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SPEECH OF CHAIRMAN OF INTERNATIONAL CONFERENCE ON GREEN WORLD IN BUSSINESS AND TECHNOLOGY 2012

With all my respect, Rector of Ahmad Dahlan University, keynotes speakers, authors, participants, and other guests of International Conference on Green World in Business and Technology 2012.

The issue of global warming, increased CO_2 emissions in the air, high air temperature, climate change, deforestation, flooding, energy crisis, food crisis is causing human life to be uncomfortable and crime increases. These problems are global problems require solutions that are found and solutions through a conference.

International Conference on Green World in Business and Technology 2012 designed to invite and bring together practitioners, scientists and environmentalists from various disciplines who are expected to contribute to the government of Indonesia and the world in preventing, overcoming all the consequences of environmental damage. The theme *"Technopreneurship based on Green Business and Technology"* has been chosen to support celebration of 51th Ahmad Dahlan University anniversary.

This conference is the result of dedication and commitment of many people. We are grateful to the authors who have submitted papers, to the reviewers, to the conference committee member who have been untiring in their efforts to make this conference a success. We also would like to thank our sponsors and cooperating societies who have been generous in their contributions to the conference.

Finally, I would like to extend my welcome to participants of the International Conference on Green World in Business and Technology 2012. We hope this will be an exciting meeting for everyone. We apologize if there are some unpleasant things about organizing and holding this conference.

Thank you.

Yogyakarta, March 2012
Chairman of International Conference on Green World
in Business and Technology 2012

Dr. Abdul Fadlil, M.T.

Induction of Oocyte Maturation in The Giant Gouramy (*Osphronemus gouramy* Lac.) using Progesterone and GnRH analog

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Abstract. Giant gouramy represents one of consumption fish having high economic value. Juvenile demand of Giant gouramy increase from year to year being in line with increase of its consumption. Successful Giant gouramy culture needs the knowledge and understanding concerning its reproduction biology; meanwhile information on reproduction biology remains limited. Previous study to induce the oocyte maturation of Giant gouramy using combination of 2.6 ng kg^{-1} progesterone and 0.5 mL kg^{-1} analogous GnRH showed that oocyte diameter increased. These results indicate that progesterone combined with analogous GnRH play important role in Giant gouramy oocyte maturation. This research aims to evaluate the effect of progesterone and analogous GnRH combination on oocyte maturation in Giant gouramy and to determine the combination dose between progesterone and analogous GnRH in inducing the oocyte maturation of Giant gouramy. The benefit of this research is to inform about appropriate dose of progesterone and analogous GnRH to induce oocyte maturation, hence, the information about hormonal regulation on Giant gouramy reproduction could be enriched. The information can also be developed furthermore to optimize Giant gouramy reproduction supporting then its juvenile production. This research resulted in increase of the oocyte maturation, i.e treatment A (analogous GnRH 0.7mL/body weight BW, progesterone 130 ng/BW) was statistically the best result compared to other treatments. Largest improvement of oocyte diameter was obtained with treatment D induction (analogous GnRH 0.7mL/BW, progesterone 54 ng/BW) and A (analogous GnRH 0.7mL/BW, progesterone 130 ng/BW) although did not significantly differ. Increase in oocyte diameter was not comparable with that of in progesterone dose. From parameters of the increase in oocyte maturation level and diameter, it could be concluded that treatment A (analogous GnRH 0.7mL/BW, progesterone 130 ng/BW) seem to be the best result.
Keywords : *giant gouramy, induction, analogous gnrh, progesterone.*

1 INTRODUCTION

Giant gouramy (Osphronemus gouramy Lac.) Is one of the fish that has high economical value. Demanding in Giant gouramy juvenile is raising every year equal with demanding of the consumption. Success in Giant gouramy culture needs knowledge about its reproduction, but still restricted information about that. The success of Gouramy culture requires knowledge and understanding of the biology of the reproduction, while the information on reproductive biology is still limited in Gouramy (Wijaya et al, 2009b). Aspects of reproduction in Gouramy which have been studied include the frequency of spawning (Wijaya et al., 2008), reproductive hormone profiles and gametogenesis in female or male parent (Wijaya et al., 2009a and 2009b).

The success of breeding is strongly influenced by the number of oocytes that mature at the time of spawning, while the number of eggs that mature at the time of spawning is determined by the number of oocytes that had reached the stage of maturation and ovulation (Wijaya et al., 2008). Preliminary studies to induce oocyte maturation has been done by Setiyadi (2009), showed that administration of progesterone in the fourth week post breeding can significantly increase the diameter of the oocyte. Also found that the level Gouramy oocyte maturation induced progesterone increase in induction with a combination of 2.6 ng kg-1 of progesterone and 0.5 mL kg-1 GnRH analog GnRH ovaprim. These results indicate that progesterone has the potential to be induced oocyte maturation in Gouramy.

GnRH would stimulate the pituitary to secrete gonadotropin (GtH) in fish.

GtH carried by the bloodstream to target organs, and at certain levels to stimulate growth and gonadal maturation. Final gonad maturation stimuli performed with oocytes to synthesize steroid hormones or Maturation maturation inducing substance (MIS) (Nagahama, 1994). The use of GnRH analogues to trigger Gouramy oocyte maturation is still very limited. However, the use of GnRH analogue for induction of spawning has been reported. Sunarma et al. (2007) informs that administration of 0.7 mL ovaprim as GnRH analogues capable of triggering ovulation in Gouramy oocytes that had reached the size of $\geq$ 2.8 mm. The purpose of this study is to evaluate the effect of a combination of progesterone and GnRH analogue (ovaprim) on carp oocyte maturation and know-dose combination of progesterone and GnRH analogue (ovaprim) are efficient to induce oocyte maturation carp.

2 METHOD

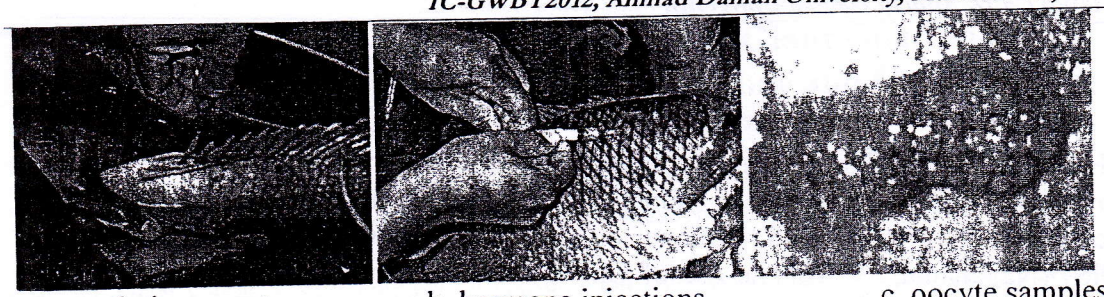
This reseacha used female Gouramy-broodstock 1,5-2 kg in weigh. Gouramy-broodstock obtained from fish farmers from the village of Pasir Wetan, District of Purwokerto Barat, Banyumas. Research materials used are: progesterone (Progesterone water soluble, Sigma P7556), GnRH analogue with a trademark ovaprim (Syndel Lab. Vancouver, Canada), chemicals for the manufacture of carp oocyte histology preparations, and chemicals to pool water quality measurement.

This research was carried out experimentally using the experimental design completely randomized design with 4 treatments and 4 replications. Total female=broodstock post breeding required is 20 fish. Variables examined in this study is to increase the diameter of oocytes and oocyte maturity level. Oocyte maturity level is determined by the parameters clarity oocyte and oocyte diameter (Wijaya et al, 2009).

Maintenance performed on the Gouramy brood stock pond soil. One month before the study, the pool maintenance which also is spawning prepared by drying pond for one week and then partitioned into map-sized pool 3x3 m.

Table 2.1. The concentration of hormone induction of the carp. Ovaprim (ml / kg BW) Progesterone (ng / kg BW) Description

Ovaprim (ml/Kg BB)	Progesteron (ng/Kg BB)	Treatmens
0	0	Control (C)
0,7	52	Treatmen I (D)
0,7	78	Treatmen II (B)
0,7	104	Treatmen III (E)
0,7	130	Treatmen IV (A)



a. cannulation oocytes b. hormone injections c. oocyte samples

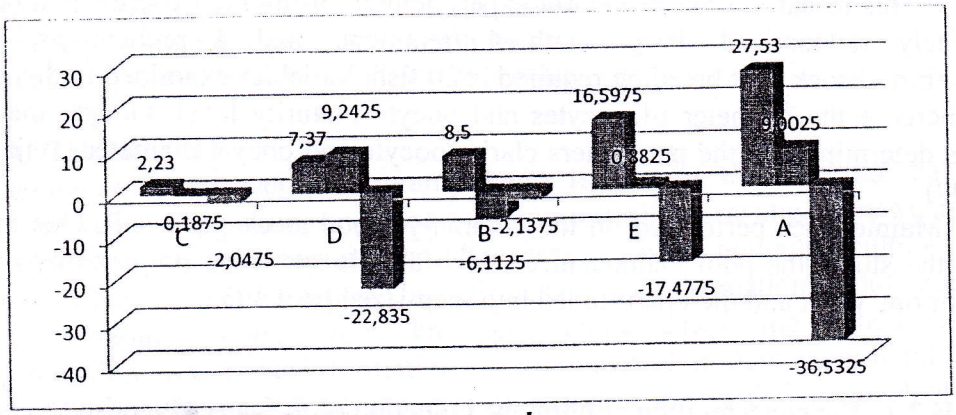
Figure 2.1. The process of sampling the oocyte and the hormone induction.

3 RESULTS AND DISCUSSION

The effectiveness of GnRH analogues and progesterone induces oocyte maturation in carp in the study, evaluated by analyzing the proportion of oocytes that had been added to reach maturation. Carp oocyte that has matured morphologically characterized by the appearance of a clear yellow and histologically characterized by no detection of the oocyte nucleus (GV).

3.1 The proportion of oocytes Mature

Data proportions (%) changes in the mature egg after induction are presented in bar chart below (Figure 3.1.).



**Blue : clear Red : half-clear green : not clear*

C = Control D = 0.7 Ml.kg GnRH and progesterone-1 BB-1 BB 52ng kg
B = 0.7 mL kg GnRH-1 BB and progesterone 78ng kg-1 BW
E = 0.7 mL kg GnRH-1 BB and progesterone 104ng
A = 0.7 mL kg GnRH-1 BB and progesterone 130ng kg-1 BW

Figure 3.1 shows that the increase in the proportion of mature oocytes and a half clear is the implication of a reduction in the proportion of oocytes that are still opaque. Except in the treatment of B which increases the proportion of mature oocytes are the implications of reduced oocyte half clear and opaque. The development of oocytes for 48 hours post-induction showed that the higher dose induced propesteron the higher the proportion of oocytes mature wear. To find out where the real differences that increase the percentage of mature oocytes between treatments, then followed by post hoc tests using Duncan test. Duncan test results are shown below.

Table 3.1. The proportion of Clear After Duncan's test treatments.

Treatments	N	Subset for alpha = .05	
		1	2
D	4	9.4525	
C	4	9,9700	
B	4	18,9750	
E	4	24,2850	24,2850
A	4		38,3900
Sig.		,077	,071

Based on table 3.1. the numbers on the same column indicate no significant difference, whereas the different columns showed no significant difference among the treatments. Control compare with treatment D, B, E, there is no real difference. Treatment A (GnRH analogue 0.7 mL / BB, progesterone 130 ng / BW) produced a statistically better result compared with other treatments.

Ovaprim induction in Gouramy has not been much done. In this study doses of GnRH analogues used by 0.7 mL / BB is ideal, because in preliminary studies by Sunarma et al. (2007) 0.7 mL dose of GnRH analogues to induce ovulation oocytes carp well, with a size of 0.28 cm diovulasikan oocytes. In the study Jamroz et al. (2008), induction ovaprim (0.5 mL / BB) and the combination ovaprim (0.5 mL / BB) with ovopel (0.2 mL / BB) in *Leuciscus* IDUs produce the best results (82 and 85% of embryos alive .) A recent study by Taufek et al. (2009) found that induction with salmon GnRH (20 ug / kg) combined with chicken GnRH (200 ug / kg) to get more efficient results in the induction of oocyte maturation African catfish *Clarias gariepinus* (Burchell).

The role of GnRH analogue (ovaprim) on oocyte maturation process is indirect. GnRH analogues stimulate the fish to secrete endogenous GtH. of GtH I, which plays a role in vitellogenesis and GtH II plays a role in oocyte maturation. Increase in GtH II as Maturation inducing substance (MIS). result from the induction of GnRH analogues is expected to spur the maturation of oocytes. Oocytes must be prepared by GtH to be ready at induction with Maturation inducing hormone (MIH).

Induction of progesterone as prekusor of 17a, 20b dihydroxy-4-pregnen-3-one which is the MIH is expected to increase the process of oocyte maturation in carp. Matsuyama et.al (2002), revealed that the in vitro steroid 17.20 β-dihydroxy-4-pregnen-3-one (17.20 β-P) and 17.20 0.21 β-trihydroxy-4-pregnen-3-one (20β-S) is effective and strongly induce GVBD in oocytes Kyusen wrasse (*Halichoeres poecilopterus*). 17.20 Levels of β-P and 20 β-S in the blood increases at the time of GVBD and ovulation indicates that 17.20 β-P and 20 β-S act as MIH in Kyusen wrasse (*Halichoeres poecilopterus*). Basu et al. (2004) reported that in *Anabas testudineus*, freshwater fish species, 17a, 20b dihydroxy-4-pregnen-3-one as the MIH with a dose of 1 mg / mL to induce GVBD completely 21 hours after induction. According to Liu et al. (2005), there are two main functions of progesterone are mediated by two forms of progesterone receptor (PR) is a work XPR2-dependent

transcription in the oocyte and ovulation XPR1 who work in transcription-independent on oocyte maturation.

In this study Gonadotropin as a mediator I oocyte maturation will increase because the induction of GnRH analogue ovaprim. Induction ovaprim will stimulate the pituitary to secrete GtH I and GtH II, which plays a role in the final stages of oocyte maturation and ovulation and oviposition. Ovaprim also cause the ovaries to synthesize active 17α, 20β-DP which will trigger oocyte maturation and ovulation. While the combination of induction of progesterone as a MIS will accelerate the process of oocyte maturation carp. Progesterone as a MIS can directly trigger the formation of MPF by stimulating the synthesis of cyclin-B. So the combination of induction of oocyte maturation and progesterone ovaprim carp using proven to work synergistically (increase carp oocyte maturation).

3.2 Diameter of oocytes.

Changes in oocyte diameter can be seen in the diagram below (cm):

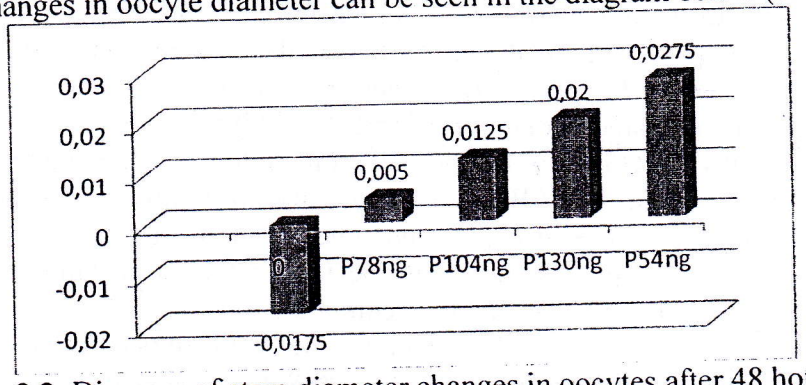
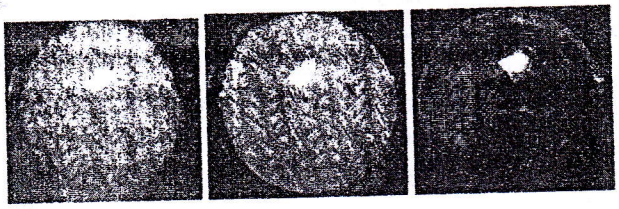
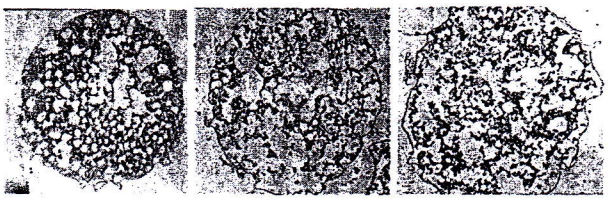


Figure 3.2. Diagram of stem diameter changes in oocytes after 48 hours of induction.

Based on Figure 4.4 in the treatment D is a combination of GnRH analogue 0.7 mL to 52 ng / BB progesterone although not significant, but the increase is seen at high (0.0275 cm). When viewed from the initial state, the carp in treatment D the average diameter of oocytes initially the lowest, which is about 0.1475 inches or 1475 μm (Figure 4.5). Oocyte developmental stages of carp criteria set out Wijaya et al. (2008) in Table 2.2 (page 7-8), the oocytes are still in the stage V-VI vitelogenesis. Increased diameter of oocytes in treatment D (0.7 mL of GnRH analogues with 52 ng / BB progesterone) are high because the study used GnRH analogues would stimulate the secretion of GtH I, which plays a role in increased vitellogenesis. Progesterone induced internalization vitelogenin also helps increase the diameter of the oocyte so that the oocytes in treatment D is the biggest (although not statistically significant). Confirmation carp oocyte maturation in this study with histological preparations and comparison was made clear oocytes with oocytes that have not been clear (opaque) and half clear.





a. b. c.

Figure 3.3. Histology confirmation carp oocytes showed that the opaque oocytes (a), half-clear (b) is an immature egg, and eggs are clear (c) is an egg that has been cooked (having GVBD).

Figure 3.3. which clearly shows that the oocytes are already mature oocyte, so that fish farmers if you want to know the level carp oocyte maturation percentage menganulasi stay and see the percentage of clear eggs. If the percentage has more than 80% then ready for spawning carp. Knowledge of gonad maturity level is very important and very supportive of success in carp germinate because the parent is closely related to the selection of fish that will be cultivated. The higher the level of development of gonads, eggs contained in it getting bigger as a result of the accumulation of yolk, hydration, and formation of beads of oil.

Histologic evaluation showed that the oocyte egg yolk filled by the longer will be more homogeneous so that a physical would seem clear. So this study strengthen previous research (Setiyadi, 2009) that a clear yellow carp oocyte is a mature carp oocytes. So if done cannulation on the female parent and obtained a clear egg is 80% then the breeders are ready for spawning.

4 Conclusion and Implications

Based on the results of data analysis and discussion that is done then it can be concluded as follows:

1. Induction of the hormone GnRH (0.7 ml / kg) in combination with progesterone can increase the percentage of oocyte maturation.
2. Induction of the hormone GnRH in combination with progesterone in general had no significant effect on increasing the diameter of the oocyte.

The results of this study need to be developed further in order to be used as a protocol increase oocyte maturation in carp. Necessary to investigate the use of hormonal induction of GnRH analogues and progesterone kobinasi with higher doses.

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