CMFRI

Course Manual

Winter School on

Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish

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Compiled and Edited by

Dr. K. Madhu, Senior Scientist and Director,
Winter school
&
Dr. Rema Madhu, Senior Scientist and Co-ordinator
Central Marine Fisheries Research Institute

Central Marine Fisheries Research Institute
(*Indian Council of Agricultural Research*)
P.B.No.1603, Marine Drive North Extension,
Ernakulam North ,P.O.
Cochin, KERALA – INDIA - 682018

PHYSIOLOGY IN AQUACULTURE WITH SPECIAL REFERENCE TO PENAEID SHRIMP AQUACULTURE

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C.P. Balasubrsmanian,
Senior Scientist, Central Institute of Brackishwater Aquaculture , Chennai-28.
E -mail : balunavaneeth@gmail.com

Aquaculture has been practiced in some societies for many centuries. However, the transition from low-input, pond-based capture system to more intensive and industrialized method has been done only during the last few decades. Contrary to terrestrial agriculture systems, aquaculture is a new industry with limited scientific knowledge. This is especially true for shrimp aquaculture, the fast growing and most valued food production systems of the world. Shrimp aquaculture has been developed without sufficient understanding of basic physiology of species of interest. Physiology is a powerful science, which has potential to contribute positively for the development and growth of aquaculture. The more understanding of the physiological processes that underlie crop performance leads to the improvement and optimization of aquaculture production. Further, as knowledge of physiological process expands, desirable traits can be identified, and that can be utilized as selection criteria. Incorporation of physiological traits for selection in breeding programme, thus, would help to achieve results more quickly and efficiently than selecting for yield performance alone. This lecture note provides an overview of various aspects of reproductive physiology that have direct application in optimizing aquaculture production. The possibilities of selecting physiological traits in breeding programme have also been discussed.

Reproductive science

The first investigator to provide direct proof for hormone involved in reproduction was Panouse (1943). His experiments on caridean shrimp, *Palaemon serrata*, showed that eyestalk ablation results rapid maturation of the ovaries. Later, he concluded that this effect is due to the hormone, Gonad Inhibiting Hormone (GIH), present in the sinus gland. Sometimes it was called as vitellogenin inhibiting hormone as it is inhibiting vitellogenesis in females. Subsequently GIH was also reported in male crustacean as well. Therefore GIH is considered to be preferred term than VIH. This scientific principle is used by earlier shrimp aquaculturists, and it had far reaching impact on aquaculture in general and shrimp farming in particular. This technique made almost similar impact as ‘hypophysation’ (injection of pituitary extract) procedure that revolutionized finfish breeding and culture. However, hypopysation technique has been refined and more sophisticated hormonal treatment procedure has been introduced based on latest findings from reproductive endocrinology of fin fishes.

Physiology of reproduction

Although research on shrimp reproductive endocrinology has been carried out for a half a century, information on control of reproduction is still far from well understood. Thus development or refinement of reproductive technology is still far from commercial application. The most accepted model for shrimp reproduction is: vitellogenesis (accumulation of vitellin or yolk protein; generally termed as ovarian maturation) is stimulated by gonad stimulating hormone secreted by neuro endocrine and endocrine tissues and inhibited by gonad inhibiting hormones of eyestalk. The antagonism of the eyestalk reduced by a decline in the titre of GIH as shrimp grows or moves into an environment suitable for spawning or sufficient nutrient reserves attained for vitellogenesis. The following section describes various hormones involved in the reproduction of crustaceans.

Gonad inhibiting hormone (GIH)

GIH is synthesized and stored in the X-organ sinus gland complex housed in the optic peduncle of the eyestalk. Although GIH was known as early as 1940 and used the ‘inhibiting principle’ in shrimp aquaculture industry, the hormone was first isolated after forty years later (Soyez et al 1987); it is partially due to the complexity of the procedure for isolation. So far GIH has been identified only from few species (Table 1). With the introduction of molecular biological technique to the aquaculture research, separation and structural elucidation of GIH made possible as in the case of many other hormones. Recently GIH from the eyestalk was cloned using RT PCR and RACE technique (Treerattrakool et al 2008, Vijayan et al 2008). It was found that GIH is a peptide with 79 amino acids. This study also shows that GIH is expressed in brain, thoracic nerve cord in addition to the eyestalk. Interestingly GIH is also found to be expressed in males, females, adults and adolescent shrimps. The studies on the mode of action of GIH is rather scarce; Jugan(1985) found that inhibitory effect of GIH is due to the competition of vitellogenin and GIH molecule for the same receptor on the oocyte membrane.

Gonad stimulating hormones/gonadotropins

It is generally accepted that identification of gonad stimulating hormones (GSH) in crustaceans would greatly expedite domestication and closing of life cycle of commercially important aquacultured crustaceans that do not reproduce readily in captivity. Research on GSH has been started in 1960s. A substance known to stimulate vitellogenesis has been found in brain and thoracic ganglion of several crustacean species (Otsu, 1963; Gomez, 1965). But the chemistry of this species is not yet elucidated. De Kleijn and Van Herp (1998) suggested that this hormone might be a crustacean hyper glycemic hormone.

Methyl Farnesoate: Parallel to the search for GSH, studies on other gonad stimulating hormones (gonadotropins) have been carried out by several researchers. These investigations results in the discovery of a crustacean hormone, Methyl farnesoate (MF) (Laufer, 1987). MF is an intermediate compound produced during the juvenile hormone biosynthetic pathway (Fig 1) juvenile hormone is well known and extensively studied in insects where they play several regulatory roles both as gonadotropin in adults and morphogens during development. Juvenile hormone as such is not found in crustaceans, but MF is isolated from mandibular organs (Fig 2) of crustaceans. Mandibular organ actively synthesize MF during vitellogenesis and become less active during non-reproductive periods. The secretion of MF is tissue specific and circulated through the blood, and circulating levels are positively correlated with the reproductive state of females.

It has also been shown that mandibular organ is negatively regulated by an eyestalk neuro hormone, mandibular

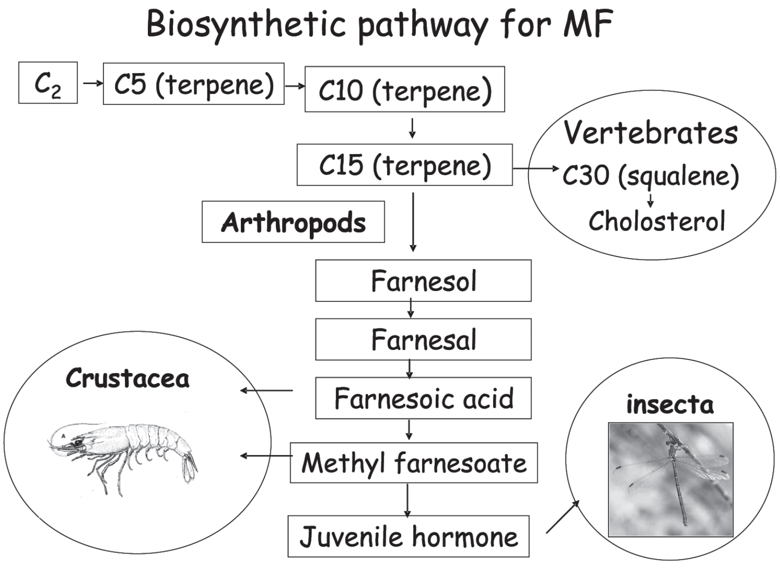

Fig 1 Biosynthetic pathway of methyl Farnesoate

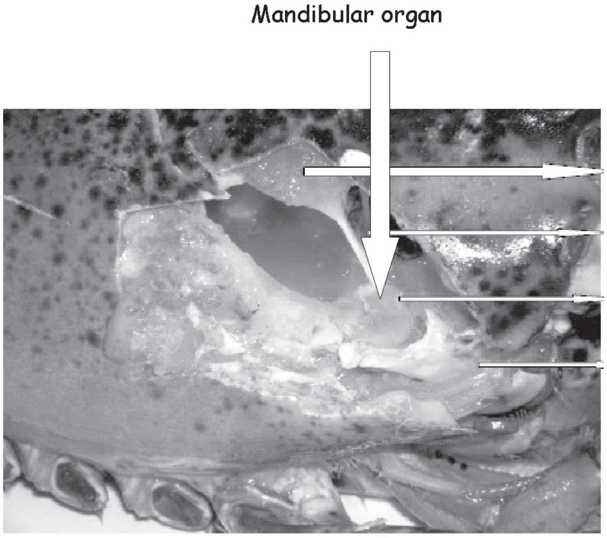

Fig 2 Mandibular organ in lobster (Nagaraju 2007)

organ inhibiting hormones (MOIH). The inhibition is artificially reversed *in vivo* by eyestalk ablation. However, MF can be applied directly, and in many cases successful ovarian maturation and spawning have been achieved as well. Table 1 shows that experimental results of MF treatment to study the effect of this hormone on reproduction of females.

Table1: Effect of MF on ovarian maturation species wise information

Species	*In vivo*	*In vitro*	Reference
Homarus americanus	Positive	—-	Borst *et al.* 1987
Libinia emarginata	Positive	—-	Vogel and Borst 1990
Litopenaeus vannamei	Positive	positive	Laufer 1992; Tsukimura 1991
Macrobrachium malcomsonii	Positive	—-	Nagaraju *et al.* 2003
Penaeus indicus	Positive	—-	Nagaraju *et al.* 2002
Procambarus clarkia	Positive	—-	Rodrigues *et al.* 2002
Homarus americanus	No effects	—-	Tsukimura *et al.* 1991
Macrobrachium rosenbergii	No effects	—-	Wilder *et al.* 1994
Triops longicaudatus	No effects	—-	Linder and Tsukimura 1999
Penaeus monodon	Inhibition	—-	Marsden *et al.* 2008

It was found that, effect of MF on maturation is still inconclusive, although in many species a positive effect has been observed.

Ecdysteroid: Ecdysteroid is a polyhydrated keto steroid, found in most arthropods and have a primary function as molting hormone. In crustacea ecdysteroids are synthesized in ‘Y’ organ (=ecdysal gland), and the alternative sources of Ecdysteroid is the epidermis, and ovary. It has been reported that Ecdysteroid has a possible role in reproduction and maturation as in the case of insects (Subramoniam 2000). It has been suggested that crustacean hormones are multifunctional in nature, a single hormone can mediate different functions. Ecdysteroid mediate as hormone that promote protective membrane in embryos, then they function as molting hormone from larvae to adult life. In adult they function as gonadotropin. Chang (2001) called it as an ‘amazing economy of nature’.

Vertebrate-like steroids: Several decapod crustaceans have the ability to synthesize the vertebrate-type steroids (e. g. progesterone, 17-â- estradiol, and testosterone). Some of them fluctuate during the reproductive cycles, and therefore, indicates their role in the reproduction. Many crustaceans have been found to be responding to the injections of these steroids. In additions to the steroids, biogenic amines such as 5 hydroxy tryptamine (serotonin) are also found to have ovarian stimulatory effect. Recently Wongprasert (2006) reported that serotonin injected *P. monodon* had ovarian maturation and spawning similar to that of unilateral eyestalk ablated females. Although the mode of action of these hormones/neuro transmitters is not properly understood, there seems to be a tremendous potential in using these hormones to stimulate gonadal maturation in the aquacultured species.

In summary, the crustacean egg production is controlled by a cascade of hormonal activities which is triggered by environmental and nutritional factors (Fig 3). Therefore, all these factors should be considered when any reproductive technology is developed. Replacement of eyestalk ablation procedure with more potent and non destructive procedure has been one of the elusive goals of shrimp aquaculturists and researchers. Although several hormones were evaluated to induce maturation, no commercially viable procedure has been developed so far. This is mainly due to the more intense nature of gonad inhibiting hormone than other gonadotropins. Recently researchers have been tried to develop antibodies against GIH. It is hoped that shrimp can be immunized against GIH by injecting these antibody and it would neutralize the effect of hormone. This will be more specific than eyestalk ablation, and further it is physiologically non destructive.

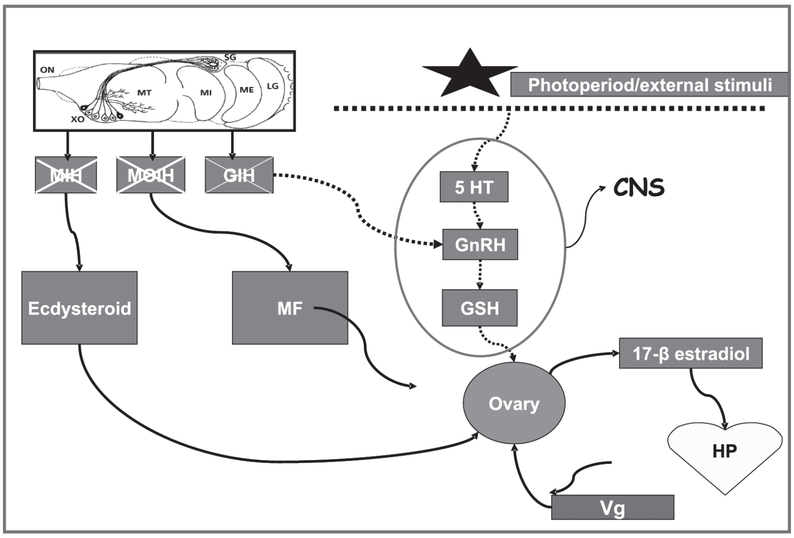

Figure 3 Schematic representation of shrimp reproduction and various factors controlling reproduction

CNS: central nervous system; GIH: Gonad inhibiting hormone; GnRH: Gonadotropic releasing hormone; GSH: Gonad stimulating hormone; 5HT: 5 hydroxy tryptamine; HP: hepatopancreas; LG: Lamia ganglioris, MF: methyl farnesoate; MT: Medulla terminalis; ME: Medulla externa: MI: Medulla interna; MIH: Molt inhibiting hormone, MOIH: Mandibular organ inhibiting hormone; Vg: vitellogenin. Dotted lines represent hypothetical effect whereas continuous lines represent proved observations

Application of reproductive traits in genetic selection programme to improve reproductive performance

It is well established that reproductive performance of penaeid broodstock are extremely variable under similar environmental conditions, and large proportion of the broodstocks are non spawners even using the stimulus of eyestalk ablation (Mc Govern 1988; Bray et al 1990, Wang et al 1990 ,Ibarra et al 2007) (Fig 4). Further, Bray et al (1990) report about 75% of nauplii were produced by 25% of females. Evidences also indicate that such of these individual variations is genetic and inherited from generation to generation. Offsprings from most fecund animals in captive maturation out perform unselected controls (Wyban and Sweeny, 1991)(Fig 5). Recently many reports have been published on the heritability of various reproductive traits by applying tools of quantitative genetics. A Mexican research group demonstrated heritability of various reproductive traits of *L. vannamei* (Fig 6).

Coman *et al.* (2006) report significant variation in reproductive performance between different families and these variations are consistent for age group and rearing system (Fig. 7). They further suggest that family based genetic selection in breeding programme to improve the reproductive performance of *P. monodon*

Recently, Macbeth et al (2007) reported heritability estimates of reproductive traits in *P. monodon*. They measured heritability estimates of days to spawn (0.47± 0.15), egg number (0.41± 0.18), nauplii number (0.27± 0.16) and proportion hatched (0.18± 0.16). It is concluded that improvement in reproductive performance of *P. monodon* is possible by selectively breeding those with genetically superior reproductive traits. Further, there is no evidence to suggest that selection of growth will adversely impact on reproductive performance.

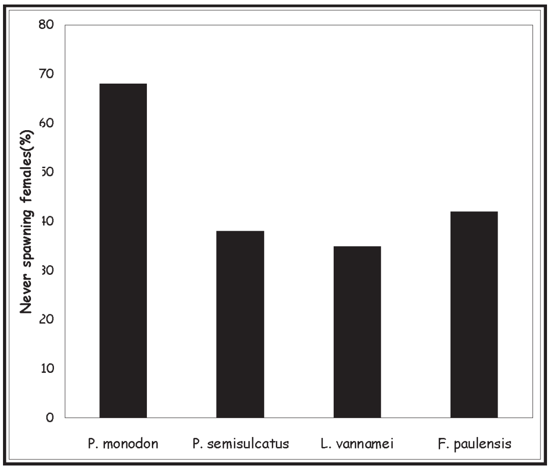

Fig. 4 Proportion of never spawning females in hatchery production of commercially important shrimps

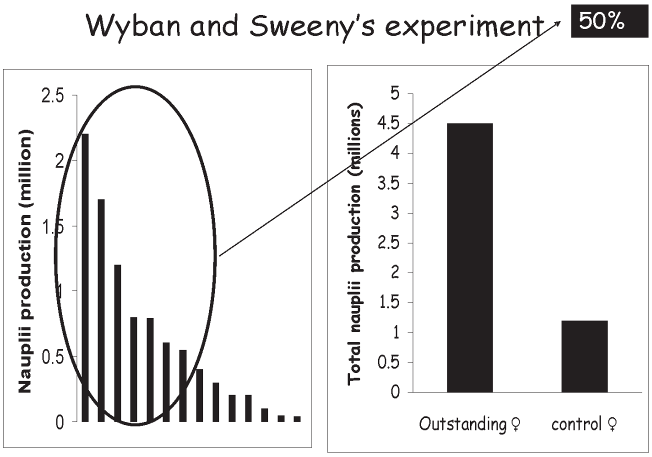

Fig.5 Nauplii production per females during hatchery production of Litopenaeus vannamei; 50% of total nauplii is produced by few outstanding females(a) and it was found that broodstock developed from theses nauplii also showed higher reproductive performance

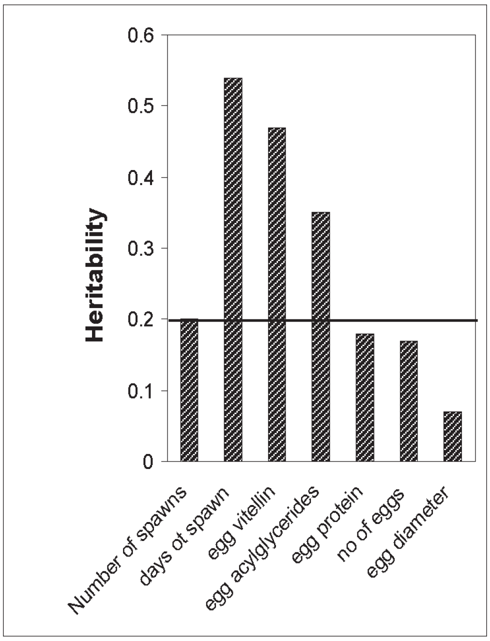

Fig. 6 Heritability estimates of reproductive traits of *Litopenaeus vannamei* (Ibarra *et al.,* 2007); the horizontal line at 0.2 level indicates the cut of level between heritable and non heritable variables.

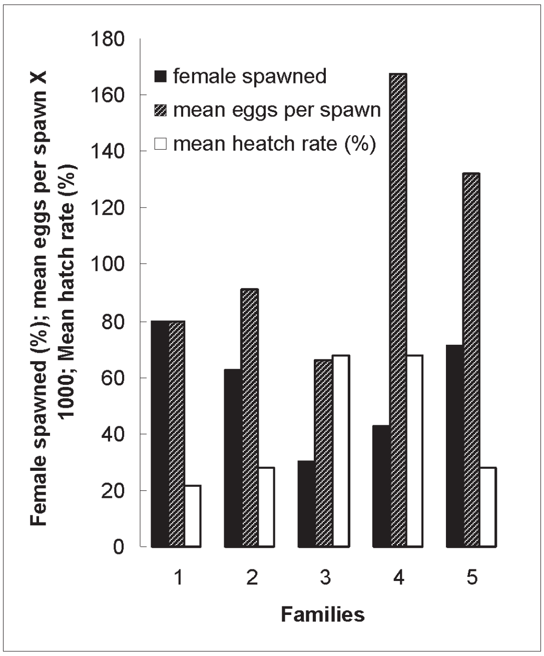

Fig.7 Family wise variations in reproductive performance of *P. monodon*

Male reproductive physiology

Failures in mating in captivity and low success in fertilization have often been encountered in commercial penaeid hatchery. Although female reproductive physiology has been addressed by several authors, physiology/biology of male reproduction has received little attention. This is partly due to the belief that males are continuous breeders or capable of mating without interruptions. The earlier crustacean workers mainly focused on internal morphology of male reproductive system and spermatophore formation. Male genital system of penaeid shrimp described as early as 1940s (King, 1948). It comprises a pair of testes, a pair of vas deference and a paired terminal ampoule. In addition to these internal organs, a paired petasma and a paired appendix musculina are found externally. Sperms are transferred to female genital system as specialized packets called spermatophores. Spermatophores are evolved to minimize sperm loss in aquatic invertebrates. Spermatophores are either deposited externally onto the female body (e. g. penaeid shrimps) or inserted into the seminal receptacles by intromittent organ (e. g. Brachyuran crabs). Spermatophores of the crustacea are never discharged freely into the medium. Spermatophores are used as the mode of sperm transfer in decapod crustacea because decapod sperms are aflegellate and non motile. In the penaeoid shrimp the spermatophores show remarkable variability. The complexity is closely related to the nature of thelyca (= the external modification of females posterior thoracic sternite). Females with open thelyca generally receive a spermatophore with complicated structures whereas in closed thelycum forms males produce simpler spermatophores. Here, spermatophores have two subdivisions: namely main body and the wing. Main body is composed of a bulky and viscous sperm mass surrounded by a thick envelope, the sperm sac. The membranous wing is attached to the sperm sac is slimy and more transparent.

A description of spermatogenesis in comparison with oogenesis is given in Box 1

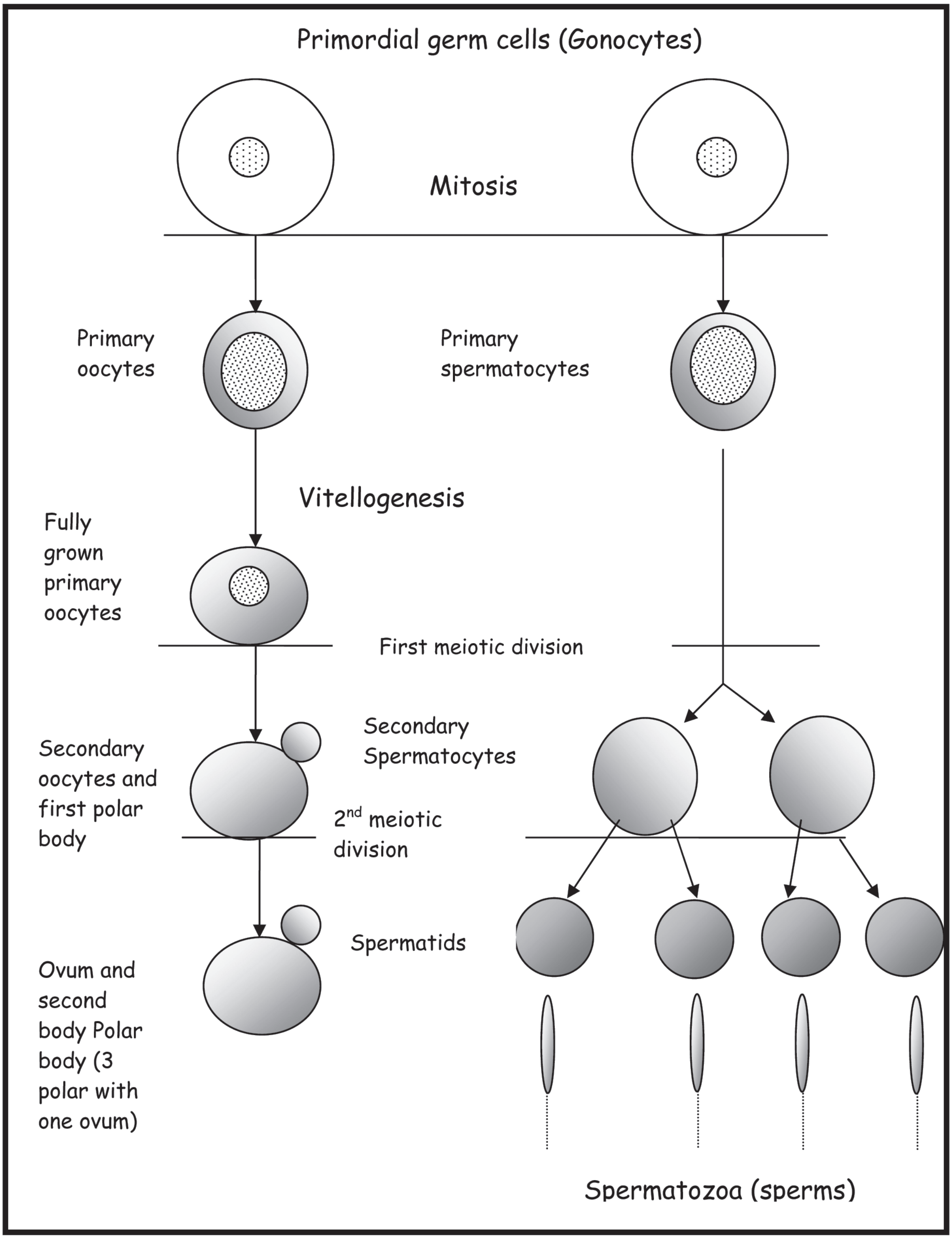

Fig. 8 Oogenesis and spermatogenesis: diagrammatic representation.

Box 1

The first phase in the sexual reproduction of animals is gametogenesis, a process of transformation whereby certain cells become the highly specialized sex cells: spermatogenesis in the male and oogenesis in the female. In both male and female, the primordial germ cells originate outside the gonad. The primordial germ cells are motile and invasive, and migrate to arrive at the gonadal ridges and colonize the indifferent gonad, a mass of mesoderm on the dorsal body wall.. When the primordial germ cells have completed their migration, they lose their motile characteristics and proliferate rapidly, dividing by mitosis to increase their number. This proliferation is followed by a period of cell growth, which is much more significant in the female gamete than in the male gamete. The key event of gametogenesis, in both sexes, is the halving of the number of chromosomes during meiosis. Meiosis is a specialized cell cycle consisting of two successive rounds of chromosome segregation following a single round of DNA replication, producing progeny cells with half as many chromosomes as their parents.. However, the similarity between oogenesis and spermatogenesis ends at this point. In the male, each primary spermatocyte divides meiotically to produce four spermatids, each destined to become a functional spermatozoon; in the female, of the four cells produced from each primary oocyte, only one develops into a viable oocyte. An unequal distribution of cytoplasm at division results in the production of three small cells, the polar bodies, which eventually degenerate. A further distinction between the two gametes is that the spermatozoon acquires the ability to fertilize the oocyte only following the completion of meiosis; in the majority of animals, the oocyte is capable of interacting with the spermatozoon before meiosis is complete. Meiosis in oocytes is arrested at various stages of the division cycle, depending upon the species, and is reinitiated as a result of fertilization. However, the sea urchin and some coelenterates are exceptions to this rule, in that their oocytes have completed meiosis before fertilization. Strictly speaking only in these two cases may the female gametes at the time of fertilization be described as ‘ripe eggs’; in all other cases they should be considered as oocytes. The process whereby the oocyte attains the ability to interact with spermatozoa, described by Delage in 1901 as cytoplasmic maturation, seems to be independent of the nuclear division cycle. It should be noted, however, that, in oocytes that are normally fertilized before the completion of meiosis, the male nucleus remains quiescent in the cytoplasm until meiosis is completed.

Captivity induced reproductive dysfunction in males

Sterility in male shrimps has been reported after having the animals were reared in captivity for 3-4 weeks. Spermaophores of males held in captivity become progressively melanized and eventually the animals become unsuitable for breeding. It was also reported that melanization can be delayed or even reversed as long as males remain sexually active. Intact inter molt spermatophores disappeared about 2 h after pre molt, and new pair of spermatophores appeared in the day after the males had molted. A marked difference in the sperm quality is recorded in the males kept in constantly low temperature, where male sperm quality remain significantly higher than the animals reared in higher temperature. The temperature induced sensitivity has been reported in many higher organisms as well.

Remarks: The research on physiology of reproduction has great potential to improve aquaculture production. However, eyestalk ablation procedure still remains the most important contribution of basic physiology to the applied aquaculture. Most of the research on reproductive physiology is still hidden in the literature.

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