Effect of ovaprim and prostaglandine $F_2\alpha$ on semen volume and sperm quality in Sepat siam (*Trichogaster pectoralis* Regan)

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ABSTRACT

The effects of ovaprim and prostaglandine $F_2\alpha$ (PG $F_2\alpha$) on semen volume and sperm quality in sepat siam (Trichogaster pectoralis Regan) were observed. Eighteen wild cought male fishes were injected with three combinations of ovaprim and prostaglandine $F_2\alpha$ (0,250 ml ovaprim + 1250 µg PG $F_2\alpha$/kg body weight, P1), 0,375 ml ovaprim + 625 µg $PGF_2\alpha$ /kg body weight, P2), 0,125 ml ovaprim + 1875 µg PG $F_2\alpha$/kg body weight, P3), and two other individual hormones, ovaprim (0,50 ml ovaprim/kg body weight, P4) and PG $F_2\alpha$ (2500 µg PG $F_2\alpha$/kg body weight, P5), and control (1 ml NaCl 0.9%, P6). The best result was presented by the combination of 0.250 ml ovaprim + 1250 µg prostaglandine $F_2\alpha$ per kg body weight (P1), which the semen volume, spermatozoa concentration, spermatozoa viability and spermatozoa motility were 0.163 ml, 18.167 x 10^9/ml, 81.50% and 79.833 % respectively.

Keywords *:* semen, spermatozoa, concentration, viability and motility

INTRODUCTION

Sepat siam (*Trichogaster pectoralis* Regan) is one of the most important local fresh water species in Riau. The fish inhabit in the rivers, oxbows and reservoirs of the area. Sepat siam is currently being investigated as a species for aquaculture in Riau as the fish has high economic value, fast growth with adaptable to artificial feeding; and their availability in the nature is decreasing due to environmental defect. However, one of difficulty working with a new candidate for culture is obtaining reliable quantities of viable eggs due to poor semen quality and ovulation.

Some exogenous hormone treatments have been widely used to improve semen quality as well as ovulation. Successful manipulation of reproductive process depends on some level of understanding of reproduction in the target species. At present, there is no published data on endocrine function in sepat siam ; and little is known of its general reproductive biology.

Ovaprim and prostaglandin $F_2\alpha$ combination has been used to improve semen volume and spermatozoa quality in some fresh water species, such as *Clarias gariepinus* Burcheel (Sukendi, 1995), *Leptobarbus hauvey* Blkr (Putra dan Sukendi, 1998), *Oxyeleotris marmorata* Blkr (Putra dan Sukendi, 2000), *Mystus nemurus* CV (Sukendi, 2001), *Thynnicthys thynnoides* Blkr) (Sukendi, Putra and Yurisman, 2011). Our prelimanary experiment indicated that there was a spermilation respon toward ovaprim and prostaglandine $F_2\alpha$ which were injected separately, however, the semen qualities were poore. Combinations of ovaprim and prostaglandine $F_2\alpha$, therefore were practiced to improve semen volume and spermatozoan quality in sepat siam.

MATERIALS AND METHODS

Fish broodstock

Two hundred fishes were cought from the Kampar River and transported to laboratory at the Faculty of Fisheries and Marine Science Universitas Riau, Pekanbaru. The fishes were conditioned for three months in cage which were fed ad libitum with artificial diet (28% protein) twice a day.

Injection and milt collection

The fishes were randomely grouped into six groups, three fihes each group. The fish were injected with three combinations of ovaprim and prostaglandine $F_2\alpha$ (0,250 ml ovaprim + 1250 µg PGF $_2$ α/kg body weight, P1), 0,375 ml ovaprim + 625 µg $PGF_2\alpha$ /kg body weight, P2), 0,125 ml two other individual hormones, ovaprim (0,50 µg $PGF_2\alpha$/kg body weight, P5), and control (1

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For P1, P2 and P3, the fishes were firstly injected with ovaprim and reinjected after six hours with $PGF_2\alpha$, while for P4 and P5, the fishes were firstly injected with half dose and reinjected after six hours with another half dose.

The milt colection was made by stripping the fish after six hours of injection. The sperm sampels obtained were analysed for sperm quality

Sperm Quality

The sperm quality was evaluated for semen volume, spermatozoa concentration, spermatozoa viability and motility. The semen volume was measured by sucking the semen using scaling syring. The spermatozoa concentration was quantified using haemositometer illustrated by Toelihere (1985). Ten milliliters of the semen sample was pipeted and diluted with distilled water up to $2x10^{-2}$, then was mixed gentally. The semen solution were put into Neubeur counting room and covered with cover glass. The spermatozoa cells were enumerated under microscope; and the number was calculated using the following formula:

$$X(400/80)(10 \times 200) = X(0.01 \text{ million spermatozoa})/mm^3$$
$$= X(10^7 \text{ spermatozoa})/ml$$

The spermatozoa viability was enumerated by staining the cells with 2% eosin. Two hundred stained spermatozoa cells of each treatments were sampled and enumerated under microscope for the viable cell. The viable cells were charecterized by unfixed eosin by the cells which was expressed in percent. The motility of the cells moreover, was based on the progresive movement of the viable cells which was also expressed in percent. The spermatozoa viability and motility were calculated as bellow:

$$\text{Sperm Viability} = \frac{\sum \text{Viable spermatozoa}}{\text{Total spermatozoa}} \times 100\ \%$$

$$\text{Sperm Motility} = \frac{\sum \text{Motile spermatozoa}}{\text{Total spermatozoa}} \times 100\ \%$$

Statistical analysis

One-way analysis of variance (ANOVA) and Duncan's multiple ranges test (Steel and Torrie, 1993) were used to compare the differences between the means of treatments; and the analysis of correlation was made to prove the correlations between semen volume and spermatozoa concentrations, semen volume and spermatozoa motility, spermatozoa concentrations and spermatozoa viability, spermatozoa concentrations and spermatozoa motility, and spermatozoa viability and spermatozoa motility. The analyses were performed using the GLM and REG procedures in the statistical analysis system software (SAS Institute, 1990).

RESULTS

The semen volume, spermatozoa concentration, spermatozoa viability and spermatozoa motility of fish resulted from different hormonal injection doses were presented in Table 1. The semen volumes from the highest to the lowest levels were 0,163 ml (P1), 0,153 ml (P2), 0,143 ml (P4), 0,133 ml (P3), 0,123 ml (P5) and 0,107 ml (Control) respectively. Overall, the fish injected with 0,250 ml ovaprim + 1250 µg $PGF_2\alpha$/kg body weight (P1) produced the highest level of the semen volume ($p < 0,05$), although the level was not statistically different ($p > 0.05$) from the fish injected with 0,125 ml ovaprim + 1875 µg $PGF_2\alpha$/kg body weight (P2).

The spermatozoa concentration from the highest to the lowest were 22,167 x 10^9/ml (Control), 21,500 x 10^9/ml (P5), 20,167 x 10^9/ml (P3), 19,833 x 10^9/ml (P4), 19,00 x 10^9/ml (P2), and 18,167 x 10^9/ml (P1) respectively. Statistical analysis indicated that the spermatozoa concentration was lower ($P<0.05$) for the fish injected with P1, P2 and P4 than that injected with control. However, there was no significantly different in the spermatozoa concentration ($P>0.05$)

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The spermatozoa viability was higher for the fish injected with hormonal doses than that of control (P<0.05). The values from the highest to the lowest were 81.500 % (P1), 79.167 % (P2), 77.800 % (P4), 75.33 % (P3), 72.667 % (P5) and 70.667 % (Control) respectively.

The spermatozoa motility was also higher for the fish injected with hormonal doses than that of control (P<0.05). The values from the highest to the lowest were 79.833 % (P1), 77.000 % (P2), 72.833 % (P4), 71.500 % (P3), 69.167 % (P5) and 67.333 % (Control) respectively.

Table 1. Semen quality of fish (triplicate) injected with different hormonal doses

Rep.	Semen volume (ml)					
	P1	P 2	P3	P4	P5	Control
Semen volume (ml)	0.163 ± 0.0153 ^{ef}	0.153 ± 0.0058 ^{de}	0.133 ± 0.0058 ^{bc}	0.143 ± 0.0058 ^{cd}	0.123. ± 0.0058 ^{b}	0.1067 ± 0.0116 ^{a}
Spermatozoa concentration (10^9/ml)	18.167 ± 0.5774 ^{a}	19.000 ± 0.8660 ^{ab}	20.167 ± 0.7638 ^{bcd}	19.833 ± 0.7638 ^{ac}	21.500 ± 1.000 ^{ce}	22.167 ± 0.5774 ^{ef}
Spermatozoa viability (%)	81.500 ± 1.000 ^{de}	79.167 ± 0.7638 ^{cd}	75.333 ± 0.7638 ^{b}	77.800 ± 1.1269 ^{c}	72.667 ± 2.5658 ^{a}	70.667 ± 0.7638 ^{a}
Spermatozoa motility (%)	79.833 ± 1.2583 ^{f}	77.000 ± 1.000 ^{e}	71.500 ± 2.1794 ^{bc}	72.833 ± 1.408 ^{cd}	69.167 ± 0.7638 ^{ab}	67.333 ± 1.6073 ^{a}

Values within rows followed by different superscripts are significantly different (P<0.05)

A negative correlation was shown between semen volume and spermatozoa concentration, R^2=0.977 (Fig 1). The more the semen volume the lower the spermatozoa concentration. However, the semen volume (Fig 2 and 3) had a positive correlation with spermatozoa viability (R^2=0.986) and spermatozoa motility (R^2=0.948). The more the semen volume the higher the spermatozoa viability as well as the spermatozoa motility.

The spermatozoa concentration (Fig 4 and Fig 5) correlated negatively with the spermatozoa viability (R^2=0.984) and spermatozoa motility (R^2=0.959). The lower the spermatozoa concentration the higher the spermatozoa viability as well as the spermatozoa motility. The spermatozoa viability, however, had a positive correlation with spermatozoa motility (Fig 6). The more the spermatozoa viability the higher the spermatozoa motility, R^2=0.949.

DISCUSSION

Various hormonal injections, either individual or combination of two hormones with single or double doses have been successfully practiced in most species of fish to improve semen quality (Kruger *et al.,*1984; Nurman, 1995); Putra dan Sukendi, 1998; Putra dan Sukendi, 2000; Sukendi, 2001). In this study, semen quality of sepat siam were affected by ovaprim and prostaglandine $F_2\alpha$ injections, either as an individual or combination of the two hormones. The effects were indicated by an increase of the semen volume up to 65% for those injected with hormones as compared to those injected with saline (control).

However, spermatozoa concentration decreased as the semen volume increased. This was because the hormonal injections only stimulated the production of the semen plasma fluid but not spermatozoan cells. As the semen volume increased, while the number of spermatozoan cells were constant, therefore, the spermatozoa concentration per ml semen volume decreased. This result was an agreement with the research findings conducted on male cyprinid carps injected with hypofisis extract, which the semen volume increased from 7,2 ml to 13,2 ml and the spermatozoa concentration decreased from 22 x 10^9/ml to 20 x 10^9/ml with increasing of the hormonal injection [illegible]).

[illegible]vely with sperm motility and viability. As the [illegible]ozoa motlity and viability increased. The low

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spermatozoa concentration, therefore was disirable as the spermatozoa motility and viability increased with the lowering spermatozoa concentration. The highest spermatozoa motility and viability was shown by the fish injected with 0,375 ml ovaprim + 625 µg $PGF_2\alpha$ /kg body weight. This phenomenon was also demontrated by some previous studies on *Clarias gariepinus* (Nurman, 1995), *Leptobarbus hoeveni* (Putra dan Sukendi, 1998), *Oxyoleoterus marmorata* (Putra dan Sukendi, 2000) dan *Mystus nemurus* (Sukendi, 2001).

The fact that the low spermatozoa concentration inproved the spermatozoa motility and viability was because the lower the spermatozoa concentration the more the glucose could be used for energy, which in turn increased the rate of spermatozoa motility and viability (Minkittrich dan Moncia (1987).

CONCLUSION

Semen volume, spermatozoa concentration, spermatozoa motility and viability were affected by ovaprim and $PGF_2\alpha$ injections, either individual or combinations An injection of 50 % ovaprim + 50 % $PGF_2\ \alpha$ (0,250 ml ovaprim + 1250 µg $PGF_2\ \alpha$ /kg fish body weight resulted in the best spermatozoa motility and viability. The lower the spermatozoa concentration the higher the semen volume, spermatozoa motility and viability rates.

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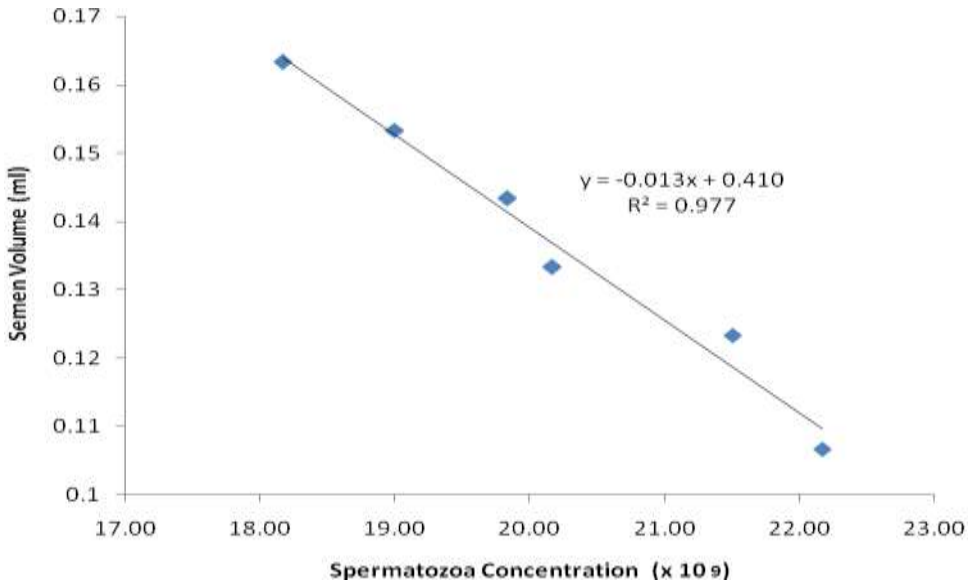

Figure 1. Correlation between semen volume and spermatozoa concentration

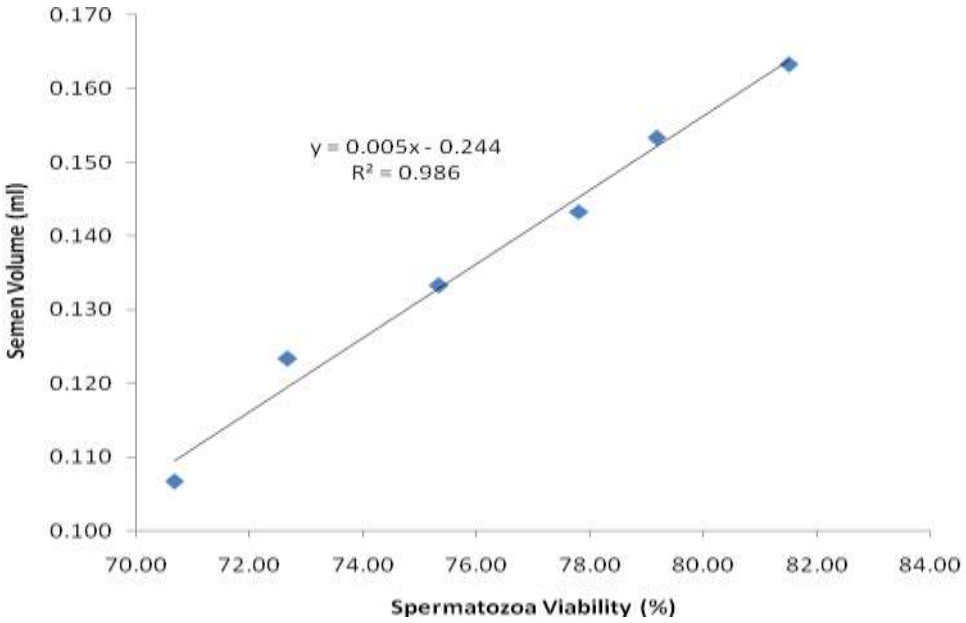

Figure 2. Correlation between semen volume and spermatozoa viability

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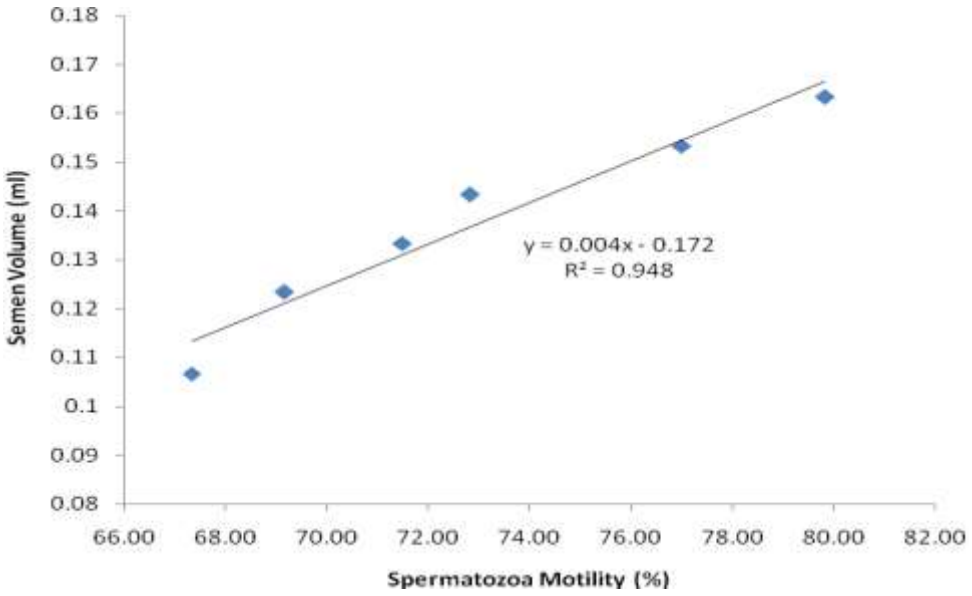

Figure 3. Correlation between semen volume and spermatozoa motility

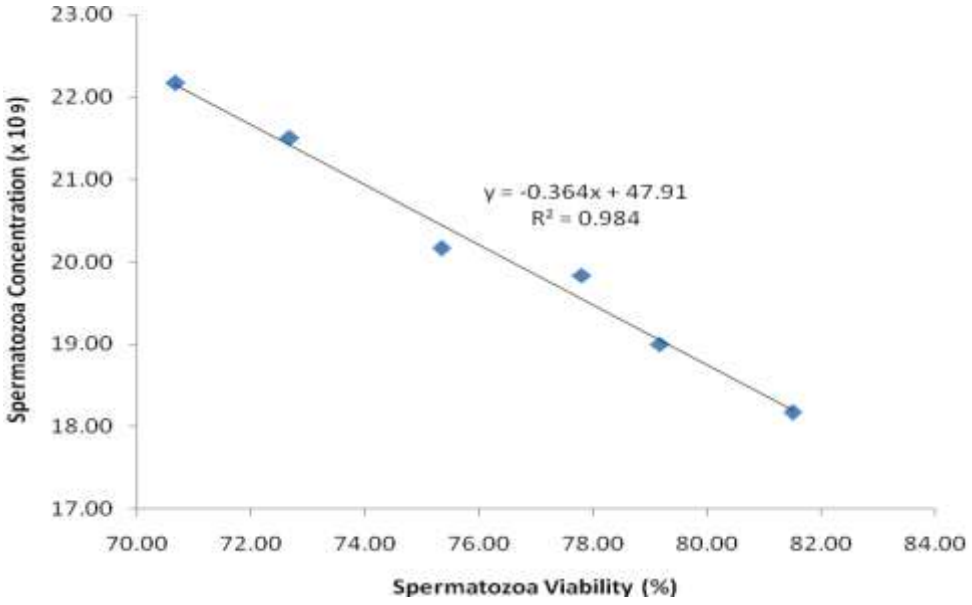

Figure 4. Correlation between spermatozoa concentration and spermatozoa viability

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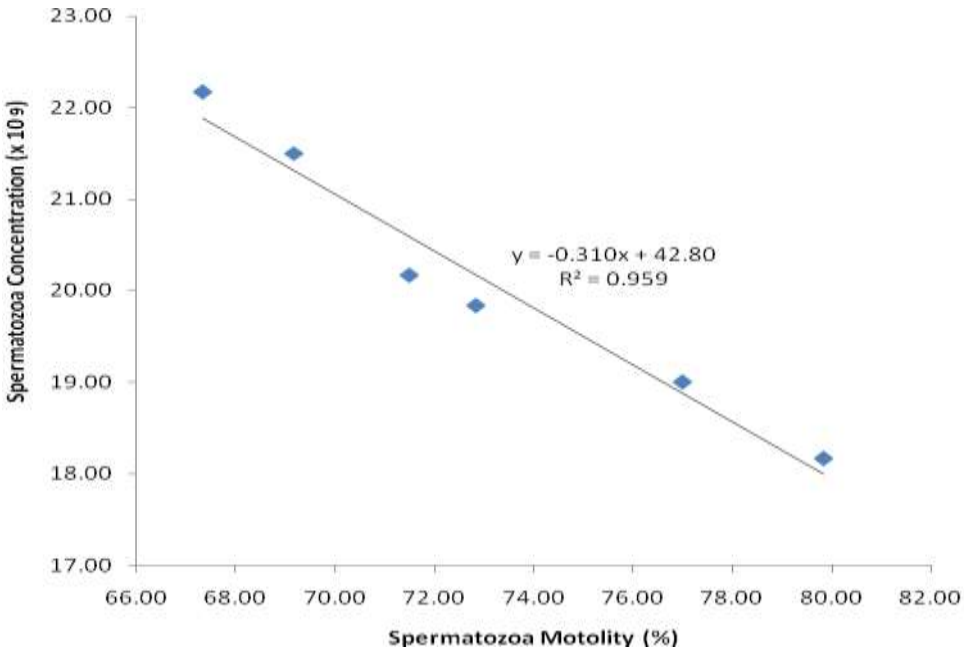

Figure 5. Correlation between Spermatozoa concentration and spmermatozoa motility

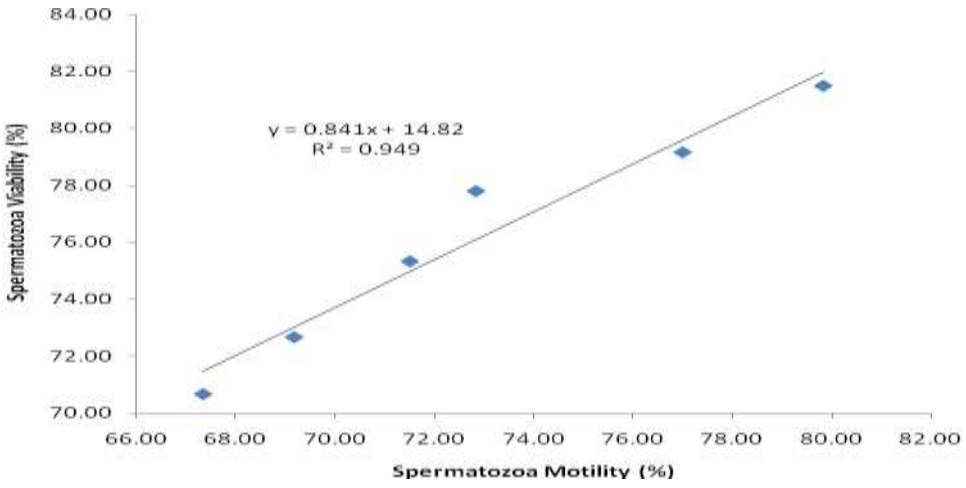

Figure 6. Correlation between Spermatozoa viability and spmermatozoa motility

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