International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Volume 3, Issue 4 - 2016

Research Article

SOI: http://s-o-i.org/1.15/ijarbs-2016-3-4-24

In vitro effectiveness of hCG on the induction of steroidogenesis in the oocyte of *Cirrhinus mrigala*

Uma T[1]., Saravanan N[2]., Inbaraj R.M[2]., & Jothi Narendiran N[1].

[1]PG- Research Department of Advanced Zoology and Biotechnology, Govt Arts College, Nandhanam, Chennai-35.
[2]Endocrinology Unit, Department of Zoology, Madras Christian College, Chennai-59.
*Corresponding author: *umathangavel.t@gmail.com*

Abstract

Ovarian maturation is regulated by several factors. Hormone action is main factor for the oocyte development and maturation of fishes. Gonadotropins are well characterized in fishes, they are follicle stimulating hormones and luteinizing hormone. Oocyte maturation in fish is initiated by the release of luteinizing hormone secreted from pituitary. LH stimulates the production of 17 α-hydroxyprogesterone (17α-HP), which is converted to 17α, 20β-dihydroxyprogesterone (DHP) the maturation inducing hormone (MIH). Not much of the work reported the *in vitro* studies in the hCG action of oocyte maturation in the fish species of *C. mrigala*. Hence the present study focused on the incubation studies to know the levels of various steroids profiles during the various developmental stages of oocyte.

Keywords: Gonadotropin, Maturation, *Cirrhinus mrigala*, Steroidogenesis.

Introduction

Oocytes incubated in culture medium have been demonstrated to secrete steroid hormones (Petrino *et al.*, 1989a) in the presence of steroid precursors. In teleost fishes C18 and C19 steroids are generally secreted during gonadal growth and C21 steroids during spawing period (Fostier *et al.*, 1983; Goetz, 1983; Scot and Canario, 1987). A Shift in the steroidogenic pathway from E2 to 17,20β-P occurs in ovarian follicle cells prior to oocyte maturation (Nagahama, 2000; Planas *et al.*, 2000). Investigations in oocyte maturation in fishes indicate that estrogens are generally not effective in inducing final oocyte maturation (Goetz, 1983). In Salmonids and Cyprinids, 17,20β-P was indentified in vivo and in vitro as Maturation inducing steroid (MIS) (Nagahama *et al.*, 1983; Fostier *et al.*, 1983; Scot and Canario, 1987). Kime (1993) suggested that other steroids could be implicated in final oocyte maturation in other fish. For instance, 17,20β-P and 17,21-P were the main metabolites in vitro in Pleuronectiforms and Siluriforms, respectively (Upadhya and Haider, 1986; Scott and Canario, 1990). 17,20β-P was identified as MIS in *Clarias batrachus* (Inbaraj *et al.*, 2001).

Fish gonadotropins are not easily available hormones from mammalian sources are commonly used as alternatives in various studies in fish (Kwok *et al.*, 2005). Oocyte maturation is first initiated by a LH surge from the pituitary which triggers the production of 17α-hydroxyprogesterone from the thecal cells. 17α-hydroxyprogesterone is then converted into 17α,20β- dihydroxyprogesterone (17α,20β-P) known as maturation inducing hormone in the granulosa cell layer of cyprinid fishes like goldfish and zebrafish

(Nagahama and Yamashita, 2008; Patino *et al.,* 2001). MIH activates MPF by binding to membrane progestin receptor leads to final oocyte maturation which includes meiotic resumption of oocytes and breakdown of germinal vesicle (Kondo *et al.,* 2001; Patino *et al.,* 2001; Thomas *et al.,* 2004; Miura and Miura, 2008; Nagahama and Yamashita, 2008).

This study was the first to examine the *in vitro* synthesis of steroids by the regulation of hCG in the oocyte sample of *C. mrigala* by *in vitro* method. Patino and Thomas, 1990 reported that in many teleosts, oocyte maturation is promoted by LH in two ways. The first step is to increase the oocyte maturational competence and the second step is to promote the MIH production. hCG induces oocyte maturation in a similar fashion to fish LH, and interact with fish LH receptors (Pinter and Thomas, 1999). Tsai *et al.,* 2010 studied the *in vitro* cultures of early stage zebrafish ovarian follicles for the first time. Ovarian follicles of croaker were treated with gonadotropin showed upregulated mPRα protein level, accompanied the development of oocyte sensitivity to 20β-s and completion of oocyte maturation (Tubbs *et al.,* 2010). These findings consistent with those obtained in spotted seatrout and goldfish (Tokumoto *et al.,* 2006; Zhu *et al.,* 2003b). Studies of Pang and Ge, 2002; Patino and Kagawa 1999; Zhu *et al.,* 1989; Kagawa *et al.,* 1994, reported that in teleosts hCG promotes the oocyte maturation competence. Wang and Greenwald *et al.,* 1993; Wang *et al.,* 2005 revealed that hCG induces significant increase in the growth of primary follicles in mice. Results of Kumar *et al.,* (2001a, b) reported that hCG activates FSH and LS receptors of channel catfish. Pang and Ge, (2002) studies supported the significant maturation of zebrafish stage III oocytes by hCG. Progesterone and gonadogropins interaction and their effects on folliculogeneisis in cat were proposed by Roche, 1996; Monniaux *et al.,* 1997; McGee and Hsueh, 2000.

In atlantic croaker spontaneous ovulation of fully grown follicles and germinal vesicle breakdown of intra follicular oocytes were significantly higher at a pH of 8.5 than 7.5 (Patino *et al.,* 2005). hCG activated both FSH and LH receptors in catfish (Kumar *et al.,* 2001a, b). In mice hCG showed significant increase in the growth of primary follicles (Wang and Greenwald, 1993; Wang *et al.,* 2004). However, the lacuna of information about the hormones induction of ovarian steroidogenesis in difference during the development of ovary in *C. mrigala*. Hence, incubation studies were carried out to identify the steroidogenesis difference during the hormonal induction of ovarian development.

Materials and Methods

Collection of Fish

Female fish of *Cirrhinus mrigala* were collected during the reproductive period from Saathanur reservoir located in Thiruvannamalai district. Thiruvannamalai is located at 12.22°N′79.07, E°′. It has an average elevation of 171 meters (561 feet) and is situated 185 km from Chennai and 210 km from Bangalore. Saathanur Dam across Thenpennai River is a tourist place near Thiruvannamalai.

Incubation studies

The female fish used for the study showed Latevitellogenic oocytes. The ovaries were dissected out after sacrificing the fish. The ovarian fragments were introduced into culture vials each containing 3 ml of the incubation medium.

The culture medium was prepared by dissolving 7.3 gm NaCl, 0.18 gm KCl, 0.07 gm $MgSO_4$, 0.18 gm $MgCl_2$, 0.29 gm $CaCl_2$, 0.95 gm HEPES and 1.0 gm Glucose in 1 litre of distilled water and the medium was maintained at 18^0C. pH was maintained at 7.2. Incubation of oocytes was carried out in culture vials. 3ml medium was used for each incubation. Incubations were carried out using hCG. Oocytes were incubated with these two hormones in different time intervals of 30 minutes, 1hour, 2 hours, 4 hours, 8 hours and 16 hours in the concentration of 1µg/ml. In the experimental incubation vials each containing 3ml of medium and 3µg of hCG. Three replicas were maintained for same concentration in different time intervals to get the concordant result. The incubation was maintained for 16 hours at 18^0C in a modified BOD incubator. The incubated medium was stored in separate vials at -70^0C for further processing to know the synthesis of steroids from the incubated oocytes.

Steroid Extraction

The incubated medium was extracted thrice with dichloromethane - 3ml, 2ml and 2 ml respectively. The mixture was vortexed and centrifuged at 4000 rpm and the supernatant was separated. The supernatant collected was pooled and dried. The dried extract was then dissolved in 50 µl of dichloromethane and methanol (9:1).

High-performance Liquid Chromatography

Acetonitrile and water (40:60) were used as the solvent with a flow through rate 1ml/minute. The C18 column (ODS 0.2µ) used for separation. The UV-visual detector used to identify the synthesis of steroids from in vitro oocytes incubated medium used at 244nm and 254nm. 17α,20β, 21-P; 11-KT; 17,21-P; 21-P; 17,20β-P; 17α,20α-P; T; 11-DOC; 17α-P and P4 (the order mentioned here as per the retention time) were used as reference.

Results

In vitro sysnthesis of steroids by the induction of hCG

HPLC analysis revealed the presence of all the steroid metabolities identified by the incubations carried out with latevitellogenic oocytes with hCG and LH at different time intervals in the concentration (1µg/ml). The standards of steroids were run separately the peak chromatogram and tables were observed and the entire steroids standard collectively mixed together then these standards were run too analyzed by HPLC, the peak chromatogram results represent in (Table. 1), standards steroid graph and 30 mins control peak chromatogram in (Fig. 1 and 2.). Oocytes incubated without hCG for experiment control 30minutes yielded 17, 21-P, 17,20β-P, 11-KT, T and 21-P peaks were observed. 30 minutes incubated oocytes with hCG yielded 17, 21-P, 17,20β-P, 11-DOC, 11-KT, 21-P, 17,20α-P, P4 and 20β-P peaks were observed. The synthesis of steroids 17, 21-P, 17,20β-P, 11-KT, T, 21-P, 17,20α-P, P4 and 20β-P peaks were observed from oocytes incubated with hCG at 1hour. 2 hours incubated oocytes incubated with hCG yielded 17,21-P, 17,20β-P, T, 21-P, 17,20α-P, P4 and 20β-P peaks were observed. Oocytes incubated 4 hours with hCG to synthesized the steroids of 17,21-P, 17,20β-P, 11-KT, T, 21-P, 17,20α-P, P4 and 20β-P peaks were observed. The synthesis was carried out with hCG at 8 hours yielded the steroids 17,21-P, 17,20β-P, 11-KT, 17,20β,21-P, T, 21-P, P4 and 20β-P peaks were observed. 16 hours oocytes incubated with hCG yielded the steroids of 17,21-P, 17,20β-P, 11-KT, 17,20β,21-P, T, 21-P, P4 and 20β-P peaks were observed. Oocytes of control maintain without hCG at 16 hours incubation yielded the steroids of 17,21-P, 17,20β-P, 11-KT, T, 21-P, 17,20α-P, P4 and 20β-P peaks were observed.

Table 1. Shows the *in vitro* synthesis of steroids in the ovary of *C. mrigala* by the induction of hCG in different time durations.

Steroids Name	Ovary incubated with hCG hormone in different time intervals						
	30 mins. control	30 mins. hCG	1 hour hCG	2 hour hCG	4 hours hCG	8 hours hCG	16 hours hCG
17α,21-Hydroxyprogesterone	++++	++++	++	+++	++++	++++	++++
17α,20β,21-Trihydroxyprogesterone	-	-	-	-	-	++	++
11-Deoxycorticosterone	-	+	-	-	-	-	-
11-Ketotestosterone	+	++	++	-	+	++	++
Testosterone	++	++	++	+++	++	+++	+++
Progesterone		+	+	+	+	+	+
21-Hydroxyprogesterone	++++	++	++++	+++	++	++++	+++
20β-Hydroxyprogesterone	-	+++	++	++	++++	++	+++
17α,20α-Dihydroxyprogesterone	-	++	+	++	++	-	+
17α,20β-Dihydroxyprogesterone	+++	++	+++	++	++	+++	++++

(+)- indicates the presence of the steroids in trace levels, (++)-indicates the moderate levels, (+++)-indicates the increased levels, (++++) - indicates the highly increased levels of steroids in the incubated oocytes and (-)-indicates the absence or non-detectable range of steroids.

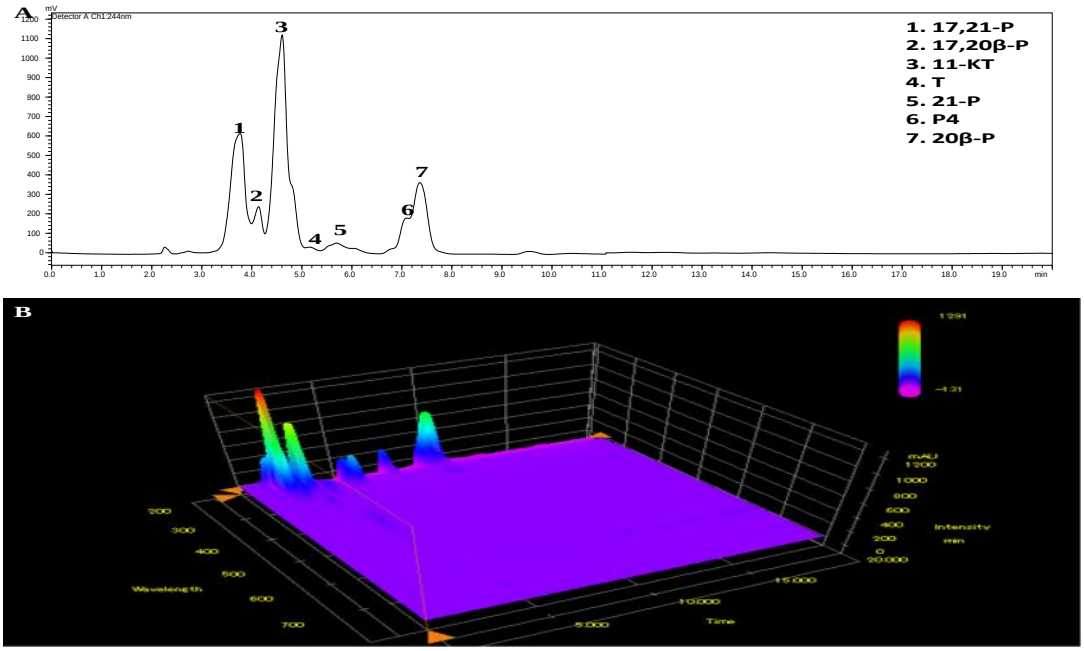

Fig. 1 shows the standards of various steroids peak detected by HPLC A and B shows the peaks chromatogram of standards steroids and 3D graph respectively.

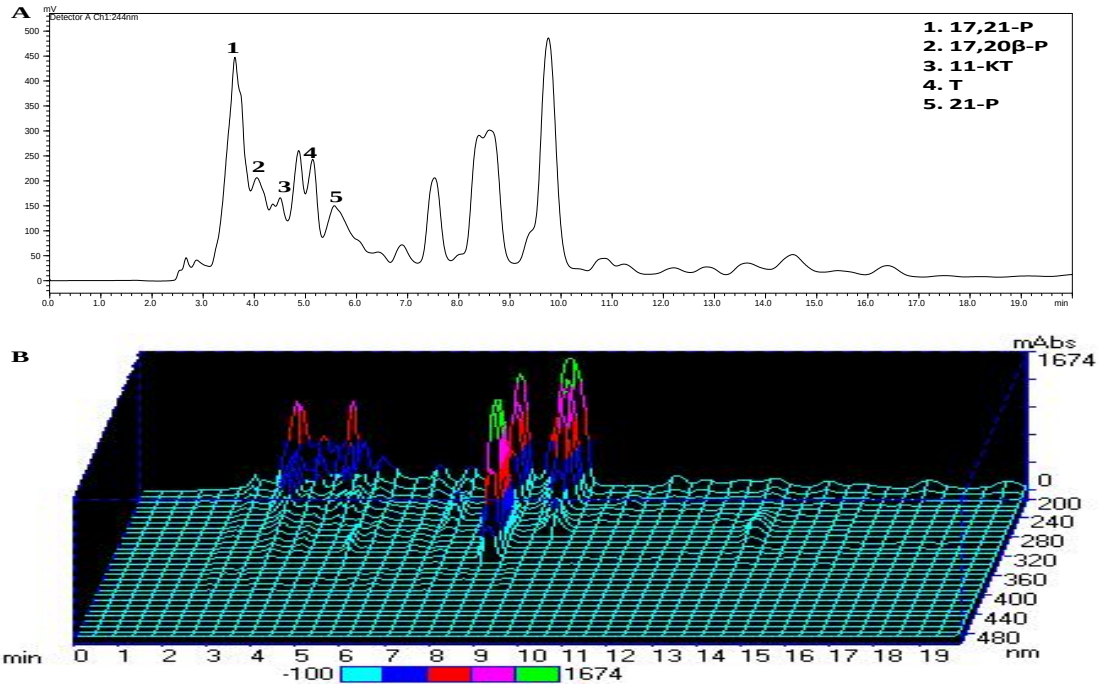

Fig. 2. The *in vitro* synthesis of steroids in the 30 minutes control tissue of ovary in the *C. mrigala.* A and B shows the chromatogram of steroids and 3D graph of steroids respectively.

Discussion

The present results revealed the *in vitro* synthesis of steroids in the incubated oocytes in the *C. mrigala* by the induction of hCG at different time durations. Several steroids 17,21-P; 17,20β-P; 17,20β,21-P; 11-KT; T; 21-P; 17,20α-P; 20β-P and P4 peaks were observed by HPLC and confirmed the steroids were involved in the oocyte steroidogenesis in the *C. mrigala*.Nelson and Kraak, (2010) report suggested that the insulin like growth factor IGF is involved in

oocyte maturation and that follicles become responsive to IGFs at an earlier stage compared to 17,20β-P. IGF-I also increased the responsiveness of the follicle to 17,20β-P, they are suggesting a role in promoting maturational competence. IGF-I alone and in combination with hCG stimulated the production of 17,20β-P by ovarian follicles incubated *in vitro*. More recently Pramanick *et al.,* 2014 suggest the 17,20β-P produced in follicle cells by the induction of gonadotropin is able to induce oocyte maturation in *Tenualosa ilisha*. 17,20β-P induced the oocyte maturation in this fish is mediated through activation of Phosphatidylinositol 3 kinase (P13) kinase pathway. According to Algriany *et al.,* (2004) factors present in follicles at later stages of follicular growth play an important role steroidogenesis in oocyte maturation mechanisms and thereby enhance developmental competence of oocytes. Chen *et al.,* 2013 reported and suggest no evidence for the dihydroxyprogesterone mediated increase of 11-KT production, In contrast present findings given ideal results of hCG induction of oocytes steroidogenesis in the *C. mrigala* was not drastically changes the 11-KT and T synthesis. This is the first report of identifying the steroidogenic profile patterns of several steroids involved in the mechanism of oocyte maturation by the action of hCG incubated in the oocytes of *C. mrigala*. Incubation studies were carried out different time intervals with a reduction of changes observed in the patterns of expression in the periods of 30 minutes, 1hour, 2 hours, 4hour, 8hours but slightly varied in the several ovarian steroids peaks were observed in oocytes of *C. mrigala* incubated hCG of 16 hours.

Results from our study reported the variations of steroids in the various developmental stages of oocyte of *C. mrigala.* Present study deals with hCG incubation were given at 30 mins, 1hour, 2hours, 4hours, 8hours and 16hours time interval of the ovarian steroids observed. This action of hCG to increase steroids levels in 17,20β,21-P binding to ovarian membrane of *C. mrigala* and the ability of oocytes to respond to 20β-S, the maturation inducing steroid in this species (Trant and Thomas, 1986, 1988) and undergo meiotic maturation (Thomas *et al.,* 2001). Results of Tsai *et al.,* 2010 indicates that diameter of stage II follicles increased after hCG treatment. hCG promotes growth in early stage follicles of zebrafish. This result supports their function in regulating the oocye maturational competence. hCG tiger to increases the oocyte maturation of steroids level in the fish species of *C. mrigala*. Gonadotropins upregulated membrane progestin receptor protein levels in oocytes. Meanwhile, the sensitivity of ooocytes to MIS in several fish species including seatrout, goldfish, atlantic croaker and zebrafish has been increasing concomitantly (Tan *et al.,* 2009; Tubbs *et al.,* 2010; Zhu *et al.,* 2003b). According to the results of Tubbs *et al.,* 2010 the first stage of follicular development in croaker is gonadotropin dependent but steroid independent and marked by the development of oocyte maturational competence. They also reported that the upregulation of mPRα protein in respond to hCG treatment is based on time course. Results of Hanna and Zhu, 2011 reported that numbers of oocytes undergoing final oocyte maturation were increased, when mPRα is overexpressed. These results were consistent with the results of Zhu *et al.,* 2003a, Hanna *et al.,* 2006).

Results of our studies were consistent with the report of Zhu and Hanna, 2008 which showed that the effect of membrane progestin receptors various levels. Interestingly, an evaluation of individual incubated time durational in vitro synthesis of steroid profile in the *C. mrigala* revealed modest elevations in MIS steroids approaching the delineation between the baseline and elevated values. Future, study required focused on the specific role and mechanisms of hormones for the ovarian steroidogenesis of *C. mrigala*.

References

Algriany, O., Bevers, M., Schoevers, E., Colenbrander, B., Dieleman, S., 2004. Follicle size-dependent effects of sow follicular fluid on in vitro cumulus expansion, nuclear maturation and blastocyst formation of sow cumulus oocytes complexes. *Theriogenology* **62**: 1483–1497.

Chen, S.X., Bogerd, J., Schoonen, N.E., Martijn, J., de Waal, P.P., Schulz, R.W., 2013. A progestin (17α,20β-dihydroxy-4-pregnen-3-one) stimulates early stages of spermatogenesis in zebrafish. *General and Comparative Endocrinology*. **185**: 1–9.

Fostier, A., Jalabert, B., Billard, R., Breton, B., Zohar, Y., 1983. The gonadal steroidogenesis. In: Hoar, W.s., Randall, D.J., Donaldson, E.M. (Eds.), *Fish physiology Academic Press*, New York, **9**A: 277-372.

Ge, W., 2005. Intrafollicular paracrine communication in the zebrafish ovary: the state of the art of an emerging model for the study of vertebrate folliculogenesis. *Mol. Cell. Endocrinol.* **237**: 1–10.

Hanna, R., and Zhu, Y., 2009. Expression of membrane progestin receptors in zebrafish (*Danio rerio*) oocytes, testis and pituitary, *Gen. Comp. Endocrinol*. **16**: 153–157.

Hanna, R.N., Zhu, Y., 2011. Controls of meiotic signaling by membrane or nuclear progestin receptor in zebrafish follicle-enclosed oocytes, *Mol. Cell. Endocrinol*. **337**: 80–90.

Hanna, R.N., Zhu, Y., 2008. Expression of membrane progestin receptors in zebrafish (*Danio rerio*) oocytes, testis and pituitary. *Gen. Comp. Endocrinol. Epub*.

Inbaraj, R.M., and S. Haider, 1988. In vitro effectiveness of estradiol-17b, androgens, corticosteroids, progesterone and other pregnen derivatives on germinal vesicle breakdown in oocytes of the exotic common carp *Cyprinus carpio* (L.), *Ind. J. Exp. Biol.*, **26**: 583–585.

Inbaraj, R. M., Haider S. and Baqri, S. S. R., 2001. Dynamics of 1*7a*,20β-dihydroxy-4-pregnen-3-one and 17α,20β,21-trihydroxy-4-pregnen-3-one in plasma and oocyte incubation media of catfish *(Clarias batrachu*s) in response to salmon gonadotropin, *Curr. Sci*, **80**(3): 455 – 58.

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

Kazeto, Y., Goto-Kazeto, R., Thomas, P., Trant, JM. 2005. Molecular characterization of three forms of putative membrane-bound progestin receptors and their tissue-distribution in channel catfish, *Ictalurus punctatus*. *J Mol Endocrinol*., **34**(3): 781-191.

Kime, D.E., 1993. "Classical" and "nonclassical" reproductive steroids in fish. *Reviews in fish biology, and Fisheries*, **3**: 160-180.

Kondo, T., Kotani, T., Yamashita, M., 2001. Dispersion of cyclin B mRNA aggregation is coupled with translational activation of mRNA during zebrafish oocyte maturation. *Dev. Biol.*, **229**: 421-431.

McGee, E.A., Hsueh, A.J., 2000. Initial and cyclic recruitment of ovarian follicles. *Endocr. Rev*. **21**: 200–214.

Monniaux, D., Huet, C., Besnard, N., Clement, F., Bosc, M., Pisselet, C., Monget, P., Mariana, J.C., 1997. Follicular growth and ovarian dynamics in mammals. *J. Reprod. Fertil. Suppl*. **51**: 3–23.

Nagahama, Y., Hirose, K., Young, G., Adachi, S., Suzuki, K., Tamaoki, B., 1983. Relative in vitro effectiveness of 17 alpha, 20 beta-dihydroxy-4-pregnen-3-one and other pregnene derivatives on germinal vesicle breakdown in oocytes of ayu (*Plecoglossus altivelis*); amago salmon, (*Oncorhynchus rhodurus*); rainbow trout, (*Salmo gairdneri*) and goldfish, (*Carassius auratus*). *Gen Comp Endocrinol*. **51**: 15-23.

Nagahama, Y., Yoshikuni, M., Yamashita, M., Sakai, N., Tanaka, M., 1993. Molecular Endocrinology of oocyte growth and maturation in fish, *Fish. Physiol. Biochem*. **11**: 3–14.

Nagahama, Y., and Yamashita, M., 2008. Regulation of oocyte maturation in fish. *Dev. Growth Differ*. **50** (1): 195–219.

Pang, Y., and Thomas, P., 2011. Progesterone signals through membrane progesterone receptors (mPRs) in MDA-MB-468 and mPR-transfected MDA-MB-231 breast cancer cells which lack full-length and N-terminally truncated isoforms of the nuclear progesterone receptor. *Steroids*, **76**: 921-928.

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

Patino, R., and Thomas, P., 1990b. Chracterization of membrane receptor activity for 17α,20β,21-trihydroxy-4-pregnen-3-one in ovaries of spotted seatrout (*Cynoscion nebulosus*). *Gen. Comp. Endocrinol.* **78**: 204 - 217.

Patino, R., Yoshizaki, G., Thomas, P., Kagawa, H., 2001. Gonadotropic control of ovarian follicle maturation: the two-stage concept and its mechanisms. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* **129**: 427–439.

Petrino, T.R., Greeley, Jr. M.S., Selman, K., Lin, Y.W.P. and Wallace, R.A., 1989a. Steroidogenesis in *Fundulus heteroclitus* II. Production of 17α, 20β, dihydroxy-4-pregnen-3-one, Testosterone and 17β, Estradiol by various components of ovarian follicles. *Gen. Comp. Endocrinol.* **76**: 230-240.

Pinter, J., and Thomas, P., 1997. The ovarian progestogen receptor in the spotted seatrout, *Cynoscion nebulosus*, demonstrates steroid specificity intermediate between progesterone and glucocorticoid receptors in other vertebrates. *J Steroid Biochem Mol Biol*., **60**(1–2): 113–9.

Pinter, J., and Thomas, P., 1999. Induction of ovulation of mature oocytes by the maturation-inducing steroid 17α, 20β,21-trihydroxy-4-pregnen-3-one in the spotted seatrout. *Gen. Comp. Endocrinol*. **115**: 200–209.

Planas, J.V., and Swanson, P., 1995. Maturation-associated changes in the response of the salmon testis to the steroidogenic actions of gonadotropins

(GTH I and GTH II) *in vitro*, *Biol Reprod.*, **52**(3): 697-704.

Planas, J.V., Athos, J., Goetz, F.W. and Swanson, P., 2000. Regulation of ovarian steroidogenesis in vitro by follicle-stimulating hormone and luteinizing hormone during sexual maturation in salmonid fish. *Biol. of Reprod*., **62**(5): 1262-1269.

Pramanick, K., Kundu, S., Paul, S., Mallick, B., Roy Moulik, S., Pal, P., Mukherjee, D., 2014. Changes in plasma steroid levels during oocyte development in Indian shad, Tenulosa ilisha (Hamilton, 1822): role of gonadotropins on *in vitro* steroid production and development of oocyte maturational competence. *Anim. Reprod. ScI*. **141**: 177–188.

Roche, J.F., 1996. Control and regulation of folliculogenesis - a symposium in perspective. *Rev. Reprod*. **1**: 19–27.

Scott, A.P. and Canario, A.V.M., 1987. Status of oocyte maturation-inducing steroids in Teleost. *In:* Idler, D. R., Crim, J. W., and Walsh, J. M (eds.), *Proceedings of the 3rd International Symposium on the Reproductive Physiology of Fish. St. John's, Nfld*. Memorial University of Newfoundland, St. John's, Nfld, pp224-234.

Scott, A.P., Mac Kenzie, D.S., Stacey, N.E., 1984. Endocrine changes during natural spawning in the white sucker, (*Catostomus commersoni).* II. Steroid hormones. *Gen Comp Endocrinol.*, **56**: 349-359.

Tan, Q., Zagrodny, A., Bernaudo, S., Peng, C., 2009. Regulation of membrane progestin receptors in the zebrafish ovary by gonadotropin, activin, TGF-beta and BMP-15. *Mol. Cell. Endocrinol*. **312**: 72–79.

Tang, Y.T., Hu, T., Arterburn, M., Boyle, B., Bright, J.M., Emtage, P.C., 2005. PAQR proteins: a novel membrane receptor family defined by an ancient 7-transmembrane pass motif. *J. Mol. Evol.* **61**: 372-380.

Thomas, P., Brown, N.J. and Trant, J.M., 1987. Plasma levels of gonadal steroids during the reproductive cycle of female spotted seatrout, *Cynoscion nebulosus*. In Idler, D.R., Crim, L.W. and Walsh, J.M. (eds.). *In the proceedings of Third International Symposium of Reproductive Physiology of Fish. Memorial University Press,* St. John's, p219.

Thomas, P., and Trant, J.M., 1989. Evidence that 17α,20β, 21 trihydroxy-4-pregnen-3-one is a maturation Inducing steroid in spotted sea trout. *Fish. Physiol. Biochem.,* **7:** 185-191.

Thomas, P., Breckenridge-Miller, D., and Detweiler, C., 1997. Binding characteristics and regulation of the 17α,20β,21-trihydroxy-4-pregnen-3-one(20β-S) receptor on the testicular and sperm plasma membranes of spotted seatrout (*Cynoscion nebulosus*). *Fish Physiol. Biochem.* **17:** 109–117.

Thomas, P., Pinter, J., and Das, S., 2001. Upregulation of the maturation-inducing steroid membrane receptor in spotted seatrout ovaries by gonadotropin during oocyte maturation and its physiological significance. *Biol. Reprod*., **64**: 21-29.

Thomas, P., Pang, Y., Zhu, Y., Detweiler, C., Doughty, K., 2004. Multiple rapid progestin actions and progestin membrane receptor subtypes in fish. Steroids **69**: 567–573.

Thomas, P., 2008. Review: characteristics of membrane progestin receptor alpha (mPRα) and progesterone membrane receptor component 1 (PGMRC1) and their roles in mediating rapid progestin actions. *Front. Neuroendocrinol*. **29**: 292–312.

Thomas, P., Harris, C., Pang, Y., 2008. The roles of different types of progestin receptors in steroid induction of oocyte maturation in zebrafish. *Biol Reprod.,* (Sp Iss S1):220 [Abs. 705].

Thomas, P., Tubbs, C., Garry, V.F., 2009. Progestin functions in vertebrate gametes mediated by membrane progestin receptors (mPRs): identification of mPRα on human sperm and its association with sperm motility. *Steroids* **74**: 614–621.

Thomas, P., 2012. Rapid steroid hormone actions initiated at the cell surface and the receptors that mediate them with an emphasis on recent progress in fish models. *General and Comparative Endocrinology*. **175**: 367–383.

Tokumoto, M., Nagahama, Y., Thomas, P., Tokumoto, T., 2006. Cloning and identification of a membrane progestin receptor in goldfish ovaries and evidence it is an intermediary in oocyte meiotic maturation, *Gen. Comp. Endocrinol.* **145**: 101–108.

Trant, J. M. and Thomas, P., 1988. Struc- activity relationships of steroids in inducing GVBD of Atlantic croaker oocytes *in vitro. Gen. Comp. Endocrinol*. **71**: 307-317.

Trant, J. M. and Thomas, P., 1989. Isolation of a novel maturation inducing steroid produced *in vitro* by ovaries of Atlantic croaker, *Gen. Comp. Endocrinol*. **75**: 397 - 404.

Tsai, S., Rawson, D.M., Zhang, T., 2010. Development of in vitro culture method for early stage zebrafish (*Danio rerio*) ovarian follicles for use in cryopreservation studies. *Theriogenology* **74**: 290–303.

Tubbs, C., Pace, M., Thomas, P., 2010. Expression and gonadotropin regulation of membrane progestin receptor alpha in Atlantic croaker

(*Micropogonias undulatus*) gonads: role in gamete maturation, *Gen. Comp. Endocrinol*. **165**: 144–154.

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

Wang, Y., and Ge, W., 2004. Cloning of epidermal growth factor (EGF) and receptor from the zebrafish ovary: evidence for EGF as a potential paracrine factor from the oocyte to regulate activin/follistatin system in the follicle cells. *Biol. Reprod*. **71**: 749-760.

Wang, H., Jiang, J.Y., Zhu, C., Peng, C., Tsang, B.K., 2005. Role and regulation of nodal/activin receptor-like kinase 7 signaling pathway in the control of ovarian follicular atresia. *Mol. Endocrinol*., **20**: 2469-2482.

Zhu, Y., Rice, C.D., Pang, Y., Pace, M., Thomas, P., 2003a. Cloning, expression and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. *Proc Natl Acad Sci*., USA, **100**: 2231-2236.

Zhu, Y., Bond, J., Thomas, P., 2003b. Identification classification and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. *Proc Natl Acad Sci.*, USA, **100**: 2237-2242.

Zhu, Y., and Hanna, R., 2008. Characterization of membrane progestin receptors in zebrafish oocytes. *Cybium. **32**(2) suppl.*: 264-265.

Access this Article in Online	
Quick Response Code	Website: www.ijarbs.com
	Subject: Endocrinology

How to cite this article:
Uma T., Saravanan N., Inbaraj R.M., & Jothi Narendiran N. (2016). *In vitro* effectiveness of hCG on the induction of steroidogenesis in the oocyte of *Cirrhinus mrigala* . Int. J. Adv. Res. Biol. Sci. 3(4): 168-175.