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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)
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 fofemale 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 species[1] 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]

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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 Radioimmuniassay, 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.

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 Philpswegl, 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 delipidizated 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).[17]

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*.

Parameter	Minimum	Maximum	Mean	Standard deviation
Age (year)	13	25	16.9	3.44
Fork Length (cm)	126	177.5	153.4	15.05
Weight (kg)	15.5	48	25.1	11.34

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).

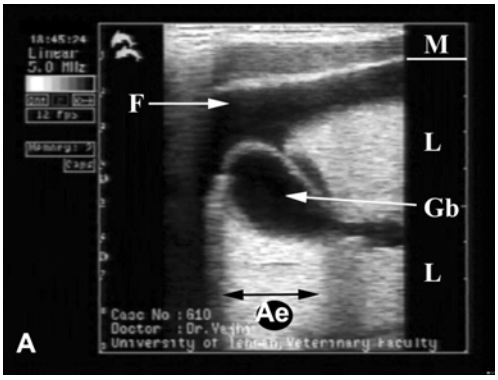

Figure 1- Stage V female adult *Acipenser persicus* organs. Sagittal sonogram of the liver lobes region shows plenty amount of anechoic fluids (F) surrounding liver (L) and gallbladder (Gb); M: Muscle; Ae: Acoustic enhancement artifact (black arrow).

- 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).

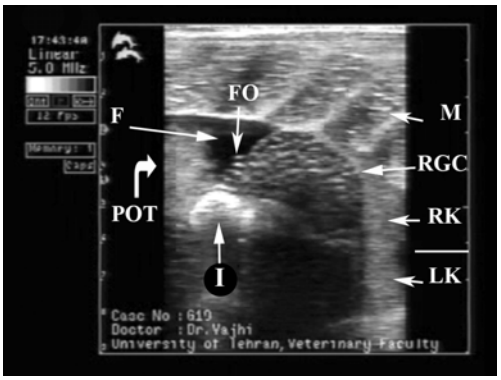

Figure 2- Ultrasonographic appearance of ovary in stage V. transverse view of the post ovulated ovarian tissue (POT) and the right oocyte-filled genital canal (RGC). F: fluid, M: muscle, RGC: right genital canal, I: intestine, RK: right kidney, LK: left kidney; FO: free oocyte.

- Genital canal was detectable by its distinct layer; fluid and oocytes were obvious inside it. The junction of the left and right genital canals was observed in the left and right frontal and sagittal images. Oocytes were set concentrically in the genital canals (Fig.3).

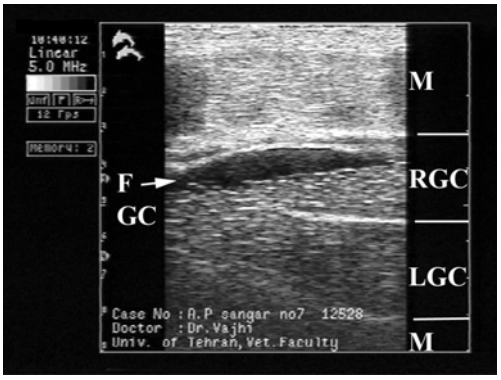

Figure 3- Frontal view of the genital canal (GC) where left and right genital canals meet each other. F: fluid, M: muscle, LGC: left genital canal, RGC: right genital canal.

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).

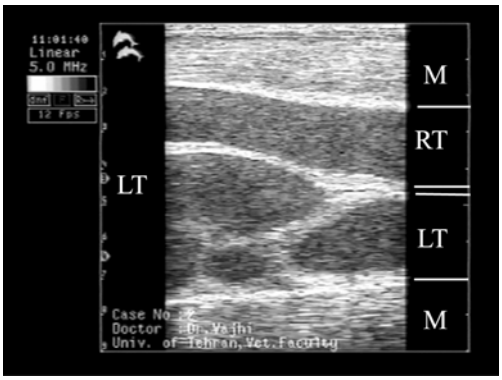

Figure 4- Ultrasonographic findings of stage V male adult *Acipenser persicus* testis, Frontal view which shows lobulation in the left testis; M: muscle, LT: left testis, RT: right testis.

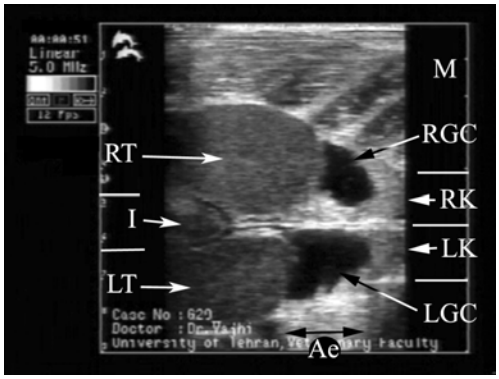

Figure 5- Ultrasonographic findings of stage V male adult *Acipenser persicus* testis, Transverse view: LGC: left genital canal, RGC: right genital canal, M: muscle, LT: left testis, RT: right testis. Ae: acoustic enhancement artifact, I: intestine, LK: left kidney, RK: right kidney.

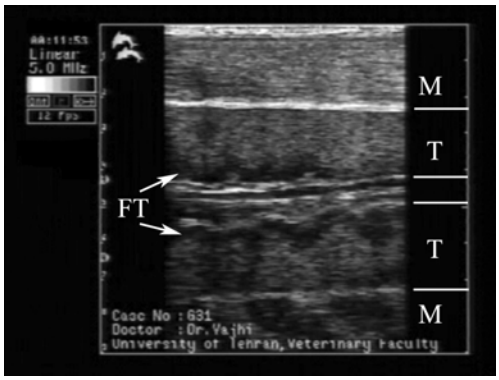

Figure 6- Ultrasonographic frontal view of adult *Acipenser persicus* testis in stage V with several sperm collections. FT: Spermal fluid in testis, M: muscle, T: testis.

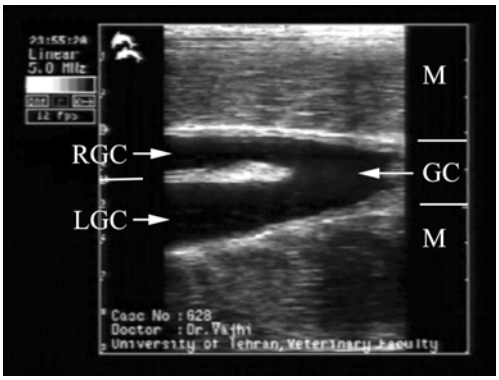

Figure 7- The frontal view ultrasonographic findings of adult *Acipenser persicus* testis in stage V which shows the junction part of the left and right genital canals; GC: genital canal, LGC and RGC: left and right genital canals, M: muscle.

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).[18] 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.[14, 15]

Rommens and Martin-Robichaurd (2001) claimed that differencing between male and female fish is not possible during stage V, because of water absorption by the ovaries which decrease their echogenecity in females and spermatozoids which make the texture of the testes more granulated and hyperechoic in males.[19] They added that there was a similarity in echogenecity and shape between genital canal 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 ultrasonographicaly. It could be more echogenic, possibly due to the presents of hemorrhage out of the gonads while it is highly congested. Increasing of the echogenecity 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. [19]

Images showed that stage V ovaries were completely different from stage IV ones according to the previous reports.[15, 19-21] 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 spermatic 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;[14, 15] in these 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".[22] Ultrasonography can be an accurate, non-invasive, and fast technique to assess the gonads of sturgeons in propagation centers.

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چکیده

یافته‌های اولتراسونوگرافی بیضه و تخمدان در قره برون بالغ در طی تکثیر مصنوعی (مرحله پنجم رسیدگی جنسی)

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هدف- قره برون یکی از مهمترین ماهیان تولیدکننده خاویار در دریای خزر و یکی از گونه‌های در خطر انقراض ماهیان خاویاری می‌باشد. برای پرورش ماهیان خاویاری، تعداد زیادی از آنها باید صید و بررسی شوند تا اینکه تعداد مناسبی ماهی بالغ به دست آید تا ظرف چند ماه در اسارت تخم‌ریزی کنند. در این مطالعه گنادهای قره برون‌های بالغ در طی تکثیر مصنوعی به وسیله اولتراسونوگرافی برای مشخص کردن ظاهر و تغییرات تخمدان و بیضه مورد بررسی قرار گرفتند.

طرح مطالعه- تحقیقی

حیوانات- تعداد ۶۶ قره برون بالغ (۴۰ ماده و ۲۶ نر)

روش کار- در طی برنامه تکثیر مصنوعی در این مطالعه مورد بررسی قرار گرفت. این مطالعه با استفاده از دستگاه اولتراسونوگرافی Pie Medical 200 VET انجام شد. نماهای پاراساژیتال راست و چپ و عرضی بین باله‌های سینه‌ای و مخرجی اخذ شدند. نمای اولتراسونوگرافی تخمدان و بیضه در قره برون بالغ طی تکثیر مصنوعی (مرحله پنجم رسیدگی جنسی) مشخص شد.

نتایج- اولتراسونوگرافی داده‌های مفیدی در مورد ساختار، اکوتکسچر و اکوژنسیته گناد در طول دوره تکثیر مصنوعی فراهم می‌آورد.

بحث و نتیجه گیری- اولتراسونوگرافی می‌تواند یک روش دقیق، غیر تهاجمی و سریع برای ارزیابی گنادهای ماهیان خاویاری در مراکز تکثیر باشد.

کلیدواژگان- قره برون، اولتراسونوگرافی، ماهیان خاویاری، تخمدان، بیضه، تکثیر مصنوعی