scar. By a pair of scissors, fine forceps and needle the pearl formed are carefully removed without cutting or damaging the intestine or other internal tissues. The mussel may also be sacrificed to extract a gonadal pearl. The layers of the gonad are cut open and the pearl is removed easily. In this process regular, round (3.5-5.5 mm diameter) unattached pearls are obtained⁶.

9.2: Surgery:

Methods of freshwater pearl culture vary depending on the surgery done in the internal structure of the pearl mussel and the type of pearl products targeted. A shell bead constitutes the essential input in the pearl culture operations. The certain locally available, inexpensive and bio-compatible acrylic material can be employed as nuclei in freshwater pearl culture. It has been observed that the pearl mussels of the size 8 to 10 cm in shell length and weight of 50 gm and above are ideal for pearl culture operation¹⁹.

The mussels before surgery are segregated into two groups, the mussels to be operated upon the operation mussels or recipient mussels and those to be sacrificed, is the cell mussels or the donor mussels. The live donor mussel is sacrificed and the pallial mantle ribbon of 0.5 mm wide and 0.7 cm long is collected on a pre-cleared moist wooden board. The strip is then cut into appropriate sized graft pieces (2-4 mm) and implanted alone or along with small nucleus (2 mm diameter) into the mantle tissue of the recipient mussel. Using a shell opener, the recipient mussel is carefully opened (1 cm wide). The inner side of the mantle is exposed by gently pushing aside the gills and the foot. Using a specialized needle few pockets are cut in the inner mantle. The previously prepared live graft pieces are inserted with care into these pockets (one graft piece/pocket) with the outer side of the graft facing the inner side of the operation mussel shell. Such grafting is done on both sides of the mantle lobes. It has been observed that the implanted mantle graft epithelium leads to enveloping the nucleus in the form of a pearl sac in about 15 days and the microvillus of the pearl sac epithelium constituted the cellular basis for crystallization of aragonite calcium carbonate, the first step in pearl formation. The number of implantation can vary between 2-8 depending upon the size and mantle thickness of the recipient mussel. In nucleated operations, a small nucleus is inserted along with the live graft piece in close association into these mantle pockets. The

surgeries must be performed during winter that's the main criteria. The temperature can be anywhere between 25°C to 28°C. Avoid performing surgeries in warm climate²⁰.

9.3: Preparation of Graft Tissues:

The mussel to be used as graft should be properly selected; it should be young and healthy. The two mantles of one mussel will be sufficient for use in operating on 10 to 15 mussels. To prepare the graft tissues, one mussel will be opened to remove its mantles. This can be done by slipping the pointed, narrower end of the graft knife between the shells to cut the adductor muscles (anterior and posterior). With the shells widely opened, the two mantles can then be cut and separated from the body of the mussel and placed on a wooden board. The slimy fluid on the mantle should be wiped off with a wet synthetic sponge. They should be divided into long strips by cutting or eliminating the outer margin of the mantles which produce the prismatic layer of the shells. They are then cut into 2 to 4 mm squares and are ready for use as graft tissues^{21, 23}.

9.4: Culture of Pearls on the Mantle:

Pearls can be grown on the mantle of the mussels. Only pieces of graft tissues can be inserted between the layers of the mantle, without any nucleus or irritant. The graft tissue will easily become attached to the mantle; while a nucleus is inserted will be expelled. The following is the procedure for operating on the mantle of mussels for seed pearls; first, the graft tissues should be prepared (the same way as when used in the operation of the gonad). Insert the end of the shell opener into the gaping mussel to make a 7 mm opening. Insert a wooden peg between the shells to prevent them from closing and remove the shell opener. Place the mussel in a holder. With the use of a spatula, push the gills upward for a clear view of the lower mantle lobe. Then, with the use of the incision knife, make slits on the mantle, taking extra care not to cut through the other side. The mantles are usually bulging due to the presence of a watery fluid, so when slit, fluid will ooze out. Two or three slits can be made on the mantle. Using the graft carrier, insert one mantle tissue in each of the slits. Turn the mussel in the holder upside down so that the other mantle can be operated on. Repeat the procedure for this mantle. For operating on two mantle lobes, four to six graft tissues may be inserted in one mussel. In some mussels, especially if the size is large, it may be possible to insert up to 10 graft tissues in one mussel. The use of larger-sized graft tissues is preferable as pearls will develop faster and the culture period will be shorter. It will be difficult, however,

to insert these larger sized graft tissues and the expulsion or rejection rate of the tissues will be higher. The graft tissue will join with the inner epithelial cells or with the connective tissue between the external and then internal epithelial cells of the mantle. This will have the same function as when it is part of the mantle, and will, therefore, secrete the shell substance or calcium carbonate and eventually result in seed pearls²².

10: Biomineralization:

Those mollusks which have a pearl lining of nacre on the interior of the shell surface can produce lustrous pearls. It is an abnormal process in the normal biological system of the animal. The mantle with its outer epithelial cells is the tissue responsible for producing pearl nacre. When an external stimulus such as accidental trapping of a hard foreign body or a parasite or a lesion occurs in the outer epithelium of the mantle tissue, it leads foreign body resulting in a pearl. Initially, the epithelial cell of the mantle forms a pearl-sac surrounding the irritant foreign body forming the cellular basis for crystallization of calcium carbonate. This process of pearl formation is well known to the scientific community as Pearl Sac Theory. This abnormal response to a foreign body in the normal biological processes that build up the shell in certain mollusks constituted the base for pearl culture operations. The shape of the pearl is governed by the irritant foreign body and its quality by the nature of secretions of the pearl sac. Thus, the outer epithelium of the mantle tissue is the key-note in the orchestra of biomineralization of a pearl²⁴.

11: Requirements for Pearl Culture:

11.1: Fixed Costs (One Time Investment):

- 1. Operation Shed:** A place or room where surgeries can be performed.
- 2. Mussel Holding Tanks:** Ferro-cement/FRP tanks, or any kind of large container that can hold mussels before and after surgeries.
- 3. Culture Units:** PVC pipe and floats, bamboos, ropes – this is needed when the shells dump in the pond.
- 4. Surgical Sets:** Set of tools needed to perform the surgeries.

5. Furniture for Surgical Facilities: Basic furniture like chair and table is needed to aid workers to perform surgeries.

11.2: Variable Costs:

1. Pond Lease Value: In case if one doesn't have a pond then it has to be taken on lease. But if one possesses ponds then this expense will be excluded from the list.

2. Mussels: Mussels need to be bought or caught from rivers, canals etc. one can also approach fish farmers as they possess lots in their needs.

3. Pearl Nucleus: Nucleus is a bead inserted in the mussels.

4. Skilled Workers for Implantation: This is one of the most important requirements. If the workers are not trained properly then the results can be different from expectations. So, the cost of training is incurred.

5. Wages: Money has to be paid to labour towards the maintenance of the ponds and farm.

6. Fertilizers, Lime and Other Miscellaneous Costs: If the pond is new and not used for fish farming then bringing it to the proper condition with algae and planktons and phytoplanktons are important. If one possesses a pond with this facility then this cost will not be added.

7. Post-Harvest Proceeding of Pearls: Generally bleaching, polishing, cutting pearl from the shell etc. can be done after pearls are harvested.

12: Important Parameters for Freshwater Pearl Culture:

12.1: Water:

1. pH: 7.5 to 8.5
2. Total alkalinity: 75 to 150 ppm
3. Total hardness: 40 to 75 ppm
4. Dissolved calcium: 25 to 50 ppm

12.2: Soil:

1. pH: 6.5 to 7.5
2. Organic carbon: 1.0% to 2.5%
3. Available nitrogen: 25 to 75 mg/100 gm of soil
4. Hydrogen sulphide: Nil

13: Important Facts about Freshwater Pearl Culture:

The cultivation period of pearls can be longer by using younger mussels. In the case of 8 mm, cultured pearls need at least six years. When the pearl cultivar starts tissue nucleation with a larger mussel, it will grow too old to produce a large pearl during the cultivation period required. Therefore, the use of younger mussels for pearl culture of large size can be taken (about one half-year-old mussel) as hosts. The pearls can grow larger along with their hosts.

Another factor, for obtaining a larger size of pearls, mussels can be moved from one pearl farm to another pearl farm during the cultivation period. It takes approximately two years to cultivate 4 mm tissue-nucleated pearl. Literature shows that six years are needed for the production of 8 mm size pearl. That's why after two and four years, mussels can be transferred to another farm. The change in the environment (*i.e.* water temperature and water conditions) stimulates the mussels, causing them to secrete more nacre. The change in the environments may also have some impact on the internal growth structure of these cultured pearls, such as changes in colour from growth in one pond to growth in another. Changes in the farm places make the mussels under a type of "stress" that activates their respiration so that they produce more carbon dioxide. The carbon dioxide ions that combine with the calcium ions to form more calcium carbonate crystals. This improves the nacre growth, thus promoting good lustre and colour as well as larger pearls⁷.

Pearl culturers identified that the posterior mantle lobe of the mussel, where the mother-of-pearl has the desired colour and lustre, is the key location to produce a better quality pearl with fine colour and lustre. So, pieces cut from the tissue from the posterior part of the sacrificed "donor" mussel and then placed them into pockets in the same posterior mantle lobes of the host mussel. With this new technique, pearl culturers can improve the colour and lustre of the cultured pearls⁸. Japanese scientists have recently demonstrated that pearl lustre

is caused by certain genes found in the mollusk body. The corresponding protein has been isolated from the mollusk which name is nacrein. That's why scientist believed that in the nearest future it will be possible to produce pearls for the commercial purpose outside the body of the mollusk because of the isolation of the specific gene and the resultant protein. The biological parameters which are very important need to be checked before starting of pearl cultures such as water quality, water source, water depth, substratum type, nutrient load, temperature and superior quality of recipient as well as donor mussels. Site selection has to be convenient for operational activities. Mussels which are collected from the wild environment are ideal; however pathological parameters of the indoor produced animals need more attention before selection.

14: *In-Vitro* Pearl Culture:

In-vitro mussel culture and the induction of nacre secretion eventually open the doors to new screening methods of mussel quality and *in-vitro* pearl production. Nacre crystals can be formed by using mussel's mantle epithelium cell culture. Generally, nacre is secreted by the mussel epithelium cells as the reaction against foreign particle. Nacre can be produced by the mixture of calcium carbonate and some proteins, in which major protein is conchiolin. This protein is produced by mantle epithelial cells. Under *in-vitro* conditions, nacre can be formed by mussels when they contact with some stress-causing agents, which can be included in the culture medium. Nacre will be formed by mantle epithelial cells expressed matrix protein and calcium carbonate as a component of the culture medium⁹.

The *in-vitro* culture of nacre-secreting pallial mantle explants of *L. marginalis* can be performing by preparing the sterile explant from the ribbon and then they can be transferred for culturing into petri-dishes. Special synthetic tissue culture media enriched with additives such as inactivated calf serum with antibiotics into petri-dishes having explant can be used. Incubate the culture plates in the CO₂ incubator at 5% CO₂ and 30°C. After incubating 12 hours of epithelial-like cells are formed, a complete cell sheet takes 7 to 10 days for preparation surrounding the explant. Cells indicate functional viability in the culture medium and after 38 to 40 days of culture typical aragonitic crystals of CaCO₃ will be observed throughout the culture medium.

15: Commercial Value of Freshwater Pearl Culture:

The freshwater pearl is produced by the freshwater mussel, which has a high commercial value. Mussel flesh and shell also have some commercial value. Mussels are important to both human and the environment. In one hand, they are filter feeders and act as natural water cleaner and indicators of water quality conditions. On the other hand, they are edible food to both human and animals. Shell is the primary raw material for the button industries. It can be used as nuclei for pearl culture, jewellery, crafts and animal food additives. Pearl powder contains 17 amino acids and many mineral elements; it can be used as nutrient and medicine, which is useful in treating various heart disease, sore throat, heartburn, eye disease etc. pearl powder can also be used as a vital content of skin protection cream^{13, 17}.

If pearl culture is undertaken professionally, then according to experts profit value will be more than 50% to 75%. Even 200% of returns are also possible. Cost for the first year will be slightly more compared to the subsequent years as the fixed cost will be incurred. There are no taxes applicable for pearl culture as it comes under the agriculture sector. But in the case of export, there will be taxes. 50% of minimum returns can be extracted from pearl culture in the first year.

15.1: Pearl Value:

The values of a pearl depend on the following factors:

- 1. Lustre:** Higher the lustre higher the value. The brightness or radiance of light from a pearl is called lustre. A high-quality pearl will be brilliant and bright, and people should be able to easily see their reflection on its surface.
- 2. Surface:** Cleaner the surface higher the value.
- 3. Shape:** The more round shape, better value. However, it depends on clients that what shape they want. Otherwise, shapes are various including round, off-round, oval, baroque and ringed.
- 4. Colour:** There are various tones of colour available. However, the black pearl fetches the highest price in the market.

5. Size: The bigger the size the higher the value. Pearls range in size from a couple of millimeters to over 15 millimeters depending on the type. Larger pearls are rarer and its price is very high.

6. Nacre: Nacre is the thickness of the pearls coating and it is a layer of aragonite and conchiolin which form around the centre of the pearl. The thicker the nacre, the longer a pearl will typically last.

16: Genetic Improvements:

Sustainable aquaculture depends on the constant breeding of cultured stocks of bivalve mollusks. The principle advantages rising from the genetics and biotechnology applications to aquaculture for pearl culture are habitually identified as optimization of production cycle through control of development and maturation, gamete production and storage, sex and ploidy manipulation, transgenesis, selective breeding and marker-assisted selection.

Variation in the colouration of nacre is very important in the pearl industry because nacre colour is closely related to the pearl colour; this is one of the important factors for setting the price of pearls. The mechanism behind the nacre colouration appears to be a very complicated process involving genetics and physiological factors. Only the yellow colour has been studied extensively as this is important for the pearl market. The mussels contain yellow colour in their nacre as a pigment. Experimental transplantation of parts of the mantle showed that yellow pearls were mostly produced from the pearl sac formed by the mantle tissue from the animal with yellow nacre. This trait may be inherited. Transplantation of the mantle tissue, which was conducted using the populations, revealed that yellow pearls were produced at a higher rate in the mussels selected for yellow nacre than another mussel that had nacre without yellow pigment. The weight of pearls is another factor which affects the price of pearls. External colour of the bivalve shell is mainly associated with the pigments contained in the prismatic layer of the shell. In bivalve mollusks, triploid can be produced by suppressing meiosis I and II with chemical or temperature treatment of the fertilized egg. Cytochalasin B is the most commonly used chemical to induce triploid of bivalve mollusks. Triploid bivalves exhibit three sets of genomes^{17, 19}.

16.1: Molecular and Biotechnological Studies:

Sequences of cDNA which encode proteins that relate to formation of the shell and which exist in the organic matrix of the bivalve shell or pearls have been studied by using the methods of molecular biology such as cDNA cloning, characterization and purification of nacrein protein, which is a soluble organic matrix protein in the nacreous layer of the mussels. Nacrein function as a matrix protein whose repeated Gly-Xaa-Asn domain possibly binds calcium and as carbonic anhydrase which catalyzes HCO^{-3} formation that participating in calcium carbonate crystal formation in the nacreous layer⁹.

17: CONCLUSION

The results of the proper business plan with good marketing strategy establish or generate substantial revenue to support running costs and provide a share of the dividend. It is more effective to work in favour of the set objectives to achieve the desired goal by sharpening skills and adapt the market cost-effectively and practically. Pearl production can be established as a small scale based pearl farms but is providing to become a significant means for income generation among the communities. Furthermore, the pearl industry provides an opportunity for the involvement of women and provides the raw materials for local handicrafts manufacture, which may include lower grade pearls or pearl shells. As the business grows, additional pearl cultivators are on the horizon, the existing farms will enjoy the first-mover advantage in the local market. Pearl culture can be possible with a minimum budget to exclude the business plan; pearl farming should be carried out efficiently and cost-effectively targeting both tourists and domestic market.

The budget of both small and medium-size pearl culture farm, it can be possible that by scaling up to the medium size farm, a farmer can earn or make higher returns. Essentially, the returns double, but the costs increased only slightly since many of the costs are similar for small and medium-size farms. Startup costs are slightly higher in the medium-size farm, but this increase in scale modest, the startup costs are recovered by the first harvest. That's why it is advisable to establish a larger farm when possible.

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