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Research Article

The Effect of Sea Fish Powder on Development and Growth of Fetal Teeth Mice

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Abstract

Growth and development occur in human teeth, which have experienced growth and development since pregnancy. Teeth are formed from two types of cells: oral epithelial cells, which form the enamel organ, and dental papilla, which produce dentine and pulp. Pregnant women who lack nutrition will impact the growth and development of the fetus, including the teeth. Nutrition is an essential component of dental and oral health. The nutrients needed for dental and oral health are calcium, fluorine, phosphorus, and vitamin D. This study aims to determine the effect of sea fish powder on the development and growth of the fetal toothing parent mice. This research used a true experimental design with a post-test-only control design. Pregnant mice were divided into two groups: the control group, which was given distilled water, and the treatment group, which was given seafish powder. The mouse fetuses were taken at 18 days of gestation, and dentition was observed between the control and treatment groups. Histological preparations of the fetal teeth were observed using hematoxylin-eosin (HE) staining. Fetuses of mice in the treatment group showed tooth development conditions in the early bell stage, while fetuses in the control group showed the initiation stage. There were differences in the growth and development phase of the fetus in mice in the control group and the treatment group given sea fish powder. Providing sea fish powder can accelerate the process of odontogenesis in mouse fetuses.

Keywords: development growth; sea fish; teeth

INTRODUCTION

The gestation period is a period of growth and development lasting 40 weeks or nine months. Pregnancy can occur because a sperm cell fertilizes an egg after it is released from the ovary during ovulation.[1] The fertilized egg will go to the uterine wall, and the implantation occurs. Physiological changes experienced by pregnant women include increased uterine blood flow, fetal weight, plasma volume, and food reserves so that the body weight increases by at least 15 kg. During pregnancy, pregnant women will need additional nutrition and calories of 300 calories compared to before pregnancy.[2]

During pregnancy, the fetus in the stomach will continue to experience changes, such as the appearance of teeth. Teeth are formed from two types of cells: oral epithelial cells, which form the enamel organ, and dental papilla, which produce dentin and pulp.[3] Odontogenesis is the process of tooth formation, which begins in the 6th intrauterine week. The process of tooth development stages is divided into initiation (bud stage), proliferation (bud stage and cup stage), histodifferentiation

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(early bell stage), morpho-differentiation (late bell stage), and apposition.[4]

Early oral cavity development begins with ectodermal cells consisting of columnar cells that turn into squamous and stratified cells. Next, the tooth buds appear, as seen from the initial thickening of the oral epithelial cells called the primary epithelial band. The direct epithelial band is divided into two vestibular laminae, which lie buccally, and a dental lamina, which lies lingually. This process is called initiation and takes place between weeks 6 and 7.[5] The next process is the cup stage, marked by the enamel organs forming florets (cups). At the proliferative phase, differentiation and morpho-differentiation processes also occur; ectomesenchymal cells will condense to form dental papillae, which will form dentine and pulp. During the morpho-differentiation stage, cells are arranged to form the size and shape of the tooth. Histodifferentiation is the stage of cellular differentiation; ameloblast cells will differentiate into the enamel, and odontoblasts will differentiate into dentine. Next is the "bell stage," forming dental hard tissue. The last is apposition, characterized by the deposition of the matrix of the hard tooth structure. After the corona of the tooth is formed, the tooth is ready to penetrate the alveolar bone, but the root has not yet started. A tooth eruption usually occurs in babies who are born at the age of 6 months.[6] Pregnant women who lack nutrition will impact the growth and development of the fetus, including the teeth. Therefore, nutrition is an important component of dental and oral health.[7] Examples of nutrients needed for dental and oral health are calcium, fluorine, phosphorus, and vitamin B8. This study aims to determine the effect of fish powder on the stage of tooth development. The fish powder contains calcium, omega 3, and other proteins that can optimize the formation of inorganic materials and organic collagen during the prenatal period.[8]

MATERIALS AND METHODS

The post-test-only control design was used in this study of mice (*Mus musculus L.*) at various stages of tooth development. This study was divided into a seafish powder treatment group and an aqua dye control group. The population selected in the study was female mice (*Mus musculus L.*). The sampling design for pregnant mice was a simple random sampling by taking the giant fetus. The sample size used in this study was 14 female mice in each research group, aged 2-3 months and weighing 20–35 grams.

The research phase began with the acclimatization of test animals at the Sultan Agung Islamic University Biology Experimental Animal Laboratory for seven days before treatment, with certificate number 401/B.1-KEPK/SA-FKG/VIII/ 2022. Female mice aged 2-3 months were placed in a 40x30 cm cage, and the room temperature was set to range from 23–27ºC with light-dark cycles lasting 12 hours each. Feeding and drinking were carried out ad libitum. During acclimatization, the estrus cycle was determined by observing the results of vaginal swabs under a microscope. The mice used were observed for morphological appearance. Namely, the vagina was red and open. The smear method was carried out by rinsing a cotton bud with distilled water and inserting it into the vagina of the female mouse at an angle of 45°, rotating it 2-3 times, and then making a smear preparation. The smear preparation was placed in a 70% alcohol fixative solution for 5 minutes, then removed and air-dried. The practices were observed for the morphology of the epithelial cells using a microscope with a magnification of 100x and 400x. The mice experiencing the estrous phase were then mated by combining four female mice and two male mice in one cage in the afternoon and left for up to 12 hours. An observation of the vaginal plug was carried out the next morning, and if a plug was found, it indicated that the mice had mated and entered the 0^{th} day of pregnancy. The

pregnant mice were separated into separate cages. If the sample was not fulfilled, then the mice that were not pregnant were again mixed with male mice.

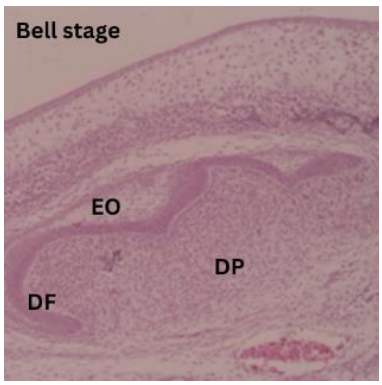
The next stage was grouping; all pregnant female mice were separated into separate cages and divided into 2 treatment groups, namely the control group, where six pregnant female mice were not given sea fish powder and six pregnant female mice were given fish powder. Furthermore, the stages of tooth development were observed on the 18th day of gestation.

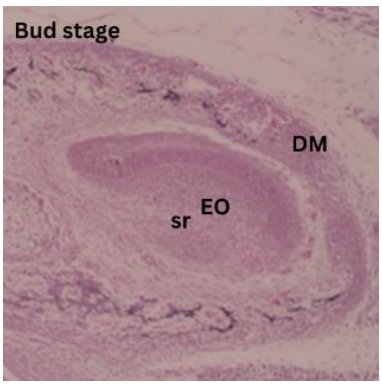
The dose of nanoparticles of sea fish powder given to mice was 2.17 mg. The materials used in this study consisted of dehydrated saltwater fish derived from sardines (*Sardinellafimbriata*), splendid ponyfish (*Leiognathussplendens*), and cobs (*Euthynnusaffinis*), an extraction of saltwater fish in powder form and emulsifier CMC (*Carboxy Methyl Cellulose*). The procedure for making fish powder comprised the following steps: weighing the fish according to dosage and dissolving it in hot water at 70°C. The saltwater fish powder was homogenized, crushed, and softened using an ultraturax device (IKA, Germany). 1% CMC material was stirred for about 15 minutes until homogenous. The calcium value of saltwater fish powder was one of 5.56% b/b using an ICP (Inductive Coupled Plasma) method, and the omega 3 level was 3.34% as measured utilizing gas chromatography. 10-20 ml of chloroform (Henan Haofei Chemical Co., Ltd, Indonesia) was used to euthanize the subjects. In group I, mice were only given food and distilled water ad libitum without adding seafish powder. In group II, mice were given food and drink ad libitum and seafish powder based on a dose of 2.17 mg dissolved in 0.5 ml of distilled water. The consumption of seafish powder was carried out orally in the morning (09.00 WIB) and in the afternoon (16.00 WIB) from day 0 to day 18 of pregnancy. Surgery on female mice was carried out using a set of surgical tools on the 18^{th} day of pregnancy. First, all mice in the control and treatment groups were anesthetized using chloroform. Furthermore, the mice underwent surgery on the abdomen to remove the fetus from the uterus. The fetuses were cleaned, and the heads were taken and then divided sagittally to take the tooth germs. The tissue was then put into a fixation solution containing 10% formalin. The container was labeled with the type of organ or tissue, collection date, animal species, and preservatives.

Furthermore, preparation and staining with hematoxylin and eosin (HE) allow visualization and imaging of tissue preparations to determine the condition of the preparation, which can represent the visualization of the histological presentation of the observed tissue and help in studying histology subjects in terms of body structure as well as those related to their function.

RESULT

The stages of tooth development in mice children were measured using HE staining in this study. The results obtained are shown in Figures 1 - 4.

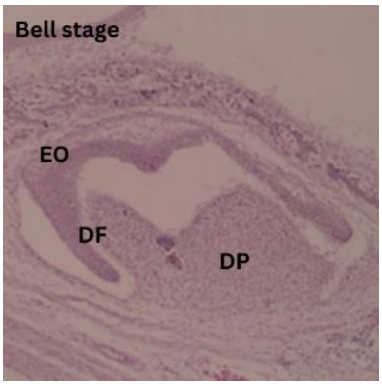


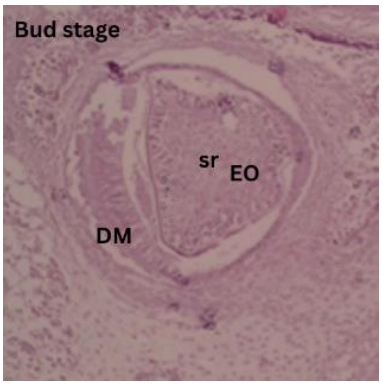


Treatment group (a.1) **Control group (a.2)**

Figure 1. Historical images of the tooth germ in the treatment and control group in mice 1; DM, dental mesenchyme; DP, dental papillae; EO, enamel organ; sr, stellate reticulum

Figure 1 showed the developmental stages of tooth germs that have reached the bell stage in the treatment group given fish powder (a.1) and the bud stage in the aquadest control group (a.2).

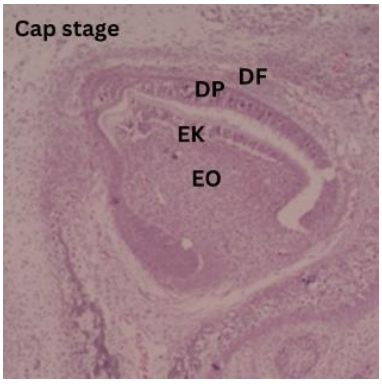




Treatment group (b.1) **Control group (b.2)**

Figure 2. Historical images of the tooth germ in the treatment and control group in mice 2. Histological images of the tooth germ in the treatment and control groups in mice 1; DM, dental mesenchyme; DP, dental papillae; EO, enamel organ; sr, stellate reticulum

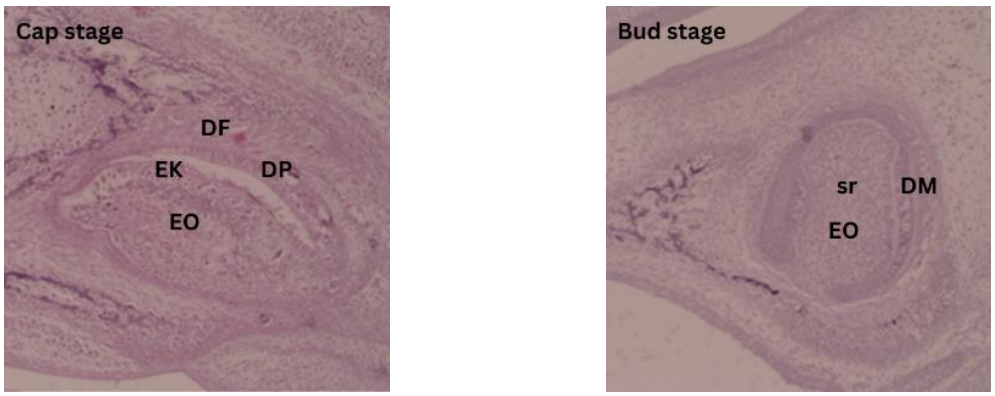
Figure 2 shows the developmental stages of tooth germs that have reached the bell stage in the treatment group given fish powder (b.1) and the bud stage in the aqua dest control group (b.2)



Treatment group (c.1) **Control group (c.2)**

Figure 3. Historical images of the tooth germ in the treatment and control group in mice 3; DM, dental mesenchyme; DP, dental papillae; EO, enamel organ; sr, stellate reticulum; EK, enamel knot

Figure 3 shows the developmental stages of tooth seeds that had reached the cap stage in the treatment group given fish powder (c.1) and the bud stage in the aquadest control group (c.2).



Treatment group (d.1) **Control group (d.2)**

Figure 4. Historical images of the tooth germ in the treatment and control group in mice 4; DM, dental mesenchyme; DP, dental papillae; EO, enamel organ; sr, stellate reticulum; EK, enamel knot

Figure 4 shows the developmental stages of tooth seeds that had reached the cap stage in the treatment group given fish powder (d.1) and the bud stage in the aquadest control group (d.2).

DISCUSSION

This study used sea fish nanoparticle powder given to mice. The dose of nanoparticles of sea fish powder given to mice was 2.17 mg. The materials used in this study consisted of dehydrated saltwater fish derived from sardines (*Sardinellafimbriata*), splendid ponyfish (*Leiognathussplendens*), and cobs (*Euthynnusaffinis*), an extraction of saltwater fish in powder form and emulsifier CMC (*Carboxy Methyl Cellulose*).[9] This fish powder contains omega-3 fatty acids and high calcium to affect fetal mouse teeth' growth. The content of omega 3 can penetrate the placenta and promote the growth of fetal teeth. These two ingredients play an active role in helping the growth of fetal teeth so that the tooth matrix can be properly filled. [10]It is proven that giving this nanoparticle powder makes the teeth progress faster than in the control group, which does not receive it. The stages that have been completed are to reach the cap and bell stages.[11]

Fetal formation and development are the two most important during pregnancy. Diet during pregnancy has an impact on fetal development outcomes.[12] Diet is an important requirement for pregnant women. Proper and adequate nutrition can help keep the mother and fetus healthy. A pregnant woman's nutritional intake can be achieved through a healthy diet and additional sources of nutrients in her breast milk, consisting of calcium, iron, phosphorus, folic acid, and various vitamins.[13]

The fulfillment of nutrients and nutrition in pregnant women is critical to the mother's and fetus's health. It is known that as much as 30 grams of calcium intake are transmitted from mother to fetus. Due to the rapid mineralization process, calcium absorption will always increase to meet bone development in the fetus, especially in the third trimester. Based on the research that has been done, it is proven that there is a significant relationship between the milk of pregnant women and the level of calcium adequacy. Calcium is one of the mineral elements that all humans, especially pregnant women, need. Calcium in the body is mostly found in hard tissues, bones, and teeth. Calcium density in the body increases in early life and decreases gradually as an adult. The body needs mineral calcium to regulate cell functions such as nerve transmission, muscle

contraction, blood clotting, and maintaining cell membrane permeability; it is also the main ingredient in the formation of bones and teeth.[14]

Tooth mineralization starts at various stages of fetal life, so later, when a child is born, mostly primary dental crowns have undergone mineralization. Intake Inadequate nutrition can make it difficult for teeth to resist bacterial invasion, making tooth decay common.[15] Teeth are organs where seed formation begins since the fetus is still 6 weeks old inside the mother's womb. Several studies show that maternal nutrition affects the growth of primary teeth.[15] Calcium is a very important nutrient, especially during pregnancy, to achieve optimal growth and development in children, including teeth. Along with the fetus growing in the uterus, the need for calcium also increased.[14] The fetus can take a supply of calcium from the mother when calcium intake is unfulfilled, resulting in the mother being at risk of developing bone fragility disorders. The fetus may be affected by the formation of teeth, so increasing caries is often found.[16]

Various factors affect the stages of tooth development. These factors are heredity, race, gender, socioeconomic conditions, nutrition, disease, and local factors. Nutritional factors are crucial as the mother and fetus consume them during pregnancy.[17] Good nutrition is important for healthy teeth and gums. Tooth calcification occurs at three to four months of gestation. Tooth germ development begins when the fetus is eight years old. Inadequate nutritional intake will cause problems such as caries, enamel hypoplasia, abnormal tooth size, and delays in the growth process. After calcium, phosphorus is the second most abundant mineral in the body. Phosphor is generally found with calcium inside the body.[11] The function of phosphorus is the formation of minerals such as bones and teeth. One of the first steps in the mineralization process is to apply phosphorus to the matrix bones and teeth. Phosphorus deficiency can also cause calcification disorders during the formation of bones and teeth, so susceptibility to caries increases.[18]

Consumption of this nanoparticle powder will affect the enamel's calcification and mineralization stages, which will be faster and better than those who do not consume it.[16] The content of vitamin D in saltwater fish powder functions in fetal growth through calcium metabolism, maintaining cell integrity, bone mineral metabolism, and maintaining placental function.[19]

The sea fish powder is turned into shaped nanoparticles so that the body can absorb more easily. Specifically, the mechanism of action of nanoparticles increases the surface area available to interact with biological support, prolongs the presence of compounds in the gut, reduces the effects of intestinal cleansing mechanisms, and penetrates deep into the fine capillaries of tissues. Permeable, fenestrated epithelial linings such as the liver allow for more efficient absorption into cells, allowing complete transport of the active ingredient to target locations in the body.[17]

The fish powder contains omega 3. Omega-3 consists of several parts, namely ALA (alpha-linolenic acid), EPA (eicosapentaenoic acid), and DHA (docosahexaenoic acid). ALA can be found in two forms, namely EPA and DHA. EPA and DHA play a role in the regulation of osteoblasts and osteoclasts.[20] DHA can affect RANKL production so that it does not bind to RANK. It results in a decrease in osteoclasts and an increase in osteoblasts. In addition, the content of omega 3 can also increase the density of enamel by forming a tissue matrix resulting from the deposition of ameloblasts, odontoblasts, and formative cells. The content of omega 3 can help the migration of proteins, which plays an important role in amelogenesis and apoptosis.[21]

Calcium and phosphorus content in fish powder will affect the balance of proteins such as amelogenin and calbindin-

28 kDa during tooth development. The calbindin-28 kDa protein has a high affinity and can bind more calcium.[21] Giving fish powder aims to improve the process of mineralization and calcium transport into the enamel matrix. Pregnant mice given fish powder at the beginning of the process will experience the merging of several crystals, making the enamel denser and larger. Furthermore, the process of degradation of the amelogenin protein will occur, and it will be replaced by other proteins.[19]

The results of the histological picture showed that there were differences between the fetuses of mice given fish powder and those given aquadest. The composition of the enamel matrix in fetal mice that were given fish powder was mostly filled with organic material, which was dominated by amelogenin protein. It will accelerate the development of teeth in mice given sea fish pollen to reach the cap and bell stages compared to the control group.[11]

CONCLUSION

The stages of development and growth of the fetal teeth of mice were affected by the consumption of sea fish powder by their mothers. The average stages of development and growth of fetal rat teeth were provided to the mother mice on the 18th day after administration of the sea powder.

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