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Ultrasonic imaging-assisted artificial reproduction of critically endangered Sichuan taimen (*Hucho bleekeri* Kimura)

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Ultrasonic imaging-assisted artificial reproduction of critically endangered Sichuan taimen (*Hucho bleekeri* Kimura)

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Abstract: The critically endangered Sichuan taimen (*Hucho bleekeri* Kimura), one of the largest salmonids in the world, has been found in most of the tributaries of the Upper Yangtze River in China. In this study, ultrasonography was used to assist the artificial reproduction of Sichuan taimen. A Treason T3000 portable ultrasonography with a model 12L5 (12-5 MHz) transducer was used to determine the sex of Sichuan taimen before spawning and after hormone induction and spawning. Two scanning areas with two central points were located to easily distinguish between the ultrasound images of females and males before and after spawning. The ultrasound images showed the maturation of the ovary after hormone induction, which is a key for determining the right time for sampling oocytes or sperm and adjusting the dose of the hormone. The transverse ultrasound images of the ovary can also be used to evaluate reproductive characteristics such as potential breeding fertility and relative gonadosomatic index. Thus, ultrasonography may be helpful in dynamically understanding the artificial reproduction of Sichuan taimen and observing the response of the ovary to exogenous hormones, which will benefit the artificial reproduction and conservation of endangered fish species.

Keywords: ultrasonography, Sichuan taimen (*Hucho bleekeri* Kimura), hormone induction, reproduction, gonadal maturation

Introduction

Sichuan taimen, *Hucho bleekeri* Kimura, is widely distributed in Minjiang River, Daduo River, and marine habitats around Shanxi Province in China (Ding et al. 1995; Chen et al. 2017). Although Sichuan taimen has a high economic and ecological value, it is a critically endangered species. Wild populations of Sichuan taimen have decreased because of the habitat fragmentation caused by hydropower development, industrial pollution, and illegal fishing (Du et al. 2014; Hu et al. 2008). Fish seed resources depression in Sichuan taimen is the main effect of wild Sichuan taimen resource in conservation, which in turn reduces population quantity as well as population type (Campton et al. 1995; Hard et al. 2000; Verspoor et al. 1988). The maintenance of population diversity is a high priority for the conservation of Sichuan taimen (Allendorf et al. 1996; Ryman et al. 1991). An understanding of fish resource distribution is important for conservation and management (Zhang et al. 2018).

Fish proliferation and release activities are effective for the restoration of fish resources (Liu et al. 2018; Zhu et al. 2009). Release programs may increase the productivity of fisheries, accelerate the recovery of fish stocks, and confirm the survival of endangered stocks (Ortega-Villaizan et al. 2011). Supportive breeding may increase the population stocks of wild Sichuan taimen, so artificial propagation technology has been used to restore natural Sichuan taimen resources. Fish need to be examined quickly and accurately under limited resource conditions. When compared with methods such as computer tomography, which requires large and heavy equipment (Li et al. 2015), a simple method like ultrasonic imaging can be used to promptly and effectively analyze fish in the wild and quickly and accurately obtain information (Tayal et al. 2004). Thus, ultrasonic imaging technology may be suitable for the artificial propagation of Sichuan taimen.

Ultrasonic imaging has been widely used in clinical treatment (Carr et al. 1987; Bamber et al. 1981); it is an important screening tool that uses simple signal processing. Ultrasonic scattering can be used to receive a signal that provides important information on tissue properties (Mo et al. 1994; Huang et al. 2007). In fact, ultrasonic scattering has been used to detecting various hematocrits (Huang et al. 2010). However, few ultrasonic imaging studies have examined fish reproduction patterns. Therefore, ultrasonic imaging may be a potentially effective method for the detection of Sichuan taimen reproduction.

The use of ultrasound imaging equipment has some advantages (Tayal et al. 2004;

Li et al. 2015). such as high resolution, ease of transport, and convenience for real-time imaging. Ultrasound imaging can be used to detect Sichuan taimen reproduction and obtain more information on the reproduction characteristics and change patterns of fish reproduction. Ultrasonic imaging has been implemented as a powerful tool for inspection. To investigate the feasibility of ultrasound imaging in the application of Sichuan taimen, ultrasound imaging is established to perform the routine examination of Sichuan taimen reproduction, dynamically observed the imaging patterns of Sichuan taimen reproduction can be dynamically observed, and analyzed the image results of the corresponding counterparts, and this method may be used to be of benefit for the evaluation of fish reproduction in the future.

Materials and methods

Ethical Statements

All experiments were followed the experimental animal management law of China and approved by the Animal Ethics Committee of Chinese Academy of Fishery Sciences.

Materials

All experiments were performed in accordance needed to obey the Laboratory Animal Management Law document of China. animal protection, we obtained permission from the animal protection committee of China animal protection. The mean weights of the female fish and male fish were 0.93 ± 0.27 kg and 1.35 ± 0.43 kg, respectively.

Establishment of fish reproduction model

Sichuan taimen were fasted for 7 h and then anesthetized, after anesthetization with an aqueous solution of MS-222 at an adequate concentration of ($120 \text{ mg}\cdot\text{L}^{-1}$). Fish was placed on suitable position and fixed on the tank. All experiments process was executed by professionals' people. Before conducting the ordinary experiments, ultrasound images were used to identify the response of the ovary to an exogenous hormone.

Ultrasonography examination

For ultrasound examination, ultrasonography ultrasound equipment instrument was used to probe with frequency of 7–17 MHz. All ultrasound image information was were stored on the hard disk of the computer. After successfully accomplishing the

experiment, the fish was maintained in a vertical position, and the corresponding region was subjected into ultrasound scanning center to identify and analyze different regions of the ovary to exogenous hormone, and the ultrasound examination was used to analyzed optimum cross-sectional ultrasound images were analyzed. Observing timely position of fish gonad identification was examined by ultrasound image.

Results and Discussion

The fish ultrasonography images are detected and collected in ultrasound equipment. Scanning areas for optimum cross-sectional ultrasound images of Sichuan taimen was showed in Fig.1. Scanning areas are names as p1 and p2 position. left area (p1) covering below the lateral line and from the end of the pectoral fin to the end of the dorsal fin will covers the swimming bladder for, which will bring mirror ultrasound images; right area (p2) covering below the lateral line and from the end of the dorsal fin to the beginning of the anal fin is easier for gonad identification.

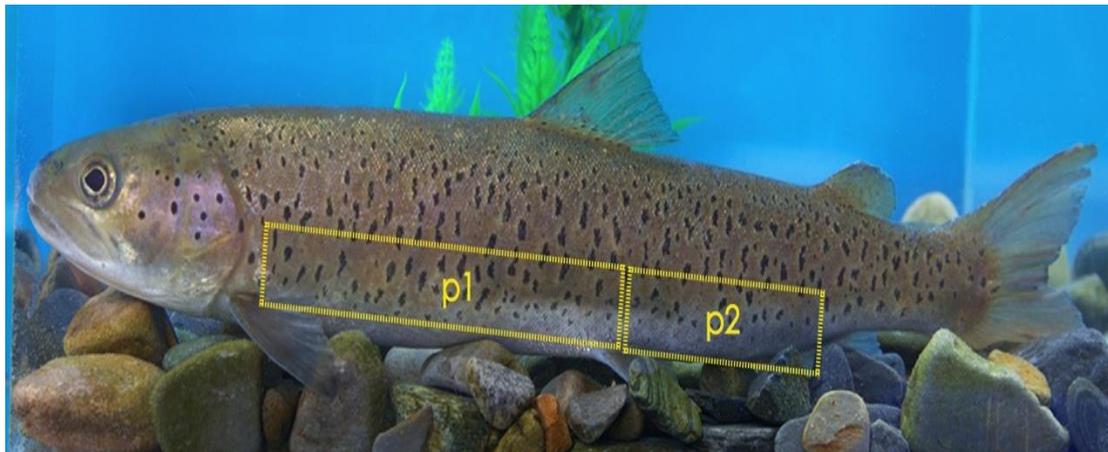


Fig. 1 Scanning areas for optimalum cross-sectional ultrasound images of Sichuan taimen, *Hucho bleekeri*: p1: central point of the left area for ultrasound images sampling; p2: central point of the right area for ultrasound images sampling.

The Sichuan taimen specimens are dissected to reveal the ovary, liver, intestine, testis, pyloric caecum, and swimming bladder (Fig. 2). To our surprise, female with stage IV ovary filed to ovulation shows an adverse bad response of ovary to the gonadotropin-releasing hormone GnRH hormone (Fig. 2a). Fig. 2b is presented with post spawning female with stage II ovary. Fig. 2c and 2d showed that the testis at the center in central of the left scanning area is degenerated after spawning and Hadley identified with the mirror images of the swimming bladder. Especially for gonadal, gonadal development of Sichuan taimen relies on sex determination and gonad differentiation. Sex determination leads to a choice of the sexual characteristics and the

formation of an ovary. Ovary acts in fetal gonads, the ovary and induces gonadal development of gonads (Piferrer et al. 2009). On the other hand, the Ovary is also essential to maintain testis determination and regulate network regulations. The Sichuan taimen specimens possessed abundant ovary, indicating Sichuan Taimen gonad shared a good gonadal development situation. The gonad plays an essential role in the fertility status of the offspring (Ramkissoon et al. 1996). Therefore, Sichuan taimen exhibited a better view of sex determination, which in ultrasound imaging remains to be unknown.



Fig.2 Dissection views of *Hucho bleekeri* broodstocks: a) prespawning female with stage IV ovary; b) postspawning female with stage II ovary; c & d) postspawning male with degenerated stage II testis

Fig.2. Dissection views of Sichuan taimen broodstocks: a) prespawning female with stage IV ovary; b) postspawning female with stage II ovary; c & d) postspawning male with degenerated stage II testis.

To dynamically identify a clear image of fish ovary and testis dynamically, ultrasound imaging technology was used to examine the detections with dissected ion of the dead Sichuan taimen specimens. As shown in Fig.3. Ovary scanning image was showed, and the ovary exhibited a presented homogeneous appearance with bright reflections at the p1 and p2 scanning points, whereas the mature testes were usually dark and displayed well-defined lobules with distinct margins, especially in the transversal section views. The extension of the ovary and testis had significant sonographic features: the extension was slightly tortuous, and shared a different diameters and irregular edges. After changing the ovary view, the continuity of the testis was interrupted when the ovary view was changed, and several fragments were produced which were mutually unconnected and located at some distance from each other were produced, and the corresponding region exhibited obvious movement. After examining the testis and ovary views, ovary resumed to a continuous tubular echo.

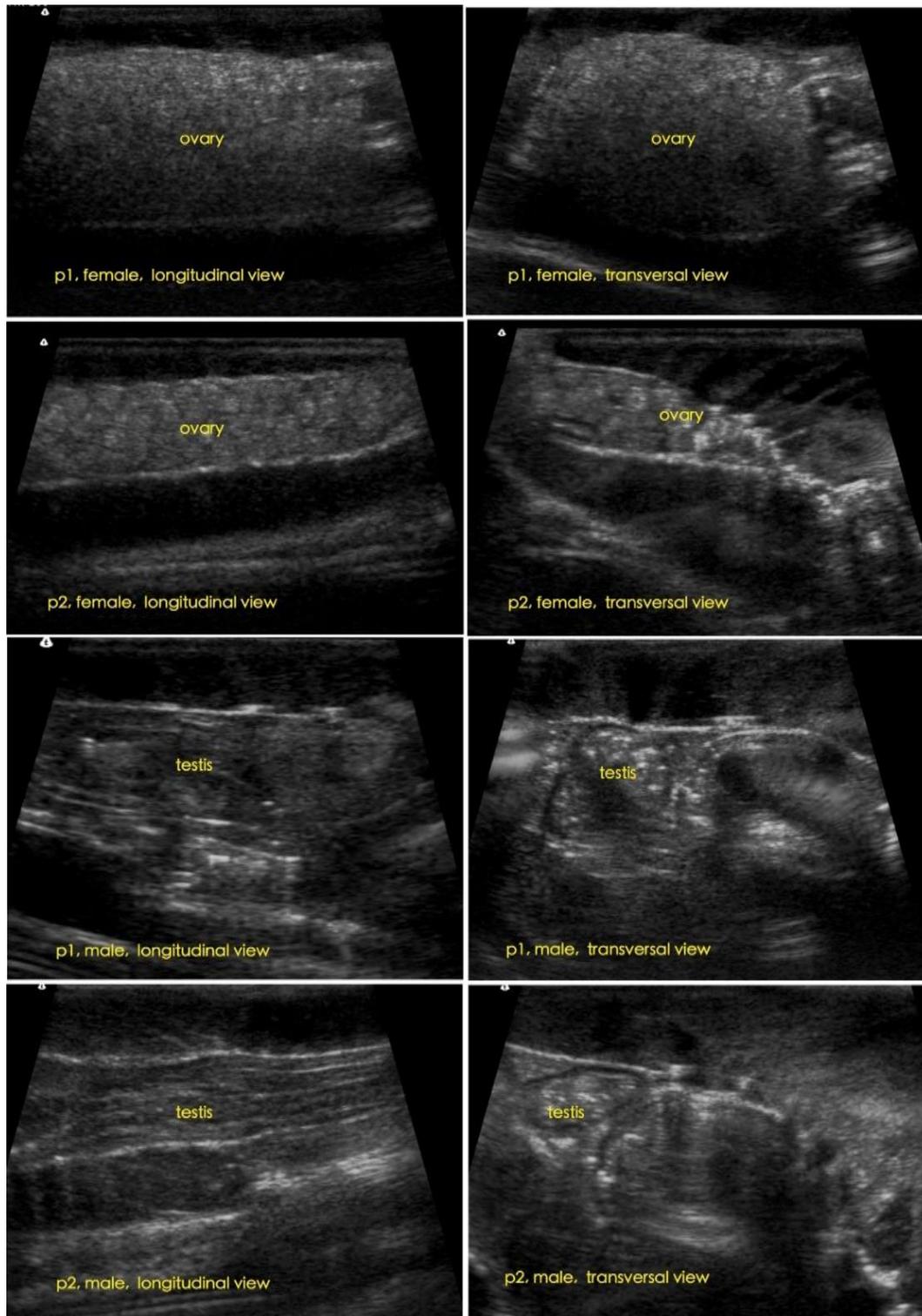


Fig.3 Distinctive ultrasound images of mature female and male *H. bleekeri*.

With the response of ovary to the exogenous GnRTH, the vacuous oocytes appeared (Fig. 4 c & d), augmented (Fig. 4 e & f), well-distributed and arranged (Fig. 4 e & f). Only these ovaries with such ultrasound images (Fig. 4 e & f) were ready for ovulation and reproduction, which is a key point for egg making decisions on artificial

collection of the eggs. The line array probe was used to probe upwards from the mouth of the fish body, and then to the left and right, respectively. Obvious gonadal structures of gonad may be clearly distinguishable at various time points. The ultrasound image showed some ultrasound imaging characteristics. Immediately after the following part, the core region of the ovary appeared to share an important part in some regions, and some regions may appear as dark areas. The ultrasound images were clearer with the development of GnRTH treatment; ultrasound image may become more clear, and the results showed a variety of clear visible echoes in the upper part of the fish tissue with normal echoes, which may be detected on both sides; the anatomical position of the ovary could show the size of the image, and the pertissue space of the ovary exhibited a strong scattered echo distribution of echo; at approximately 48 h after ovary development comes into existence, the bright region gradually increased. When the range of the bright region significantly increased at 90 h, and while the middle and low echoes started to increase. After spawning, the ovary mainly showed irregular hypo echogenicity, whereas the other situation disappeared. In addition to species specificity, the functions of GnRTH are responsible for the hypothalamic secretion of the GnRH gonadotropin-releasing hormone, which has an important role in gonadal development (Mechaly et al. 2013; Mohamed et al. 2017; Pinilla et al. 2012).

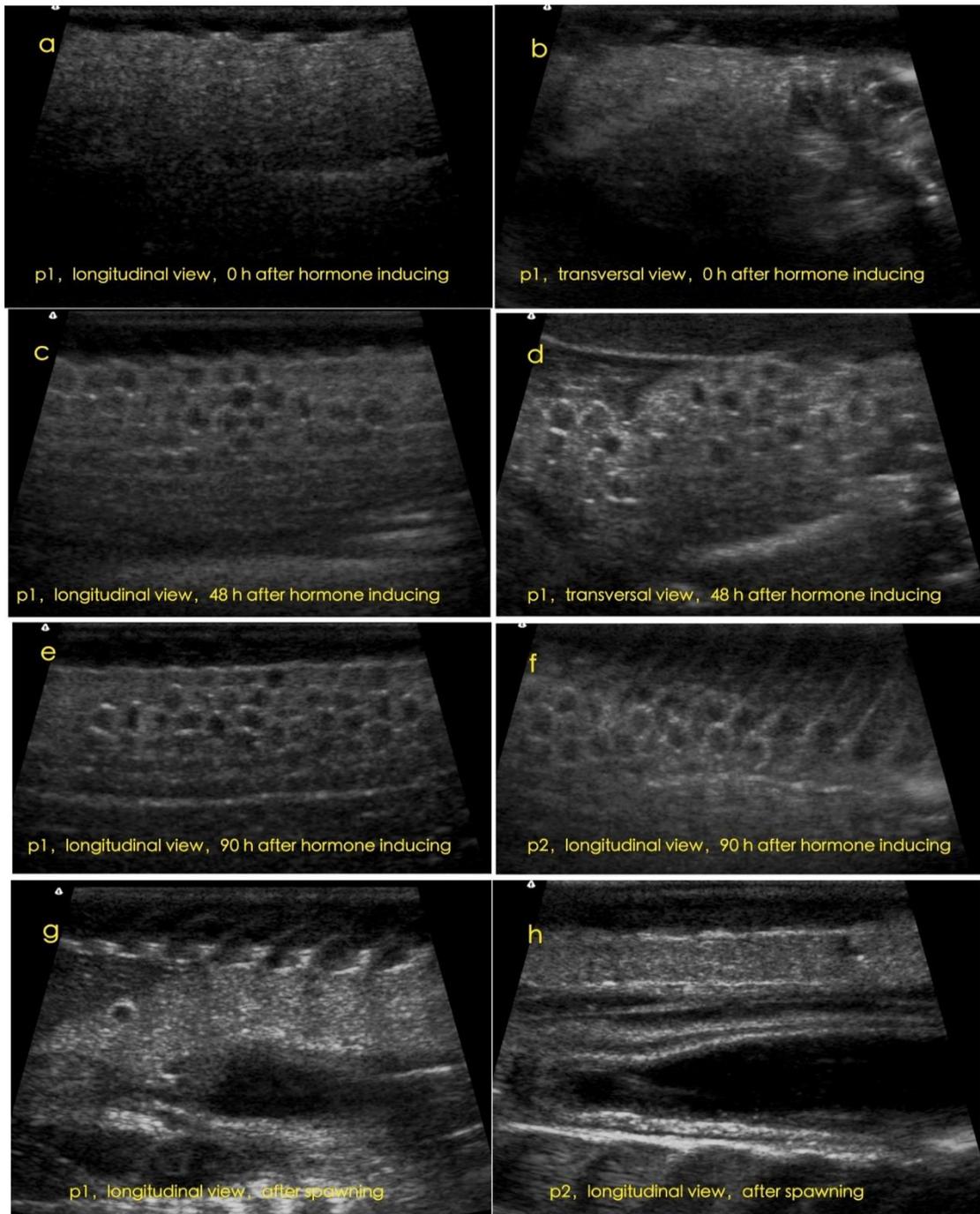


Fig. 4 Typical ultrasound images of the ovary during hormone induction.

Ultrasound images from the p1 and p2 points of views of the testis before and after spawning were easily distinguishable. At point of p1, after 80 h after hormone induction, the testis became very dark, which indicated represent the presence of seminal fluid and that means ready for spermiation. After spawning, the testis degenerated to a single line (Fig.5 a & c). At point of p2, the testis was not full of seminal fluid after 80 h after hormone induction. And after spawning, the testis at point of p2 was still visible in the

ultrasound image, and it can be imaged with a comparatively bright reflection (Fig.5 b & d).

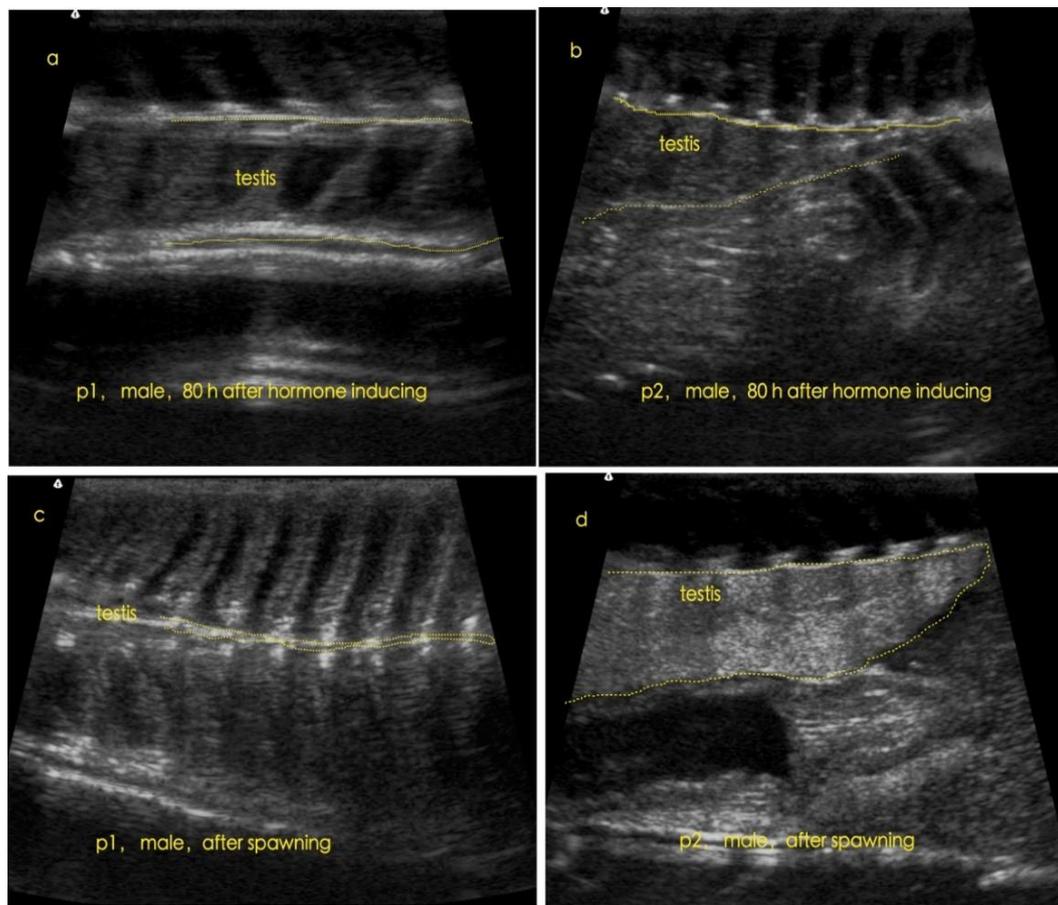


Fig. 5 Typical ultrasound images of the testis during hormone induction.

As shown in Fig. 6, the different views of the ultrasound images exhibited the reproductive capacity of Sichuan taimen, so it was possible to evaluate the reproductive characteristics. The reproductive capacities of the eggs harvested from four female fish were evaluated and compared.

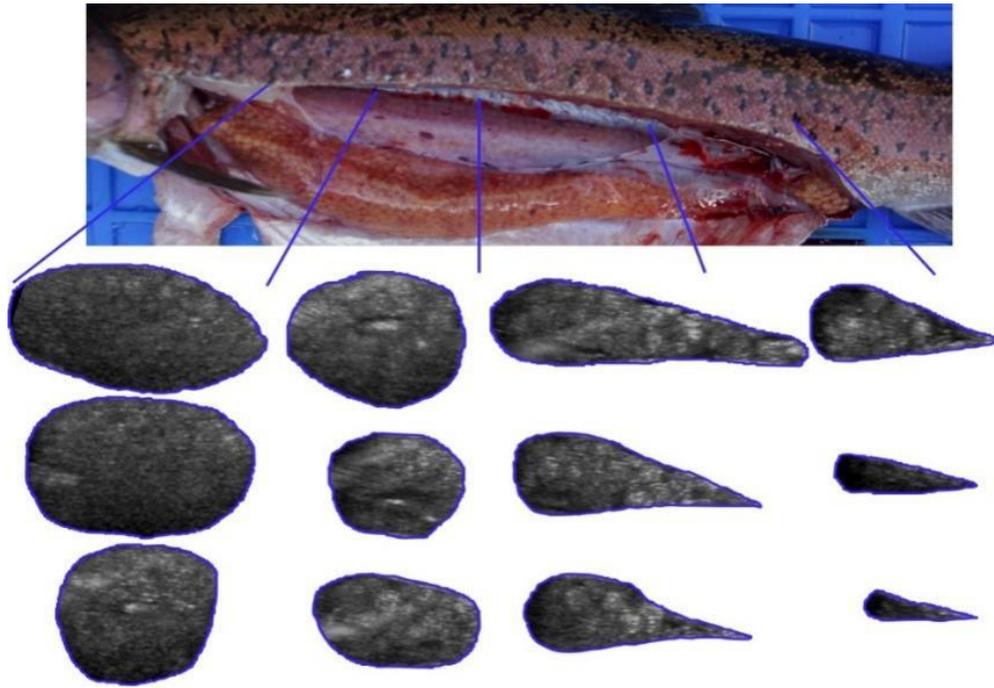


Fig. 6 Transverse ultrasound images of the ovary.

In summary, ultrasonography has been widely used to detect fish tissues in aquaculture, and ultrasound imaging analysis is difficult without a suitable standard. Thus, it remains to require a long time to apply to the basic study. In the present study, ultrasound images were used to obtain images of the ovary and testis at different time points. All images that showed a corresponding region shared a normal echo. It is easy to identify the sex of a fish by using ultrasound images.

Conclusion

Ultrasonography can be used to obtain more information on the gonadal development of Sichuan taimen, so gonad image libraries need to be established. Thus, they are applied to practical introduction. More data need to be collected to establish a basic foundation for the artificial reproduction and effective conservation of Sichuan taimen. The ultrasound images show the maturation process of the ovary after hormone induction, which is essential for determining the right time to sample an oocyte or sperm and adjusting the dose of a hormone. For the artificial reproduction of Sichuan taimen, it is necessary to determine a suitable period for ovary and testis development.

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