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SEAHORSE (*HIPPOCAMPUS SP*): OPPORTUNITIES IN ASSISTED REPRODUCTION WITH NANOTECHNOLOGY APPROACH

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ABSTRACT

Seahorse (Hippocampus sp) is the unique fish, because the male is pregnancy. The seahorse not only given an economic value but also have a potential drug for medical use, which is have a steroid an aphrodisiac for fertility. The previous studies have reported the function seahorse extract and have been studied both in vitro and in vivo to know the effects from the natural product. Nowadays, infertility caused many factors, such as a factor from the male including spermatogenesis and testis disorder. In decade, there is the treatment for infertility used GnRH therapy, but it still controversy between the impact in reproductive system and needed the study. GnRH in vertebrate has a G receptor from kisspeptin gene to transcription promote and suggested a key regulator reproductive with a kiss 1 gene, which is the same function in mammals and fishes. In this condition, it is suggested a bioactive compound in seahorse can be investigated to replace a treatment infertility in male with GnRH therapy. It can be done through drug delivery systems with several carrier systems, such as nanoparticles. In the future, bioactive compound from seahorse needed the study to explore a potential drug in medical use including the efficiency and efficacy for fertility in human with nanotechnology approach.

Keywords*: Fertility, GnRH, Kisspeptin, Nanotechnology, Seahorse (Hippocampus sp)*

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INTRODUCTION

Indonesia is an archipelago country and have more biodiversity, including the seahorse. The marine aquaculture centers have a cultivated this animal, because they have high economic value which is made Indonesia as one of the largest exporting countries in Asia (Nasution et.al, 2019; Wang et.al, 2020).

Seahorses used in traditional medicine which is have a several effects, such as increasing stamina and hematopoesis, anti-fatigue, anti-oxidant, anti-tumor, anti-inflammatory, anti-aging, and important for fertility because have an aphrodisiac effect (Adam et.al, 2014; Kim et.al, 2016; Nasution et.al, 2019; Safryna, 2020; Sanaye et.al, 2014; Wang et.al, 2020; Zhang et.al, 2017).

The function in fertility, it is suggested that bioactive compounds is steroid testosterone, which in animal tissue it is usually used in aphrodisiacs for male. The function of this hormone is to stimulate the spermatogenesis, increasing the development and activity from genital organ (Safryna et.al, 2020). In addition, for pharmacology this hormone can be effect hypothalamus to release Gonadotropin Releasing Hormone (GnRH) in reproductive system (Zitzmann et.al, 2013).

Nowadays, one of the health problems in Indonesia is infertility, which is one of the factors from the male. Male infertility influences from many factors, including from spermatogenesis and testis disorder (Li et.al, 2020; Zitzmann et.al, 2013). Nowadays, the treatment for infertility used GnRH therapy, which is used to influences the maturation of cells, and increasing testosterone levels, and increasing the germ cells (Biers, 2010; Li et.al, 2020).

GnRH therapy can use an analogues GnRH (GnRHa) which is reported that 3100 patients through 50 publications given the GnRHa. It is still controversy between the impact in reproductive system and needed the study (Li et.al, 2020; Schwentnr et.al, 2005). It is caused by several things, such as a large doses and long periods, and it can make a mutation in gene receptor GnRH when the therapy is failure (Zitzmann et.al. 2013).

Seahorse have an active hormone steroid testosterone from natural product which is suggested an alternative therapy for GnRH. Furthermore, the bioactive compound steroid in seahorse can be optimally to target organs, which is the reproductive system. To evaluate the function of bioactive compound, in this decade it can be using modern science and technology. It can be done through drug delivery systems with several carrier systems, such as a nanoparticle (Schwentner et.al, 2005). The target organ achievements by delivery through nanoparticles are evaluated using transmission electron microscopy (TEM).

Seahorses (Hippocampus sp)

Seahorse is one of the unique fish, because not only about the body's morphology, it is also the male is pregnancy (Blumenfeld, 2019). The female will give the eggs to male individu and spermatids will be included in the pore that fertilization occurs (Zhang et.al, 2017). The Male will incubate the eggs in the anteriomesial /central pore near the abdomen (Koldewey, 2010).

The species of seahorses in Indonesia are Hippocampus barbouri, H. comes, H. histrix, H. kelloggi, H. bargibanti, H. spinosissimus, H. trimaculatus and H. kuda (Meikasari et.al, 2020). Seahorse is a natural product and promising used to be the potential drug for medical use (table 1) (Chen et.al, 2014; Kumaravel et.al, 2012; Meikasari et.al, 2020).

Table 1. Bioactive Compound in Seahorse for Medical Used

No	Bioactive Compound	Medical Used	Ref
1	Amino acids	Anti-inflammatory, inluence the histology vesicular seminalis	1, 9
2	Protein	Increasing Hb	3
3	Alkalase and pepsin	Increasing testosterone and weight testis	6
4	Steroid, taurine	Aphrodisiac	7
5	Amino acid, teroid, fatty acid, microelement	Potential to healthy product	17
6	Steroid, cholesterol	Fertility	19
7	Steroid and fatty acid	Treatment BPH	20
8	Steroid and saponin	Vitality and immune respone	21
9	Trace element	Medical used	22

Gonadotropin Releasing Hormone (GnRH)

GnRH is decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2) produced from hypothalamus as the main hormone reproductive in Hypothalamus-Pitutary Gonad axis (HPG Axis) in vertebrate (Gopurappilly et.al, 2013; Jin, 2014; Sakhteman et.al, 2016). GnRH hormones has 30 isoforms, 15 in vertebrates and 15 invertebrates (NCBI, 2020). GnRH influences the spermatogenesis, steroidogenesis, and feedback mechanism in gonads. In mammals, it still consists GnRH1, GnRH2 and GnRH3, but only the GnRH1 and GnRH2 have the same amino acids in vertebrate. In humans, GnRH have a similar name as protein a progonadoliberin. It is located on chromosome 8p.2-p.21 with 276 bp which is translates from 92 amino acids (Jin, 2014; Sakhteman et.al, 2016). This gene encodes to stimulate the release of LH and FSH, and when this gene has a mutation it can be caused a hypogonadotropic hypogonadism in human (Uniport, 2020). GnRH in seahorse has a similar name protein as a gonadoliberin (NCBI, 2020).

GnRH has a G protein receptor which is transmembrane proteins to regulating fertility (Jin, 2014; Sakhteman et.al, 2016). GnRH receptors can be found in humans and the teleost such as seahorse. In vertebrate, HPG Axis will be expressing a various GnRH. In mammals it carried out by GnRH in hypothalamus which binds the G receptors stimulate to release a gonadotropin, FSH and LH in gonad (Zhang et.al, 2018). Nowadays, there is an G receptor from Kisspeptin gene as a transcription promote of the GnRH (Jin, 2014; Sakhteman et.al, 2016).

Structure of GnRH in human and seahorse have a different structure from swissmodel.expasy tools (fig.1), but they have the same Kisspeptin gene which is regulate the hypothalamus in vertebrate (Zhang et.al, 2018).

The Kisspeptin gene is also found in the teleost group of fish, such a seahorse which is a key regulator of reproduction and sensitive with steroid (Gopurappily et.al, 2013; Zhang et.al, 2018) Most teleost fishes have kiss 1 and kiss 2, furthermore in mammals there is only one Kisspeptin gene (kiss 1). Kisspeptin gene in seahorse directly regulates GnRH, although it is needed the study. However, the previous study reported the function is the same with mammals as an activator the reproductive axis through the stimulation of GnRH secretion (Zhang et.al, 2018).

The treatment for male with infertility is being developed, for example with testosterone therapy. One of them is with GnRH therapy. But now, several studies were reported have an several problems. There are about the uncomfortable in patients, cost therapy is too high, long period, and influenced from antibody from each individual (Zitzmann et.al, 2013).

Zhang et.al, (2018) reported that in teleost, there is still widely underexplored, because the Kisspeptin is still debate, but the previous study suggests that fish Kisspeptin have a similarwith mammalian Kisspeptin, which is the key to activator the reproductive axis. It can be investigated to be an alternative as a substitute therapy for infertility in male. But, the gene Kisspeptin between in humans and seahorse have a different structure from pymol tools (fig.2). In this condition, it is needed the study to answer the question is there a seahorse extract as a natural product can be a medical use for male infertility to replace a GnRH therapy, and it canbe a novel therapeutic targets and interventions.

The previous study has reported by Zhang et.al (2018) that Kisspeptin gene suggest involved the regulation in reproductive function of pubertal onset and gonadal development, and also in seahorse male pregnancy there is regulating testosterone synthesis. In assisted reproduction, study used next generation sequencing was found the Kisspeptin (kiss 1) suggest as a gene which is modulate hormone levels and reproductive outcome and can be a novel

therapeutics targets (Blasco et.al, 2020; Patel, 2020; Trevisan et.al, 2020). Another study by Hestiantoro et.al (2019) reported that Kisspeptin concentration was associated with postmenopausal because the serum concentration was lower. In the other study was reported, Kisspeptin was reported as a potential biomarker in across pregnancy and can be a future study in reproductive system such as for Polycystic ovarian syndrome (Hu et.al, 2019; Rodrigues et.al, 2019; Talbi, 2019). Khamis et.al (2019) also reported that undergoing Kisspeptin treatment as in vitro fertilization and approaches to increasing the reproductive role. Kisspeptin also reported by Oride et.al, (2020) that promising to increasing overcoming diabetic testicular dysfunctions which is enhancing spermatogenesis, as well as reducing the testicular inflammation and apoptosis. The fertility drugs may act on kiss 1 expressing neurons becauseit was modulating the HPG axis (Chenthamara et.al, 2019).

In this decade, the technology in science have been develop. There is a drug delivery system which is combine between a medicine and high technologies. When we want to know about the bioactive compound in seahorse can be use in medicine as a male infertility, one of them technology we can used the nanotechnology.

Nanotechnology in Reproductive System

Nanotechnology is the design, characterization, production and application of structures, devices and compilation systems between sciences and engineering with a nanometer scale (1-100nm) or one millionth of a meter (Albanese et.al, 2012; Brohi et.al, 2017).

Study about nanotechnology is conducted to determine the interaction of nanoparticles with biological systems is a nano-bio interaction. Nanoparticles (NP) interact with cell surface membranes. Once bound to the receptor, nanoparticles in the cell through endocytes mediated by the receptor (Falchia et.al, 2018).

Nanoparticles is synthesized from various organic or inorganic materials such as lipids, polysaccharides, metals, proteins, and synthetic or natural polymers. Formulation of the drug with nanoparticles are currently developing to increasing efficiency, prolongation of drug in circulation, targeting specific tissues, therapeutic reactions, and side effects or toxicity (Albanese et.al, 2012; Falchi et.al, 2018).

The effects of nanoparticles in cellular and tissue level are not clearly. They are some effect, that used in male and female reproductive systems at the clinical, cellular and molecular levels. In the male reproductive system, it is can be influences fertility with increasing the quality of sperms from in vivo or in vitro (Falchia et.al, 2018; Falchi et.al, 2018).

The previous studies by Safaa et.al, (2016) reported that function of nanoparticle in reproductive system, study about efficiency of NP cerium oxide (CeO2) can protect viability and increasing the motility of sperm. In the other study, they reported with addition of 5 µg/mL of vitamin E combined with 1% Nano-Se can be improve semen quality in chickens (Rezvanfar et.al, 2013). Study about supplementation from SeNPs can protect the quality of spermatozoa (motility, DNA integrity) and spermatogenesis from oxidative damage caused by anticancer agents in the male reproductive system (Saraf, 2010).

Nanoparticles is one of the technologies which is increasing the bioavailability of active substances in seahorses. It can be influences about the smaller size can increasing the delivery of active substances with low solubility because they contain lipids and cholesterol. Nanoparticles will be transferred into cells through endocytosis transitions. Nanonization of natural products provides benefits by increasing active substances, small therapeutic doses, and increasing absorption and bioavailability in the body (Kumar et.al, 2019).

To evaluating the mechanism from nanoparticle in human body, we can use a gold standar for this with a transmission electron microscopy.

Evaluation of Nanoparticles in Reproductive System

Transmission electron microscopy (TEM) is the most technique used to characterize nanoparticles, and to assess the safety and toxicological potential of a nanoparticle. TEM is a gold standard for nanoparticle because such as a reason, there are about the resolution in nanometer sizes in the range 1-100 nm, image display with atomic dimension, it can describe the physical properties quantitatively (size, shape, and surface morphology), and also can determine the agglomeration of a material (Lina et.al, 2013; Masta et.al, 2020).

In addition, TEM also has a limitation, because the analysis process depends on the transfer of representative fractions of samples containing sufficiently large amounts of particles to the specimen carrier (TEM grid) which is affected by the purification process and the concentration of the sample and the specimens must be very thin (Walther, 2017).

TEM produces an electron beam from transmitted to the specimen. The electron interacts with the specimen and transformed into a scattered electron. Electrons will be focused by electromagnetic lenses that are projected the screen to produce diffraction, contrast-amplitude images, phase-contrast images or various image variations (Hu et.al, 2019). Interpretation data from the TEM obtained interactions between electrons and matter, giving rise to a dynamic diffraction effect involving X-rays from high-voltage cathode tubes or synchrotron sources (Walther, 2017).

CONCLUSION

Seahorse (Hippocampus sp) as a natural product with bioactive compound steroid testosterone has the opportunities to investigate the further study as a substitute for GnRH therapy for male infertility, it is because the same structure and receptor protein in human and needed the study. The treatment can be evaluation with nanotechnology to know the efficient and efficacy to use in reproductive system.

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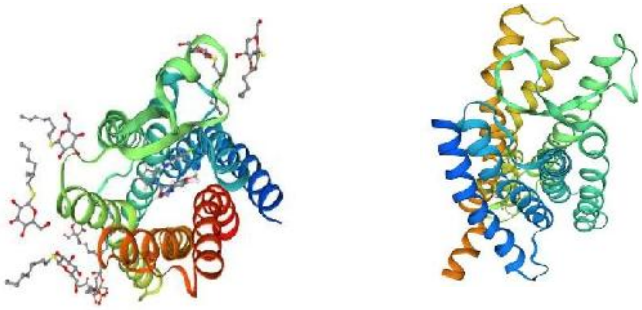
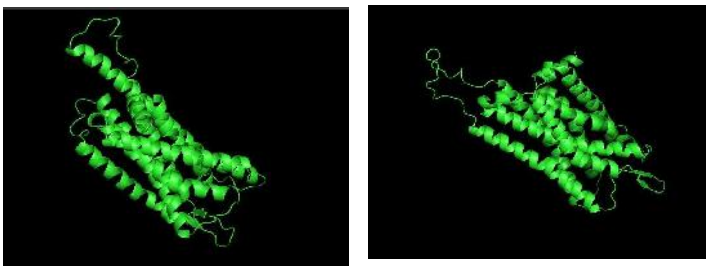
Figure:



A B

Figure 1: A. GnRH in Human, B. GnRH in Seahorse
Source: Swissmodel, 2020



A B

Figure 2: A. Kisspeptin Gene in Human, B. Kisspeptin Gene in Seahorse
Source: Pymol, 2020

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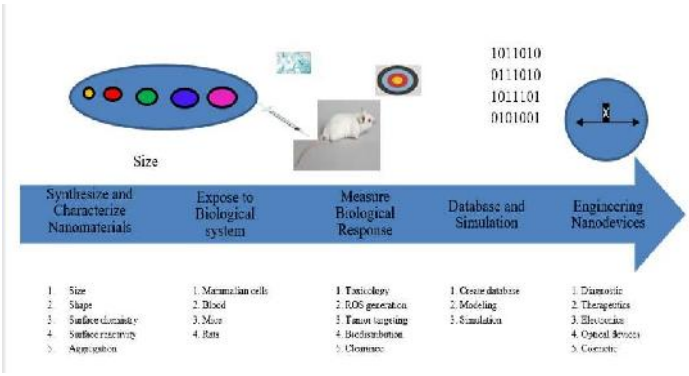

Figure 3: Overview the Nanoengineering Process
Source: Brohi et.al, 2017

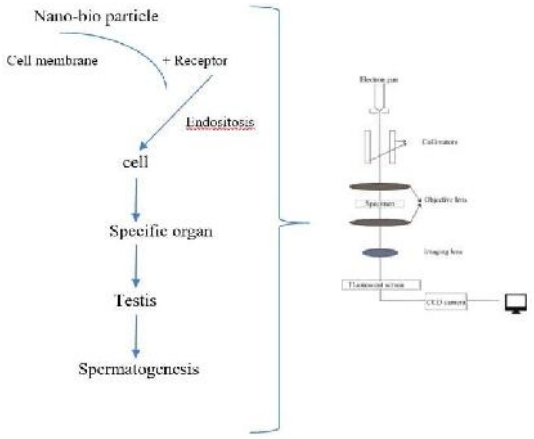

Figure 4: Schematic of TEM