New Chapter

Ultrasonographic Monitoring of Channel Catfish Ovarian Development

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Introduction

Gonadal development throughout the life cycle of channel catfish *Ictalurus punctatus* from karyogamy during fertilization, through gonadal differentiation, juvenility, and reproductive activity in adults is controlled by genetic factors (Tiersch et al. 1992, Wolters and Tiersch 2004), and the endocrine system in concert with the environment (Silverstein and Small 2004). Ovarian development in mature channel catfish and the physiological processes directing it are directly affected by oocyte development, starting from recruitment of oogonia (12–15 μm in diameter), transitioning to pre-vitellogenic (15–240 μm), and vacuolated (240–650 μm) and vitellogenic (650–3,000 μm) oocytes. These become secondary oocytes, complete the first meiotic division, undergo meiotic arrest at metaphase of the second meiotic division, and are ready to be ovulated and fertilized (Grizzle 1985). Oocyte development and maturation for spawning in channel catfish begins at 2 to 3 yr of age, although most producers use 3-yr-old catfish for induced spawning (Barrero et al. 2007, 2008). Overall, ovarian and oocyte development is complex, involving environmental, hormonal, cellular, and molecular processes leading to ovulation. Various reproductive indices exist for interpreting these interdependent processes, and for assessing the state of ovarian maturity to select channel catfish for induced spawning. These reproductive indices are obtained using invasive and non-invasive methods.

Invasive methods for evaluating ovarian and oocyte development in channel catfish populations have included ovarian catheterization (Markmann and Doroshov 1983), germinal vesicle visualization (Stoeckel 2000), monitoring of serum hormonal profiles, gross examination of the ovary and oocytes, measurement of gonadosomatic index (gonad weight/body weight x 100) (Brauhn and McCraren 1975), and preservation of ovaries for histological analysis (MacKenzie et al. 1989). The most commonly used non-invasive method for assessing channel catfish ovarian development is visual examination of external morphology, which includes monitoring for a soft, rounded, distended abdomen extending past the pelvic fin and a swollen, reddish urogenital orifice (Clemens and Sneed 1957). Other non-invasive methods for identifying reproductive females are direct observation of active spawning behavior (Bates and Tiersch 1998, Phelps et al. 2007, Lang and Tiersch 2007), and measurement of thermal exposure (degree-days) for prediction of spawning in ponds (Pawiroredjo et al. 2008).

Ultrasonography, a non-invasive technology, has been used in as many as 19 fish species for sex identification (Matsubara et al. 1999, Columbo et al. 2004, Wildhaber et al. 2005), and for development of reproductive indices such as cross-sectional ovarian and testes diameter, gonad volume, and egg diameter (Bryan et al. 2007, Wildhaber et al. 2007, Newman et al. 2008) (Table 1, next page). Two catfishes, the Neosho madtom *Noturus placidus* and the African catfish *Clarias gariepinus,* were studied for monitoring the ovarian reproductive condition before and during the natural spawning season (Bryan et al. 2005, Lazlo et al. 2008).

Although ultrasonography has been used to estimate fillet yield in channel catfish (Bosworth et al. 2001), no ultrasound imaging procedures exist for viewing the ovary of channel catfish. This chapter describes ultrasound imaging of ovaries of channel catfish at different stages of gonadal development, and corresponding histological profiles.

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Table 1. The species (n = 19) and references (n = 23) on use of ultrasonography in fish reproduction were listed. These fishes were grouped below into two main categories (i) freshwater, and (ii) marine and anadromous, according to family, genus and species, with corresponding citations. It was possible to view ovaries and testes in 85% of the species listed.

Common name*	Scientific name*	Citation
Freshwater		
Stellate sturgeon	*Acipenser stellatus*	Moghim et al. 2002
Shovelnose sturgeon	*Scaphirhynchus platorynchus*	Colombo et al. 2004, Wildhaber et al. 2005, 2007, Bryan et al. 2007
Pallid sturgeon	*Scaphirhynchus albus*	Wildhaber et al. 2005, Bryan et al. 2007
Neosho madtom	*Noturus placidus*	Bryan et al. 2005
African catfish	*Clarias gariepinus*	Lazlo et al. 2008
Murray cod	*Maccullochella peelii*	Newman et al. 2008
Marine/Anadromous		
Pacific herring	*Clupea pallasi*	Bonar et al. 1989
Atlantic cod	*Gadus morhua*	Karlsen and Holm 1994
Barfin flounder	*Verasper moseri*	Matsubara et al. 1999
Atlantic halibut	*Hippoglossus hippoglossus*	Shields et al. 1993, Martin-Robichaud and Rommens 2001
Winter flounder	*Pseudopleuronectes americanus*	Martin-Robichaud and Rommens 2001
Yellowtail flounder	*Limanda ferruginea*	Martin-Robichaud and Rommens 2001
Haddock	*Melanogrammus aeglefinus*	Martin-Robichaud and Rommens 2004
Atlantic salmon	*Salmo salar*	Mattson 1991
Coho salmon	*Oncorhynchus kisutch*	Martin et al. 1983
Rainbow trout	*Oncorhynchus mykiss*	Evans et al. 2004a,b
Striped bass	*Morone saxatilis*	Blythe et al. 1994, Will et al. 2002, Jennings et al. 2005
Red hind	*Epinephelus guttatus*	Whiteman et al. 2005
Broadnose sevengill shark	*Notorynchus cepedianus*	Daly et al. 2007

*According to Nelson et al. 2004.

Ultrasound Procedures for Viewing of Channel Catfish Ovaries

The natural spawning season for channel catfish in Baton Rouge, Louisiana (30º 22' 3.3" N, 91º 10' 54.1" W) typically starts in mid-late April when ambient water temperatures remain within a range (21–30 ^{o}C) conducive to spawning (Lang et al. 2003, Pawiroredjo et al. 2008), and continues through May and sometimes into July (Bates et al. 1996). Adult (3–4 yr old) female channel catfish were held in 0.1-acre ponds with blower-driven aeration, and were sampled during early, middle, and late periods of the natural spawning season in 2008. Fish were captured by seining the ponds, and were held in concrete raceways at a salinity of 5 ppt (solar salt, Cargill Inc. Minneapolis, Minnesota, USA) to reduce osmotic imbalances due to stress for 1-3 d before ultrasound imaging and ovary sample collection for histological processing.

Channel catfish were caught with polyethylene dip nets from the concrete raceways, and placed in a portable, 49-L cooler (Igloo, 52 quart Sportsman™) filled with water. The fish maintained an upright swimming position (ventral recumbency), while the left side was scanned anterior to the base of the pelvic fin, and posterior to the dorsal fin (Figure 1). B-Mode ultrasound images were obtained using a laptop-computer ultrasound unit (TELAVET 1000, Classic Medical, Tequesta, Florida) with a multi-frequency (5–8 MHz) linear probe (model LV7.5/60/96Z) set at 8 MHz. During the entire procedure, the probe and the catfish were completely submersed in water, which provided the sole ultrasound transmission medium. The location of the ovary and the largest cross-section were determined by scanning the left side of the abdomen between the pectoral and dorsal fins with the probe, with the probe tip aligned to the bottom (ventral) side of the fish, and the cable end of the probe aligned to the top (dorsal) aspect of the fish. Ultrasound images were recorded on the hard drive of the laptop computer, with each image labeled with the corresponding identification number (i.e., Floy tag) of the fish.

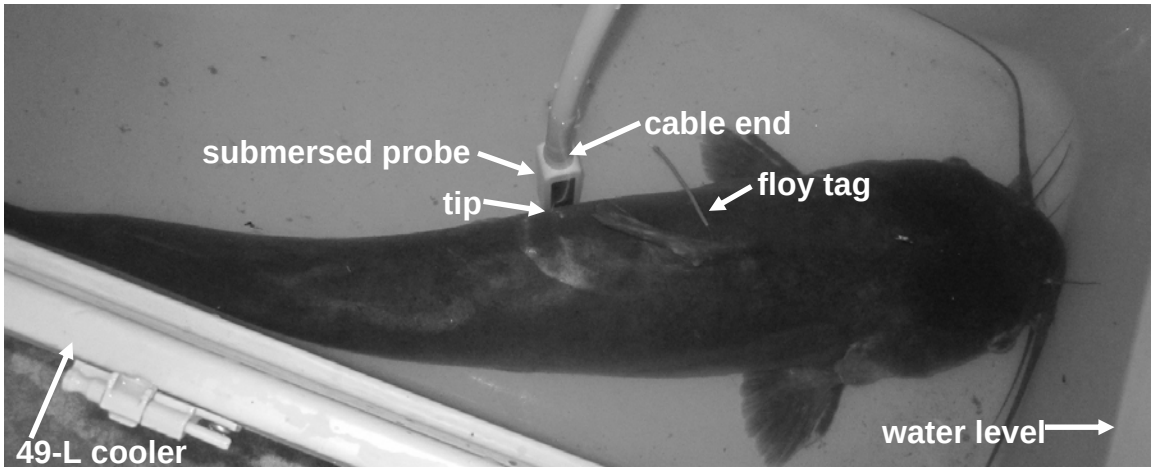

Figure 1. For viewing, the channel catfish were completely submerged in water, with the ultrasound probe placed on the left side, the cable end (connected to the ultrasound unit) located dorsally (adjacent to the spine), and the probe tip located ventrally (adjacent to bottom of the abdomen).

B-Mode ultrasound images of the ovary were created with the emission of ultrasound waves (in this case, 8 million cycles of ultrasound waves per sec) by piezoelectric crystal elements inside the linear array probe. These ultrasound waves were transmitted into the water, which acted as a transmission medium (a similar function is served by application of ultrasound

gel to eliminate the air interface between the probe and the surface of the anatomy being scanned). The emitted acoustic waves made contact first with the skin, and subsequently with muscle and ovarian tissues in the area at which the probe was positioned (Figure 1). The return of these ultrasound waves to the probe was displayed in a rectangular, two dimensional gray-scale image on the laptop monitor. The ultrasound echoes were recorded as dots along a vertical axis, with the dots located in the near view field of the image (top of the display image) creating ultrasound images of anatomical structures (skin and muscle) closest to the probe, and the dots in the far view field of the image (bottom of the display), creating ultrasound images of anatomical structures (ovarian structure) furthest from the probe (Figure 2).

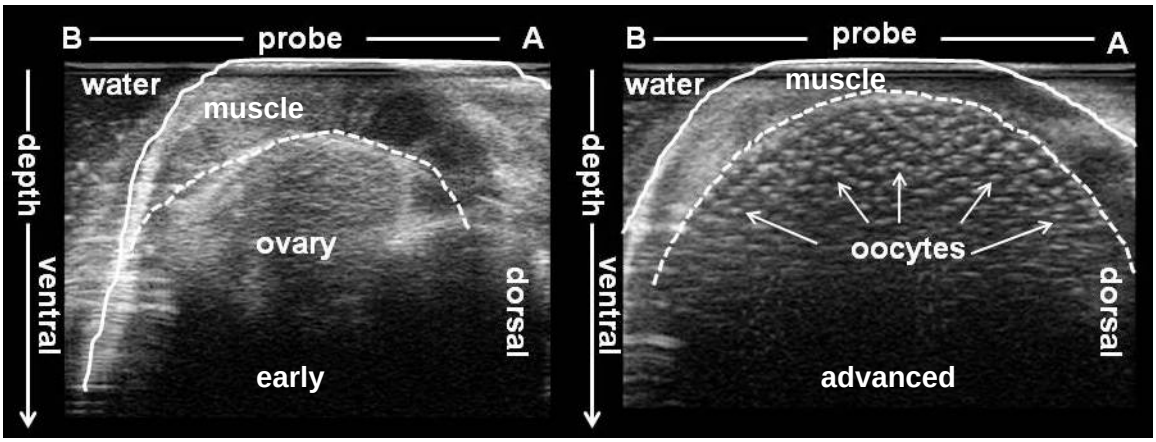

Figure 2. The image produced by a linear array probe is a rectangular, gray-scale ultrasound image, with the top of the image corresponding to the position of the probe. The cable end of the probe (A) corresponds to the top of the fish (right side of the image), and the tip of the probe (B) corresponds to the bottom of the fish (left side of the image). The top of the image (near field) corresponds to anatomical structures closest to the probe, with the skin depicted by the outermost solid line delineating the curving external perimeter of the fish, followed by muscle tissue, the ovary (dashed curved line), and oocytes (indicated by arrows). The image on the left represents early ovarian development in channel catfish, and the image on the right a more advanced stage.

The position of the dots along the vertical axis of the ultrasound image display represents the depth (mm) of the internal anatomical structures from which the echo originated. The brightness of the dot is proportional to the strength of the returning echo, and corresponds to an intensity within a 256 gray-scale range, with the brighter grays representing echoes of greater intensity. These vertical axis lines, when aligned, represent parallel scan lines produced by acoustic pulses and echoes at different points on the linear array of elements, which are rectangular in shape, arranged in a straight line, and produce a cross-sectional gray-scale image of the tranverse scanning plane of the ovary.

Thus, one of the key elements in interpreting the ultrasound image irrespective of gonadal condition of the fish is to understand the relationship of the physical position of the probe on the external anatomy of the channel catfish (Figure 1), and the corresponding probe and anatomical structures in the resulting display image (Figure 2). When the probe was placed on the lateral aspect of the abdomen (Figure 1), the orientation of the ultrasound image in the monitor was displayed with the top of the image (the near field view) representing the nearest distance to the probe, and with the bottom of the image (the far field view) representing the furthest distance from the probe. In these ultrasound images, the cable end corresponded to the right side of the

image (dorsal aspect of the fish), and the tip of the probe corresponded to the left side of the image (ventral aspect of the fish) (Figure 2), but this orientation of the fish anatomy can be switched by using the ultrasound software controls such that the left side of the image corresponds to the dorsal aspect of the fish and the right side of the image corresponds to the ventral side of the fish. The relationship of the orientation of the probe with respect to the external anatomical positioning of the probe and the internal anatomy of the fish should be clearly defined for basic interpretation of ultrasound images (Figure 3).

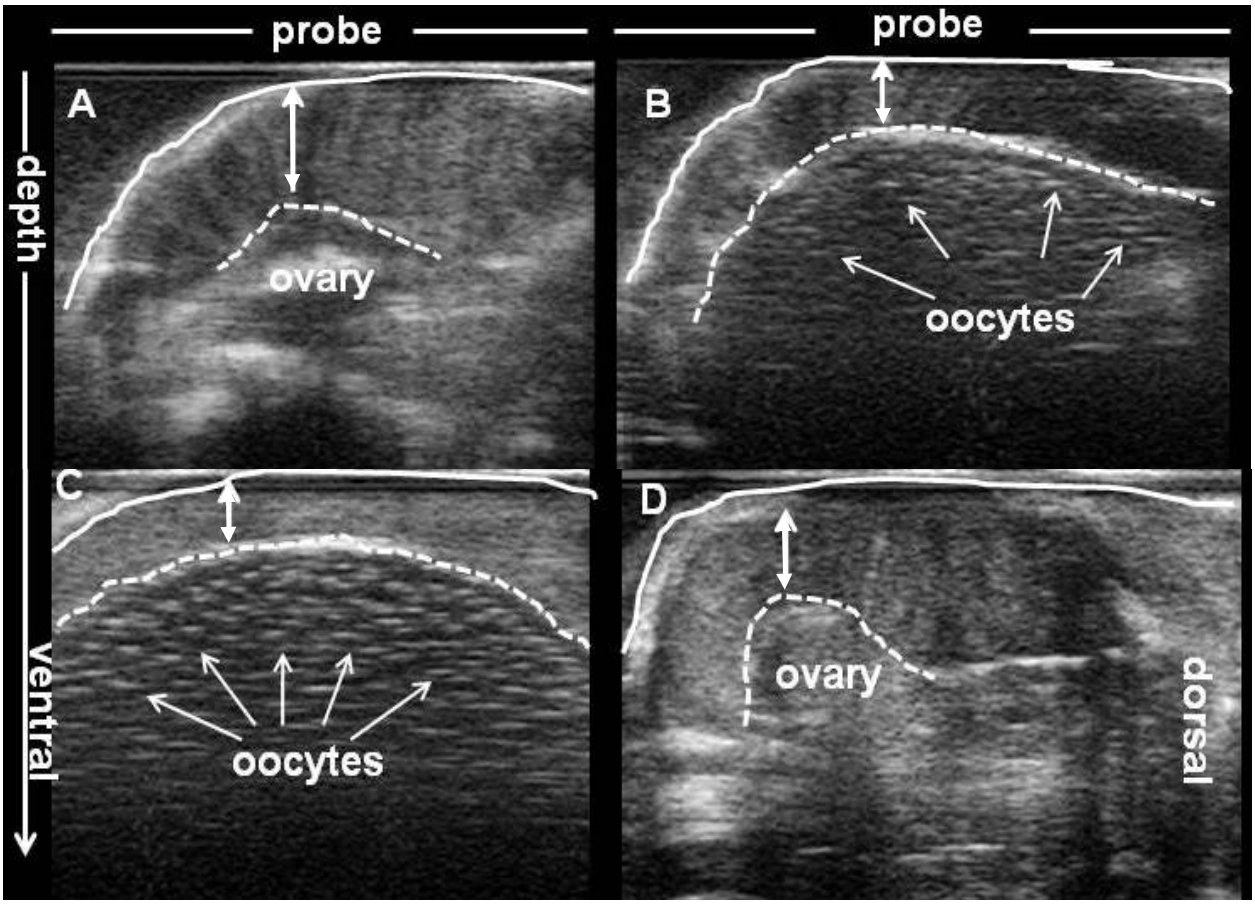

Figure 3. Ultrasonography provided direct images of channel catfish ovaries during the natural spawning season of channel catfish, revealing distinct gonadal appearances for early (A), developing (B, C) and atretic (D) ovaries. The curved solid white line depicts the skin, the anatomical structure closest to the probe. The dashed line depicts the contour of the ovary, with the dorsal and ventral aspect of the fish on the right and left side of each image, and the double-headed arrows between the skin and the ovary showing the changes in thickness of the body wall in each image. Oocytes appear flattened rather than rounded probably due to the polar distributions of aqueous and lipid compartments.

Early gonadal development (Figure 3A) was seen with more frequency early in the natural spawning season (i.e., early April), with ultrasound images displaying a small ovarian size. At this time the shape of the ovary was frequently not clearly defined, or not clearly distinguished from surrounding internal structures, and there were no visible oocytes. The

distance of the abdominal muscle between the periphery of the ovary and the skin (the body wall) was at its widest point.

Ovarian growth was noticeable during the middle and late period of the natural spawning season (i.e., late April to early July), with ultrasound images displaying a progressively enlarging ovarian size (Figure 3B and C). The ovarian structure was immediately visible and did not require multiple abdominal scans to be identified. The shape of the ovary was clearly visible, with the outermost periphery of the ovary curved and clearly defined, similar to the skin margin of the fish which was always visible during ultrasound scans. Oocytes were immediately visible during ultrasound scans. Individual oocytes and a high degree of organization within the ovary were discernible as the spawning season and gonadal growth advanced. The thickness of the body wall between the periphery of the ovary and the skin progressively narrowed with increased ovarian growth.

Towards the end of the spawning season (i.e., July), ultrasound images revealed gonads that were drastically reduced in size, and undergoing atresia (Figure 3D). Atretic ovaries could be identified by images displaying a small, disfigured ovarian wall, and disorganized, disintegrating oocytes that lacked a clear perimeter and regular shape. The body wall thickened with the reduced size of ovaries undergoing atresia.

Histological Profiles of Ultrasound Images

Fish were placed into a lethal dose of MS-222 for removal of ovaries, which were preserved in 10% neutral buffered formalin (NBF). After storage (≥ 1 month), the ovaries were sectioned through the largest cross-section, corresponding to the position of the ultrasound probe, and sent for histological processing to the Histology Laboratory of the Louisiana State University School of Veterinary Medicine, Baton Rouge, Louisiana. The section widths of the ovary samples on the histology slides were 3 – 4 μm, and the chemical stains used were hematoxylin and eosin. Digital images of histology slides were obtained using a stereoscope (Nikon SMZ-U, Tokyo, Japan). A mm-increment ruler was positioned in the upper left corner of each histology slide to provide a standard size reference.

Histology profiles (Figure 4) corresponding to the ultrasound images collected during the spawning season (Figure 3) revealed the microscopic biological progression of ovarian development which was not visible in the ultrasound images. Ultrasound images of early and atretic ovaries (Figure 3A, D) depicted small ovaries with no visible oocytes (Figure 3 A), or small ovaries with disfigured oocytes (Figure 3 D). In contrast, the histology profiles of early and atretic ovaries revealed a large ovarian cross-sectional area, with numerous oocytes (Figure 4A) and distinct primary and atretic oocytes (Figure 4D) enclosed in a thick ovarian wall. The larger ovarian cross-sectional area depicted in the histology profiles was directly related to the sampling of small ovaries, corresponding to early-developing or regressing ovaries (Figure 3 A, D). Ultrasound images of developing ovaries (Figure 3 A, D) depicted a larger cross-sectional area occupied by enlarging ovaries and visible, enlarging oocytes. In contrast, the histology profiles of developing ovaries (Figure 4 B, C), prominently displayed a large oocyte cross-sectional area, rather than a large ovarian cross-sectional area. Consequently, internal oocyte processes such as the formation and coalescence of yolk globules (Figure 4 B, C) were depicted.

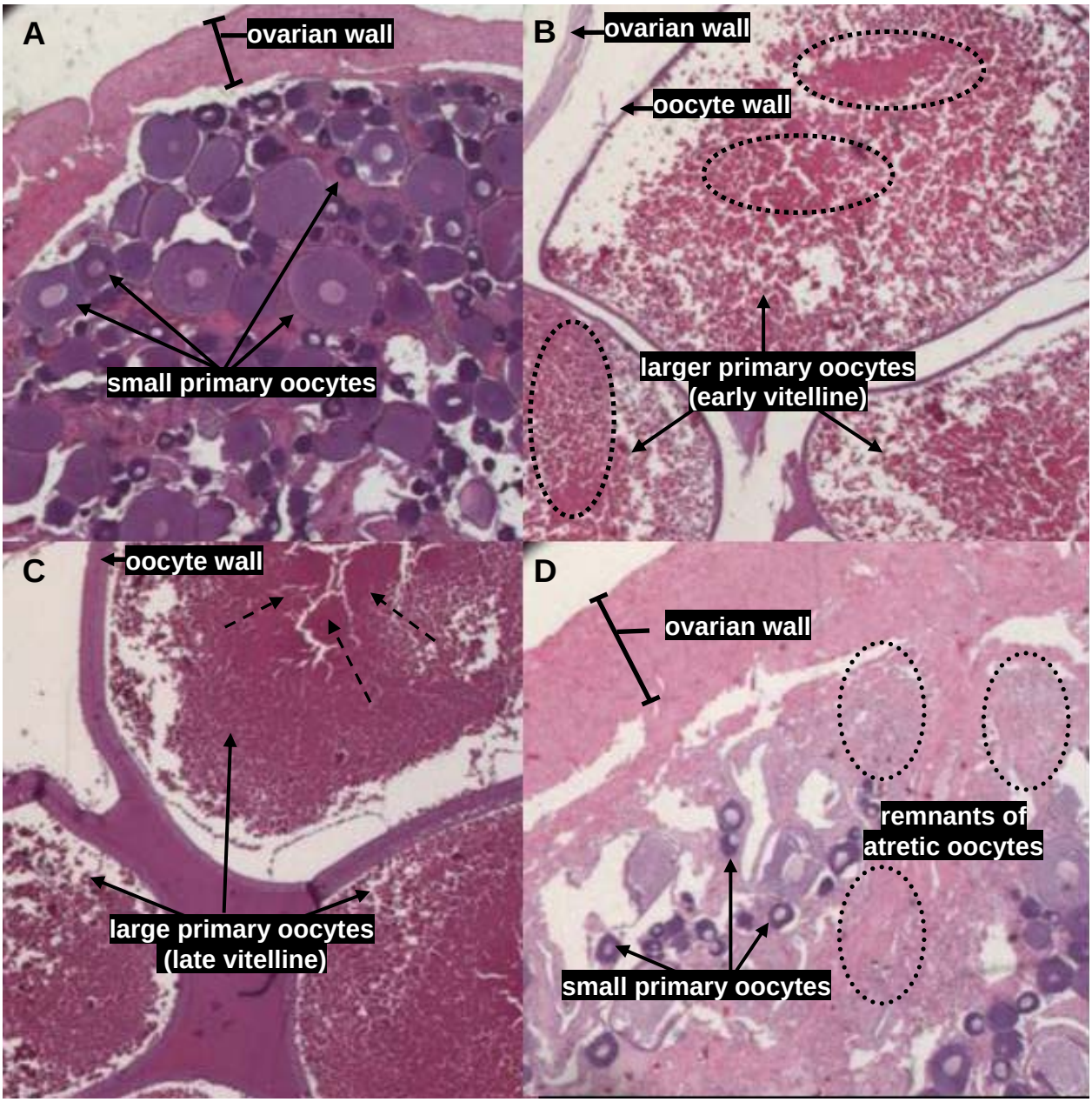

Figure 4. Histology corresponding to ultrasound images of early (A), developing (B, C) and atretic (D) ovaries of channel catfish during the natural spawning season revealed distinct profiles. Histology corresponding to early (A) ovarian development displayed a thick ovarian wall enclosing numerous small primary oocytes with no discernible wall. Histology of developing ovaries (B, C) displayed large vitelline oocytes with a thin ovarian wall, a visible, thin oocyte wall (B), yolk globule formation (B, dotted oval shapes), a thickened oocyte wall (C), and coalescing yolk globules (C, dotted arrows). Histology of atretic (D) ovaries showed remnants of atretic oocytes, and few small primary oocytes.

Conclusions

Application of ultrasound technology provides a direct, non-invasive, visualization method that can be used for evaluation of the reproductive condition of channel catfish females during the spawning season. Histological profiles corresponding to ultrasound images revealed microscopic processes that were not visible with ultrasonography, but which corroborate ultrasound imaging of ovarian development, demonstrating a strong potential utility of ultrasonography in channel catfish reproduction. Linking the ultrasound images with histology of gonadal development provides a comprehensive view of ultrasound images representative of different gonadal stages ranging from developing and developed, to advanced and atretic ovaries.

The ability to use ultrasound technology and corroborate its application with histology and other biometric indices is important in understanding the biological development of the channel catfish ovary. Identification of females in late vitellogenesis is critical for efficient hormonal induction of spawning in the hatchery. This is especially important in the application of cryopreservation to the production of hybrid catfish (channel catfish females x blue catfish *Ictalurus furcatus* males) at a commercial scale. To adequately assess and improve cryopreservation of aquatic species, technologies for understanding gonadal biology need to be incorporated into selection of females with the highest chance of ovulating fertilizable oocytes. Further studies are needed for addressing qualitative and quantitative analysis of fish gonadal development based on ultrasonography and histology to fully explore the potential application of ultrasound as a standard, non-invasive, informative means of commercial-scale assessment of a variety of fish species.

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