

Full Length Research Paper

Effect of human chorionic gonadotropin (hCG) on *in vitro* oocyte maturation in freshwater cyprinid, *Barilius vagra*

Amina Zuberi^{1*}, Muhammad Naeem² and Samina Jalali¹

¹Department of Animal Sciences, Quaid-i-Azam University, Islamabad, 45320 Pakistan.

²Institute of Pure and Applied Biology, Zoology Division, Bahauddin Zakariya University, Multan 60800, Pakistan.

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***In vitro* exposure of *Barilius vagra* ovarian follicles to human chorionic gonadotropin (hCG) influenced the position of germinal vesicle and resulted in a maturation of oocyte, although the response was time dependent. There was slight but significant ($P < 0.05$) increased in germinal vesicle breakdown (GVBD, 12.42 vs. 2.94%) at 24 h, whereas there was significant ($P < 0.0001$) decreased in oocyte having central germinal vesicle (CGV, 59.6 vs. 85%). Prolonged incubation further increased GVBD (29.8% at 72 h) and decreased central position germinal vesicle (CGV, 8.7%). Moreover, hCG treatment at 72 h enhanced the synthesizing capability of the vitellogenic oocytes. There was significant increase in the secretion of estradiol-17 β ($P = 0.0001$) and non significant effect on the secretion of testosterone ($P = 0.07$). 17 α -hydroxyprogesterone (17-OHP: 17 α -hydroxypregn-4-ene-3, 20-dione) and 17 α -20 β -dihydroxypregn-4-ene-3-dione (17,20 β P: 17, 20 β -dihydroxypregn-4-ene-3-one) were detected and identified only in the hCG treated incubation medium.**

Key words: hCG, *in vitro*, oocyte maturation, germinal vesicle breakdown, maturation inducing steroid (MIS).

INTRODUCTION

Developmental events in the ovaries of teleosts are regulated by steroids synthesized under the direct influence of two gonadotropins; GtH1, GtH2 (Patino et al., 2001; Nagahama and Yamashita, 2008; Mishra and Joy, 2006; Rehman et al., 2001; Skobolina, 2009). Oocyte growth follows oocyte maturation in fish (the resumption of meiosis), and require two steroidal mediator, estradiol-17 β (E₂) and 17 α , 20 β -dihydroxy-4-pregnen-3-one (17 α , 20 β P), which are under the direct control of gonadotropin. In the period of oocyte growth, estradiol-17 β regulates the synthesis of hepatic yolk that is then sequestered into the oocyte. GtH1 has been shown to activate the enzyme P₄₅₀ aromatase that converts testosterone and androsteronedione to estradiol-17 β and to estrone; the entire process occurring respectively, in the theca and granulosa cells of the follicle (two-cell model) (Nagahama et al., 1994) where the theca cells

supply the precursor steroids and the granulosa cells produce the steroidal mediators under the direct influence of gonadotropin. On completion of vitellogenesis, a distinct shift in steroidogenesis; from Estradiol to maturation inducing steroid (MIS) as well as the steroidogenic enzyme genes from ovarian cytochrome P₄₅₀ aromatase to 20- β -hydroxysteroid dehydrogenase (20 β -HSD), occurs in the granulosa layers of ovarian follicles prior to oocyte maturation (Senthilkumaran, 2004). Gonadotropins stimulates synthesis of maturation inducing hormone (MIS) via 20 β -hydroxysteroid dehydrogenase (20 β -HSD), which in most species appears to be 17 α , 20 β -dihydroxy-4-pregnen-3-one (17, 20 β P) (Nagahama et al., 1997; Haider and Inbaraj, 1989; Patiño and Sullivan, 2002; Matsuyama et al., 2002; Garcia-Alonso et al., 2004; Zuberi et al., 2011).

Like other vertebrates, final oocyte maturation in teleosts occurs prior to ovulation and consists of the migration and breakdown of the germinal vesicle (GVBD), chromosome condensation and formation of the first polar body (Nagahama and Yamashita, 2008; Patiño and

*Corresponding author: E-mail: zuberi_amina@yahoo.com.
Tel: +92 51 9064 3199.

Sullivan, 2002). Final maturation terminates in ovulation involving release of the oocyte from the enveloping follicular tissue into the ovarian lumen. Several studies have reported the morphological and cytoplasmic changes in the follicular compartment, including vitelline envelope, granulosa cell layer and theca cell layer during final oocyte maturation (Kayaba et al., 2001; Srijunngam et al., 2005; Unal et al., 2005). Nagahama et al. (1994) described that during oocyte growth and maturation, follicular somatic cells undergo cytodifferentiation and cooperate in the production of steroidal mediators, whereas Kayaba et al. (2001) and Srijunngam et al. (2005) reported the increased number of organelles including mitochondria, Golgi bodies, endoplasmic reticulum and free ribosomes during final maturation. Moreover, gonadotropin induction of final oocyte maturation is thought to be mediated by follicular production of maturation inducing hormone (MIS) (Pattino et al., 2001; Senthilkumaran, 2004)

Several studies have determined the effectiveness of various gonadotropin and pituitary preparations to induce oocyte maturation *in vivo* (Haniffa et al., 2000; Rehman et al., 2001; Chuda et al 2002; Mishra and Joy, 2006) and *in vitro* (Skoblina, 2009; Sorbera et al., 1999; Kagawa et al., 1994; Patino & Thomas, 1990a; Rehman et al., 2001) in many species. Gonadotropin plays its role in induction of oocyte maturation, first by making the oocyte sensitive to MIS and second by inducing MIS synthesis in follicle cells surrounding the oocyte (Kobayashi et al., 1988; Zhu et al., 1989; Patino and Thomas, 1990b). In majority of teleost 17, 20 β P appeared as the most potent maturation inducing hormone (MIH) (Nagahama et al., 1983; Haider and Inbaraj, 1989; Patiño and Sullivan, 2002; Matsuyama et al., 2002; Garcia-Alonso et al., 2004; Zuberi et al., 2011), whereas a number of other ovarian steroids including estradiol, corti-costeroids and deoxycorticosteroids have also been found to have equal and in some cases even greater potency as MIS *in vitro* in several species (Hirose, 1976; Goswami and Sundraraj, 1977; Nagahama and Yamashita, 2008; Goetz, 1983; Milla et al., 2009).

Human chorionic gonadotropin (hCG) found in mammals is a structurally related variant of fish gonadotropin, which acts as an effective inducer of oocyte maturation in several teleost (Skoblina, 2009). It stimulate the *in vitro* steroidogenesis of granulosa cell and release the MIS in the incubation medium (King et al., 1995; Sorbera et al., 1999; Hakan et al., 2005), although there are some other species it has no effect (Patino and Thomas, 1990b).

The diversity of the teleostean group and the reproductive adaptation unique to its various taxa, warrant a wider examination of the gonadotropin responses in a variety of species. *Barilius vagra*, an important local freshwater species for both basic and applied research in Pakistan was selected as a model for this experiment. The basic aim of this work was twofold; firstly, to determine the efficacy of human chorionic gonadotropin in the induction of oocyte maturation of *B. vagra*,

and secondly, to investigate the role of hCG on the steroid synthesizing capability of oocyte specially MIS.

MATERIALS AND METHODS

Chemicals and hormones

The steroids used in this study were estradiol-17 β (E₂: estra-1,3,5 (10)-triene-3,17 β -diol), testosterone (T:17 β -hydroxy-4-androsten-3-one), progesterone (P₄: pregn-4-ene-3, 20-dione), 17 α -hydroxyprogesterone (17-OHP: 17 α -hydroxypregn-4-ene-3, 20-dione), 17 α -20 β -dihydroxypregn-4-ene-3-dione (17,20 β P: 17, 20 β -dihydroxypregn-4-ene-3-one) and human chorionic gonadotropin (hCG). These were purchased from Sigma Chemical Co (St. Louis Missouri, USA). RIA kits for estradiol-17 β , testosterone, progesterone, 17-OHP and 17,20 β P were purchase from IBL, International.

Experimental fish

B. vagra, belonging to the family Cyprinidae, is commonly found in the hill streams of Islamabad, Pakistan where surface water temperature ranges during the year between 14°C in the coldest months to 29°C in the warmest months. During early breeding season (late March to early April), mature females were transported live to the experimental fish laboratory of the Department of Biological Sciences, Quaid-i-Azam University, and stocked one week for acclimation to the ambient laboratory conditions in glass aquaria containing aerated water. They were given tropical fish food twice daily.

In vitro assay

For the *in vitro* study, mature fish were sacrificed, the ovaries placed in ice-cold basic salt solution (BSS), and then reduced to small pieces. Individual ovarian follicles were separated using fine forceps without disrupting the integrity of the theca and granulosa layers. A sub sample of the 20 largest ovarian follicles was treated with oocyte clearing fixative (Zhoa and Wright, 1985), which revealed that such follicles contained oocytes with mostly central to nearly CGV.

The culture technique for *in vitro* incubation of the ovarian follicles was adapted from Upadhyaya and Haider (1986) and Zuberi et al. (2002, 2011). The culture medium (BSS) consisted of 3.7 g NaCl, 0.32 g KCl, 0.16 g CaCl₂, 0.1 g NaH₂PO₄, 0.16 g MgSO₄.7H₂O and 0.8 g glucose in 1 L of double distilled water. The medium was autoclaved, cooled and its pH was adjusted to 7.5 with sterilized 1.0 N NaHCO₃. Bacterial and fungal contamination was controlled with penicillin-streptomycin (100 IU/ml) and fungizone (2.5 μ g/ml). The medium was sterile filtered through a millipore filter of GS 0.22 μ m (Bedford, Mass, USA). Filtration was usually done in a laminar flow cabinet. If necessary, the medium was stored in a refrigerator at 4°C.

In vitro experiments were conducted inside a sterile room. Samples of 30 to 35 ovarian follicles were transferred to individual culture tubes containing 3.0 ml fresh culture medium and hCG was added to each tube with a micro syringe to obtain concentrations of 20 IU/ml of the culture medium. The control cultures received an equal volume of saline. Viability of the oocytes was checked in preliminary tests by trypan blue in samples of cultured oocytes at various time intervals. According to this test, viable oocytes remain clear following the treatment, while the dead oocytes take up the stain. The incubations for each test system were run in triplicate. The replicate cultures were maintained at 22 \pm 0.2°C in a humidified temperature-controlled incubator for 24, 48, and 72 h each. Upon

completion of the incubations, the follicles were examined under an inverted microscope for the position of germinal vesicle (GV) and breakdown (GVBD) by treating them with egg clearing solution (ethanol, formalin, and glacial acetic acid mixed 95:10: 5 by volume) (Zhoa and Wright, 1985). Incubation medium from the 72 h control and treated groups were either directly applied on extraction cartridges or store at -80°C for the extraction of estradiol-17 β , testosterone, progesterone, 17-OHP and 17 α , 20 β P.

Hormone extraction

The extraction procedure was based on the methods described by Khan et al. (1996) and Zuberi et al. (2002). The steroid hormone metabolites generated by the oocytes and released in the incubation replicates were extracted using an activated LiChrolut® RP-18 solid phase extraction cartridge (500 mg, 3 ml, and 40 to 63 μM). Extraction columns were primed using two consecutive washes with 2 ml of 100% methanol followed by two consecutive washes with 2 ml de-ionized water (DI). The incubation mediums were directly applied and then pushed through the columns using the vacuum manifold. After pumping, the cartridges were washed with 5ml DI and free (unconjugated) steroids were eluted from the columns into 10 ml borosilicate test tube (12 x 75 mm) by two consecutive 3 ml washes with diethyl ether. The 6 ml of eluted solvent was evaporated at 45°C under stream of nitrogen gas and the residue was re-dissolved in 500 μl of RIA buffer and stored frozen until assayed.

Radioimmunoassay (RIA)

Free estradiol, testosterone, progesterone, 17-OHP and 17 α , 20 β P concentrations were measured using RIA kit obtained from IBL, International. All samples were run in duplicate. The RIA kits were validated for measuring 17 β -estradiol, testosterone, progesterone, 17-OHP and 17 α , 20 β P by verifying the slopes obtained when serial dilutions of these samples were parallel to the curve created with kit standards (compare slopes, 17 β -estradiol: slope = 0.978 ± 0.034 , $r^2 = 0.995$, $P = 0.97$; testosterone: slope = 0.932 ± 0.026 , $r^2 = 0.998$, $P = 0.96$; progesterone: slope = 0.922 ± 0.021 , $r^2 = 0.968$, $P = 0.97$; 17-OHP: slope = 0.955 ± 0.023 , $r^2 = 0.985$, $P = 0.97$ and 17 α ,20 β P: Slope = 0.90 ± 0.029 , $r^2 = 0.978$, $P = 0.98$). The precision of the method was determined by comparing the results from repeated assays and assay of samples at different time and subsequently calculating the coefficient of variation (%CV). The intra-assay coefficient of variation for 17 β -estradiol, testosterone, progesterone, 17-OHP and 17 α , 20 β P were 6.3, 5.9, 7.1, 10.2 and 9.3%, respectively, whereas inter-assay coefficient of variation (%CV) were 9.8, 7.6, 9.3, 11.1 and 10.4%, respectively.

The extraction efficiency (% recovery) from incubation medium was assessed by adding an equal volume of the 10, 50 and 100 ng/ml standards supplied with the RIA kit to basic culture medium. Basic salt solution was then pumped through extraction cartridges. The steroids hormones were retrieved from the cartridges using 2 x 3ml volumes of diethyl ether and respective hormone concentration quantified using RIA as aforementioned. Minimum observed recovery of the methodology was 94.9, 94.6, 92.3, 93.8 and 91.9% for 17 β -estradiol, testosterone, progesterone, 17-OHP and 17 α , 20 β P, respectively. All steroid hormones values are reported as pg oocyte $^{-1}$.

Statistical analyses

Percentage position of germinal vesicle breakdown was calculated and expressed as mean \pm S.E. The percentage (%) positions of germinal vesicle, followed by treatment with various hCG and

incubation periods were analyzed by appropriate ANOVA models using SPSS for Windows (Software version 10.0 Chicago, IL).

RESULTS

In vitro induction of maturation

Spontaneous breakdown of the germinal vesicle in untreated (control) oocytes was observed in a low percentage and its proportion varied according to the duration of incubation (Figure 1). At the start of the experiment, GVBD percentage in the controls culture was 1.83 ± 1.83 and was significantly increased at 72 h (7.97 ± 0.87). Majority of the oocyte in the control groups had centrally located germinal vesicle.

Treatment of the ovarian follicles with hCG resulted in the significant effect on the behavior of germinal vesicle (Table 1). At 24 h, compared with the control, a substantially significant ($P < 0.0001$) smaller percentage of oocytes contained centrally placed germinal vesicle and much larger percentage contained migratory and peripheral germinal vesicle (Figure 1). In respect to GVBD, treated group differed significantly $P < 0.01$ from the control (12.42 vs. 2.94%). There was also significant treatment by time interaction (Table 1). With prolonged incubation, further increase in GVBD (19.91% at 48 h and 28.89% at 72 h) and decrease in centrally located germinal vesicle (19.01 and 8.67% at 48 and 72 h, respectively) occurred (Figure 1). Moreover, linear regression analysis of individual data of hCG treated group revealed positive correlation between percentage of germinal vesicle breakdown and time period ($y = 0.3623x + 3.3234$, $R^2 = 0.953$), whereas negative correlation ($y = -1.0604x + 79.98$, $R^2 = 0.892$) existed between centrally located germinal vesicle and incubation period (Figure 2).

Steroid production from incubation of follicle-enclosed oocytes of *B. vagra*

hCG enhanced the steroid synthesizing capability of oocyte and stimulated the production of estradiol-17 β , 17-OHP and 17 α , 20 β P at 72 h (Figure 3). In the control incubation medium at 72 h, both free 17-OHP and 17 α , 20 β P were below the detection limit, whereas 3.6 ± 0.4 , 6.8 ± 0.65 and 5.3 ± 0.49 pg oocyte $^{-1}$ for estradiol-17 β , testosterone and progesterone, were respectively detected. The concentration of testosterone and progesterone were statistically comparable ($P = 0.14$) and significantly ($P < 0.01$) higher than estradiol-17 β . The presence of hCG in the incubation medium stimulated the estradiol-17 β secretion ~ 6 fold (23.4 ± 1.19 pg oocyte $^{-1}$; $P \leq 0.0001$), while there was no significant increase in the concentration of testosterone (8.8 ± 0.53 ; $P = 0.07$). In addition, the culture medium supplemented with hCG did not differ in P_4 secretion when compared to the control.

Table 1. Results of multivariate analysis of variance examining the effect of hCG (20 IU/ml) at different time periods on the induction of oocyte maturation in freshwater cyprinid, *B. vagra*.

Source	Position of Germinal vesicle	DF	F value	P value
Treatment	Central	1	1954.402	0.000
	Migratory	1	580.276	0.000
	Peripheral	1	131.293	0.000
	GVBD	1	271.388	0.000
Time	Central	2	336.567	0.000
	Migratory	2	90.221	0.000
	Peripheral	2	26.873	0.000
	GVBD	2	48.195	0.000
Treatment × time	Central	2	108.527	0.000
	Migratory	2	38.672	0.000
	Peripheral	2	4.749	0.030
	GVBD	2	14.606	0.001

On the other hand, the levels of 17-OHP and 17 α , 20 β P that were below the detection limit in the control cultured medium were stimulated and concentration reached 3.9 ± 0.32 and 11.15 ± 0.28 pg oocyte⁻¹, respectively at 72 h.

DISCUSSION

The result of this study shows that *B. vagra* oocytes responded to gonadotropins in a manner similar to what has been described for other species *in vitro*. In teleost fishes, final oocyte maturation consists of the migration and breakdown of GVBD, chromosome condensation and formation of the first polar body (Goetz, 1983; Thomas, 1994), while hCG is capable of stimulating dose and time dependent oocyte maturation (Sorbera et al., 1999; Rahman, 2002; Matsuyama et al., 2002). Although, in the control incubation medium there was significant decrease ($P < 0.01$) in number of oocyte having central germinal vesicle and slight increase ($P \leq 0.05$) in germinal vesicle break down at 72 h; the shifting of germinal vesicle from central towards periphery and its breakdown (GVBD) was much more pronounced in response to exogenous hCG. At 24 h, 60% oocytes had central germinal vesicle and only 12.42% showed GVBD. However, with prolonged incubation (72 h), there was significant decrease (8.67 ± 0.22 ; $P < 0.0001$) in % oocytes with CV and increase in % GVBD (29.81 ± 0.62 vs. 4.97 ± 1.03 ; $P < 0.0001$) (Figure 1). These results reveal positive linear relationship between incubation period and % GVBD and negative relationship between time and % oocytes having central germinal vesicle (CV) (Figure 2).

These findings are in agreement with previous studies where hCG was tested as a possible maturation inducing agent *in vitro* (Sorbera et al., 1999; Patino and Thomas, 1990a; York et al., 1993). The response latency of *B.*

vagra oocytes to exogenous gonadotropins was also comparable with that observed in other teleosts. It has been demonstrated *in vitro* in several species that gonadotropin-stimulated final maturation has longer response latency than steroid-induced maturation. For example, while steroid-induced maturation can be completed within 20 h of incubation for *Fundulus heteroclitus* L., gonadotropin-induced maturation proceeded more slowly and was completed within 48 h (Wallace and Selman, 1980; Petrino et al., 1993). The longer latency may be due to the fact that *in vitro* gonadotropins do not act directly on the oocyte to induce resumption of meiosis, but instead initiates maturation of the oocyte by synthesis of a MIS by the ovarian follicles (Senthilkumaran et al., 2004). In most teleost species, progesterone derivatives 17 α , 20 β -dihydroxy-4-pregnen-3-one (17, 20 β P) were detected in the incubation medium in response to gonadotropin and appeared as a MIS (Semenkova et al., 2006; Matsuyama et al., 2001; Rahman et al., 2002; Nagahama, 1997; Garcia-Alonso et al., 2004).

In addition to incubation period, the dose of hCG during treatment also had greater influence on oocyte response latency. The incubation of sea bass oocyte with 100 IU/ml stimulated the stronger maturation response rate of 86.9% with shorter response latency (50 h), whereas there was longer response latency (100 h) and an average maximal response rate of 60.4% with 50 IU hCG/ml (Sorbera et al., 1999). The lower dose of 10 IU/ml had no significant effect on maturation in sea bass, but there was significance (26% GVBD) at 72 h with 10 IU/ml hCG in *B. vagra* (Zuberi et al., 2002). The % GVBD with 20 IU/ml at 24, 48 and 72h observed in the study was slightly higher and confirmed the view that species variation exist in the response of gonadotropin. For example, there were some species like hybrid Sturgeon, where hCG did not show any response at any

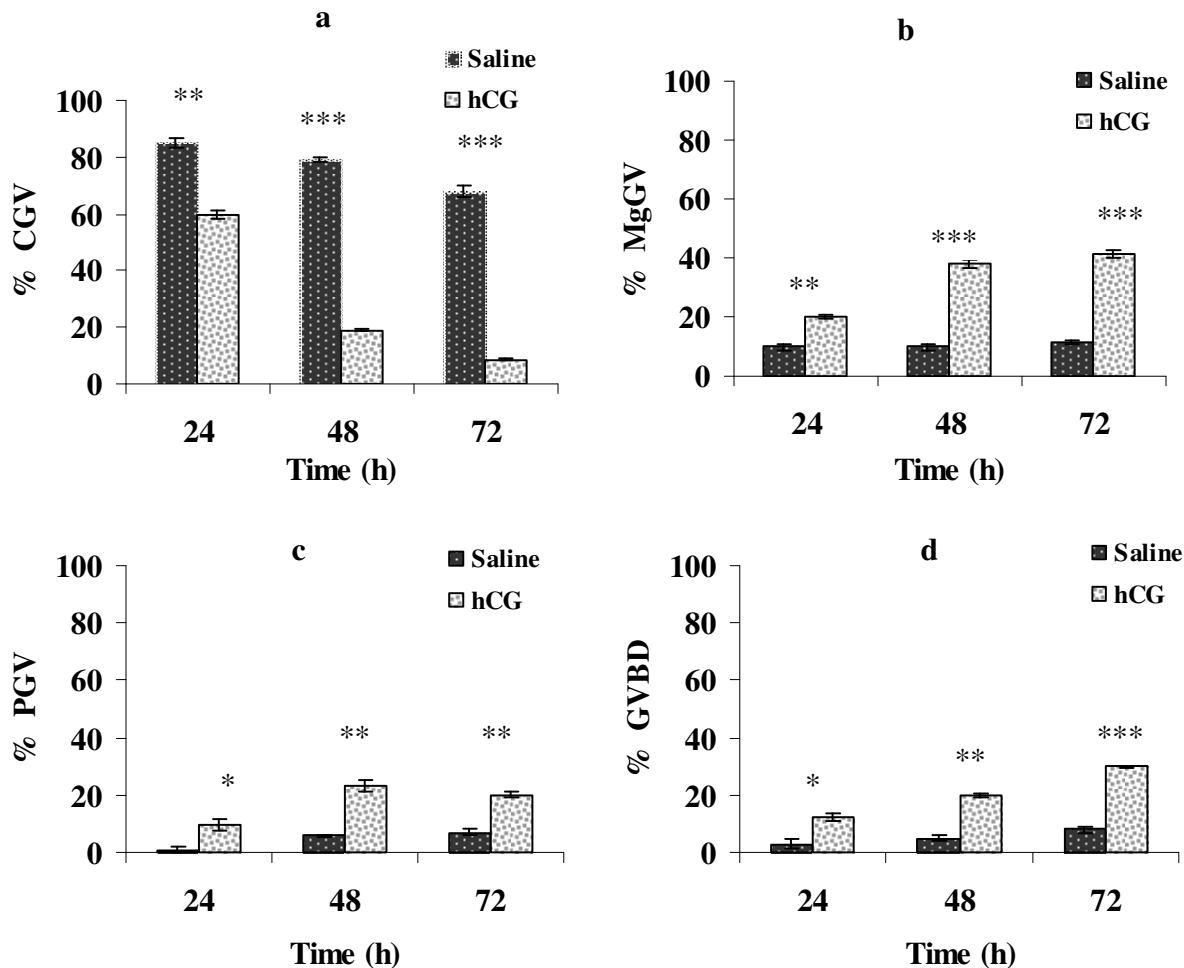


Figure 1. Position of germinal vesicle in the control (saline) and gonadotropin (hCG; 20 IU/ml) treated oocyte of *B. vagra* at different incubation period (* = P < 0.01; ** = P < 0.001; *** = P < 0.0001).

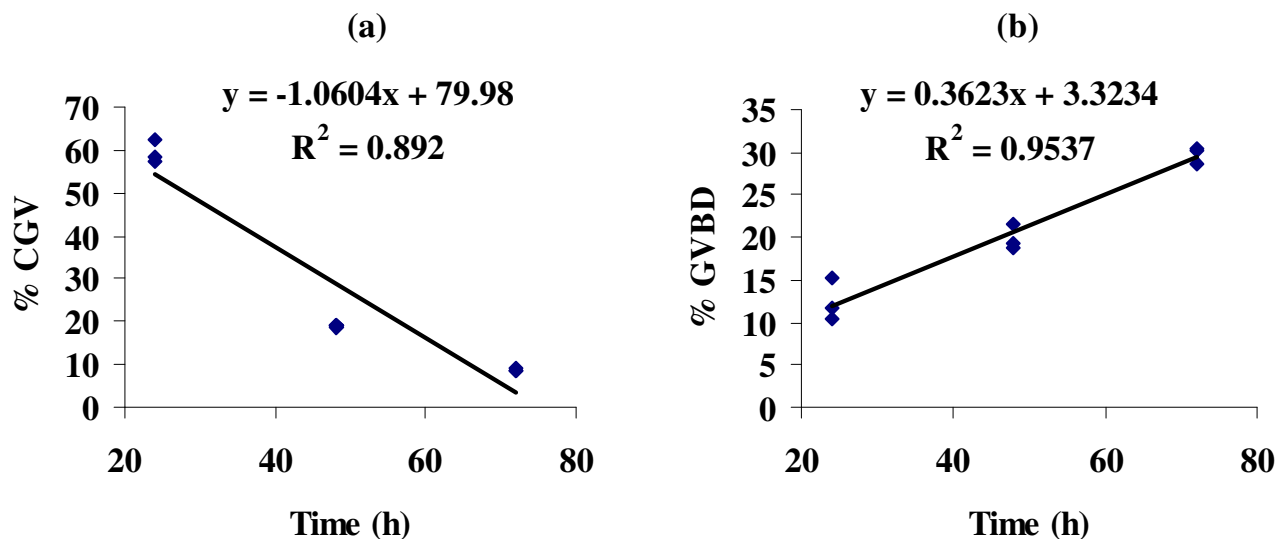


Figure 2. Relationship between time period and % position of germinal vesicle of oocyte incubated with hCG (20 IU/ml). (a) Negative linear relationship between % CGV and incubation period; (b) positive linear relationship between % GVBD and incubation period.

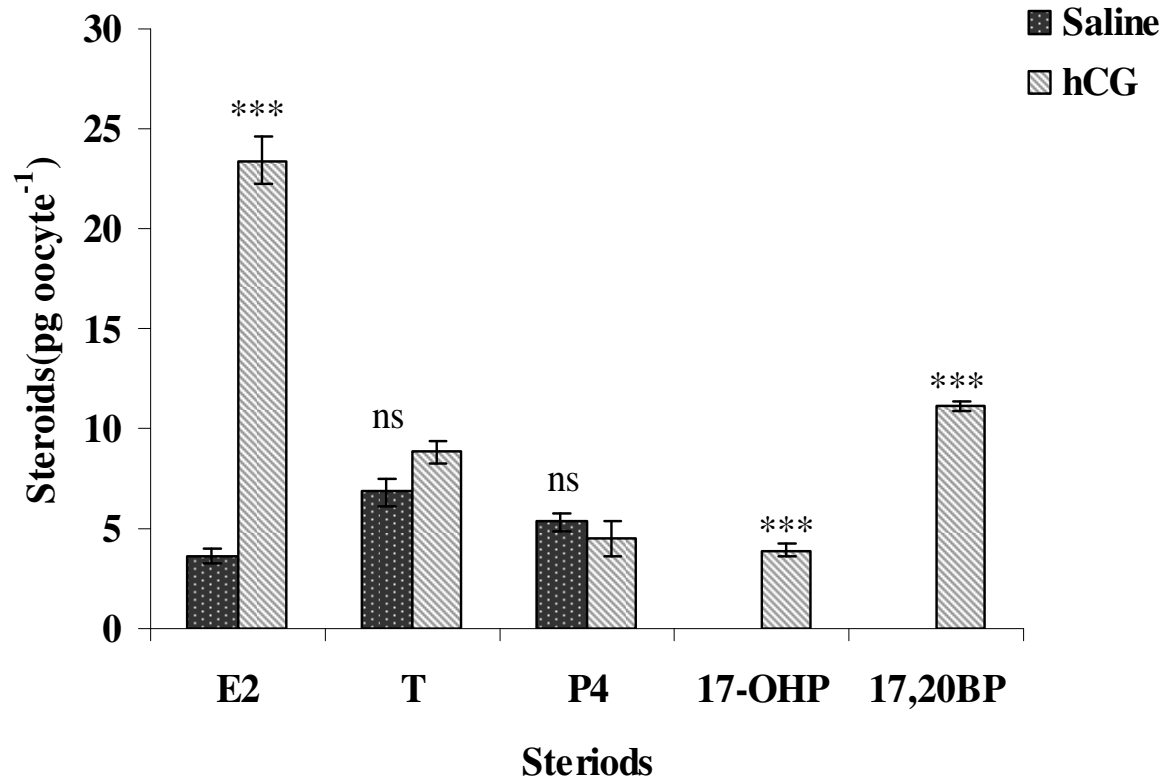


Figure 3. Steroid production from follicle-enclosed oocytes of *B. vagra* incubated *in vitro* for 72 h with and without gonadotropin (hCG; 20 IU/ml). T, Testosterone; P4, progesterone; E2, estradiol-17 β ; 17- α -hydroxyprogesterone (17-OHP); 17 α -20 β -dihydroypregn-4-ene-3-dione (17,20 β P) were only found in the cultured medium of the hCG treated follicles (***) = $P < 0.0001$; ns = non significant).

concentration of gonadotropin (Amiri et al., 2001), whereas follicle enclosed oocyte of some other species having oocyte of definite size did not respond to MIS until they were primed with gonadotropin (*Sillago japonica*: Kobayashi et al., 1988; *Repomucenus beniteguri*: Zhu et al., 1989; *Micropogonias undulatus*: Patino and Thomas, 1990c; *Cynoscion nebulosus*: Pinter and Thomas, 1999). The “priming” effect of gonadotropin on oocyte maturation induced by steroids suggest the dual role of gonadotropin in the induction of oocyte maturation in some teleost species: first, to make the oocyte competent for maturation (sensitive to MIS), and second, to induce MIS synthesis and oocyte maturation (Kobayashi et al., 1988; Zhu et al., 1989; Patino and Thomas, 1990c).

Human chorionic gonadotropin has been confirmed to stimulate maturation of the gonads of several fish species (Barannikova et al., 1975; Scott and Canario, 1990; Kagawa et al., 1994; Lubzens et al., 2010) and stimulated steroid production in vitellogenic and full grown ovarian follicle (Skoblina, 2009; Senthilkumaran et al., 2004). Incubation of the follicles of *B. vagra* with hCG resulted in increased production of estrogens. Testosterone was a dominant hormone in the control group, while there was several fold increased in the production of E2 as compared to testosterone in the treated group (Figure 3).

This high level of E2 resulted from the interaction of both granulosa and theca cells.

It is thus evident that in response to hCG, theca cells produce and release testosterone, a precursor of E2, which readily diffuse into the granulosa cells layer and is converted in E2 by the stimulation of steroidogenic enzyme P₄₅₀ aromatase.

Pankhurst and Ripie (2000) reported the same increase in E2 and T production by vitellogenic follicles of greenback flounder (*Rhombosolea tapirina*) in response to hCG at 18 h. In carp, *Cyprinus carpio*, hCG also enhances synthesis of T and E₂ *in vitro* in the vitellogenic oocytes (Galas et al., 1999).

As regard progesterone, an important metabolite in steroidogenesis, the amount was nearly similar in the control and hCG supplemented incubation media in *B. vagra* (Figure 3). This might be related to activation of 20 β -hydroxysteroid dehydrogenase (20 β -HSD) and intense conversion to 17 α , 20 β -dihydroxy-4-pregnen-3-one (17, 20 β P). In this study, 17-OHP and 17, 20 β P were below the detection limit in the control incubation of *B. vagra*, while there was three fold increase in the amount of 17, 20 β P as compared to of 17-OHP in response to hCG (Figure 3). The low level of 17-OHP in the incubations were probably due to its rapid metabolism

(Kime, 1994). Enhanced production of 17-OHP and 17, 20 β P under the influence of hCG has also been observed in other species, but the level of the individual metabolites produced depends on the stage in which the oocytes were at the time of incubation (Galas et al., 1999). The increased production of 17, 20 β P is associated with oocyte germinal vesicle migration and GVBD such as found in *Morone Americana* (King et al., 1995), *Acipenser transmontanus* (Amiri et al., 2001) and *Micropogonias furnieri* (Garcia-Alonso et al., 2004). In Atlantic salmon, *Salmo salar* (Zhoa and Wright, 1985), gonadotropin markedly stimulated the steroid output in general, where 17, 20 β P was released in significantly greater amount. Apparently increased production of 17, 20 β P in response to hCG is correlated with stimulation of P450 c17 and 20 β -hydroxysteroid dehydrogenase (20 β -HSD) (Nagahama and Yamashita, 2008; Senthikumar et al., 2004). The low or non detectable production of 17-OHP and 17, 20 β P in the control incubation of *B. vagra* suggests that the enzyme 20 β -HSD could not be activated fully in oocytes that were still mostly in central GV stage (Zhao and Wright, 1985; Galas et al., 1999; Amiri et al., 2001).

In conclusion, this study reveal that hCG treatment of post vitellogenic oocyte of *B. vagra* induced oocyte maturation to some extent by the stimulation of P450 c17 and 20 β -hydroxysteroid dehydrogenase (20 β -HSD), as well as the production of MIS, which in turn affected the behavior of the germinal vesicle of the oocyte. The effect of hCG was quite consistent and promoted time-dependent improvement in GVBD.

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