JLSB
Journal of Life Science and Biomedicine

J. Life Sci. Biomed. 3(6): 444-449, 2013
2013, Scienceline Publication
ISSN 2251-9939

Original Article

Exploration Laser Punctures Exposure Effect on Reproductive Point to Increasing Number of Leydig Cells Catfish (Clarias sp.)

Vyona Mantayborbir*, Mohamad Fadjar, Agung P. W. Mahendra

[1]*Graduate student of Faculty Fisheries of Brawijaya University, Lecturer Faculty Fisheries of Brawijaya University, Lecturer Faculty of Mathematics and Natural Sciences of Brawijaya University, Jalan Veteran, 65145, Indonesia*

*Corresponding author's e-mail: vyonamantay@ymail.com

ABSTRACT

The main problems faced by in farmers catfish is still conventional of cultivation method is they based on the by nature conditions of the reproductive cycle. For reach adult size catfish male takes short time of about 8-12 months. Therefore, required the application of appropriate technology for accelerate provision of channel catfish brood stock and seed mass and continuous. Excitatory laser technology as a biostimulasi reproduction is a relatively new approach and has been shown to stimulate gonadal maturation and spawning types of female tilapia and catfish (Clarias sp). This indicates that the laser exposure can increase the performance of a hormone that plays an important role in the control of the reproductive system. The purpose of this study is: To determine the level of gonadal maturation, increased number of Leydig cells, determine sperm quality (motility and viability), and for determine the fertilization of sperm and egg hatching (hatching rate) male catfish (Clarias sp) which is exposed to the frequencies laser puncture exposure is different compared to control (no treatment). The experiment was conducted in April and July 2013 in the Laboratory of Reproductive and Breeding Fish Hatchery, Faculty of Fisheries and Marine Sciences, University of Brawijaya. This experimental study consisted of two treatments: control and treatment with exposure laser puncture in 2/3 part of the parent vessel governoer male catfish for 15 seconds with 3 replications. Laserpuncture treatment, each male parent catfish from each treatment was given maximum exposure laser puncture with power 5 mW and a wavelength of 632.8 nm. Frequency of exposure varied between treatments laser puncture the first treatment frequency laser puncture 1-week exposure, frequency of exposure laser puncture II treatment once every two weeks, and the frequency of exposure treatment III laser puncture one time in 1 month. To control the parent male catfish laser puncture maintained without treatment exposure. Data were analysed descriptively and diversity analysis or test F. If the F values were significantly different or significantly different then tested LSD (Least Significant Difference) and continued regression of the data for relationships between variables. Frequency of exposure laser puncture best for speed up the process of gonadal maturation, increasing the number of Leydig cells, motility and viability of sperm is laser puncture exposure frequency is once a week. Within one month has reached TKG IV, the number of Leydig cells 60.2%, 43% sperm motility and viability of sperm 89%. When compared with two weeks of exposure frequency, exposure frequency of one month, testis empty (post-spawning), and control (no treatment). After are exposed laser puncture sperm produced has proven capable for fertilization and egg hatchability. With an 86.2% fertilization results and 84.2% hatchability of eggs. The conclusion of this study is laser puncture can be used as an alternative to accelerate and to greater male reproductive system catfish (Clarias sp).

Keywords: Laserpuncture, Catfish Clariassp, Reproduction Acupoints, Biostimulation

INTRODUCTION

Laser puncture technology is an acupuncture point stimulation technique by using a laser as a tool that has a stimulatory effect. Effect caused by laser beam called laser beam elektrobioluminsencel which means the cells electrically stimulate tissue [1].

In a study conducted by Kusuma [2, 3], has been able to find the point of reproduction and reproductive cycles in female black tilapia varieties GIFT (Genetic Improvement of Tilapia Farmer). Laser exposures of the fish for 6 seconds to the point of reproduction on 2/6 ventral body (governor vessel) can be optimally affect gonad maturity stages (GMS), the GMS IV. Hariani study (2009) showed that catfish (Clarias sp) in the control group they in GMS I in the past 10 weeks, while in the group exposed to the laser for 15 seconds in 2/3 of the body ventral (governor vessel) for optimally affect GMS GMS IV reached 15 days after exposure to the laser was [4]. This

indicates that the laser exposure can increase the performance of a hormone that plays an important role in the control of the reproductive system (Kusuma, 2012) [5].

The purpose of this study is: To determine the level of gonadal maturation, increased number of Leydig cells, determine sperm quality (motility and viability), and for determine the fertilization of sperm and egg hatching (hatching rate) male catfish (Clarias sp) which is exposed to the frequencies laser puncture exposure is different compared to control (no treatment) [6].

MATERIALS AND METHODS

Animals used in this study are the parent catfish (Clarias sp) that mature gonads, aged 8-9 months. Total of 20 males and 20 females samples were used in this research. Female fish was only used for immunization processes including spawning, with the aim that the treatment of laser puncture FUNDS exposure to the condition of spawning male parent (Gonad who was empty).

Maintenance Parent

Parent male and female catfish (Clarias sp) is selected and mature gonads, adapted separately in the tub for 14 days for avoid spawning before treatment is given. During maintenance, the fish fed with fish feed types Pokphan 782 with 36% protein content and is given every day in the morning and evening around 5% body weight .

Research Tools

The tools used in the study is a soft laser unit, tub maintenance, dissecting sets, syringes, plastic bowls, microscopes, petri dishes, flasks, pipettes, heater. And water quality instruments (pH meters, DO meters).

Work Procedures

Shooting Laser Treatment

Laser puncture exposure in 2/3 parts governoer vessel for 15 seconds. With exposure frequency varies between treatments laserpunktur i.e. first treatment frequency exposure laserpunktur 1 week for 1 month, treatment exposure laserpunktur II twice in 1 month, and III treatment exposure laserpunktur one time in 1 month. For the control the parent-parent male catfish reared in tubs laserpunktur maintenance without treatment .

Histology Making

Every male tail catfish for be taken gonadnya then dissected. Fixation into the gonads in Bouin solution for histological preparations was then made. By using the light microscope histological picture obtained microscopic morphology and abundance of Leydig cells from spermatogonia network in catfish .

Dilution Motility

Sperm samples were taken 10 ml included in the Eppendorf. As much 990 ml of water mixed into the Eppendorf contains 10 ml of sperm (comparison between samples with a solution of 1:9 dilution), Stirred using a micropipette until completely homogeneous. Sperm is a sperm diluted with dilution of 1000 X .

Testing Sperm Motility

Sperm that has been diluted 1000x taken using pipette. Sperm dripped on glass objects. Covered with a cover glass and observed using a microscope. Movable or immovable, is determined by the percentage motility.

Testing Sperm Viability

Sperm sample was diluted 1000 X taken as many as 10 mikrolit hatched on preparations. Added 10 mikrolit dyes (eosin) (comparison between samples with a solution of 1:1 dilution). Touched a drop of sperm and eosin using the other end of the object glaas, sperm droplets leveled with another glass object was away from the drop point. Spermatozoa smears allowed to air dry for 5 minutes. Observed with a microscope, spermatozoa sought. Normal spermatozoa and abnormal spermatozoa drawn. Spermatozoa counted in 5 different visual field. The percentage of live and dead sperm is determined .

Fertilization

Fertility is the ability of sperm is able to fertilize eggs for fish. In the process of fertilization occur spermatozoa merging with the core nucleus in the cytoplasm of the egg to form a zygote [8]. Fertilized egg is not fertilized and is calculated by counting the percentage of fertility with the following formula:

$$Fertility = \frac{\sum Fertilized\ egg}{\sum Total\ egg}\ x\ 100\%$$

Hatching Rate (HR)

Hatching Rate (hatchability) shows the percentage of initial egg fertilization until the eggs are hatched. Hatchability of eggs can be calculated using the following formula:

$$HR = \frac{\sum hatching\ eggs}{\sum egg\ fertilization\ initial}\ x\ 100\%$$

Data Analysis

Data were analyzed descriptively that include morphological observation of gonad maturation and anatomy, and an increase in the number of Leydig cells. And presented in the form of graphs and photos microscopic picture of gonadal morphology and number of Leydig cells in the visual analysis of histological samples and test variability or F. If the F values were significantly different or significantly different then tested LSD (Least Significant Difference) for determine which provide significantly different treatment and continued regression of the data for relationships between variables.

RESULTS AND DISCUSSION

Exposure role Laserpunktur on Point to Reproductive Development Maturity Level Males gonads Catfish (Clarias sp). Of research findings about bio stimuli lasepunktur the parent for see catfish male morphological appearance of testes (gonads) can be seen in (Table 1).

These results indicate that the reproductive cycle of the male parent catfish laserpunktur that are exposed at the point of reproduction with a frequency exposure once a week shorter than the fortnightly frequency, frequency of once a month, or to a control group.

At the point of reproduction (governoer vessel) found many active cells have a low resistance properties and high potential. That is, if the cells are getting bio stimuli of the laser, then the cell will undergo a process of cell polarization, ion regulation and formation reactions also occur Adenosine Tri Phosphate (ATP) which is distributed intracellular and eventually lead to potential changes in other active cells to target cells (gonads) in the form of Energy [7]. Furthermore cAMP stimulates mitochondrial cristae tubular section to encourage the conversion of cholesterol into gonadotropin hormone (GTH), this hormone consisting of GTH I and GTH II of the anterior pituitary. Gonadotropin hormone (GTH) and then into the blood flowed to induce final gonadal maturation through simulation to synthesize the maturation of steroid hormones (such as testosterone and estradiol hormone) in the ovaries or testes, and influence the development of secondary sex [8].

Exposure role Laserpunktur in Reproductive point to Total Leydig cells Catfish (Clarias sp). Number of Leydig cells obtained can be seen in Table 2. Test results using One Way ANOVA Data Leydig catfish spermatozoa in Table 3 shows that the testis is empty, control and treatment gave a significantly different effect on catfish Leydig cells. It is based on p-value (Table 3 in the column Sig. Amounted to 0.001) is smaller than α (0.05) so that H0 is rejected and we can conclude the control and treatment have different effects on Leydig cells of the catfish. Table 4 shows that the blanks and control testes with 1-week exposure frequency was significantly different to each other, whereas the empty testes and controls the frequency of exposure 1bulan once while the frequency of exposure of 2 weeks with 1-week exposure frequency gives the average fish Leydig cells catfish were not significantly different. Leydig highest average obtained at a frequency of 1-week exposure laserpunktur by 60.2%, while the lowest average was found in testicular Leydig vacant by 2.4%.

Table 1. Determination of Levels Kemantangan gonads Catfish (Clarias sp) based on morphology Appearance testes

Parent treatment Catfish Males	GMS	Morphology
Control group (witho treatment laser puncture)	II	¼ gonad filling the body cavity. The color is gray or white, flat shape
Laser group		
Frequency Shooting 1 week	IV	¾ fill the body cavity of the gonads. Gonadal white
Frequency Shooting 2 weeks	III	Gonadal ½ fill the body cavity. white
Frequency Shooting 1 month	III	Gonadal ½ fill the body cavity. white

Table 2. Number of Leydig cells

Treatment	Fish			Total	Average (%)
	1	2	3		
Empty (Post-spawning)	11	14	11	36	2,4
Control (without treatment Laserpunktur)	30	28	33	91	6,06
Frequency Exposure once a week	400	232	271	903	60,2
Frequency Exposure of two weeks	283	283	176	742	49,46
Frequency. Exposure once a month	81	47	44	172	11,46

To cite this paper: Mantayborbir, V., Fadjar, M. Mahendra, A.P.W. 2013. Exploration Laser Puncture Exposure Effect on Reproductive Point to Increasing Number of Leydig Cells Catfish (Clarias sp). *J. Life Sci. Biomed.* 3(6): 444-449.
Journal homepage: http://jlsb.science-line.com

Table 3. One Way ANOVA Treatment of Leydig cells Catfish

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6582.960	3	2194.320	18.323	0.001
Within Groups	958.080	8	119.760		
Total	7541.040	11			

Table 4. Test results for LSD Leydig cells Catfish

Treatment	Average	Notation
Empty (Post-spawning)	2,4	A
Control (without treatment Laserpunktur)	6,07	A
Frequency Exposure once a week	11,47	A
Frequency Exposure of two weeks	49,47	B
Frequency. Exposure once a month	60,20	B

From the results obtained it turns out laserpunktur exposure with exposure frequency of once a week Leydig cells produce a number of pretty good compared to other treatments. Number of Leydig cells are pretty good at the exposure frequency of once a week allegedly due to information received after dipapari laserpunktur then following through meridian points through two channels, namely ionotropic pathways and pathways simultaneously metabotropic. Ionotropic path that the information obtained is received by a collection of cells that are active throughout the body surface of living things which will then communicate with other similar active cell and to be associated with target organ. Active cells will undergo the process of cell polarization, ion regulation and also occurs on the mitochondrial ATP-forming reaction into electrical energy in the form of electron flow, then distributed intracellular. While metabotropik through the information received by means of the senses and of keotak forwarded here passed through the nerves to the hypothalamus which is located under the center of the brain. The hypothalamus receives stimuli from the central nervous system (brain) will issue a release factors (GnRH) to stimulate glands Hypophysa (Adenohypophysa) that secrete FSH and LH. Gonadal hormones FSH will go (cyste seminiferi) to accelerate the process of spermatogenesis and produce inhibin which suppresses the hypothalamus and adenohypophysa. While going towards the gonadal LH (Leydig cells) to spur spending testosterone (androgen) coming stimulate the development of secondary sex organs, and can increase sexual behavior (libido) which will be fed back into the work process hypothalamus.

Exposure role Laser punctures At Point to Sperm Quality Reproduction Catfish (Clarias sp).
Sperm motility Catfish (Clarias sp) Post Exposure Treatment Laserpunktur.

Test results using One Way ANOVA Data catfish sperm motility in Table 6 shows that the control and treatment gave a significantly different effect on sperm motility catfish. It is based on p-value (Table 6 in the column Sig. Amounted to 0.070) is smaller than α (0,1) so that H0 is rejected and we can conclude the control and treatment have different effects on motility of spermatozoa from the channel catfish at 10% confidence level.

Table 5. Sperm Motility (%)

Treatment	Replay			Total	Average
	1	2	3		
Control (without treatment Laserpunktur)	25	25	25	75	25
Frequency Exposure once a week	50	30	50	130	43
Frequency Exposure of two weeks	40	30	30	120	33
Frequency. Exposure once a month	30	40	40	110	36

Table 6. One Way ANOVA Treatment of Catfish Sperm Motility

Item	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	522.917	3	174.306	3.486	0.070
Within Groups	400.000	8	50.000		
Total	922.917	11			

Table 7 shows that between controls, the frequency of exposure of 2 weeks and 1 month gives the average sperm motility catfish are not significantly different, whereas among controls, the frequency of exposure of 2 weeks and 1 month to 1-week exposure frequency gives average the average sperm motility was significantly different catfish.

From the data obtained also turns motility motility rate of fish that are exposed to the laser frequency exposure laserpunktur once a month higher than the frequency of exposure two weeks. This is presumably due to changes in cell volume alter intracellular ion concentrations can also affect sperm motility activation. In some fish, sperm motility is activated by an increase in pH, ionic calcium and cAMP, which is suppressed by the low pH in

the epididymis [9]. Laser puncture is an electromagnetic wave that can cause inhibition and biostimulasi biological networks, such as cellular aktiitas increases, the regeneration of both central and peripheral nerve and hormone and enzyme production [10], as it also affects the Trans membrane ion transport and membrane polarization change cell [11]. From the results of this study laserpunktur causes a series of reactions that can speed up the process of gonadal maturation and the highest increase sperm motility 43% catfish (Clarias sp).

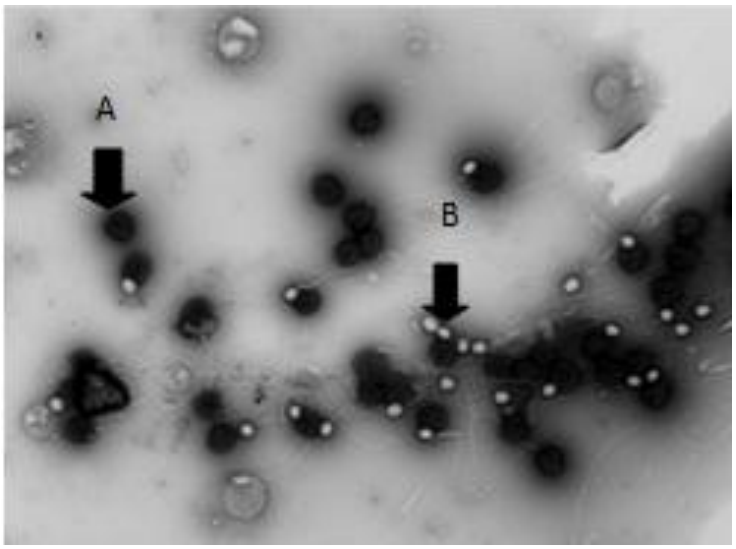
Table 7. Test results for the LSD Catfish Sperm Motility

Treatment	Average	Notasi
Control (without treatment Laserpunktur)	25.000	a
Frequency Exposure once a week	33.333	a
Frequency Exposure of two weeks	36.367	a
Frequency. Exposure once a month	43.333	b

Sperm viability Catfish (Clarias sp) Post Exposure Treatment Laserpunktur

Test results using One Way ANOVA catfish spermatozoa viability of the data in Table 9 shows that the control and treatment gave a significantly different effect on the viability of spermatozoa catfish. It is based on p-value (Table 9 in the column Sig. Amounted to 0.062) is smaller than α (0, 1) so that H0 is rejected and we can conclude the control and treatment have different effects on the viability of spermatozoa of catfish.

From the results obtained it appears that the frequency of exposure laserpunktur influence on sperm viability. The shorter the exposure time eating the higher value of viability. Sperm membrane permeability is closely related to the viability of spermatozoa because as is well known membrane permeability is associated with transport of nutrients essential role in cell metabolism. Added by Jones and Stewart (1979) in Rustidja, (2000) that changes in the infrastructure in the plasma membrane, the loss of several mitochondrial matrix and decrease the electron density of the mitochondrial matrix leads to loss of viability of spermatozoa [12]. From the results of this study laserpunktur exposure frequency of once a week led to a series of reactions that can improve sperm viability catfish (Clarias sp) reached 89%.



Gam bar 1. Spermatozoa using eosin staining. Arrows (A) is a dead sperm and an arrow (B) is a live sperm

Table 8. Sperm Viability (%)

Treatment	Replay			Total (%)	Average (%)
	1	2	3		
Control (without treatment Laserpunktur)	79,5	70	76,3	225,8	75,2
Frequency Exposure once a week	90,5	96	80,5	267	89
Frequency Exposure of two weeks	81,5	91,5	85	258	86
Frequency. Exposure once a month	78	82	83	243	81

Table 9. One Way ANOVA Treatment of Catfish Sperm Motility

Item	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	326.010	3	108.670	3.688	0.062
Within Groups	235.727	8	29.466		
Total	561.737	11			

Table 10. Test results for the LSD Catfish Sperm Viability

Treatment	Average	Notasi
Control (without treatment Laserpunktur)	75.267	a
Frequency Exposure once a week	81.000	ab
Frequency Exposure of two weeks	86.000	b
Frequency. Exposure once a month	89.000	b

Power fertilization with sperm that are exposed laserpunktur exposure frequency of once a week.

Table 11. Sperm Fertilization Power

Treatment	Replay				Total (%)	Average (%)
	1	2	3	4		
Frequency Exposure once a week	81	96	77	91	345	86,2

Table 12. Hatchability of eggs

Treatment	Replay				Total (%)	Average (%)
	1	2	3	4		
Frequency Exposure once a week	86	84	78	89	337	84.2

From the data in Table 11, note that the average percentage of sperm fertilitasi power that has been given exposure laserpunktur, seen a decline is not too high of a number of fertilized eggs. Revealed that the success of fertilization of the egg by the sperm is influenced by sperm motility [13]. Term viability of the state may not be able to produce high fertilization, because in this situation so requires a lot of energy spermatozoa to fertilize an egg. Hatchability of eggs (Hatching Rate) that are exposed laserpunktur with exposure frequency of once a week.

Hatchability of eggs in addition influenced by factors such as hormones and volume in the egg yolk is also influenced by external factors such as salinity, temperature, and pH [14]. Results of high hatchability are believed to be related to the level of sperm motility and viability was pretty good in the amount of 43% and 89%. Motility and viability levels that can cause fertilization pretty good so it is likely that they use the whole egg at fertilization process can be fertilized and hatch in high quantities.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

1. Cierezsko, A., K. Dabrowski., B. Piros., M. Kwasnik and J. Glogowski. 2001. Characterization of zebra mussel (Dreissena polymorpha) sperm motility: duration of movement, effects of cations, pH and gossypol. Kluwer Academic. Netherlands. Hydrobiologia. 452:225–232.
2. Didik, S. 2010. Endokrinologi Reproduksi. Ed ke 1. Jakarta: Obgynmag Press.
3. Eni. 2007. Pengaruh Lama Penembakan Soft Laser Diode Terhadap Pematangan Gonad Ikan Lele Dumbo (Clarias gariepinus). Skripsi. Tidak dipublikasi. Universitas PGRI Adibuana Surabaya. Surabaya.
4. Faqih, A.R. 2011. Penurunan Motilitas dan Daya Fertilitas Sperma Ikan Lele Dumbo (Clarias spp) Pasca Perlakuan Stress Kejutan Listrik. J.Exp. Life Sci. Vol. 1 No. 2, Feb 2011.
5. Gusrina. 2008. Budidaya Ikan Jilid 1. Jakarta: Pusat Perbukuan Departemen Pendidikan.
6. Hariani, D dan P.S.W. Kusuma. 2009. Biostimuli Reproduksi Ikan Lele Dumbo (Clarias gariepinusi) Betina Dengan Penembakan Laserpunktur. Berkas Peneletian Hayati Edisi Khusus.
7. Kusuma, P.S.W., A.P.W. Marhendra., Aulanni'am. & Marsoedi, A. 2012. Mechanism of Gonadotropin Hormone Release in Catfish (Clarias Sp.) upon Laser punctures Exposure to Reproduction Acupoint. International Journal of Basic & Applied Sciences IJBAS-IJENS Vol: 12 No: 06.
8. Kusuma, P.S.W. 2000. Pengaruh Penembakan Soft Laser He-Ne Terhadap Siklus Reproduksi Ikan Nila. Thesis. Tidak dipublikasi. Program Pascasarjana Universitas Airlangga. Surabaya.
9. Kert, J. dan Rose, L. 1989. Low Laser Therapy. Scandinavian Medical Laser. Technology. London.
10. Rustidja. 2000. Penggunaan Sinar Laser Untuk Mempercepat Kematangan Gonad Ikan Nila. Fakultas Perikanan Universitas Brawijaya. Malang.
11. Sukarta. 1992. Penggunaan Laser untuk Akupunktur. Penerbit PAKSI DPD Surabaya Jawa Timur. Meridian Indonesia, J. Acupunture. I: 49-54.
12. Saputra, K. 1997. Titik Akupuntur Sebagai Kumpulan Sel Aktif Listrik. Meridian 4: 80-87.