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The Use of OODEV® in Improving the Reproductive Performance of Male Yellow Rasbora Fish *Rasbora lateristriata* (Bleeker, 1854)

Hurul Ein[1], Bambang Retnoaji[1] and Slamet Widiyanto[1*]
[1]Biology Program, Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Sel., Sendowo, Sinduadi, Kec. Mlati, Sleman Regency, Yogyakarta Special Region 55281, Indonesia

*Correspondence :
slametbio@ugm.ac.id

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Abstract

A local commodity, Yellow rasbora *Rasbora lateristriata* (Bleeker, 1854), can potentially be used as food by the Kulon Progo community. Added to that, aquaculture is known to optimize this potential and will also prevent illegal fishing where yellow rasbora are listed as a vulnerable species in the IUCN Red list. This study induced male yellow rasbora using the hormone OODEV® injection, which comprises Pregnant Mare Serum Gonadotropin (PMSG) and anti-dopamine. Yellow rasbora was nurtured to the age of six months and treated with four different treatments (dose) for a month, namely 0 mL/Kg, 0.25 mL/Kg, 0.5 mL/Kg, and 0.75 mL/Kg. Observed parameters included motility (movement and duration) as well as sperm count, sperm viability, sperm morphology, sperm morphometry, gonad somatic index (GSI), and survival rate (SR). Data obtained were analyzed using SPSS with the One-way ANOVA test and continued with Duncan's test if the results were significant. The obtained results had no significant effect on the IGS value. In addition, administration of OODEV® also improved sperm quality with the best results at 0.75 mL/Kg in terms of motility with the highest progressive movement compared to other doses but not significant compared to the control and significantly longer movement duration compared to other doses. In terms of morphometry and tail width, it was not significant compared to other doses, longest tail length but not significant with other doses except with 0.25 mL/Kg, and significantly had the longest sperm total length. Every dose showed a 100% percentage of survival rate.

INTRODUCTION

Yellow Rasbora *Rasbora lateristriata* (Bleeker, 1854) is a vulnerable fish in the IUCN Red List. Usually, they are captured from nature by the community of Kulon Progo, Special Region of Yogyakarta, due to their ease of catching and taste. These acts led to a decrease in the Yellow Rasbora fish population. Therefore, it is necessary to continuously develop its cultivating techniques (Djumanto *et al.*, 2008; IUCN Red List, 2021).

Induced breeding has been a key to many reproduction problems in cultivated fish. OODEV® is a hormone that expresses

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anti-dopamine, folilulating hormone (FSH), and luteinizing hormone (LH). OODEV® is a hormone from a pregnant mare endometrium, namely Pregnant Mare Serum Gonadotropin (PMSG) (Kumari *et al.,* 2015; Tomasoa *et al.,* 2021). The role of anti-dopamine in the OODEV® is to inhibit the dopamine that may call off the LH secretion from the Gonadotropin Releasing Hormone (GnRH). This aligns with previous research that dopamine would prevent maturation (Levavi-Sivan *et al*., 2009).

Besides being able to induce fish gonadal maturation, it is well known that FSH and LH are responsible for stimulating the process of spermatogenesis and stimulating the production of other vital hormones (steroidogenesis) (Schulz *et al*., 2009). It is then hypothesized that the injection of OODEV® would increase gonadal maturation and increase the quality of sperm. Therefore, this research aims to know the effect of OODEV® on gonad somatic index and sperm quality, including sperm count, motility, viability, abnormalities of morphology, and morphometry.

METHODOLOGY

Ethical Approval

Ethical approval of this research with registration number KE-FK-0152-EC-2023 was approved and granted by the Research Ethical Committee of the Faculty of Medicine, Gadjah Mada University, on February 2, 2022.

Place and Time

The research occurred at the Animal Developmental Structure Laboratorium, Animal Physiology Laboratorium, and Gama Wader Fish Maintenance Site, Gadjah Mada University, from May 8 until June 20, 2023.

Research Materials

Equipments used in this research include a light microscope (Leica-DM500, Germany), a fiber pool 147 cm x 93 cm x 45 cm, which is then divided into six sections using a net, liquid filter (AT-102, China), analytical weight scale (Shimadzu Libror-AEL200, Japan), 30 G insulin syringes (Onemed-B00005523, Indonesia), small fish net, mercury thermometer, micropipette 0.5 μL – 10 μL (Biologix-01-2101, China), pipette tip, hemocytometer (Assistant-422/2, Germany), object glass (Citotest-Citoplus P/N.0303-2125, China), cover glass (Citoglas-P/N.0340-4010, China), plastic pipette, and surgical equipment.

Materials used in this research are fish feed Hi-pro-vite 781-1 (CP Prima, Indonesia), ice, OODEV® hormone (IPB, Indonesia), NaCl solution 0.9% (Widatra, Indonesia), Eosin Y 1% (Merck, Germany), Immersion oil (Merck, Germany), and Phosphate Buffer Saline (PBS) 0.1 M (vivantis, Malaysia).

Research Design

This research was conducted using five fish as a repetition in every treatment. Various treatments (doses) were used in this research, namely 0 mL/Kg (K), 0.25 mL/Kg (P1), 0.5 mL/Kg (P2), and 0.75 mL/Kg (P3). The outline methods of taking the research data were by collecting sperms from the Yellow Rasbora fish to be examined for its quality and followed by measuring its body and testis weight for the gonadosomatic index (GSI) test, where the testis was obtained by surgical method.

Work Procedure

Preparation

This research was initiated by preparing the fiber pool for Yellow Rasbora fish. The water used in this research was obtained from the tap water. Hence, it should be precipitated for one day and one night to prevent contamination of Calcium hypochlorite, and a liquid filter was installed. Fish used in this research were raised to six months old. When they reach six months old, they are stripped to differentiate between male and female. 22 male fish were selected for this research, where two fish were used to observe their gonadal maturity stage before starting the experiment. Selected six months fish were divided into four groups for four

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doses of OODEV®, namely 0 mL/Kg, abbreviated as K, 0.25 mL/Kg abbreviated as P1, 0.5 mL/Kg abbreviated as P2, and 0.75 mL/Kg abbreviated as P3(Putri *et al.,* 2019; Hutagalung *et al.,* 2015). Intraperitoneal injection of OODEV® was given with NaCl due to the small quantity given to the fish, while K was intended to be the control. Treatments were given every once a week in a month. After a month, the sperm was obtained by stripping the abdominal section to be examined for the sperm quality test.

Sperm Motility and Count

Sperm motility and count were observed using the method from Parhizkar *et al.* (2013) by adding a drop of semen mixed with cold NaCl to the counting chamber (hemocytometer). It is then recorded and captured using the Leica light microscope. This section was done concurrently with the observation of motility duration. The motility (movement) of sperms was grouped according to their categories and tabulated using the motility formula:

$$\text{Sperm percentage from various Categories (\%)} = \frac{\text{Sperm number according to their category}}{\text{Total of sperm from every category}} \text{x100}$$

Table 1. Various categories of sperm motility (Parhizkar *et al*., 2013).

Category	Characteristics
1st Category	Immotile sperms
2nd Category	Unprogressive movement with vibrating-like movement tails
3rd Category	Nonlinear movement (tend to move on a circle)
4th Category	Progressive movement

The sperm count was conducted using the multi-point tool in the Image J software and tabulated with the given formula (Parhizkar *et al*., 2013):

Sperm count = Number of sperms in 5 chambers x 50,000x100 (cells/mL)

Sperm Viability

Sperm viability was conducted by preparing a sperm using a modified method from Mahdaliana *et al.* (2022) using Eosin Y 1% as its stain. In the first step, a drop of sperm was delivered to the object glass. It is then followed by adding two to three drops of Eosin Y% to the object glass and homogenizing gently. It was then spread on the object glass gently using another object glass. The colored head of sperm indicates their death, while the uncolored one indicates their viability. The obtained preparation was then observed using image J. Results of sperm viability can be counted using the given formula (Parhizkar *et al*., 2013):

$$\text{Viability(\%)} = \frac{\text{Viable sperms}}{\text{Total number of sperms}} \text{x100}$$

Sperm Morphological Abnormalities and Morphometry

Sperm abnormalities and morphometry were observed using a modified method from Mahdaliana *et al.* (2022) by making another sperm preparation using Eosin Y 1%. Nevertheless, before adding the stain to the object glass, PBS 0.1 M was mixed with the sperm. It is then spread gently on the objective glass after adding the stain and then air-dried at room temperature. The sperm preparations are then observed using a Leica light microscope to capture six fields of view. The captured preparation was used to count the abnormal sperms and to measure different parts of the sperms with a modified method from Wibowo *et al.* (2013) using image J and tabulated using the given formula (Ardhani *et al.,* 2014):

$$\text{Morphological abnormalities (\%)} = \frac{\text{Abnormal number of sperms}}{\text{Total number of sperms}} \text{x100}$$

Morphometry was analyzed using Image J software, and the data obtained were stored in Microsoft Excel.

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Gonadosomatic Index

Since all the procedures were done, the gonadosomatic index (GSI) was conducted by measuring the fish body and testis weight. The testis weight was obtained by dissecting the fish using surgical equipment. Data were then tabulated using the given formula (Nurhidayat *et al.,* 2017).

$$GSI(\%) = \frac{\text{Weight of gonad}}{\text{weight of fish}} x100$$

Data Analysis

The data were tabulated and then analyzed by using SPSS 22 software using Analysis of Variance (ANOVA) at a real level of 5%. The post-doc analysis was carried out using the Duncan test.

RESULTS AND DISCUSSIONS

Data analysis of this research was done using IBM SPSS software ver. 23.0 with a One-way ANOVA analysis preceded by normality and homogeneity of variances test. If the results are significant, then they are tested with the Duncan test.

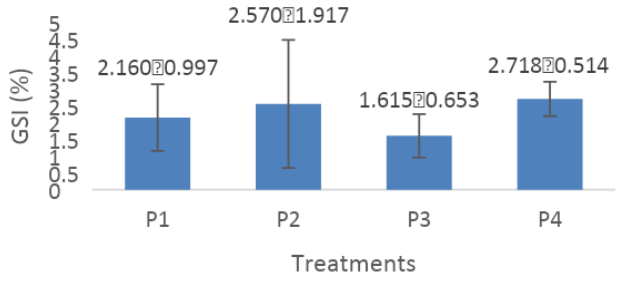

Figure 1. Percentage of IGS after various OODEV® dose treatments.

Description: Dose of OODEV® K: 0 mL/Kg, P1: 0.25 mL/Kg, P2: 0.5 mL/Kg, and P3: 0.75 mL/Kg. The average results of GSI did not show significant results ($p>0.05$) in every dose after statistical testing with ANOVA.

According to Figure 1, OODEV® treatments did not cause significant effects ($p>0.05$) on the GSI value. The first possibility that caused the insignificant result was the un-optimal dose of OODEV® to improve the GSI value—following previous research, 1 mL/Kg of OODEV® in the feed had increased the GSI value of female yellow Rasbora fish (Rey, 2022). The second possibility was due to the release of cytoplasm from the spermatid to form a mature sperm (Tang *et al.,* 2020). Cytoplasm release was believed to be the cause of the decrease in the weight of gamete cells. This event then causes the GSI to have an insignificant result where GSI itself is the comparison between testis and body weight. The second possibility was also supported by previous research where female clownfish induced with 1 mL/kg of OODEV® had higher GSI values compared to male clownfish (Tomasoa *et al.,* 2018).

Table 2. Sperm count (cell/mL) after the OODEV® treatment.

OODEV® dose (mL/Kg)	Sperm count (cell/mL)	$\bar{x} \pm sd$
K	882.000.000	882.000.000 ± 394.582.057,4
P1	994.000.000	994.000.000 ± 285.993.881,1
P2	986.000.000	986.000.000 ± 277.565.307,6
P3	1.590.000.000	1.590.000.000 ± 715.716.424,3

Description: Dose of OODEV® K: 0 mL/Kg, P1: 0.25 mL/Kg, P2: 0.5 mL/Kg, and P3: 0,75 mL/Kg. Average sperm count results between treatments did not show significant results ($p>0.05$) after the ANOVA test.

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Based on Table 2, the sperm count did not show a significant result (P>0.05). Nevertheless, it was known that the highest dose had the highest sperm count value. This event was caused by the formation of spermatogonium B from spermatogonium A to start various divisions and increase the number of cells (Wootton and Smith, 2015).

Table 3. Sperm viability (%) after different OODEV® treatment.

OODEV® dose (mL/Kg)	Sperm Viability (%)	$\bar{x}$ ± sd
K	40.972	40.972± 10.726
P1	39.330	39.330± 4.495
P2	21.505	21.505± 6.947
P3	32.818	32.818± 11.572

Description: Dose of OODEV® K: 0 mL/Kg, P1: 0.25 mL/Kg, P2: 0.5 mL/Kg, and P3: 0,75 mL/Kg. The average results of sperm viability between treatments did not show significant results (P>0.05) with the ANOVA test.

The results given in Table 3 did not show significant differences (P>0.05) among different doses. Thus, it is known that none of the treatments given in the study had any effect on sperm viability. The viability of sperm is strongly related to the existence of the plasma membrane, where damage to the plasma membrane might cause physiological and metabolic changes and end in sperm death (Armansyah *et al.*, 2021).

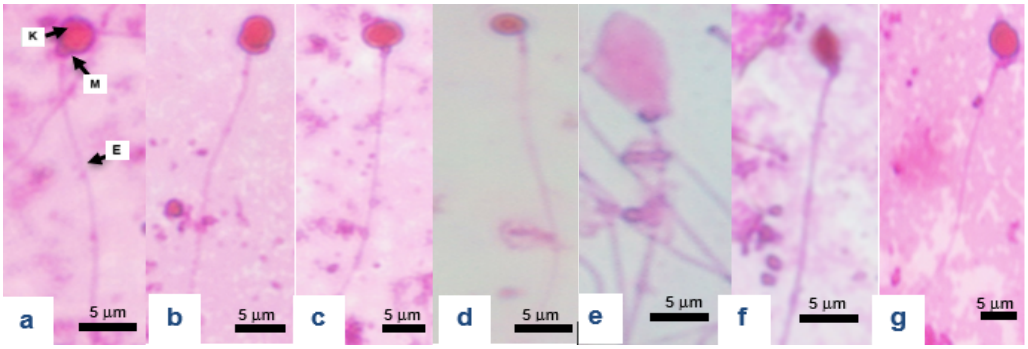

Figure 3. Various types of sperm morphology were found at each dose: (a) Normal sperm; K: Head, M: Middle piece, E: Tail; (b) Amorphous head; (c) Bent head; (d) Small head; (e) Large head, (f)Tapered head, (g) Broken middle piece.

The morphological abnormalities found in this research are presented in Figure 3. Morphological abnormalities can occur due to imperfect spermatogenesis and eventually cause fertilization failure. Failures caused by morphological abnormalities are divided into two categories. The first Category can cause failure during fertilization, while the second Category can cause failure in embryo development (Armansyah *et al.*, 2021). The late spermatid is produced throughout the last stage of spermiogenesis and is smaller than the other cells formed in the process.

This process is marked with more condensed chromatin, compared to the previous stages, and the mitochondria are present in only one of the sides of the cell. The late spermatid is similar to the matured ones but has a cytoplasm inside the middle piece. After this stage, the cytoplasm residual will be released to form a mature sperm (Tang *et al.*, 2020). Head abnormalities in Figure 3 (b-e) were influenced by chromatin abnormalities—changes during chromatin maturation and condensation cause amorphous heads. Chromatin anomaly could happen due to genetic abnormality or obtained disease (Chemes and Rawe, 2003). Bent heads are known to be caused due to

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the lack of *prm1* (protamin gene) in rats. Deficiencies of *prm1* will create abnormalities in sperm morphology, unstable DNA, chromatin decondensation, and a decrease in the membrane potential of mitochondria (Takeda *et al.*, 2016). Small and large heads happen due to the excess and lack of nuclear chromatin (Koziol and Armstrong, 2022).

The tapered head morphology in Figure 3 (f) is caused by hormonal disturbance, either systematic or local disturbance. Various kinds of stress cause a systematic disturbance, while the thermoregulation abnormality in the testes causes local disturbance. Figure 3 (g) shows an abnormality on the middle piece. The broken middle piece is caused by abnormalities in the mitochondrial sheath, where mitochondrial absence occurs and creates gaps in the middle piece. The more significant the gap formed in the middle piece, the easier it will be for the middle piece to break (Koziol and Armstrong, 2022).

Table 3. Morphological Abnormality (%) after OODEV® treatment.

OODEV® dose (mL/Kg)	Morphological Abnormalities (%)
K	7.333
P1	5.557
P2	6.548
P3	5.473

Description: Dose of OODEV® K: 0 mL/Kg, P1: 0.25 mL/Kg, P2: 0.5 mL/Kg, and P3: 0,75 mL/Kg.

Table 3 shows that OODEV® administration will reduce abnormalities that can occur. As shown in Table 3, it can be noted that the highest dose of P3 has the lowest average percentage of morphological abnormalities. In contrast, the control group, or K, has the highest average percentage of morphological abnormalities.

These results show that the hormone OODEV® can reduce sperm morphological abnormalities. OODEV® plays a role in completing spermatogenesis until normal sperm is produced, which follows the concept of the working of FSH and LH hormones. FSH and LH will stimulate the formation of 11-ketotestosterone (11-KT) that indirectly affects the proliferation of spermatogonia by producing activin B. 11-KT also plays a role as a hormone that stimulates DHP production and initiates meiosis. DHP will also induce trypsinogen to produce trypsin and induce partial spermiogenesis (Schulz and Miura, 2002; Schulz *et al.*, 2009; Miura and Miura, 2011).

Table 4. Sperm Morphometry after OODEV® treatment.

Morphometry	$\bar{x} \pm$ sd (µm)			
	K	P1	P2	P3
Head width	3.048 ± 0.452 [a]	3.356 ± 0.559 [a]	3.185 ± 0.437 [a]	3.240 ± 0.348 [a]
Head area	8.890 ± 2.158 [a]	10.562 ± 2.755 [a]	9.774 ±2.043 [a]	9.748 ± 1.768 [a]
Head length	3.274 ± 0.456 [a]	3.345 ± 0.247 [a]	3.348 ± 0.370 [a]	3.317 ± 0.314 [a]
Middle piece area	1.941 ± 0.623 [a]	1.928 ± 1.174 [a]	3.250 ± 0.380 [a]	2.037 ± 1.331 [a]
Middle piece length	2.895 ± 0.760 [b]	4.129 ± 1.143 [a]	4.956 ± 0.482 [a]	4.103 ± 0.574 [a]
Tail area	12.036 ± 1.000 [a]	9.210 ± 2.301 [b]	12.227 ± 1.023 [a]	13.844 ± 0.467 [a]
Tail length	24.857 ± 1.990 [a]	20.670 ± 4.193 [b]	23.397 ± 1.252 [ab]	26.791 ± 1.433 [a]
Total length	30.887 ± 2.616 [b]	30.394 ± 0.890 [b]	31.689 ± 1.370 [b]	34.170 ± 1.188 [a]

Description: Dose of OODEV® K: 0 mL/Kg, P1: 0.25 mL/Kg, P2: 0.5 mL/Kg, and P3: 0.75 mL/Kg. Different superscripts in the same row indicate significant differences between doses, while [ab] superscript does not indicate significant differences between doses.

Sperm morphometry plays a crucial role in determining sperm quality. In general, morphometry will significantly affect the motility of sperm. Motility is

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considered an essential aspect of the success of sperm in reaching the egg (ovum). Abnormalities in the head can affect the speed of sperm motility; for example, in macrocephaly, abnormalities tend to slow down sperm swimming speed (da Costa *et al*., 2022). Based on the results obtained in this study, it can be seen that the administration of OODEV® did not show a significant effect on the morphometry of the sperm head, which includes width, area, and length. Based on that statement, it is possible that the morphometry of the sperm head did not fully influence the motility in this study.

Like vertebrates, fish sperm consists of a head with a nucleus, a middle piece with mitochondria, and a tail consisting of axoneme flagella and an enveloped plasma membrane (Wootton and Smith, 2015). When viewed from its structure, sperm has an anatomical structure that can support sperm motility. Morphometry, in this case, the area of the middle piece, will describe the number of mitochondria in the sperm. Mitochondria itself acts as an energy source for sperm, affecting the duration of sperm motility. The width of the middle piece can then be related to the tail because the longer the tail, the faster the sperm is and the more energy it requires (Wibowo *et al.*¸ 2013). In Table 4, it can be seen that the administration of OODEV® did not show a significant effect on the middle piece area.

In contrast, the middle piece's length showed a significant effect ($P<0.05$). From the results obtained, it can be seen that K is significantly different from P1, P2, and P3, and when noticed, P2 shows that the average area and length from the middle piece is higher than the others. Table 4 shows that the average tail area at the lowest dose (P1) has a relatively low average value compared to the others. The same result happened to the tail length tail of the sperm of the P1 dose, whose tail length was significantly different from the control (K). This situation indicates an abnormality in the P1 dose. The tail length between K and P3 did not show a significant difference, but the P3 dose had the highest average tail length compared to the others. P2 dose, whose average tail length was not significantly different from P3 and K, had a higher average middle piece area than the others. This will undoubtedly affect the duration of movement (motility) of sperm from the P2 dose, which will be discussed in the next section of this manuscript.

Overall, the different doses of OODEV® showed a significant average ($P<0.05$) on total sperm length. If sorted by average, the longest to shortest sperm based on the dose, respectively, are P3, P2, P1, and followed by K. As has been mentioned before, OODEV® is a hormone that contains FSH and LH; in short, this hormone can induce the formation of testosterone as well as 11-ketotestosterone (11-KT). According to Wahyuni *et al.* (2019), the more mature a sperm, the longer the sperm morphometry. Therefore, the sperm at the P3 dose can be considered the sperm with the highest maturity. Wahyuni *et al.* (2019) also added that in aceh cow, testosterone plays a vital role in sperm maturation in the corpus and cauda, while testosterone in the head of the epididymis will change to dihydrotestosterone (DHT) with the help of the 5α-reductase enzyme. DHT will be beneficial in the maturation of sperm. In this process, testosterone indirectly binds to various cells with androgen receptors (AR) to produce various proteins that support sperm maturity. Administration of 11-KT and DHT to the fathead minnow (*Pimephales promelas*) can increase testicular maturity and reach various advanced stages of spermatogenesis at a young age. However, the administration of DHT has more potential in the process of spermatogenesis compared to 11-KT. This could be due to the stronger binding affinity of DHT to AR compared to 11-KT to AR.

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Table 5. Motility of sperm after different treatments with OODEV®.

OODEV® Dose (mL/Kg)	Motility Categories; $\bar{x} \pm sd$ (%)				Movement duration; $\bar{x} \pm$ sd (minute)
	1	2	3	4	
K	84.745±9.780[a]	2.481±1.430[ab]	0.454±0.024[a]	8.301±4.556 [ab]	5.7±2.335[b]
P1	94.000±0.617[a]	3.382±1.543[a]	0.399±0.053[a]	2.443±1.442 [bc]	2.8±0.908[c]
P2	90.976±6.059[a]	3.257±2.455[a]	0.398±0.425[a]	0.457±0.430[c]	6.2±1.824[b]
P3	83.810±9.590[a]	0.962±0.326[b]	0.438±0.333[a]	13.735±8.707[a]	9.0±2.646[a]

Description: Dose of OODEV® K: 0 mL/Kg, P1: 0.25 mL/Kg, P2: 0.5 mL/Kg, and P3: 0,75 mL/Kg. Average values with different superscripts show significantly different averages among doses.

In Table 5, it can be understood that motility in categories 1 and 3 did not show a significant difference (P>0.05), and vice versa in categories 2 and 4 (P<0.05). Motility in category 2 showed a vibrating-like movement with significantly different average results at P3. This significant difference is due to the relatively small average value of category two at the P3 dose compared to the other doses. The best motility is category 4, which shows progressive movement. When viewed from the average value, it can be seen that P3 shows the highest value compared to the other doses, while P2 is significantly different from P3, which shows the lowest average value. This indicates that OODEV® plays a role in increasing sperm motility.

The low quality of motility at P2 can be supported by previous data, where the abnormality of sperm morphology is relatively high compared to P1 and P3, which were also given OODEV®. According to the movement duration, the administration of OODEV® hormone administration showed significant results (P<0.05). P3 and P2 showed an excellent average value of movement duration compared to the control (K). P2 has the middle piece area with the highest average among the other doses. Such conditions then support the duration of sperm movement at P2 doses. However, P2 has high morphological abnormalities that affect the percentage of motility categories.

The increase in sperm motility and duration is closely related to the composition of OODEV®, which has both FSH and LH gonadotropin properties. By previous research conducted by Miura *et al.* (1992), gonadotropins in salmon (SGA) can increase the production of 17α, 20β-DP (DHP). The presence of DHP can improve the quality of motility by increasing the pH of the seminal fluid of salmon from 7.4 to 8.0. This increase was also supported by increased cyclic adenose 3'-5' monophosphate (cAMP).

CONCLUSION

In conclusion, P3 had the highest GSI value; this dose improves sperm quality without affecting sperm viability. This increase in quality is supported by lowering the level of abnormalities and the highest average tail length, accompanied by the second most expansive average area of the middle piece. This situation then affects sperm motility in progressive movements, evidenced by the most extended duration of sperm movement.

CONFLICT OF INTEREST

No conflict of interest exists among the authors who contributed to writing and publishing this manuscript.

AUTHOR CONTRIBUTION

Slamet Widiyanto and Bambang Retnoaji guided this research until it is published.

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