

# Kisspeptin stimulates final oocyte maturation in the catfish, *Clarias batrachus*: an *in vitro* approach

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**Abstract:** Kisspeptin (kiss) and its receptor (kiss1r) play a critical role in reproduction by regulating the hormonal secretions of hypothalamus, pituitary and gonadal (HPG) axis in mammals. The existence of kiss and kiss1r has also been identified in the brain and gonads of several fish species, but studies on its role are largely restricted to the hypothalamus and pituitary secretions. The role of kiss at gonadal level remains poorly studied, despite the fact that presence of kiss and kiss1r mRNA is demonstrated in ovary and testis, and also that fishes display a wide range of mode and mechanism of reproductive and spawning activities. The present study, therefore, was undertaken to evaluate the role of kiss on the post-vitellogenic oocyte maturation of *Clarias batrachus* using kiss agonist (KP-10) and kiss1r antagonist, p234. The post-vitellogenic follicles in the ovarian tissue were incubated *in vitro* with KP-10 and p234, for 24hr. The follicles were then processed to count and score germinal vesicle breakdown (GVBD), as an index of final oocyte maturation. KP-10 accelerated while p234 down regulated the occurrence of GVBD in post-vitellogenic oocytes, suggesting that KP-10 stimulates the final oocytes maturation of fully grown ovarian follicles in the catfish, *C. batrachus*.

**Index Terms:** Kisspeptin, Final oocytes maturation, GVBD, KP-10, p234

## I. INTRODUCTION

In recent decades, relevance of kiss in reproductive physiology has been strongly recognized in vertebrates, including fishes. Kiss mRNA and kiss immunoreactivity have been demonstrated in the follicular cells of the growing as well as pre-ovulatory follicles, corpus luteum, cumulus-oocytes complex, granulosa and thecal cells in several mammalian species (Castellano et al., 2006; Ciesh et al., 2017; Hsu et al., 2014; Ricu et al., 2012; Shahed & Young, 2009; Zhou et al., 2014), attributing its role in folliculogenesis. The existence of *kiss/kiss1r* transcripts has also been identified in the brain of

several fish species establishing their role of regulation of gonadotropin release hormone (GnRH) and thereby, gonadotropins (luteinizing hormone- LH, follicle stimulating hormone- FSH) (Filby et al., 2008; Gopurappilly et al., 2013; Ogawa & Parhar, 2013; Parhar et al., 2004; Selvaraj et al., 2012). The *kiss* and *kiss1r* mRNA as well as immunoreactive kiss have also been detected in peripheral tissues including gonads of some fishes (Bakshi & Rai, 2019; Saha et al., 2016; Selvaraj et al., 2010; Shahi et al., 2017; Singh et al., 2021a; Tovar Bohórquez et al., 2017). Hitherto, the studies on their role in regulation of reproductive activities at gonad level are very rare. Recently, the authors have demonstrated the local effect of kiss using kiss antagonist (p234) and agonist (KP-10) both *in vivo* and *in vitro* which suggest the pro-gonadal role of kiss in catfish (Singh et al., 2021a, 2021b). The KP-10 used in the study is a mammalian kiss homologue decapeptide is an endogenous ligand of kiss receptor (kiss1r/gpr54) while p234 is a ten amino acid kiss receptor (kiss1r/gpr54) antagonist which binds directly to kiss-10 binding sites and suppresses the release of GnRH. In mammals role of kiss in the final oocyte maturation is well established but in fish the studies are practically missing. Thus, the present study was undertaken to unravel the role of kiss in early oocyte in the freshwater catfish, *Clarias batrachus* during the post-vitellogenic phase if any.

## II. MATERIAL AND METHODS

### A. Chemicals

Kiss agonist (KP-10) and its receptors antagonist (p234) were provided by Prof. R.P Millar, Department of Immunology, University of Pretoria, South Africa. Details of KP-10 and p234 are described elsewhere (Millar & Newton, 2013; Singh et al., 2021a, 2021b). Streptomycin sulphate

(RM220) and culture Medium199 (AL094A) was procured from Himedia Laboratories Pvt. Ltd., India. The other AR grade chemicals used in routine was purchased from Merck, SRL and HiMedia through local authorized vendors.

### B. Procurement of fish

The freshwater catfish, *C. batrachus* were collected in the last week of June (early-spawning phase). Fish were acclimated for 14 days to the laboratory conditions in tanks made up of cement carrying 200L of water and were fed with chopped goat liver *ad libitum*. The fishes were provided with ambient photoperiod and temperature. The entire experiment were conducted in accordance to the guidelines of Institutional Animal Ethics and Care of Banaras Hindu University, India (approval letter No. F.Sc./IAEC/2016-17/1136).

### C. *In vitro* treatments with KP-10 and p234

To evaluate the effect of KP-10 and p234 on final oocyte maturation, catfish were sacrificed; their ovaries were excised rapidly and aseptically. The adhered fat tissues were cleaned properly. Thereafter, ovaries were cut into small fragments with approximately 50 follicles and were placed in medium199 supplemented with 0.2% NaHCO<sub>3</sub>, penicillin 100IU/ml, streptomycin 100µg/ml and 40µg/ml gentamycin and pre-incubated for 3hr under humidified atmosphere with 95% air and 5% CO<sub>2</sub> at pH 7.4 at 25°C. Then after, the culture medium was replaced with fresh medium containing various doses of KP-10 and p234 (0.0, 0.5, 5, 25 and 50 nM/ml), separately, for 24hr under similar condition (see Singh et al., 2021a, 2021b). Concurrently, the ovarian tissue fragments with around 50 follicles were incubated in medium199 without agonist and antagonist to use it as controls. Subsequently, ovarian fragments were fixed in glacial acetic acid and ethanol (5: 95, v/v) for 10min. Thereafter, the follicles were dispersed with the help of soft small brush and counted to score germinal vesicles breakdown (GVBD) as an index of final oocyte maturation as described elsewhere (see Sarang & Lal, 2005). In verily, the germinal vesicle breakdown (GVBD) is the morphological manifestation of final oocyte maturation that is the migration of the germinal vesicle (GV), nucleus of the oocyte, towards the animal pole and disappearance of the nuclear membrane is known as “GV breakdown” (GVBD) indicating the completion of the first meiotic prophase. The treatment was run in triplicate for each ovary using ovaries collected from the four *C. batrachus*, separately. The entire experiments were repeated three times. The *in vitro* protocol to study the effect of various drugs/hormones has been well established in the laboratory of author (Priyadarshini & Lal, 2018; Singh, et al., 2021a, 2021b; Singh nee Priyadarshini & Lal, 2018; Yadav & Lal, 2019).

### D. Statistical analyses

Data were analyzed by one way ANOVA followed by Duncan's multiple range tests at 95% confidence limit ( $P < 0.05$ ) for the comparison amongst different groups and are presented as Mean±SEM (n=3). All the statistical analyses were performed in SPSS16 software (SPSS Inc., Chicago, IL, USA).

## III. RESULTS

The *in vitro* treatment of oocytes with kiss agonist, KP-10, induced the increase in number of oocytes with GVBD (Fig.1) in dose-dependent manner as compared to control (Fig.2). However, kiss receptors antagonist, p234, treatment lowered the occurrence of the GVBD in post-vitellogenic oocytes significantly when compared with the control group (Fig.3).

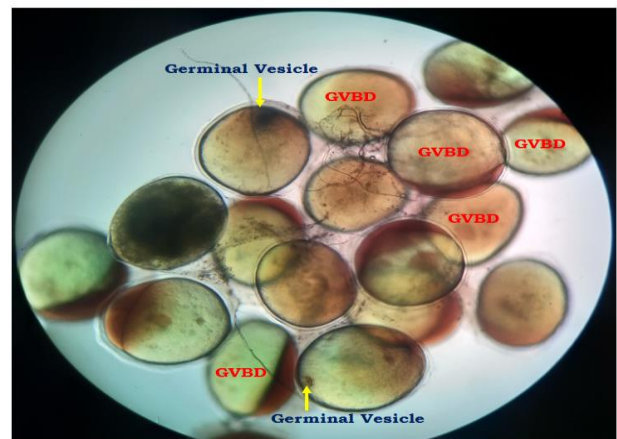


Fig.1. Diagrammatical representation of germinal vesicle breakdown

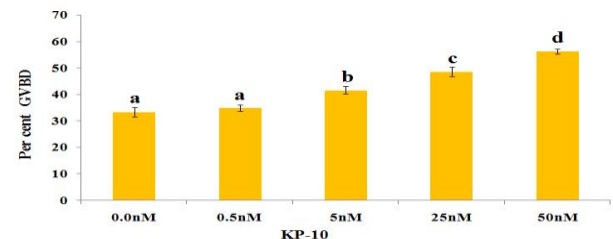


Fig.2. Percent GVBD (y-axis) in ovarian fragments of *Clarias batrachus* exposed to different concentration of KP-10 (x-axis). Each bar represents Mean±SEM (n=3). Means with same superscript do not differ from each other, while means with different superscripts are different from each other statistically at  $P < 0.05$  (Duncan's multiple range test). Superscripts a, b, c, & d are used for percent GVBD.

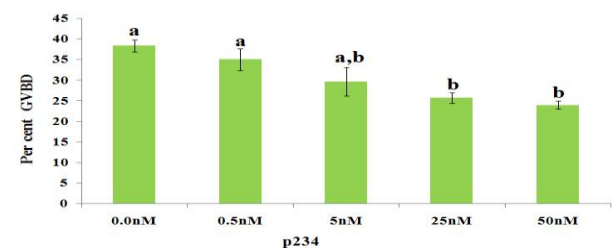


Fig.3. Percent GVBD (y-axis) in ovarian fragments of *Clarias batrachus* exposed to different concentration of p234 (x-axis). Each bar represents Mean±SEM (n=3). Means with same superscript do not differ from each other, while means with different superscripts are different from each other statistically at  $P < 0.05$  (Duncan's multiple range test). Superscripts a and b are used for percent GVBD.

## IV. DISCUSSION

Kiss emerged as an important neuropeptides that regulate vertebrate reproduction through regulation of GnRH. The arrangement of kiss neurons in the brain, and their interactive control of GnRH are different in fish from the mammals. GnRH plays a central role by stimulating the synthesis and release of the pituitary gonadotropin, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from pituitary. The LH and FSH once secreted target the gonads (Backstrom et al., 1982; Burger et al., 2004; Marshall & Kelch, 1986; Wu et al., 1990) and stimulate steroidogenesis, which is responsible for progression of gonadal growth and maturation (Nagahama & Yamashita, 2008; Yaron et al., 2003). Once oocyte is fully grown by the end of June in the present catfish the estradiol levels decreases leading to the removal of dopamine induced GnRH release, well known to cause LH surge needed for the production of maturation induced steroids ( $17\alpha$ ,  $20\beta$ -dihydroxy-4-pregnen-3-one). However, the autocrine/paracrine role of kiss is poorly studied in vertebrates including fishes.

The growth of oocyte is followed by the final oocyte maturation i.e. resumption of meiotic division. The oocyte maturation is most vital step to produce haploid egg involving the production of  $17\alpha$ ,  $20\beta$ -dihydroxy-4-pregnen-3-one (DPH), a maturation inducing steroid (MIS), and synthesis of maturation promoting factor (MPF) under the influence of stimuli-specific LH surge from the pituitary (Fig.4). During the course of final maturation, the oocytes undergo various morphological and nuclear reorganizations. The binding of LH on granulosa cells induce the production of MIS which initiates nuclear reorganization and migration of the germinal vesicle (GV) towards the animal pole where the nuclear membrane disappears, and this condition is known as "GV breakdown" or GVBD. It is also accompanied by condensation of chromosomes, formation of spindle and release of the first polar body indicating the end of the first meiotic division. The GVBD indicates the completion of first meiotic prophase. Following this, the process of ovulation starts i.e. the rupturing of follicular layer and release of eggs (oocytes) into the ovarian lumen. The movement egg is released from the ovarian follicles, meiosis is again arrested in metaphase II, and remains halted till the commencement of second meiotic division as result of egg stimulation by the sperms, which lead to the release of the second polar body.

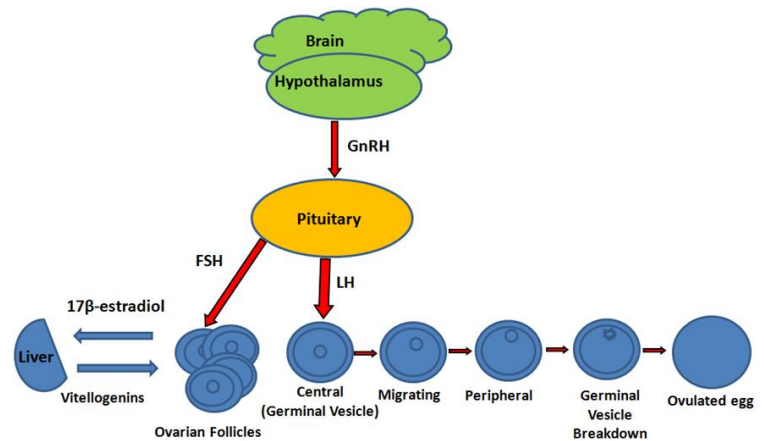


Fig.4. Diagrammatical representation of germinal vesicle breakdown

The present fish, *C. batrachus* is a seasonally breeding catfish and breeds during July and August (Singh et al., 2021a; Singh & Lal, 2016; Yadav & Lal, 2019). Generally, the present catfish develops maximum number of fully grown oocytes (oocytes- III) by end of June, and waits for the suitable environmental stimuli leading to the LH surge, which initiates ultimately the final oocyte maturation in July onwards, involving several physiological and hormonal events.

The present *in vitro* studies, using kiss agonist and antagonist, distinctly demonstrates that kiss is capable of stimulating GVBD in fully grown oocytes, independent of known extra-gonadal factors. The results from the present study show that exogenous administration of KP-10 accelerates final oocyte maturation while kiss antagonist down regulates the final oocyte maturation. The KP-10 at a dose of 25nM and 50nM was able to induce the GVBD more effectively and maximally. However, p234 treatment caused significant reduction in number of the oocytes with GVBD when compared with control group without treatment.

In mammals, although kiss has been shown to trigger the oocyte maturation under *in vivo* and *in vitro* conditions successfully (Abbara et al., 2015, 2018; Jayasena et al., 2014; Kasum et al., 2017; Rehman et al., 2020; Saadeldin et al., 2012). However, studies in sub-mammalian species, particularly in fishes, are rare and indirect. Ohga et al., (2018) have reported maximum expression of kiss1 in brain of the scombroid during the period, when oocytes undergo final oocyte maturation and ovulation and thus have corroborated its role in final oocytes maturation. Similarly, numerous other researchers have also demonstrated relatively high level of expressions of kiss and concurrent GnRH1 in different region of hypothalamus, and LH in pituitary and have attributed their high level to increased number of advanced oocytes including oocytes with GVBD in several fish species (Kanda et al., 2008, 2013; Selvaraj et al., 2012; Zmora et al., 2015). The induction in the expression of LH receptors and  $20\beta$ -hydroxyl steroid dehydrogenase genes in zebrafish oocytes following *in vitro* KP-10 treatment has also

been well documented. Based on their results they have suggested that KP-10 accelerates the final oocytes maturation. Nevertheless, the present *in vitro* studies using kiss agonist and antagonist clearly establishes the direct role of kiss in triggering the final oocytes of the fully grown oocytes in the catfish. This study also suggest that kiss acts locally in an autocrine/paracrine manner, independent of extra-ovarian factors, although underlying mode and mechanism of action of kiss at ovarian level remains to be elucidated and requires further detailed study.

## V. CONCLUSION

The present study for the first time reports the role of kiss in stimulating the final oocyte maturation directly at follicular level in the freshwater catfish, *C. batrachus*. The putative mode and mechanism of action on kiss on final oocyte maturation is presented in fig.5.

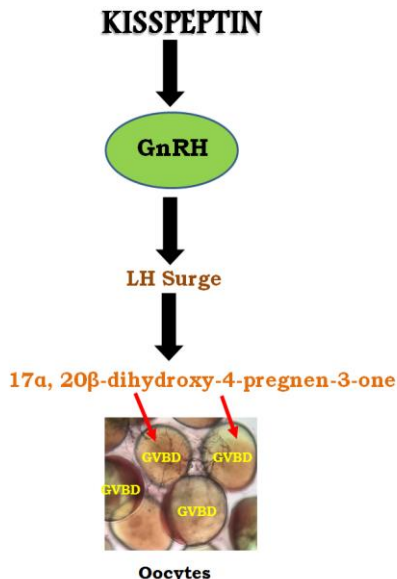


Fig.5. The figure represents the bunch of oocyte showing GVBD in response to KP-10.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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