

Prostaglandin Affects *In Vitro* Ovulation and 17 α , 20 β -Dihydroxy-4-pregnen-3-one Production in Longchin Goby, *Chasmichthys dolichognathus* Oocytes

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ABSTRACT : This study focused on the association of prostaglandins and a progestin, 17 α , 20 β -dihydroxy-4-pregnen-3-one (17 α 20 β P) during the ovulation process in longchin goby, *Chasmichthys dolichognathus*. We performed several *in vitro* experiments using 850–920 μ m diameter oocytes which were at the migratory nucleus stage. With the 890–920 μ m diameter oocytes, no significant difference in ovulation was observed in any of the prostaglandins (PGE1, PGE2, and PGF2 α) treated groups although PGE2 and PGF2 α at concentrations of 50 ng/mL increased ovulation slightly compared with controls; however, 17 α 20 β P production was stimulated with PGE1 alone at low concentrations (5 ng/mL). In 850 μ m diameter oocytes, PGF2 α at concentrations of 50 and 500 ng/mL resulted in a significant increase in ovulation. 17 α 20 β P (50 ng/mL) alone had no observable effect on ovulation, but in the combined of PGF2 α 50 or 500 ng/mL it caused the greatest effect on ovulation. The sensitivity of oocytes to the induction of ovulation varies between 850 and 890–920 μ m, it appeared to vary depending on the migration status of nucleus. These results suggest that PGF2 α (or combined of 17 α 20 β P) was more potent in inducing ovulation of the longchin goby.

Key words : *Chasmichthys dolichognathus*, Ovulation, Prostaglandins, 17 α , 20 β -dihydroxy-4-pregnen-3-one

INTRODUCTION

In most teleosts, final oocyte maturation is a prerequisite for ovulation. When final maturation is complete, ovulation begins and oocytes are released from the ovarian follicles. These processes are regulated by the teleost progestin, 17 α , 20 β -dihydroxy-4-pregnen-3-one (17 α 20 β P), is one of the main maturation-inducing steroids (MIS; Goetz, 1983; Nagahama & Yamashita, 2008). In addition, prostaglandins (PGs), are synthesized from arachidonic acid (AA) by cyclooxygenase, also have important roles in ovarian maturation, ovulation, steroidogenesis, and sexual behavior

(Goetz et al., 1991; Lister & Van Der Kraak, 2008; Chourasia & Joy, 2012). PGs induced germinal vesicle breakdown (GVBD) and ovulation in yellow perch, *Perca flavescens* (Goetz & Theofan, 1979) and in *Salvelinus fontinalis* (Goetz et al., 1982); they are strongly associated with the process of ovulation in fish (Lister & Van Der Kraak, 2008; Lister & Van Der Kraak, 2009).

There are several groups of PGs. In mammalian ovaries, prostaglandin E2 (PGE2) is involved in the process of ovulation of periovulatory follicles (Espey & Richards, 2006; Duffy, 2015), while prostaglandin F2 α (PGF2 α) plays an important role in the luteolytic events of post-

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ovulatory follicles (Sugimoto et al., 2015). Studies on many other teleost have explored the role of PGs in fish ovulation. PGF2 α serves as a regulator of ovulation in many fish species studied to date. PGE2, which is an essential mediator for ovulation in mammals, plays a role in the ovulatory process in several teleost species (Fujimori et al., 2011; Fujimori et al., 2012). In yellow perch, oocyte ovulation suppressed *in vitro* by indomethacin (cyclooxygenase inhibitor) could be restarted by prostaglandin E1 (PGE1), PGE2, and PGF2 α ; and PGE2 was the most active among them (Goetz & Teofan, 1979). Moreover, 17 α 20 β P significantly increased PGF2 α levels in culture medium of the ovarian follicles with the time of ovulation (Goetz et al., 1989). Using Eurasian perch, Henrotte et al. (2011) reported that AA induced the production of 17 α 20 β P, an effective steroid in the induction of oocyte maturation and ovulation in teleost, as well as the production of PGE2 and PGF2 α . In addition, 17 α 20 β P production was stimulated with exogenous PGE2.

The regulation of sex steroid and PG production in teleost ovaries during the breeding season is not fully understood. Using *in vitro* incubation methods, Kagawa et al. (2003) demonstrated 17 α 20 β P induced ovulation through the synthesis of endogenous prostaglandin in the follicular layers of the Japanese eel, *Anguilla japonica*. Other *in vitro* studies in *P. flavescens*, 17 α 20 β P was reported as a release inducer of prostaglandin E and F within mature follicles (Berndtson et al., 1989). Little is known about their mutual relationship.

Our previous studies reported that PGE2 possibly plays important roles in final oocyte maturation (GVBD) *in vitro* in the longchin goby, *C. dolichognathus* (Kim & Baek, 2017). This study focused on the effects of prostaglandins on 17 α 20 β P production *in vitro* during the ovulation process in the longchin goby.

MATERIALS AND METHODS

The experimental fish (4.7–5.6 cm in body length) were

collected at the coastal waters of Chongsapo, Busan, Korea, during the breeding period (March–April). The ovaries were taken from several mature females to obtain oocyte follicles (approximately 850–920 μ m in diameter) at the migratory nucleus stage (Fig. 1A). After separating the ovaries into small pieces in ice-cold balanced salt solution (132.96 mM NaCl, 3.09 mM KCl, 0.28 mM MgSO $_4$ ·7H $_2$ O, 0.98 mM MgCl $_2$ ·6H $_2$ O, 3.40 mM CaCl $_2$ ·6H $_2$ O, 3.65 mM HEPES), approximately 20–30 follicle-enclosed oocytes were incubated in each well of a 24-well culture plates containing 1 mL of Leibovitz L15 medium (Gibco) and/or 5–500 ng/mL PGs (PGE1, PGE2, and PGF2 α ; Sigma) and 17 α 20 β P (Sigma). The plates were incubated for 16–18 h at 18°C with constant gentle shaking. The pH and osmolality of the medium were adjusted to 7.7 and 300 milliosmoles, respectively.

After incubation, oocytes were fixed with clearing solution (ethanol:formalin:glacial acetic acid=6:3:1). The number of oocytes that had completed final oocyte maturation (GVBD), or had ovulated (Fig. 1B), was counted in each well under low-power magnification using a dissecting microscope. Steroids from media were extracted twice using five volumes of ethylacetate: cyclohexane (1:1), dried under nitrogen gas and resuspended in phosphate buffer (pH=7.5). Then, 17 α 20 β P levels were measured by

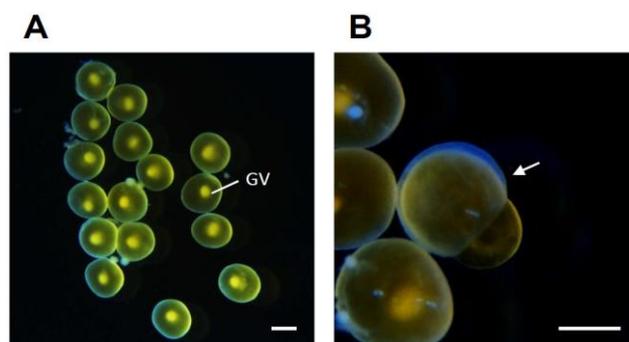


Fig. 1. Morphology of longchin goby, *Chasmichthys dolichognathus* oocytes. (A) oocyte before *in vitro* incubation, (B) oocyte that had ovulated (arrow) were observed. Scale bars indicate 500 μ m. GV, germinal vesicle.

radioimmunoassay (RIA) following Kobayashi et al. (1988). Radiolabeled $17\alpha20\beta\text{P}$ was prepared from $[3\text{H}]-17\alpha\text{-hydroxyprogesterone}$ (Amersham Life Sciences) by enzymatic conversion as described by Scott's method (Scott et al., 1982).

All data are expressed as means \pm SEM, and SPSS software (version 21) for Windows was used for the Kruskal–Wallis test followed by Tuckey's test. A value of $p<0.05$ was considered statistically significant.

RESULTS

1. Effects of prostaglandins on ovulation and $17\alpha20\beta\text{P}$ production *in vitro*

We performed several separate similar experiments using 850–920 μm diameter oocytes which were at the migratory nucleus stage. With the 890–920 μm oocytes (Fig. 2), no significant difference in ovulation was observed in any of the prostaglandins (PGE1, PGE2, and PGF2 α) treated groups although PGE2 and PGF2 α at concentrations of 50 ng/mL increased ovulation slightly (38.64 ± 4.48 and $43.25\pm5.12\%$, respectively) compared with controls ($27.47\pm1.27\%$) (Fig. 2A). Then, we analyzed $17\alpha20\beta\text{P}$ production by 890–920 μm oocytes (Fig. 3). Treatment with 5 ng/mL PGE1 alone resulted in a significant increase in the production of $17\alpha20\beta\text{P}$ compared with controls (32.22 ± 6.25 vs. 10.33 ± 0.42 pg/mL) (Fig. 3A). Whereas PGE2 and PGF2 α in all concentration tested (5, 50, and 500 ng/mL) using the 890 μm oocytes resulted in increases in the production of $17\alpha20\beta\text{P}$ compared with the controls, but there was no significant difference (Fig. 3B).

We examined the effects of $17\alpha20\beta\text{P}$ and prostaglandins (PGE1, PGE2, and PGF2 α) on ovulation using 850–900 μm oocytes (Fig. 4). No significant difference in ovulation was observed in any of the treatment groups, but it tended to increase in all tested groups except PGE1 5 ng/mL compared with controls in the 900 μm oocytes (Fig. 4A). We tested the effects of $17\alpha20\beta\text{P}$ +PGF2 α on ovulation at

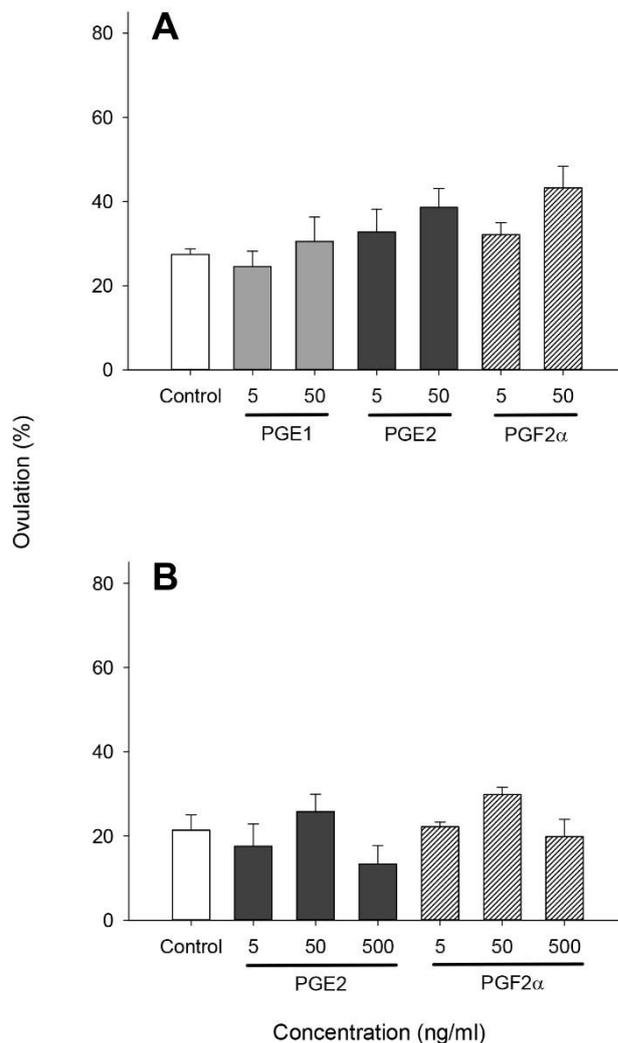


Fig. 2. Effects of different PGE1, PGE2, and PGF2 α concentrations on *in vitro* ovulation in longchin goby, *Chasmichthys dolichognathus* oocytes (A, 920 μm oocyte diameter; B, 890 μm oocyte diameter). Values are means \pm SEM of ovulation in triplicate with 20 oocytes/well. Data were analyzed using Kruskal-Wallis test.

concentrations of 50 and/or 500 ng/mL using 850 μm . Treatment with 50 and 500 ng/mL PGF2 α resulted in a significant increase in ovulation (38.90 ± 4.54 and $40.94\pm4.09\%$, respectively) compared with controls ($21.00\pm2.73\%$) (Fig. 4B). In addition, the combination groups of $17\alpha20\beta\text{P}$ (50 ng/mL)+PGF2 α (50 and 500 ng/mL) significantly increased ovulation (58.19 ± 3.44 and $62.93\pm2.86\%$, respectively) compared with controls.

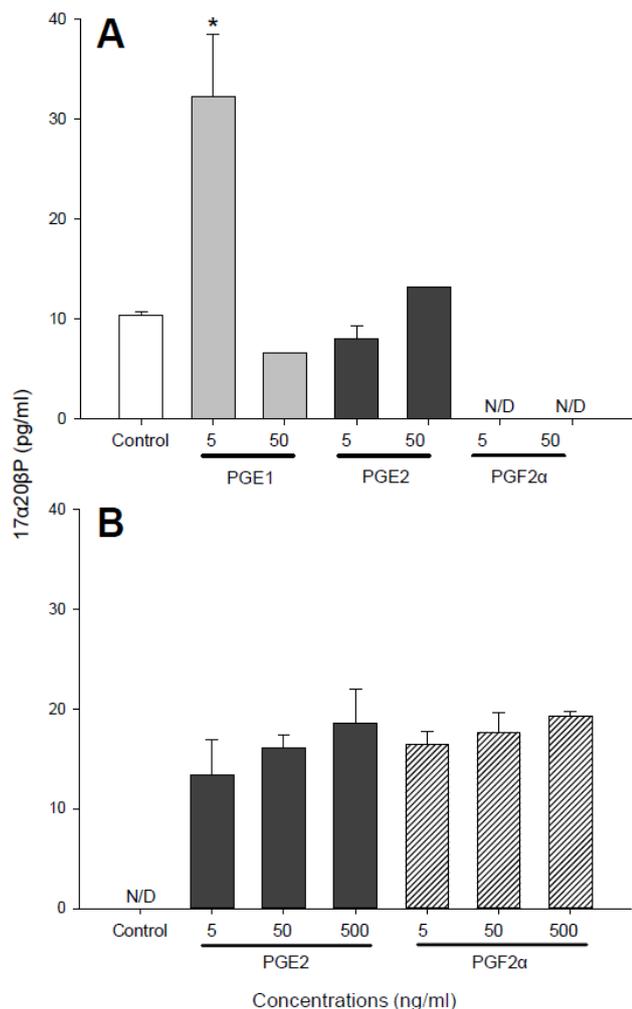


Fig. 3. Effects of different PGE1, PGE2, and PGF2 α concentrations on *in vitro* 17 α 20 β P production in longchin goby, *Chasmichthys dolichognathus* oocytes (A, 920 μ m oocyte diameter; B, 890 μ m oocyte diameter). Values are means \pm SEM of ovulation in triplicate with 20 oocytes/well. Data were analyzed using Kruskal-Wallis test. Asterisks indicate significant differences from controls ($p < 0.05$). N/D, not detected.

DISCUSSION

Oocyte ovulation as well as maturation in teleost is induced by gonadotropins and MIS (Pinter & Thomas, 1999); especially, 17 α 20 β P is one of the main MIS (Goetz, 1983; Nagahama & Yamashita, 2008). PGs play also an important role in maturation and ovulation of fish (Dongre

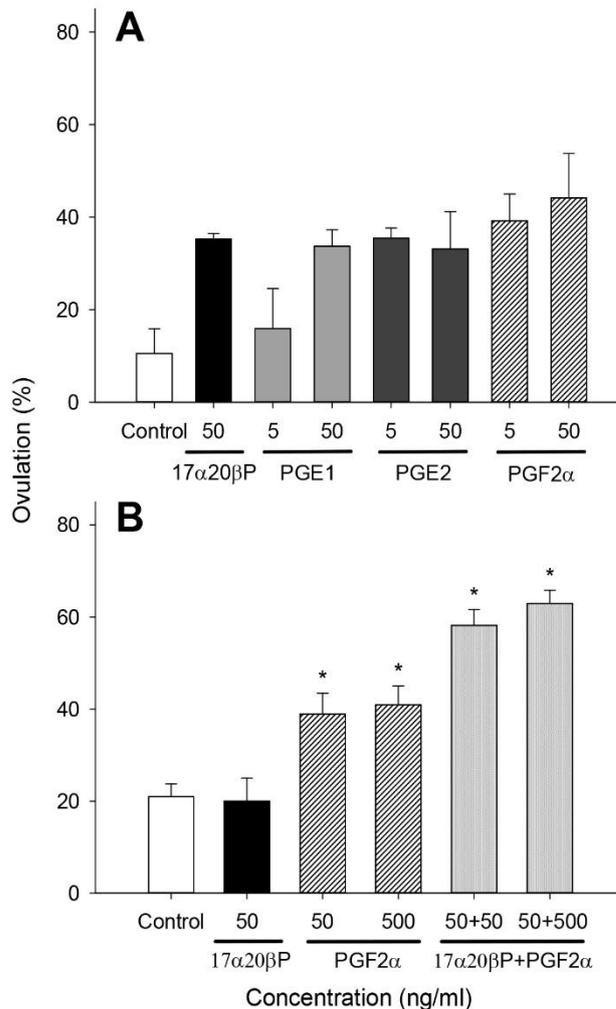


Fig. 4. Effects of different 17 α 20 β P, PGE1, PGE2, and PGF2 α concentrations on *in vitro* ovulation in longchin goby, *Chasmichthys dolichognathus* oocytes (A, 900 μ m oocyte diameter; B, 850 μ m oocyte diameter). Values are means \pm SEM of ovulation in triplicate with 20 oocytes/well. Data were analyzed using Kruskal-Wallis test followed by Mann-Whitney U-test. Asterisks indicate significant differences from controls ($p < 0.05$).

et al., 2012). In different fish species, the ovulation of oocytes matured in the presence of gonadotropins or MIS can be induced *in vitro* by different PGs. Only PGF2 α can induce oocyte ovulation *in vitro* in all fish species studied to date. Such induction was demonstrated in rainbow trout, goldfish, pike (Kagawa & Nagahama, 1981), Japanese eel (Kagawa et al., 2003), and Atlantic croaker (Patino et al.,

2003).

Our previous studies demonstrated that PGE₂ (50 ng/mL) significantly induced GVBD of matured oocytes (at the migratory nucleus stage) *in vitro* in the longchin goby, *C. dolichognathus* (Kim & Baek, 2017). In this study, we focused on the effects of PGs on ovulation and 17 α 20 β P production by oocytes *in vitro*.

This studies showed that treatment with 50 ng/mL PGE₂ and PGF₂ α increased ovulation slightly using 890–920 μ m diameter oocytes although there was no significant difference; at the same time, 17 α 20 β P production was stimulated with PGE₁ alone at low concentrations (5 ng/mL), suggesting that these substances are related to the process of final maturation in the longchin goby, which 17 α 20 β P was reported as major MIS (Baek, 2008; Baek et al., 2003).

In 850 μ m oocytes, PGF₂ α at concentrations of 50 and 500 ng/mL resulted in a significant increase in ovulation. 17 α 20 β P (50 ng/mL) alone had no observable effect on ovulation, but in the combined of PGF₂ α 50 or 500 ng/mL it caused the greatest effect on ovulation. The sensitivity of oocytes to the induction of ovulation varies between 850 and 890–920 μ m, morphological maturation of these oocytes even at the migratory nucleus stage (Fig. 1); it appeared to vary depending on the migration status of nucleus (Baek et al., 2007).

In case of perch, it is possible that 17 α 20 β P is regulating an enzyme in the follicles since PGF levels increased in the presence of 17 α 20 β P while PGE levels appear to decrease (Goetz, 1997). In Eurasian perch, 17 α 20 β P production was stimulated with exogenous PGE₂ (Henrotte et al., 2011).

Skoblina (2009) reported that if the nucleus (germinal vesicle, GV) passed 2/3 of the distance to the animal pole, mature oocytes ovulated. At low 17 α 20 β P concentrations, oocyte matured but not ovulated. However, their long-term exposure to low 17 α 20 β P concentrations eventually induced ovulation although much later. Kagawa et al. (2003)

demonstrated that 17 α 20 β P itself can induce oocyte maturation and ovulation *in vivo* and *in vitro*. Among the prostaglandins, PGE₂ and PGF₂ α induced *in vitro* ovulation in oocytes of the Japanese eel, but PGE₁ was not effective. They concluded that PGF₂ α was the most effective in inducing ovulation of the Japanese eel. Moreover, the PGE group may not play a critical role in ovulation of the Japanese eel. These *in vitro* data are similar to our results. Similarly, PGEs (PGE₁ and PGE₂) have been shown to inhibit brook trout, *S. fontinalis* ovulation *in vitro* and follicle contraction (Goetz et al., 1982; Hsu & Goetz, 1992). Results from Joy & Singh (2013) showed that both PGF₂ α and PGE₂ stimulated ovulation and that PGF₂ α was more potent in the catfish, *Heteropneustes fossilis*. Goetz & Cetta (1983) reported significant increases in plasma and ovarian prostaglandin F concentrations in naturally ovulating, compared with nonovulating, *S. fontinalis*.

When final maturation is complete, ovulation begins and these processes are regulated by hormones. 17 α 20 β P is one of the main MIS (Nagahama & Yamashita, 2008); its precursors and metabolites as well as many other substances including PGs, also have important roles in final maturation and ovulation. For successful ovulation, a coordinated action of several proteolytic enzymes is also required (Ogiwara et al., 2015; Ogiwara & Takahashi, 2017). At present, from extensive studies, Takahashi et al. (2018) reported that it is worthwhile to examine how the PGE₂ and PGF₂ α receptor systems contribute to teleost ovulation.

Very little is known about the association of PGs and a progestin, 17 α 20 β P which has been identified as a main MIS in most teleosts including longchin goby. The elucidation of the relationship between 17 α 20 β P and PGs synthesis in ovulating follicles needs further study.

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REFERENCES

- Baek HJ (2008) Production of C₂₁-steroids in longchin goby, *Chasmichthys dolichognathus* during oocyte maturation. *Dev Reprod* 12:107-112.
- Baek HJ, Hwang IJ, Kim KS, Lee YD, Kim HB, Yoo MS (2007) Effects of BPA and DES on longchin goby (*Chasmichthys dolichognathus*) *in vitro* during the oocyte maturation. *Mar Environ Res* 64(1):79-86.
- Baek HJ, Park MH, Lee YD, Kim HB (2003) Effect of *in vitro* xenoestrogens on steroidogenesis in mature female fish, *Chasmichthys dolichognathus*. *Fish Physiol Biochem* 28:413-414.
- Berndtson AK, Goetz FW, Duman P (1989) *In vitro* ovulation, prostaglandin synthesis, and proteolysis in isolated ovarian components of yellow perch (*Perca flavescens*): Effects of 17 alpha,20 beta-dihydroxy-4-pregnen-3-one and phorbol ester. *Gen Comp Endocrinol* 75:454-465.
- Chourasia TK, Joy KP (2012) Role of catechol estrogens on ovarian prostaglandin secretion *in vitro* in the catfish *Heteropneustes fossilis* and possible mechanism of regulation. *Gen Comp Endocrinol* 177:128-142.
- Dongre V, Dabhade RC, Khurad AM (2012) Effect of prostaglandin on reproduction in relation to pituitary gonadal axis in the fish, *Cyprinus carpio* (L.). *J Appl Nat Sci* 4:192-195.
- Duffy DM (2015) Novel contraceptive targets to inhibit ovulation: The prostaglandin E2 pathway. *Hum Reprod Update* 21:652-670.
- Espey LL, Richard JS (2006) Ovulation. In: Jimmy D (ed), *Physiology of Reproduction*. 3rd ed. Academic Press, Amsterdam, pp 425-474.
- Fujimori C, Ogiwara K, Hagiwara A, Rajapakse S, Kimura A, Takahashi T (2011) Expression of cyclooxygenase-2 and prostaglandin receptor EP4b mRNA in the ovary of the medaka fish, *Oryzias latipes*: Possible involvement in ovulation. *Mol Cell Endocrinol* 332:67-77.
- Fujimori C, Ogiwara K, Hagiwara A, Takahashi T (2012) New evidence for the involvement of prostaglandin receptor EP4b in ovulation of the medaka, *Oryzias latipes*. *Mol Cell Endocrinol* 362:76-84.
- Goetz FW (1983) 3 Hormonal control of oocyte final maturation and ovulation in fishes. *Fish Physiol* 9:117-170.
- Goetz FW (1991) Compartmentalization of prostaglandin synthesis within the fish ovary. *Am J Physiol* 260:862-865.
- Goetz FW (1997) Follicle and extrafollicular tissue interaction in 17 α ,20 β -dihydroxy-4-pregnen-3-one-stimulated ovulation and prostaglandin synthesis in the yellow perch (*Perca flavescens*) ovary. *Gen Comp Endocrinol* 105:121-126.
- Goetz FW, Cetta F (1983) Ovarian and plasma PGE and PGF levels in naturally ovulating brook trout (*Salvelinus fontinalis*) and the effects of indomethacin on prostaglandin levels. *Prostaglandins* 26:387-395.
- Goetz FW, Duman P, Ranjan M, Herman CA (1989) Prostaglandin F and E synthesis by specific tissue components of the brook trout (*Salvelinus fontinalis*) ovary. *J Exp Zool* 250:196-205.
- Goetz FW, Smith DC, Krickl SP (1982) The effects of prostaglandins, phosphodiesterase inhibitors, and cyclic AMP on ovulation of brook trout (*Salvelinus fontinalis*) oocytes. *Gen Comp Endocrinol* 48:154-160.
- Goetz FW, Theofan G (1979) *In vitro* stimulation of germinal vesicle breakdown and ovulation of yellow perch (*Perca flavescens*) oocytes. effects of 17 α -hydroxy-20 β -dihydroprogesterone and prostaglandins. *Gen Comp Endocrinol* 37:273-285.
- Henrotte E, Milla S, Mandiki SNM, Kestemont P (2011) Arachidonic acid induces production of 17, 20 β -dihydroxy-4-pregnen-3-one (DHP) via a putative PGE2 receptor in fish follicles from the Eurasian perch. *Lipids* 46:179-187.
- Hsu SY, Goetz FW (1992) The effects of E and F

- prostaglandins on ovarian cAMP production and follicular contraction in the brook trout (*Salvelinus fontinalis*). *Gen Comp Endocrinol* 88:434-443.
- Joy KP, Singh V (2013) Functional interactions between vasotocin and prostaglandins during final oocyte maturation and ovulation in the catfish *Heteropneustes fossilis*. *Gen Comp Endocrinol* 186:126-135.
- Kagawa H, Nagahama Y (1981) *In vitro* effects of prostaglandins on ovulation in goldfish *Carassius auratus*. *Bull Jpn Soc Sci Fish* 47:1119-1121.
- Kagawa H, Tanaka H, Unuma T, Ohta H, Gen K, Okujawa K (2003) Role of prostaglandin in the control of ovulation in the Japanese eel *Anguilla japonica*. *Fish Sci* 69:234-241.
- Kim HE, Baek HJ (2017) Effect of prostaglandins on *in vitro* oocyte final maturation (GVBD) and ovulation in the longchin goby *Chasmichthys dolichognathus*. *Korean J Fish Aquat Sci* 50:41-47.
- Kobayashi M, Aida K, Hanyu I (1988) Hormone changes during the ovulatory cycle in goldfish. *Gen Comp Endocrinol* 69:301-307.
- Lister AL, Van Der Kraak GJ (2008) An investigation into the role of prostaglandins in zebrafish oocyte maturation and ovulation. *Gen Comp Endocrinol* 159:46-57.
- Lister AL, Van Der Kraak GJ (2009) Regulation of prostaglandin synthesis in ovaries of sexually-mature zebrafish (*Danio rerio*). *Mol Reprod Dev* 76:1064-1075.
- Nagahama Y, Yamashita M (2008) Regulation of oocyte maturation in fish. *Dev Growth Differ* 50:S195-S219.
- Ogiwara K, Hagiwara A, Rajapakse S, Takahashi T (2015) The role of urokinase plasminogen activator and plasminogen activator inhibitor-1 in follicle rupture during ovulation in the teleost medaka. *Biol Reprod* 92:10, 1-7.
- Ogiwara K, Takahashi T (2017) Involvement of the nuclear progesterin receptor in LH-induced expression of membrane type 2-matrix metalloproteinase required for follicle rupture during ovulation in the medaka, *Oryzias latipes*. *Mol Cell Endocrinol* 450:54-63.
- Patino R, Yoshizaki G, Bolamba D, Thomas P (2003) Role of arachidonic acid and protein kinase C during maturation-inducing hormone-dependent meiotic resumption and ovulation in ovarian follicles of atlantic croaker. *Biol Rep* 68:516-523.
- Pinter J, Thomas P (1999) Induction of ovulation of mature oocytes by the maturation-inducing steroid 17,20 β ,21-trihydroxy-4-pregnen-3-one in the spotted seatrout. *Gen Comp Endocrinol* 115:200-209.
- Scott AP, Sheldrick EL, Flint APF (1982) Measurement of 17 α ,20 β -dihydroxy-4-pregnen-3-one in plasma of trout (*Salmo gairdneri* Richardson): Seasonal changes and response to salmon pituitary extract. *Gen Comp Endocrinol* 46:444-451.
- Skoblina MN (2009) *In vitro* stimulation of oocyte ovulation in teleosts by gonadotropic and steroid hormones. *Russ J Dev Biol* 40:191-197.
- Sugimoto Y, Inazumi T, Tsuchiya S (2015) Roles of prostaglandin receptors in female reproduction. *J Biochem* 157:73-80.
- Takahashi T, Hagiwara A, Ogiwara K (2018) Prostaglandins in teleost ovulation: A review of the roles with a view to comparison with prostaglandins in mammalian ovulation. *Mol Cell Endocrinol* 461:236-247.