Ultrasonographic Findings of Ovary and Testis in Adult Acipenser Persicus during Artificial Propagation (Stage V of Maturity)

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Abstract

Objective- Acipenser persicus is one of the most important caviar producing fishes in the Caspian Sea and one of the endangered species of the sturgeon fishes. For breeding of sturgeons, large numbers of animals have to be captured and examined to obtain sufficient numbers of mature fishes that will spawn within a few months in captivity.

Design- Original study

Animals- A total of 66 adult Acipenser persicus (40 female and 26 male) were used in this practice.

Procedures- In the present study the gonads of adult Acipenser persicus have been assessed by ultrasonography during artificial propagation to detect the features and changes of the ovary and testis. A total of 66 adult Acipenser persicus (40 female and 26 male), during artificial propagation program, were used in this practice. They underwent ultrasonographic study using a Pie Medical 200 VET ultrasonic machine. A right and left lateral parasagittal of the fish between the pectoral and anal fins and a transverse view was taken.

Results- Ultrasonographic view of ovary and testis in adult Acipenser persicus during artificial propagation (stage V of maturity) were detected.

Conclusion and Clinical Relevance- Ultrasonography prepares useful data about gonad structure, echotexture, and echogenicity during the artificial breeding period. Ultrasonography can be an accurate, non-invasive, and fast technique to assess the gonads of sturgeons in propagation centers.

Key words- Acipenser persicus, ultrasonography, sturgeon fish, ovary, testis, artificial propagation.

Introduction

Sturgeon fishery, the most valuable product of the Caspian Sea which is a major economic resource and plays a significant role in the income of the Iranian South Caspian Sea fisheries. Acipenser persicus is one of the most important caviar producing fishes in the Caspian Sea and one of the endangered species of the sturgeon fishes. Compared to other fish, all sturgeons require a much longer time to reach sexual maturity and to complete the gamatogenic cycle. The average age of fish at their first spawning varies 10 to 25 years in different species1 which is a limitation factor in their propagation quantity. With the partial destruction of natural sturgeon spawning grounds, the artificial breeding of sturgeon species and fingerling release was started in Russia in 1955.2 The same trend began in Iran in 1970.3

For breeding of sturgeons, large numbers of animals have to be captured and examined to obtain sufficient numbers of mature fishes that will spawn within a few months in captivity. Evaluation of practical, precise, fast, and non-lethal techniques for investigating the gonads status is critical to advancement of aquaculture. At present, broodstocks are checked by sampling eggs and the germinal vesicle (GV); and if they are not suitable for breeding, they will be sent back to the fisheries station for caviar processing. Other techniques for characterizing the gonad status in fish include plasma lipophosphprotein concentrations,4,5 plasma vitellogenin concentrations,6 immunoagglutination methods,7 and radioimmunoassay of blood steroid levels.8 Most of these techniques are invasive, time consuming, threaten fish health and reproductive success, besides needing expert and specialized personnel. Other maturation detecting techniques like Immunodiffusion assay and Radioimunniassyay, not only need complicated analysis, but also are useful just in specific seasons.7, 9 Ultrasonography has been proven to be useful in determining the sex and maturity status in different fishes.10-16 This technique may also help determining changes of ovaries and testes during stage V of maturity of gonads and assigning the exact artificial breeding time.

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The aim of this study was to assess the gonads changes of adult *Acipenser persicus* by ultrasonography to estimation the best period of artificial propagation.

**Materials and Methods**

A total of 66 adult *Acipenser persicus* (40 female and 26 male), during artificial propagation program, were used in this practice. They were captured from the Caspian Sea, by gillnet, during March and April of 2005 and transferred to the hatcheries by truck equipped with oxygen. They underwent ultrasonographic study using a Pie Medical 200 VET ultrasonic machine and dual-frequency (5 and 7.5 MHz) linear waterproof transducer (Model 40915,5/7.5 MHz 64E VET. Pie Medical Philipswegl, Maastricht, The Netherlands). No sedation or anastasia had been used.

The fish were kept in freshwater for as long as 5 days. A total of 66 fish were found to be suitable by Polarization Index lower than 8%. Primary ultrasonography was done to check if they had been suitable for injection. Afterward pituitary gland extract was injected to start the examination. The extract had been captured from mature fish on breeding season. It had been dehydrated and delipidized by Acetone and converted to powder. The powder was then solved in normal saline and used for the injections. One consecutive injection was used to obtain the best ovulatory response (5mg per fish, IM). Before scanning, they were submerged in a water tank. The necessary acoustic coupling was provided by the water itself through which attenuation of ultrasound was relatively low. Scanning was performed by close contact with the skin surface (1 cm apart) whereby the scanning head of the transducer was covered by a condom to protect against rough contact with scutes and star-shaped skin. A right and left lateral parasagittal of the fish between the pectoral and anal fins was obtained wherever needed. The transverse view was also taken. The echogenecity (echo rate from tissue to transducer that is shown as bright spots on the monitor), respective morphology (echotexture), and other qualitative findings of the ovaries and testes during the maturity status (stage V) were described ultrasonographicaly. All images were recorded on video tape for subsequent analysis. During the scanning, constant focus, brightness, and constant settings were used and the gain settings were fixed.

The ultrasonography results were immediately confirmed by necropsy for each of the scanned fish. Fork length, from the tip of the snout to the end of the middle caudal fin rays,(nearest 1cm) and weight measurements (nearest 0.1 kg) were carried out for each fish, and fin ray section for age determination was also collected.

Finally, probable similarities and differences of ultrasonographic findings of gonads between stage V and stage IV according to previous researches were assessed.

**Results**

The range of fork length, weight, and age of the scanned adult *Acipenser persicus* are shown in table 1.

### Table 1- Range of fork length, weight, and age of the scanned adult *Acipenser persicus*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>13</td>
<td>25</td>
<td>16.9</td>
<td>3.44</td>
</tr>
<tr>
<td>Fork Length (cm)</td>
<td>126</td>
<td>177.5</td>
<td>153.4</td>
<td>15.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>15.5</td>
<td>48</td>
<td>25.1</td>
<td>11.34</td>
</tr>
</tbody>
</table>

Ultrasonographic findings of stage V female adult *Acipenser persicus* gonad appearance were as followings:

- Plenty amount of free fluid was seen in the abdominal cavity especially between the liver lobes, and around the muscular stomach. Echogenecity of this fluid was depended on its contents but normally it was anechoic (Fig. 1).

- The liver was shown more hyperechoic due to surrounding fluid and creating acoustic enhancement artifact (Fluids do not absorb the ultrasound, so more ultrasound is transmitted to underlying structures and they seem more echogenic falsely) (Fig. 1).

![Figure 1- Stage V female adult *Acipenser persicus* organs. Sagittal sonogram of the liver lobes region shows plenty amount of anechoic fluids (F) surrounding liver (L) and gallbladder (Gb); M: Muscle; Ae: Acoustic enhancement artifact (black arrow).](image)

- The oocytes were mostly separated from the ovary and were floated in the free fluid or genital canal. They were also distinguished around the liver and all parts of the abdominal cavity (Fig 2).

- Emptied parts of the ovary from oocytes were seen as irregular hyperechoic structures (Fig. 2).
Ultrasonographic findings of stage V male adult *Acipenser persicus* gonad appearance were as following:
- The testes were enlarged, lobulated, tortuous, and completely increased in the diameter (Fig. 4).
- Stage V testes mostly had homogenic parenchymal texture with marked surrounding capsule. Echogenecity of the testis parenchyma was less than adjacent muscular layer (Fig. 4 and 5). Some shapeless and dispersed hypoechoic to anechoic parts, were often seen within testes parenchyma; which in some cases, they were present along the testis especially far away from the muscle (Fig. 6).
- Genital canals were dilated by anechoic fluid contained echogenic particles (Fig. 6 and 7). Left and right canals junction was clearly visible in caudal part of the abdominal cavity (Fig. 7).
Discussion

Ultrasonography as a fast and non-invasive technique, prepares useful data about gonad structure, echotexture, and echogenicity during the artificial breeding period. Borescope was also used to determine the sex and eggs maturity stages in sturgeon by Keynard and Kieffer (2002). This method was inefficient when genital orifice had been narrow or it was mistakable whenever the ovary had been surrounded by fat. Whereas, ultrasonography does not have the same problems and gonad detecting will be possible even if the ovary is completely covered by fat and also there is no possibility to harm the genital system which is common in using borescope. Same authors claimed that ultrasonography can not be a proper tool for sex detection in fish because it is not able to distinguish first stage oocytes from matured ones. However, this investigation and some others have shown that ultrasonography can accurately detect sex and maturity stages in sturgeon due the significant different appearances. Rommens and Martin-Robichaud (2001) claimed that differenting between male and female fish is not possible during stage V, because of water absorption by the ovaries which decrease their echogenicity in females and spermatozoids which make the texture of the testes more granulated and hyperechoic in males. They added that there was a similarity in echogenicity and shape between genital canals and vasodeferent. However, in the present study these distinctions were simply possible using ultrasonography.

In Stage V oocytes are fluted in the abdominal fluid which was seen to be anechoic ultrasonographically. It could be more echogenic, possibly due to the presents of hemorrhage out of the gonads while it is highly congested. Increasing of the echogenicity happens even more than gonad hemorrhagic period when the fat is released by ruptured oocytes in over-maturated conditions. Martin-Robichaud and Rommens (2001) also discussed that it may be possible to detect the over-maturated oocytes by ultrasonography but they did not describe any ultrasonographic feature for it. In overmature ovary, there was no acoustic enhancement phenomena in the liver and stomach which could be because of the sound attenuation by released fat and free protein. Images showed that stage V ovaries were completely different from stage IV ones according to the previous reports. Sound penetration, deep visualization, floating and mobile oocytes in free fluid, and well-differentiated oocytes which can be seen in the stage V, were some of these differences. Stage V testes were mostly hypoechoic to anechoic which could be because of sperm and spermatids fluids accumulation. If this feature appears in all part of the testis, it will show a heterogenic view through the organ which may cause making mistake in diagnostic cases. The ultrasonographic view of these testes was slightly similar to stage II ovaries according to the previous reports. These testes are not possible in cases, operator should search for genital canals to help.

Propagation of Acipenser persicus is very critical in keeping its reserves since they are mentioned in red list under "Convention on International Trade in Endangered Species". Ultrasonography can be an accurate, non-invasive, and fast technique to assess the gonads of sturgeons in propagation centers.

References

چکیده

یافته‌های اولتراسونوگرافی بیشتر و تخمدان در قره‌برون بالغ در طی تکثیر مصنوعی

(مرحله پنجم رشدگی جنسی)

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هدف: قره‌برون یکی از مهم‌ترین ماهیان تولیدکننده کوپری در دریای خزر و یکی از گونه‌ها در حالت طبیعی در خاطر افترافکت ماهیان خاویاری می‌باشد. برای پرورش ماهیان خاویاری، تعداد زیادی از آنها باید صید و بررسی شوند تا اینکه تعداد مناسب ماهی بالغ به دست آید تا در گرم‌ترین جهت‌های خاک و در طی تکثیر مصنوعی به وسیله اولتراسونوگرافی برای مشخص

درمان نظری و تغییرات تخمدان و بیشتر مورد بررسی قرار گرفتند.

طرح مطالعه: تحقیقی

راکن- تعداد ۶۶ قره‌برون بالغ (۴۰ ماه و ۲۴ تیر)

روش کار- در طی پستانه تکثیر مصنوعی در این مطالعه مورد بررسی قرار گرفت. این مطالعه با استفاده از دستگاه اولتراسونوگرافی Pie Medical ۲۰۰ VET انجام شد. نماهای پاراژنتال راست و چپ و عضوی بین بالهای سینه‌ای و مخرجی اخذ شدند. نماهای اولتراسونوگرافی تخمدان و بیشتر در قره‌برون بالغ طی تکثیر مصنوعی (مرحله پنجم رشدگی جنسی) مشخص شد.

نتایج: اولتراسونوگرافی داده‌های مفیدی در مورد ساختار، محورهای داخلی و گونه‌سازی گردان در طول دوره تکثیر مصنوعی فراهم می‌آورد.

بحث و نتیجه‌گیری: اولتراسونوگرافی می‌تواند یک روش دقیق و غیر تهاجمی و سریع برای ارزیابی گونه‌سازی ماهیان خاویاری در مراکز تکثیر باشد.

کلیدواژگان- قره‌برون، اولتراسونوگرافی، ماهیان خاویاری، تخمدان، بیشتر، تکثیر مصنوعی