ONTOGENIC EXPRESSION PROFILES OF GONADOTROPINS (*fshb*, *lhb*) AND GROWTH HORMONE (*gh*) DURING SEXUAL DIFFERENTIATION AND PUBERTY ONSET IN FEMALE ZEBRAFISH

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Introduction:

The reproductive process in teleosts is mainly regulated by the hypothalamus-pituitary-gonad (HPG) axis. As a primary factor in the axis, gonadotropin-releasing hormone (GnRH) from the hypothalamus acts at the pituitary to control the expression and release of gonadotropins (GTHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which in turn regulate gonadal activities [1]. Growth hormone (GH), another pituitary hormone, has also been demonstrated to play an important role in reproduction, so has been termed co-gonadotropin [2-4]. The timing of the appearance of FSH and LH-expressing cells during reproductive development appeared to be species-specific. In most species studied such as the salmonids [5, 6], gilthead seabream [7] and cichlids [8], the FSH cells appeared earlier than LH cells. In the gilthead seabream, both FSH and LH cells appeared prior to sexual differentiation whereas in the cichlids, the LH cells were detected at the onset of sex differentiation [7]. Both FSH and LH cells also appeared before sex differentiation in the pejerry, while LH cells were detected earlier than FSH cells [9]. Puberty is the period in vertebrate life cycle that marks the transition from sexual immaturity to maturity to enable the animal to acquire adult reproductive function. As an important point in vertebrate reproductive life, puberty has been under extensive study in the past decades. Although it is well accepted that the puberty onset requires an increased pulsatile release of GnRH which in turn activates GTHs, the two types of gonadotropins seem to have different roles in controlling puberty in different species. In mammals, the onset of puberty is associated with an increase in the amplitude and frequency of LH [10]. However, evidence in teleosts points to the importance of FSH signaling in puberty onset.

Methods:

The zebrafish were collected at different time points of early development including 4, 6, 8, 10, 13, 16, 19, 22, 25, 28, 38, 48 and 53 days post fertilization (dpf). The head of each fish including the brain and pituitary was fixed in 4% paraformaldehyde at 4℃ overnight for the Fluorescent In Situ Hybridization (FISH), whereas the bodies were fixed in Bouin's solution at room temperature overnight for histological examination of gonadal developmental stage.

Results and discussion:

Using double-colored in situ hybridization, we demonstrated that the expression of *fshb* was earlier than *lhb* with its mRNA signal detectable (~2-3 cells/pituitary) shortly after hatching (4 dpf). *Lhb* expression became detectable, albeit at very low level, at the time of sex differentiation (~25 dpf). In female zebrafish, the first morphological sign for puberty is the first wave of follicle transition from the primary growth (PG) to previtellogenic stage (PV), which is marked by the appearance of cortical alveoli in the oocytes. Our data showed that the expression of *lhb* was very low (~5-6 cells/pituitary) before this transition but increased dramatically during and after the transition. In contrast, the expression of *fshb* was abundant before puberty with only a slight increase during puberty onset. Interestingly, the location of *fshb*-expressing cells changed significantly during puberty from predominantly peripheral to central location. As a control, the expression of *gh* was abundant throughout prepubertal and pubertal periods. Our result strongly suggests an important role for LH at the puberty onset of female zebrafish, similar to the situation in mammals, and its expression could be a sign for puberty at the pituitary level. However, the significance of location change of FSH cells during this period will be interesting to investigate. The increased expression of *fshb* and *lhb* at puberty was also supported by real-time qPCR analysis.

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