African Journal of Biotechnology Vol. 10(74), pp. 16986-16993, 23 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1904
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

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

Amina Zuberi[1]*, Muhammad Naeem[2] and Samina Jalali[1]

[1]Department of Animal Sciences, Quaid-i-Azam University, Islamabad, 45320 Pakistan.
[2]Institute of Pure and Applied Biology, Zoology Division, Bahauddin Zakariya University, Multan 60800, Pakistan.

Accepted 28 September, 2011

***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- 17ß (P = 0.0001) and non significant effect on the secretion of testosterone (P = 0.07). 17α-hydroxyprogesterone (17-OHP: 17α-hydroxypregn-4-ene-3, 20-dione) and 17α-20β-dihydroxypregn-4-ene-3-dione (17,20βP: 17, 20β-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; Skoblina, 2009). Oocyte growth follows oocyte maturation in fish (the resumption of meiosis), and require two steroidal mediator, estradiol-17β (E_2) and 17α, 20 ß-dihydroxy-4-pregnen-3-one (17 α, 20ßP), which are under the direct control of gonadotropin. In the period of oocyte growth, estradiol-17β regulates the synthesis of hepatic yolk that is then sequestered into the oocyte. GtH1 has been shown to activate the enzyme P_{450} aromatase that converts testosterone and androsteronedione to estradiol-17β 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 P_{450} aromatase to 20-ß-hydroxysteroid dehydrogenase (20ß-HSD), occurs in the granulosa layers of ovarian follicles prior to oocyte maturation (Senthilkumaran, 2004). Gonadotropins stimulates synthesis of maturation inducing hormone (MIS) via 20β-hydroxytsteroid dehydrogenase (20β-HSD), which in most species appears to be 17α, 20β-dihydroxy-4-pregnen-3-one (17, 20βP) (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, 20ßP 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-17β (E_2: estra-1,3,5 (10)-triene-3,17β-diol), testosterone (T:17β-hydroxy-4-androsten-3-one), progesterone (P_4: pregn-4-ene-3, 20-dione), 17α-hydroxyprogesterone (17-OHP: 17α-hydroxypregn-4-ene-3, 20-dione), 17α-20β-dihydroxypregn-4-ene-3-dione (17,20βP: 17, 20β-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-17ß, testosterone, progesterone, 17-OHP and 17,20βP 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 $CaCl_2$, 0.1 g NaH_2PO_4, 0.16 g $MgSO_4.7H_2O$ 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 $NaHCO_3$. Bacterial and fungal contamination was controlled with penicillin-streptomycin (100 IU/ml) and fungizone (2.5 µg/ml). The medium was sterile filtered through a millipore filter of GS 0.22 µM (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 ± 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-17ß, testosterone, progesterone, 17-OHP and 17α, 20ßP.

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 × 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 17α, 20ßP concentrations were measured using RIA kit obtained from IBL, International. All samples were run in duplicate. The RIA kits were validated for measuring 17 ß-estradiol, testosterone, progesterone, 17-OHP and 17α, 20ßP by verifying the slopes obtained when serial dilutions of these samples were parallel to the curve created with kit standards (compare slopes, 17ß-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 17α,20ßP: 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 ß-estradiol, testosterone, progesterone, 17-OHP and 17α, 20ßP 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 × 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 ß-estradiol, testosterone, progesterone, 17-OHP and 17α, 20ßP, 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 ± 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-17ß, 17-OHP and 17α, 20ßP at 72 h (Figure 3). In the control incubation medium at 72 h, both free 17-OHP and 17α, 20ßP were below the detection limit, whereas 3.6 ± 0.4, 6.8 ± 0.65 and 5.3 ± 0.49 pg oocyte^{-1} for estradiol-17ß, 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-17ß. The presence of hCG in the incubation medium stimulated the estradiol-17ß secretion ~ 6 fold (23.4 ± 1.19 pg oocyte^{-1}; P≤ 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 17α, 20ßP 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 at el., 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 ($P\leq0.05$) 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 17α, 20β-dihydroxy-4-pregnen-3-one (17, 20βP) 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, were hCG did not show any response at any

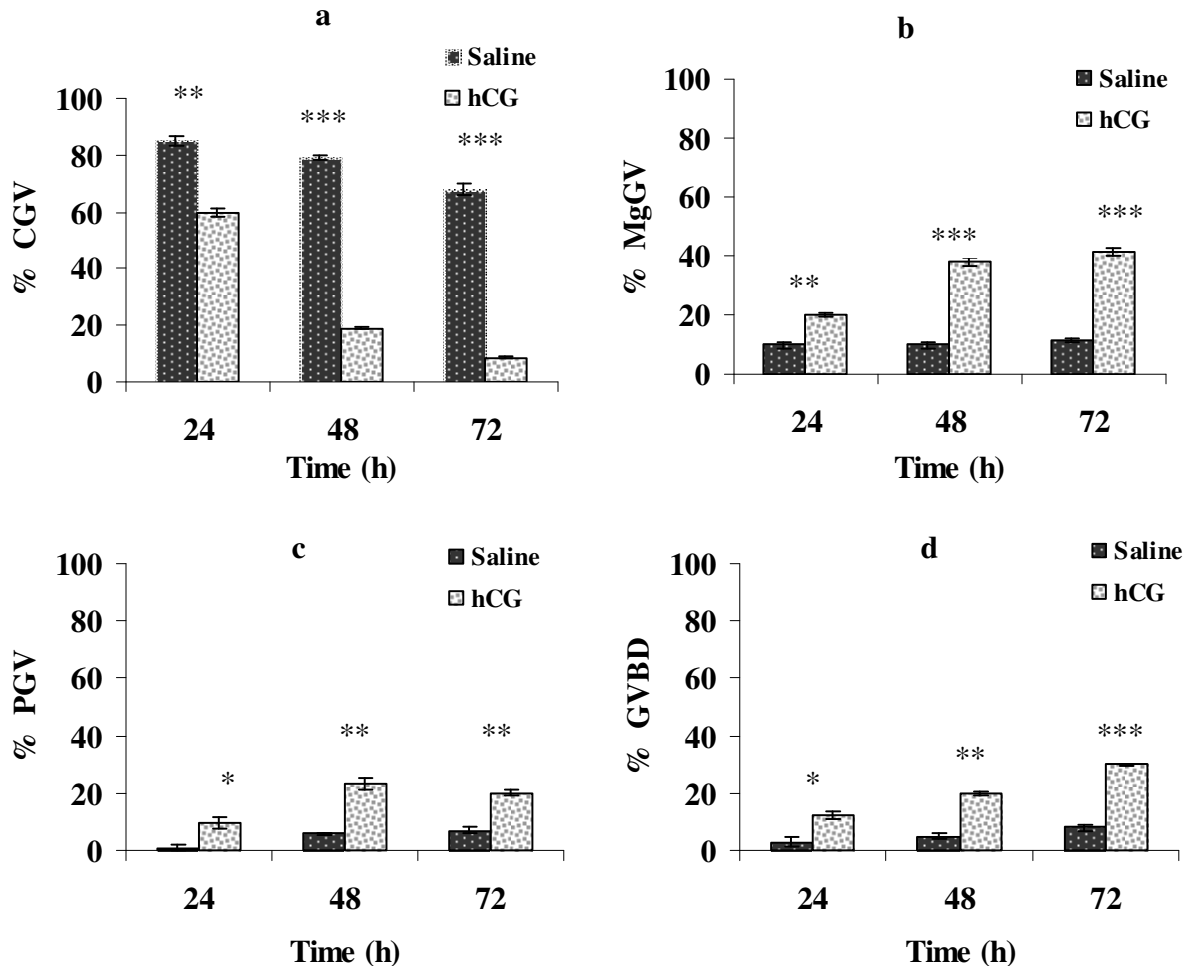

Figure 1. Position of germinal vesicle in the control (saline) and gonadotropin (hCG; 20 IU/ml) treated oocyte of *B. vagra* at different incubation period (* = P < 0.01; ** = P < 0.001; *** = P < 0.0001).

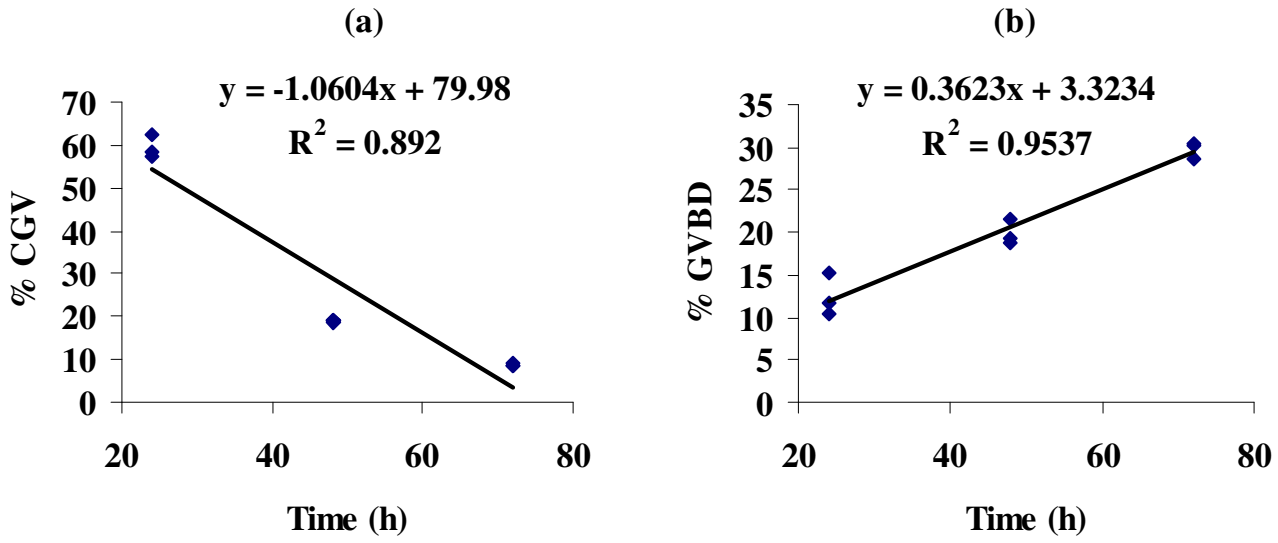

Figure 2. Relationship between time period and % position of germinal vesicle of oocyte incubated with hCG (20 IU/ml). (a) Negative linear relationship between % CGV and incubation period; (b) positive linear relationship between % GVBD and incubation period.

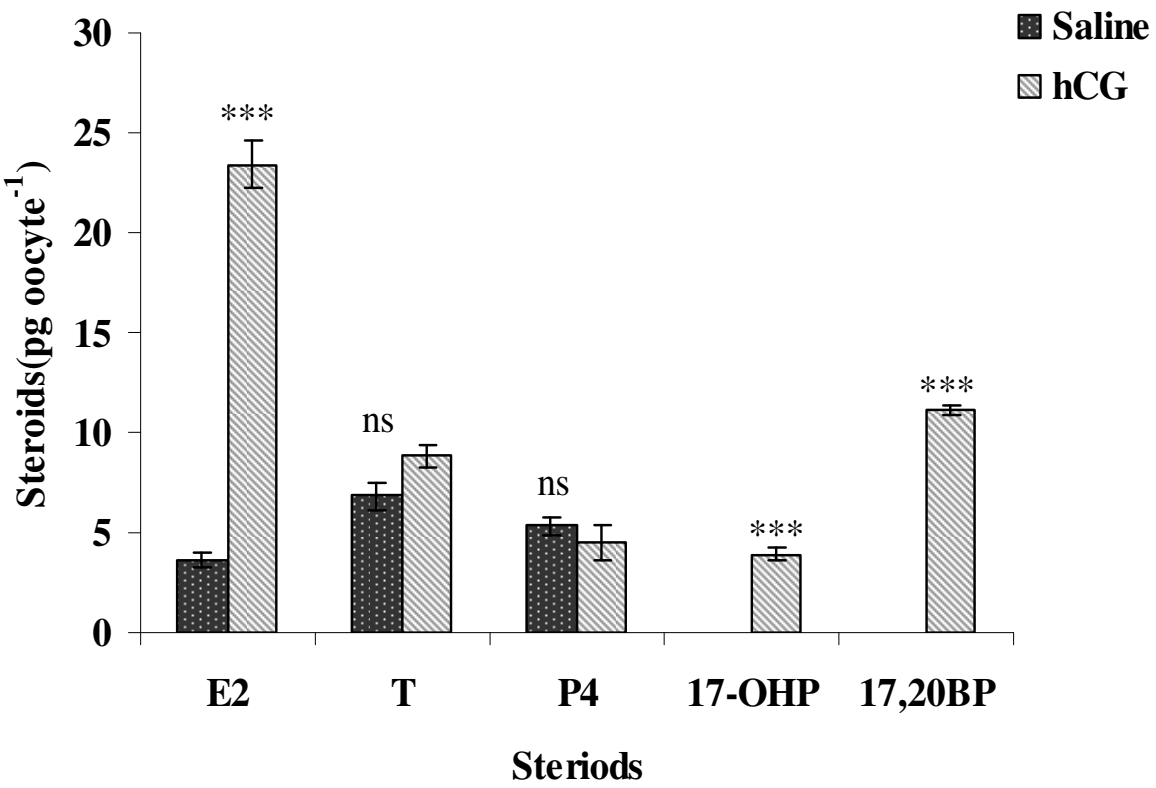

Figure 3. Steroid production from follicle-enclosed oocytes of *B. vagra* incubated *in vitro* for 72 h with and without gonadotropin (hCG; 20 IU/ml). T, Testosterone; P4, progesterone; E2, estradiol-17β;17-α-hydroxyprogesterone (17-OHP); 17α-20β-dihydroxypregn-4-ene-3-dione (17,20βP) were only found 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 P_{450} 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 E_2 *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 20β-hydroxysteroid dehydrogenase (20β-HSD) and intense conversion to 17α, 20β-dihydroxy-4-pregnen-3-one (17, 20βP). In this study, 17-OHP and 17, 20βP were below the detection limit in the control incubation of *B. vagra,* while there was three fold increase in the amount of 17, 20βP 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, 20βP 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, 20βP 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, 20βP was released in significantly greater amount. Apparently increased production of 17, 20βP in response to hCG is correlated with stimulation of P450 c17 and 20β-hydroxysteroid dehydrogenase (20β-HSD) (Nagahama and Yamashita, 2008; Senthilkumaran et al., 2004). The low or non detectable production of 17-OHP and 17, 20βP in the control incubation of *B. vagra* suggests that the enzyme 20β-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 20β-hydroxysteroid dehydrogenase (20β-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.

REFERENCES

Amiri BM, Maebayashi M, Omoto N, Adachi S, Yamauchi K (2001). In Vitro Oocyte Maturation in a Hybrid Sturgeon, Bester: Changes in the Germinal Vesicle Breakdown and 17, 20β- dihydroxy-4-pregnen-3-one Production. J. Agric. Sci. Technol. 3: 199-207.

Barannikova IA, Boev AA, Saenko JJ, Travkin BG (1975). On the possibility to use hCG for the stimulation of fish maturation (in Russian). Tr VINRO CXI, 136-140

Chuda H, Nakao T, Arakawa T, Matsuyama M (2002). Effects of HCG injection on oocyte maturation and ovulation in cultured two-year-old yellowtail, Seriola quinqueradiata) Suisanzoshoku, 50: 235-236.

Galas J, Epler1 P, Stoklosowa S (1999). Seasonal response of carp (*cyprinus carpio*) ovarian cells to stimulation by various hormones as measured by steroid secretion: tissue culture approach. Endocrine Regul. 33: 125-132

Garcia-Alonso J, Neppa A, Somoza G (2004). Steroid Metabolism in Vitro during Final Oocyte Maturation in White Croaker *Micropogonias furnieri* (Pisces: Sciaenide). Braz. J. Biol. 64: 211–220.

Goetz FW (1983). Hormonal control of oocyte final maturation and ovulation in fishes. In Fish Physiology, Vol. 1XA (Hoar WS, Randall DJ and Donaldson EM, Eds.), 117-170. Academic press, New York.

Haider S, Inbaraj RM (1989). Relative *in vitro* effectiveness of estradiol-17ß, androgens, corticosteroids, progesterone, and other pregnene derivatives on germinal vesicle breakdown in oocytes of the Indian major carps. Labeo rohita, *Cirrhinus mrigala* and *Catla catla*. Fish Physiol. Biochem. 6:289-296.

Haniffa MA, Merlin T, Mohamed JS (2000). Induced spawning of the striped murrel *channa striatvs* using pituitary extracts, human chorionic gonadotropin, luteinizing hormone releasing hormone analogue, and ovaprim Acta Icht. Piscat. 30(1): 53-60.

Hirose K (1976). Endocrine control of ovulation in medaka (*Oryzias latipes*) and ayu (*Plecoglossus altivelis*). J. Fish. Res. Bd Canada. 33: 989-994.

Kagawa H, Tanaka H, Okuzawa K, Hirose K (1994). Development of maturational competence of oocytes of red seabream, *Pagrus major*, after human chorionic gonadotropin treatment *in vitro* requires RNA and protein synthesis. Gen. Comp. Endocrinol. 94: 199-206.

Kayaba T, Takeda N, Adachi S, Yamauchi K (2001). Ultrastructure of the oocytes of the Japanese eel *Anguilla japonica* during artificially induced sexual maturation. Fish. Sci. 67: 870-879.

Khan MN (1996). Studies on steroid hormone metabolism by arctic charr (*Salvelinus alpinus*) embryos. Ph.D. Thesis, University of Guelph, UK.

King VW, Berlinsky DL, Sullivan CV (1995**).** Involvement of gonadal steroids in final oocyte maturation of white perch (*Morone americana*) and white bass (*M. chrysops*): *in vivo* and *in vitro* studies. Fish Physiol. Biochem. 14(6): 489-500.

Kobayashi M, Aida K, Furukawa K, Law YK, Moriwaki T, Hanyu I (1988). Development of sensivity to maturation-inducing steroids in the oocytes of the daily spawning teleost, the Kisu, *Sillago japonica*. Gen. Comp. Endocrinol. 72:264-271.

Lubzens E, Young G, Bobe J, Cerda J (2010). Oogenesis in teleosts: how fish eggs are formed. Gen Comp. Endocrinol. 165(3): 367-389.

Matsuyama M, Sasaki A, Nakagawa K, Kobayashi T, Nagahama Y, Chuda H **(**2001).Maturation-inducing Hormone of the Tiger Puffer, *Takifugu rubripes* (Tetraodontidae, Teleostei): Biosynthesis of Steroids by the Ovaries and the Relative Effectiveness of Steroid Metabolites for Germinal Vesicle Breakdown *In Vitro.* Zool Sci. 18(2): 225-234.

Matsuyama M, Onozato S, Kashiwagi M (2002). Endocrine control of diurnal oocyte maturation in the kyusen wrasse, Halichoeres poecilopterus, Zool Sci 19(9) *1045-1053*.

Milla S, Wang N, Mandiki SNM, Kestemont P (2009). Review;Corticosteroids: Friends or foes of teleost fish reproduction? Comp Biochem Physiol A Mol. Integr. Physiol. 153(3): 242-251.

Mishra A, Joy KP (2006). Effects of Gonadotrophin *in Vivo* and 2-Hydroxyoestradiol-17β in Vitro on Follicular Steroid Hormone Profile Associated with Oocyte Maturation in the Catfish *Heteropneustes fossilis,* J. Endocrinol. 189: 341-353.

Nagahama Y, Yamashita M (2008). Regulation of oocyte maturation in fish. Dev. Growth Differ. 50: 195-219.

Nagahama Y. (1983). The functional morphology of teleost gonads. In Fish Physiology, Vol. 1XA (Hoar WS, Randall DJ and Donaldson EM Eds.), Academic Press, New York. pp. 223-275.

Nagahama Y, Yoshikuni M, Yamashita M, Tanaka M (1994). Regulation of oocyte maturation in fish. In: Fish Physiology (Sherwood NM, Hew CL, Farrell A and Randall P Eds.) Academic Press, New York. Vol XIII, 393-439.

Nagahama Y (1997). 17 α, 20 β-Dihydroxy-4-Pregnen-3-Ones, a Maturation-Inducing Hormone in Fish Oocytes: Mechanisms of Synthesis and Action, Steroids. 62: 190-196.

Pankhurst NW, Riple G (2000). Characterization of parameters for in vitro culture of isolated ovarian follicles of greenback flounder Rhombosolea tapirina. Comp. Biochem. Phy A. Mol. Integr. Phy. 127(2): 177-189

Patino R, Thomas P (1990a). Gonadotropin stimulates 17a, 20b,21-trihydroxy-4-pregnen-3-one production from endogenous substrates in Atlantic croaker ovarian follicles undergoing final maturation in vitro. Gen. Comp. Endocrinol. 78: 474-478.

Patino R, Thomas P (1990b). Effect of gonadotropin on ovarian intrafollicular process during the development of oocyte maturational competence in a teleost, the Atlantic croaker: Evidence for two distinct stages of gonadotropic control of final oocyte maturation. Biol. Reprod. 43:818-827.

Patino R, Thomas P (1990c). Characterization of membrane receptor activity for 17α, 20β, 21-trihydroxy-4-pregnen-3-one in ovaries of spotted seatrout (*Cynoscion nebulosus*). Gen. Comp. Endocrinol. 78: 20-217.

Patiño R, Sullivan CV (2002). Ovarian follicle growth, maturation, and ovulation in teleost fish. Fish Physiol. Biochem. 26(1): 57-70.

Patiño R, Yoshizaki G, Thomas P, Kagawa H (2001). Gonadotropic

control of ovarian follicle maturation: the two-stage concept and its mechanisms Comp. Biochem.phy B. Mol. Biol. 129(2-3): 427-439.

Petrino TR, Lin YWP, Netherton JC, Powell DH, Wallace RA (1993). Steroidogenesis in *Fundulus heteroclitus* V: Purification characterization, and metabolism of 17α, 20β-dihydroxy-4-pregnen-3-one by intact follicles and its role in oocyte maturation. Gen. Comp. Endocrinol. 92: 1-15.

Rehman MA, Ohta K, Chuda H, Nakano S, Maruyama K, Matsuyama M (2001). Gonadotropin-induced steroidogenesis shift towards maturation- inducing hormone in Japanese yellowtail during final oocyte maturation. J. Fish. Biol. 58: 462-474.

Scott AP, Canario, AVM (1990). Plasma level of ovarian steroids, including 17α, 21-dihydroxy-4-pregnen-3, 20-dione and 3α, 17α, 21-trihydroxy-5ß-pregnen-20-one, in female plaice (*Pleuronectes platessa*) induced to mature with human chorionic gonadotropin. Gen. Comp. Physiol. 78:286-298.

Senthilkumaran B, Yoshikuni M, Nagahama Y. (2004**).** A shift in steroidogenesis occurring in ovarian follicles prior to oocyte maturation. Mol. Cell. Endocrinol., 215:11-18.

Semenkova TB, Canario AVM, Bayunova LV, Couto E, Kolmakov NN, Barannikova IA **(**2006). Sex steroids and oocyte maturation in the sterlet (*Acipenser ruthenus* L.) J Appl Ichthyol. 22(1): 340–345.

Skoblina MN (2009). *In Vitro* Stimulation of Oocyte Ovulation in Teleosts *by* Gonadotropic and Steroid Hormones. Russ J Dev Biol. 40(4): 191-19.

Sorbera LA, Asturiano JF, Carrillo M, Cerda J, Kime DE, Zanuy S(1999). In vitro oocyte maturation in the sea bass : effect of hCG, pituitary extract and steroids. J. Fish. Biol. 55:9-25.

Srijunngam1 J, Kitana N, Callard I, Wattanasirmkit K (2005). Ultrastructural Changes in the Ovarian Follicular Wall During Oocyte Growth in the Nile Tilapia, Oreochromis niloticus Linn. Natural History J. Chulalongkorn Univers. 5(1): 21-30.

Thomas P (1994). Hormonal control of final oocyte maturation in sciaenid fishes. In: Perspectives in Comp. Endocrinol (Davey KG, Peter RE and Tobe SS Eds.), 619-625. National Research Council of Canada, Ottawa.

Ünal G, Karakisi H, Elp M (2005). Ovarian follicle ultrastructure and changes in levels of ovarian steroids during oogenesis in *Chalcalburnus tarichi* Pallas Türk Veterinerlik ve Hayvanclk Dergisi. 29(3): 645-653

Upadhyaya N, Haider S (1986). Germinal vesicle breakdown in oocytes of catfish, *Mystus vittas* (Bloch): Relative *in vitro* effectiveness of estadiol-17β, androgens, corticosteroids, progesterone, and other pregnene derivatives. Gen. Comp. Endocrinol. 63: 70-76.

Wallace RA, Selman K (1980). Oogenesis in *Fundulus heteroclitus*, 11. The transition from vitellogenesis to maturation. Gen. Comp. Endocrinol. 42: 345-354.

York WS, Patino R, Thomas P (1993). Ultrastructural Changes in Follicle Cell-Oocyte Associations during Development and Maturation of the Ovarian Follicle in Atlantic Croaker, Gen. Comp. Endocrinol. 92: 402-418.

Zhoa WX, Wright RS (1985). The course of steroids released by intact ovarian follicles of Atlantic salmon (*Salmo salar*) incubated *in vitro* with and without gonadotropin. Gen. Comp. Endocrinol. 57: 274-280.

Zhu Y, Aida K, Furukawa K, Hanyu I (1989). Development of sensitivity to maturation-inducing steroids and gonadotropins in the oocytes of the tobinumeridragonet, *Repomucenus beniteguri*, Callionymidae (Teleostei). Gen. Comp. Endocrinol. 76: 250-260.

Zhu Y, Aida K, Furukawa K, Hanyu I (1989). Development of sensivity to maturation-inducing steroids and gonadotropins in the oocytes of the tobinumeri dragonet, *Repomucenus beniteguri*, Callionymidae (Teleostei). Gen. Comp. Endocrinol. 76: 250-260.

Zuberi A, Naeem M, Jalali S (2011).Relative *in vitro* effectiveness of several gonadal steroids on oocyte maturation in freshwater teleost *Barilius vagra*. Afr J Biotechnol.10 (55): 11772-11777.

Zuberi A, Hafeez MA, Jalali S (2002). Study of ovarian steroids by High performance liquid chromatography II: In vitro analysis of relative importance of steroids in oocyte maturation and their metabolism in local species of fresh water teleost fishes. Proc. Pak. Acad. Sci. 39(2): 151-174.