

# Understanding Gametogenesis: The First Step Toward Conservation

Gratiana E. Wijayanti

Biology Faculty, Jenderal Soedirman University, Jl. Dr Suparno No. 63 Grendeng, Purwokerto 53122 Indonesia. E-mail: bugrat\_1@yahoo.co.uk

## ***Abstract***

*Organisms that reproduce sexually arise from the gametes, the highly specialized cells that together initiate the development of a genetically new organism. The gametes develop from their precursor, the primordial germ cells, through a complex process, the gametogenesis. Failure of gametogenesis will result in infertile individual which lead to failure of reproduction. When gametogenesis fails in a commercial species, it will result in a decrease of production. When gametogenesis fails in a rare species, however, it might leads to the species extinction. In this sense, understanding gametogenesis including the stages and factor controlling the process is very crucial in biological conservation. In relation to this, a study on gametogenesis in a teleost, the hard-lipped barb (*Osteochilus hasselti*) had been conducted as a model for other teleost species.*

**Keywords:** gametogenesis, conservation, teleost, *Osteochilus hasselti*

## **Introduction**

Organisms that reproduce sexually arise from the gametes, the highly specialized cells that together initiate the development of a genetically new organism. The gametes develop from their precursor, the primordial germ cells, through a complex process, the gametogenesis. The morphological details and timing of gametogenesis greatly varied among animal species but its basic principles are relatively conserved. The gametogenesis begins after primordial germ cells reside in the gonads and determine their developmental path either as spermatogonia or oogonia. Following a series of mitotic division the spermatogonia committed to become primary spermatocytes. The spermatocytes enter the first followed by second meiotic division to form secondary spermatocytes and spermatids respectively. This event is referred as spermatocytogenesis. The spermatids then undergo morphological changes through spermiogenesis to produce spermatozoa. Homologous processes as that in spermatogenesis also occur during oogenesis. As the oogonia initiate the first meiotic division they are referred as primary oocyte. The completion of first meiotic division produces secondary oocyte. In most vertebrates, the second meiotic division is arrested at metaphase until ovulation [1] and will be resumed when the oocyte is fertilized. Thus, in such species, ootid and ovum will only be achieved after fertilization.

The unique aspect of gametogenesis is the meiosis division since this type of division only occurs in gametogenesis but not in somatic cells. There are two significances of the meiosis: 1) the reduction of the number of chromosomes from the diploid ( $2n$ ) to haploid ( $1n$ ) number so that the species number of chromosomes can be maintained from generation to generation; 2) the mixing of genetic characteristics (crossing over and independent assortment of maternal and paternal chromosomes). This paper briefly reviews the significance of gametogenesis, some aspects of gametogenesis in the hard-lipped barb and the prospect of developing a model for conservation.

## Significance of Gametogenesis

A normal gametogenesis has been a great concern in human health. The abnormal chromosomes that appear to result in abnormal development were generated during the formation of the female and male gametes [2]. The severe abnormal chromosome could abort up to 50% of all zygotes formed. Zygotes with less severe chromosomal anomalies that do not abort will result in an abnormal type of development [3]. The abnormal chromosome could be numerical (polyploidy and aneuploidy) or structural. Numerical chromosome abnormalities could be related to autosomal chromosome such as Down syndrome and Edward syndrome or sex chromosome such as Turner syndrome.

Failure of gametogenesis will result in infertile individual which lead to failure of reproduction. When gametogenesis fails in a commercial species, it will result in a decrease of production. When gametogenesis fails in a rare species, however, it might leads to the species extinction. In this sense, understanding gametogenesis including the stages and factors controlling the process is very crucial in biological conservation.

## Gametogenesis and Conservation model

Studies on gametogenesis in fish were initially conducted to promote fish production for food supply. As knowledge of fish biology improved, studies on gametogenesis in fish has been re-orientated to other purposes such as, understanding gene function in development and health [4], providing method for monitoring environmental disturbance [5] and conservation. In relation to this, studies on gametogenesis in a teleost, the (*Osteochilus hasselti*) had been conducted and developed as a model for other teleost species. This species was used as a model since this fish is an indigenous tropical fish and widely cultured in Java [6].

Since 1995 researches on reproductive aspects of the hard-lipped barb including gonadal development [7,8,9,10], the dynamic of gametogenesis [11,12,13] and fluctuation of reproductive hormones [14,15] were studied. The compiling data indicated that gonadal differentiation was detected around day 130 post hatching [8]; gametogenesis was initiated earlier in the males than females. In 4-5 month post hatching, the testis has shown spermatogenesis as indicated by the present of spermatogenic cells at various stages of development; while ovary of the same age consisted of oogonia and previtelogenic oocyte [7]. The timing of gonadal differentiation in this species was altered by environmental factor such as photoperiod [10].

Histological features of the ovary showed that hard-lipped barb is a synchronous batch spawner. This was indicated by the present of oocyte at various stages of development (Figure 1a). The diameter of oocyte increased as the oogenesis progressed and reach their maximum size of 0.9-1.2 mm prior to ovulation [11]. This increased in diameter was mainly due to the accumulation of vitelogenin protein in the ooplasm [16]. The proportion of late vitelogenic oocytes increased toward the following spawning period (Figure 1a). This leads to the increased of gonado-somatic index (Figure 1b).

Oocytes development in this species, as in other teleost, was controlled by reproductive hormones under hypothalamus-hypophysis-gonad axis. In aquaria, the level of gonadotropin (GtH) in the female was increased from week 2 to week 6 post spawning then declined up to week 8 (Figure 1c). The increased of GtH level stimulate the production of estradiol-17 $\beta$  in the ovary in a similar pattern to those of GtH level (Figure 1d). The decreased of estradiol-17 $\beta$  level could be due to the decreased of aromatase activity as

evidence in other teleost [17]. This decreased was suggested to give opportunity for progesterone synthesis needed for oocyte maturation. Studies in many teleost suggested that progesterone is the ovarian steroid responsible for oocyte maturation [18].

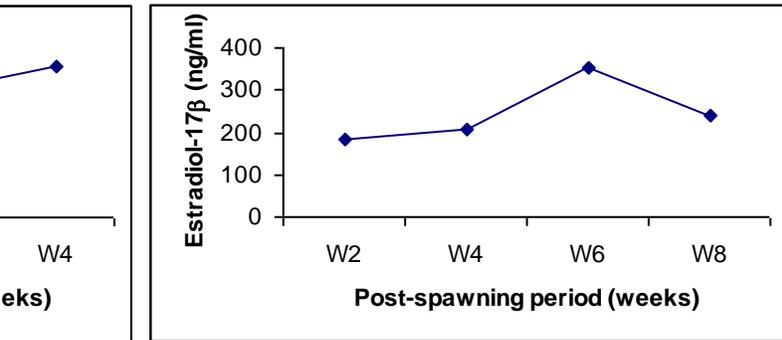
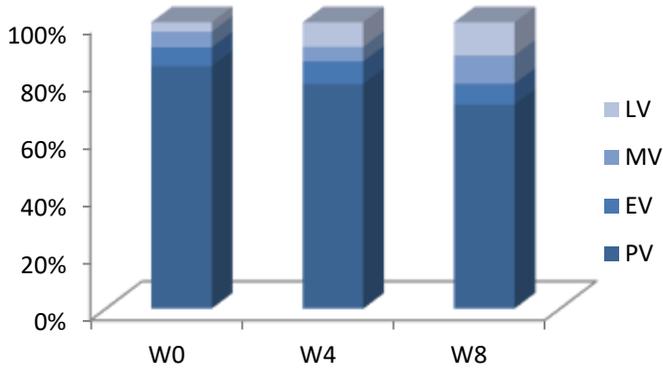


Figure 1. The gonado-somatic index the proportion of oocyte at various developmental stages, fluctuation of serum GtH and estradiol-17β in female hard-lipped barb in one spawning cycle.

Histological features of cycling testes showed that hard-lipped barb testes consisted of many spermatogenic units, the spermatocysts or lobules. Each lobule contained a particular stage of spermatogenic cells. The testis of a newly spawned fish was dominated by lobules containing early spermatogenic cells while the testis of a ready to spawn fish was dominated by lobules containing spermatozoa [11,13]. The increased of lobules number containing spermatozoa and hydration in the testis resulted in an increased of gonado-somatic index (Figure 2a). Spermatogenesis is also controlled by reproductive hormone under hypothalamus-hypophysis-gonad axis. Measurement of GtH using ELISA showed that serum GtH level steadily increased from the day of spawning to the next spawning period (Figure 2b).

Based on the available information on gametogenesis and reproductive hormone profile, a series of experiments are currently in progress to answer some critical questions such as: How do estradiol-17β, progesterone, GtH and gonadotropin releasing hormone analog exert their role on oocyte growth, development and maturation? What oogenic or spermatogenic stages are responding to hormonal induction? What sort of culture condition require for *in vitro* development oocyte or spermatocyte? The outcome of these experiments will provide a proper protocol for culturing oocyte or spermatocyte.

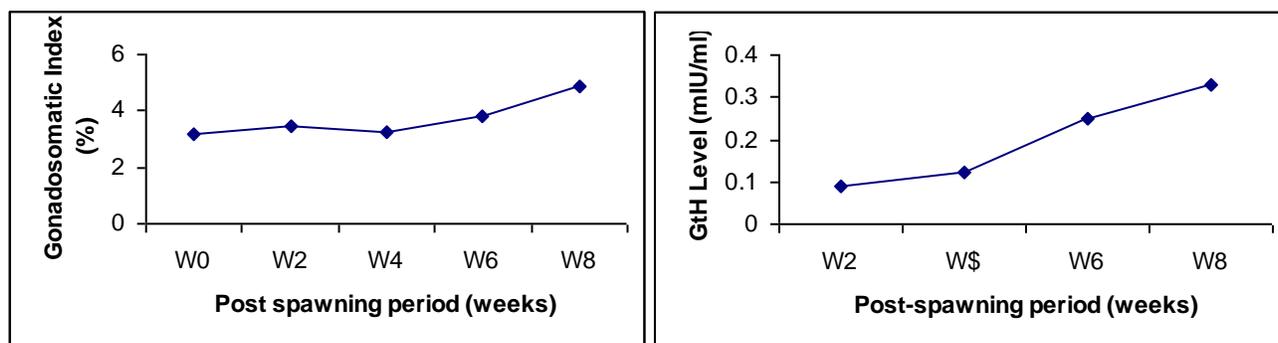


Figure 1. Fluctuation of serum GtH level and gonad somatic index in male hard-lipped barb in one spawning cycle.

### Future studies

The finding in the hard-lipped barb will be used to design a model to preserve gamete precursor including primordial germ cells, oogonia, oocyte, spermatogonia and spermatocytes. This model will be tested in other Cyprinid fish for conservation purposes.

### References

- [1] Gilbert, S.F. 2000. Developmental Biology 6<sup>th</sup> edition. Sinauer Associates Inc. Publisher, Sunderland.
- [2] Daniel, E. 2008. Significance of Gametogenesis. Undergraduate Medical Curriculum . McGill Molson Medical Informatics.
- [3] Azim, M., A.H. Khan, and Z.L. Khilji 2010. Chromosomal Abnormalities as a Cause of Recurrent Abortions: a Hospital Experience. [http://www.jpma.org.pk/full\\_article\\_text.php?article\\_id=102](http://www.jpma.org.pk/full_article_text.php?article_id=102). 16-7-2010.
- [4] Yoshizaki, G., Y. Takeuchi, T. Kobayashi and T. Takeuchi. 2003. Primordial germ cell: a novel tool for fish bioengineering. *Fish Physiology and Biochemistry* 28: 453-457
- [5] Goodbred, S.L., Smith, S.B., Greene, P.S., Rauschenberger, R.H., and Bartish, T.M., 2007, Reproductive and Endocrine Biomarkers in Largemouth Bass (*Micropterus salmoides*) and Common Carp (*Cyprinus carpio*) from United States Waters: U.S. Geological Survey Data Series 2006.
- [6] Soeminto. 2004. Viabilitas telur ikan nilam yang ditunda oviposisinya 1 hingga 24 jam. Seminar Nasional Review Tentang Perkembangan Ipteks Perikanan. UNDIP. Semarang.
- [7] Simanjuntak, S.B.I., G.E. Wijayanti, P. Susatyo dan Sugiharto. 1997. Pengadaan ikan nilam (*Osteochilus hasselti* C.V.) monosex dengan pemberian estradiol benzoate pada stadium benih. *Buletin Ilmiah Unsoed*
- [8] Soeminto, P. Susatyo dan M. Santoso. 2000. Pembentukan Jantan Homogamet (XX) Lewat Ginogenesis dan Pemberian Andriol pada ikan nilam (*Osteochilus hasselti* C.V.) Laporan hasil Penelitian (tidak dipublikasikan). Fakultas Biologi Universitas Jenderal Soedirman, Purwokerto.

- [9] Soeminto, P. Susatyo dan Y. Sistina. 2001. Percepatan diferensiasi Kelamin Larva Ikan Nilem Ginogenesis dengan Pemberian Andriol dan Kombinasi andriol-insulin. Laporan Hasil Penelitian. Fakultas Biologi Universitas Jenderal Soedirman, Purwokerto.
- [10] Habibah, A.N., Soeminto dan G.E. Wijayanti. 2009. Diferensiasi gonad ikan nilem (*Osteochilus hasselti* C.V.) pada fotoperiode berbeda. Prosiding Seminar Nasional Perikanan. Yogyakarta B1-11pp 1-9
- [11] Wijayanti, G.E., S.B.I. Simanjuntak dan Sugiharto. 2005. Optimasi potensi reproduksi pada ikan nilem (*Osteochilus hasselti* C.V.) melalui kajian gametogenesis. Seminar Nasional Hasil-hasil Penelitian Perikanan. UNDIP Semarang. 30 November 2005.
- [12] Wijayanti, G.E. dan Sugiharto. 2006. Perkembangan testis ikan nilem (*Osteochilus hasselti* C.V.) selama satu siklus reproduksi. *Omnia* 2(1): 37-43
- [13] Wijayanti, G.E., A. Wulandari and Soeminto. 2009. The dynamic of testicular activity of the nilem (*Osteochilus hasselti* C.V.) under various photoperiod. The First International seminar on Science and Technology (ISSTEC, 2009), January 24-25, 2009, Yogyakarta.
- [14] Wijayanti, G.E. dan Soeminto. 2007. Optimalisasi reproduksi ikan nilem (*Osteochilus hasselti* C.V.) melalui manipulasi fotoperiode. Laporan Penelitian. Fakultas Biologi Universitas Jenderal Soedirman.
- [15] Prayogo, N.A., I. Sulisty, Soeminto, G.E. Wijayanti. 2009. The dynamic of ovarian activity of the hard-lipped barb (*Osteochilus hasselti* c.v.) under different photoperiod regimes. *Biosfera* 25(3): 140-148
- [16] Arukwe, A. and A. Goksøyr. 2003. Eggshell and egg yolk proteins in fish: hepatic proteins for the next generation: oogenetic, population, and evolutionary implications of endocrine disruption. *Comparative Hepatology* 2:4. <http://www.comparative-hepatology.com/content/2/1/4>.
- [17] Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. *International Journal of Developmental Biology* 38:217-229
- [18] Thomas, P., J. Pinter and S. Das. 2001. Upregulation of Maturation-inducing Steroid Membrane receptor in Spotted seatrout ovaries by gonadotropin during oocyte maturation and its physiological significance. *Biology of Reproduction* 64: 21-29.