Application of ultrasound in determination gestation status in captive female freshwater stingray (*Potamotrygon* sp.)

Chayanis Daochai
Veterinary Medical Aquatic animal Research Center
Faculty of Veterinary Science, Chulalongkorn University
Bangkok, Thailand

Somsri Daochai
Asst. Prof., Center of Biomedical Instrumentation,
Institute of Molecular Biosciences,
Mahidol University, Nakhon Pathom
Asst. Prof., Faculty of public health
Western university, Kanchanaburi, Thailand

Nantarika Chansue
Assoc. Prof., Department of veterinary medicine,
Faculty of Veterinary Science, Chulalongkorn University
Bangkok, Thailand

***Abstract*— Freshwater stingray is one of the popular ornamental fish trade in Thailand. Early pregnancy diagnosis could improve reproductive performance and management in captive breeding. Therefore, the main objective of this study was to apply and develop techniques for determination of gestation status in freshwater stingray by using ultrasonography. The stingrays were placed in a fitted tank with water in ventral recumbency position. The imaging was done in B-mode. The suitable frequency was 6.5 MHz, covered all size of pregnant female stingrays. However, the frequency may be converted to 5 MHz or 8 MHz to identify implanted embryo or trophonemata. The scanning showed 3 stages of gestation; first stage, mid stage and final stage with different characteristic. The present study suggested that this application, a noninvasive technique, can be continuously used for determination the gestation status from 4-12 weeks of gestational age in captive female stingray.**

***Keywords*— freshwater stingray; gestation status; ultrasonography**

I. Introduction

Freshwater stingray (*Potamotrygon* sp.) is one of the freshwater elasmobranch, which completely restricted for living exclusively in freshwater environments [1]. The habitats are widespread throughout, several South American river systems [2]. The stingrays have been regularly captured for ornamental purpose for many years and become more popular in ornamental fish trade in Thailand, China and Asia. In recent decades, captive breeding of the freshwater stingray has rapidly increased. However, the information of reproductive management is poorly studied and limited.

Potamotrygonids exhibit some features similar to marine Elasmobranchs as low fecundity, late maturation, and slow growth [3]. Their reproductive cycle presents clearly defined periods for each of its basic steps as gonadal maturation, copulation, pregnancy, and birth. The stingray presented a reproductive mode described as matrotrophic viviparity [2], bearing 2-16 pups each parturition depending on a size of the mother. The developing embryos are nourished by yolk during the early phase of gestation. After the midpoint of gestation, specialized secretory cells in uterine endometrium, trophonemata, begin to produce a nutritious milk-like substance called histotroph, which is absorbed by advance fetuses until they complete development.

The gestation in freshwater stingray can be detected by physical changes as protruding from the abdominal area and physiological changes monitored by routine blood chemistries [4]. However, blood sampling from stingray is not suitable or practical for stingray farms, breeders, and ornamental fish owner and may cause severe stress. In addition, the visual appearance could not certainly indicate the gestation status.

Ultrasound techniques are becoming increasingly important in animal reproduction, offering both a mean of diagnosis and a useful therapeutic tool. This technology is used for a variety of reproductive purposes as determination of estrous cycle, evaluation of uterine and ovarian health or gestation status [5]. In fish, ultrasonography has been applied as a tool for fish research and management from the 2000s, while it has just been used during this decade for a health check, disease diagnostic, monitoring the gestation status and monitoring the fetal vitality in elasmobranch. The pregnancy diagnosis in captive stingrays essential for better efficacy and management of reproduction, providing information about conception success after copulation. The separation of pregnant and non-pregnant from breeding ponds prevents physiological stress of the female from rebreed and severe mating would. In addition, it reduces the losses from abortions and stillbirths [6]. Although the gestation status is unavoidably important for captive management, it has never been reported in any study using ultrasonography determination gestation status in the freshwater stingray.

II. Objective

To apply and develop techniques for determination of gestation status in captive female freshwater stingray by using ultrasonography method.

III. Method

This study was conducted on seven female freshwater stingrays (*Potamotrygon* sp.), which were maintained in breed tanks, 4x3 meters of 6,000-Liter capacity enclosures at We Mark Farm Co, Ltd., Nonthaburi, Thailand. The breed tank contained 2 males and 7 females. Female stingray had been observed in mating activities. Once the mating wound was found, the female would be moved to a nursery pond, 3x2 meters of 3,000-Liter capacity. The examination was twice during April 2016 to July 2016. These stingrays had 43.12±5.88 centimeters disc width. Water parameters were maintained at: temperature 26-28˚C, pH 7.0-7.6, salinity 0 ppt, ammonia < 0.25 ppm, and nitrites < 0.05 ppm. The tanks were located in an area of the facility where natural sunlight is filtered through slope shed and shading net. They were fed at 2-3% of body weight with yellow-stripe scads and also supplemented with multivitamin once weekly. Uneaten food was removed daily to maintain optimal water quality.

*A. **Material and equipment***

Sonography was conducted using the mobile ultrasound machine: digital ultrasonic diagnostic imaging system (DP6600 vet) and electronic micro-convex array transducer: 65C15EA. This transducer was allowed for the use of high frequency (5.0, 6.5 and 8.0 MHz) that provided extremely detailed images. The end of this ultrasound transducer was placed with a small amount of ultrasonic gel, then, wrapped by a commercial condom when using in water.

*B. **Procedure and Scanning techniques***

To perform ultrasound examination and morphometric measurements, a stingray was gently captured by fish net and placed into a 100-Liter fiberglass tank. No anesthesia was used during examination and venomous spines were left intact and uncovered. This tank was floated in the enclosure in order to prevent physiological stress from transportation. Each stingray was placed in ventral recumbency and remained submerged throughout the 10-minute process. Moreover, disc width was evaluated by measuring tape.

The imaging was done in B-mode. The transverse dorsoventral view (TDV) was performed by placing the transducer over a mid-abdominal area. If the stingray showed protruding area, the scanning could proceed directly whereas the non-protruded female, the transducer could locate within 2 centimeters from axial vertebrae or the beginning of pelvic girdle. The extended left uterine portion was located from pelvic girdle to 6th thoracolumbar vertebrae while the right located from pelvic girdle to 10th thoracolumbar vertebrae. Appearance of extended uterus and embryo implantation were attentively focused and recorded. In addition, characterization of trophonemata was noted.

The transducer frequency, position of focus, zoom and depth were adjusted to maximize image resolution due to variety of body depth and uterine status. In addition, size of uterine and embryos were recorded for further studies.

IV. RESULTS

Although the depths of both sides of uterine were varied, the optimal frequency adjusted to maximize resolution was 6.5 MHz. To identify the early stage of implanted embryo, using 5 MHz. of frequency might be appropriate due to the implanting at the bottom of the endometrium. In addition, trophonemata which suspended on the top of endometrium could be focused using 8 MHz of frequency. The depth and the position of focus image were alternately adjusted between 3-8 centimeters.

Distinctly increase of the uterine size and length of trophonemata were observed in two female with implanted embryo at the bottom of the endometrium, referred to the first stage of gestation. The embryo revealed anechoic regions with hypoechoic rim. No vital sign or movement of the embryo was clearly noticed. Moreover, the typical echogenic properties of the fluid were found dispersed inside the uterus might indicate a yoke (Fig.1B).

Three females revealed the developed characteristic with central radiating areas of anechoic area surrounded with hypoechogenicity. The embryo showed the vital sign by visually documenting fetal wing and respiratory activity. Moreover, independence tail structures were identified (Fig.1C).

The complete development fetuses were manifested in one of the females. Ultrasound appearance and structure of the fetuses were similar to the above. However, there are no anechoic areas left on both sides of the uterus while the strong movement of the fetuses was performed (Fig.1C). Surprisingly, parturition of the stingray occurred after the scanning 3 hours.

Only one female stingray showed inactive uterus with non-movement trophonemata (Fig.1D).

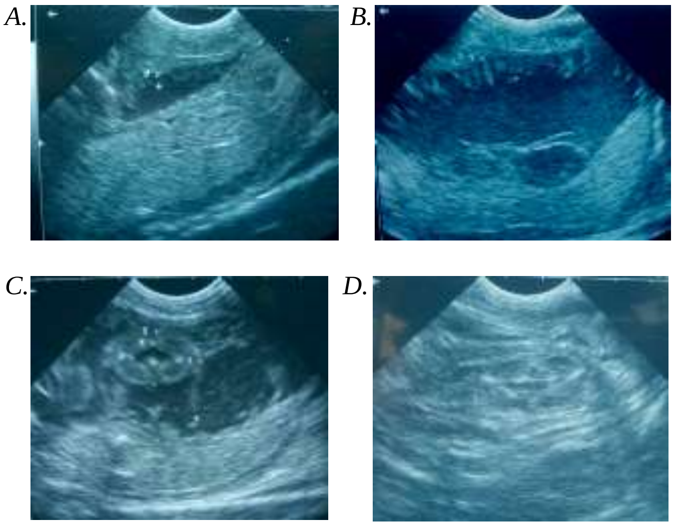

Figure.1 Ultrasonography of uterine portion in female freshwater stingray (*Potamotrygon motoro*) in TDV; A: ultrasonography of inactive uterus with no movement of trophonemata. B: ultrasonography of early stage of gestation. C: ultrasonography of mid-stage of gestation. D: ultrasonography of late stage of gestation.

V. DISSCUSSION

Ultrasonography was introduced in animal reproduction for depicting pregnancies and has rapidly been adopted as a routine clinical technique to assess physiological and pathological structures of the genital tract in all domestic animals. There are several methods for pregnancy diagnosis in mammals such as palpation, radiography, ultrasound and biochemical blood tests [7]. Since elasmobranch has a unique physiology and reproductive mode, gestation diagnostic has limitations [8]. Although the radiograph and blood test are known to be precise in evaluating gestation status, the limitations of its procedure can cause unavoidable physiological stress. Therefore, ultrasonography method according to this study has been considered to be the most suitable and practical technique using in stingray farms and public ornamental fish facilities for routine monitoring and determination gestation status in captive freshwater stingray.

From this study, the suitable frequency for confirmation a gestation status was 6.5 MHz, covered all size of pregnant female stingrays. However, the frequency may be converted to 5 MHz to identify implanted embryo located at the bottom of the uterus in an early period of gestation. If a structure of trophonemata has to be focused, the 8 MHz could be applied. In case of selecting proper ultrasound and transducer for freshwater stingray, the system and model should be allowed to appropriately frequency as regarding to the result. However, depth and position of focus may unavoidably be adjusting continuously depended on purpose of scanning and movement of the structures.

In general, captive female stingray will be observed in mating activities. Once the mating wound is found, the female would be suggested a reproductive success and moved to a nursery pond or a nursery net. Without any specific diagnosis, the protrude abdominal area indicated pregnancy will perform within 8-10 weeks. If the indication has not proceeded, a non-pregnant female will be returned to the breeding pond losing at least 2 months in a separate pond without rebreeding. In this study, the females showing 3-4 weeks mating wound were discovered the implanted embryo at the bottom of the endometrium. This can imply that ultrasonography could detect the 4 weeks stage of gestation in female stingray. However, the earlier stage could possibly be detected due to alteration of the uterine size and length of trophonemata. This may be very useful for practical determination of gestation status. Early pregnancy diagnosis of non-pregnant stingrays post breeding can improve reproductive performance by decreasing the interval between successive copulation and separating a non-pregnancy diagnosis. It may play a role in management strategies to improve reproductive efficiency and profitability on commercial captive farms or prevent excessive expense on ornamental fish owners [9]. In addition, further scientific reproductive research can be applied from the technique reported in this study.

The capture and release of elasmobranch induces various degrees of physical trauma and physiological stress, the magnitude of which is thought to be dependent on the capture method and capture duration [10]. The response could be rapid, with fluctuations in the blood occurring within 15 min of the initial disturbance [11]. Therefore, all procedures on the animals held in the submerged fiberglass tank should be done within 10 minutes in order to prevent physiological stress. The females in this study were observed cautiously for stress response at least a month after the ultrasound. In the time, there was no evidence of depressing, inappetence, fetal absorption or stillbirth. Surprisingly, one of the female gave birth of 13 pups after the ultrasound 3 hours. The mother showed over breathing until the end of the day. Although this is the final stage of gestation, the process could possibly be one of acute stressor induced the early parturition. Fortunately, all pups have no sign of stress response or abnormal behavior. However, it has been suggested that ultrasonography after 12 weeks of gestational age should be seriously concerned.

The advantage observed from this study, ultrasound is a noninvasive technique. The animals do not even require sedation for this procedure and there is no recovery time. The procedure can be done quickly without any intensely restrained or transportation. Hence, this application can be continuously use for determination the gestation status. However, limitations of the technique are the high cost of machine and sonography experience required.

VI. Conclusion

The present study suggested that this application can be continuously used for determination the gestation status from 4-12 weeks of gestational age in captive female stingray. Ultrasonography method is a noninvasive technique and the animals do not require sedation. Early pregnancy diagnosis by ultrasound can improve reproductive performance and management including reduced excessive expense.

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