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Oocyte Staging in Paddlefish, *Polyodon spathula*

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Abstract

Oocyte size, germinal vesicle position, and pigment distribution of ova were examined as indicators of progress toward ovulation for hormonally injected paddlefish, *Polyodon spathula*. A boil and cut procedure to identify germinal vesicle position in oocytes was useful for estimating stage of oocyte maturation. Pigment pattern was a functional alternative for identifying the germinal vesicle position, because it could be substituted for the boil and cut procedure. Ova size was of no practical significance in relation to estimating potential spawning success or ovulation time. Latent time to ovulation for females injected with Luteinizing-Hormone–Releasing Hormone analogue was shorter than that of females injected with paddlefish pituitary extract. Information on the rate of germinal vesicle migration relative to water temperature would facilitate prediction of ovulation time following hormonal injection.

Introduction

Artificial propagation of paddlefish is an important tool for resource management. It may be used to enhance natural populations (1) or to produce stock for aquaculture (2). Natural stocks have been impacted by environmental perturbations and by recent exploitation for the caviar market (3, 4). Some governmental agencies are assessing the status of various natural populations, and are developing plans to revitalize the affected populations.

In most warmwater species, ova viability deteriorates relatively quickly after ovulation. Consequently, the quality of ova produced by induced spawning can be improved by the ability to accurately predict time of ovulation. Convenience to hatchery personnel can also be enhanced. Techniques used to examine intra-ovarian eggs are related to species specific characteristics. During final maturation of oocytes, cytological reorganization is usually associated with visible changes. The usefulness of these changes as predictors of ovulation depends on the ability to relate identifiable stanzas with time-related sequences (5). An important event associated with these changes is migration of the germinal vesicle (GVM) toward the periphery of the oocyte to complete the first meiotic division (6, 7, 8).

Coalescence of oil globules and related redistribution of cellular components results in spontaneous ova clearing in many marine fishes. For example, this has been used as an important tool to predict ovulation in striped bass, *Morone saxatilis* (9). However, cytological redistribution and germinal vesicle (GV) position are not easily viewed in ova of most freshwater species because their oocytes are opaque.

The yolk of some fish oocytes can be cleared with one of several solutions so that the germinal vesicle can be seen (10). However, this method is not satisfactory for oocytes of fish species that have pigmented eggs. Lutes et al. (11) located GV positions in the eggs of white sturgeon, *Acipenser transmontanus* by heat-hardening the oocytes and cutting them along the animal-vegetal axis. The objectives of this study were to test the applicability of the technique used to identify GV position in sturgeon oocytes, in paddlefish oocytes, and to examine other functional alternatives.

Materials and Methods

Initially, a seasonal series of oocytes was sampled from wild adult paddlefish in Grand

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Lake, Oklahoma (12). Oocytes and pituitaries were collected from fish being processed for caviar by licensed commercial fishermen. Pituitaries were frozen for later use in spawning, while ova were examined fresh.

Ten oocytes from each mature female were measured along the vertical axis, the pigment distributions were noted, and then the oocytes were boiled for 3–5 minutes to harden them. Similar to the methodology used for sturgeon by Lutes et al. (11), the boiled oocytes were bisected through the animal-vegetal pole axis, and the position of the GV was recorded. Some ova were preserved in formalin (5–10%) for several weeks to see if they would harden sufficiently for cutting. Several clearing solutions were also used with fresh oocytes to test their effectiveness to provide a view of the GV. In a few tests, commercial bleach was used as a follow-on treatment to a clearing solution. The solutions included an alcohol-xylene treatment (13), Stockard's solution (14), and a serra solution (15). Stoeckel and Neves (10) found these solutions to be effective in clearing oocytes of 9 teleosts. The serra solution has been widely used in Europe for clearing oocytes of common carp, *Cyprinus carpio* (16).

Artificial propagation trials were conducted at the Aquaculture Research Center, Kentucky State University. Broodstock in spawning condition were netted and transported to the hatchery facility, where they were hormonally injected following the procedures of Graham et al. (17) and Semmens (18). Fresh-frozen paddlefish pituitaries or super-active analog of Luteinizing Hormone–Releasing Hormone, LH-RHa (Sigma) were used as inducing agents. A priming dose (1/10 of the total) and a resolving dose (9/10) were administered 12–24 hr apart. Intra-ovarian eggs were sampled through a small ventral incision as described by Doroshov et al. (19) at the time of priming and in some instances at the resolving injection. Pigmentation patterns and GV positions of these oocytes were examined.

Results and Discussion

Oocyte Treatments

Ova were examined from 93 mature (>15 kg) females monthly from November through April (Table 1). Oocyte size spanned nearly the same range in November as in April just prior to spawning. There was a slight increase in mean size from November through April, but this change would be of no practical value in staging oocytes for induced ovulation. Ova size during the prespawning period was similar to that reported for paddlefish in Louisiana (20).

Table 1. Paddlefish oocyte size collected from fresh ovarian samples during the prespawning period.

Month	Mean height (mm) (n = 10 ova)	Range	Number of fish
November	2.46	2.30–2.72	20
December	2.49	2.35–2.77	18
January	2.55	2.44–2.89	14
February	2.63	2.48–2.94	20
March	2.70	2.31–2.92	15
Spawning	2.71	2.56–2.79	6

Heat-hardening of paddlefish oocytes and subsequent bisection resulted in 2 egg halves that were satisfactory for locating the GV. The GV could be clearly seen as a fine-textured, round object within the more granular yolk. None of the formalin-fixed oocytes were hardened enough to be cut without distorting the egg. Further, none of the solutions cleared paddlefish ova so that the GV could be seen because the dark pigment screened any change in yolk opacity. Treatment with bleach somewhat reduced the pigment intensity, but also distorted the oocytes so that they could not be used for staging.

Oocyte Staging

In the period just preceding natural spawning, the GV was in a central position (Fig. 1, right couplet of Stage I) and pigment was unequally distributed (Fig. 1, left couplet of Stage I). Pigment appears to be more concentrated near the oocyte periphery rather than being dispersed in the yolk. Yolk in the animal hemisphere is more uniform in particle size, than that of the vegetal hemisphere. The animal hemisphere also has a much greater concentration of pigment than the vegetal hemisphere. The latter is whitish-grey, while the former is charcoal colored. Transition of pigment distribution at the equatorial circumference is distinct, and often appears as a shadowy ring at the equator. Position of the centrally located GVs usually corresponds to the zone of transition between the vegetal and an-

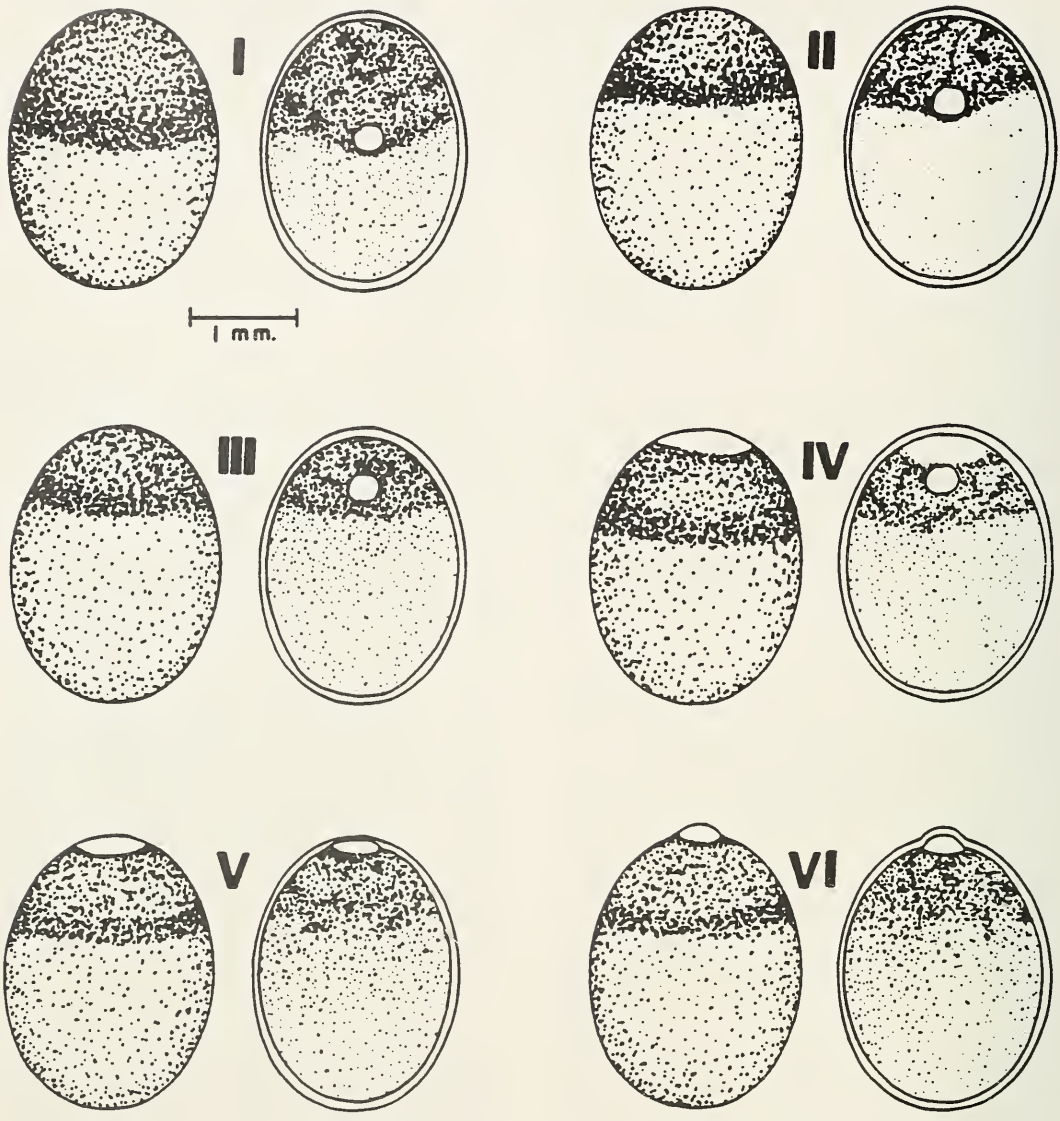

FIG. 1. Paddlefish oocyte stages (I–VI); the left illustration of each pair is representative of the surface pigmentation and the right illustration of each pair is of a bisected oocyte showing yolk distribution and germinal vesicle (GV) position. Stage I typifies an oocyte with a centrally located GV prior to resumption of meiosis-I; Stage II illustrates the slightly displaced GV at the initiation of germinal vesicle migration (GVM) and pigment redistribution after the resumption of events leading to meiosis-I; Stage III shows the GV in a position in the upper region of the oocyte near the animal pole; Stage IV depicts the clearing of the animal pole region in relation to the GV position; Stage V is characterized by further pigment change and the disappearance the GV at germinal vesicle breakdown (GVBD); Stage VI shows the appearance of an ovulated egg.

imal hemisphere pigmentation. This relationship is maintained as the GV migrates toward the animal pole.

The GV was in the central position (Stage I) throughout the fall and early spring. Migration toward the animal pole occurred rapidly, and just prior to ovulation. As the GV migrates (GVM), the pigment discontinuity also moves poleward (Fig. 1, Stage II). Pigment near the transition is often more concentrated, giving the appearance of a ring. The position of the pigment margin does not continue to move as

Table 2. Paddlefish oocyte stage, inducing agent, and temperature related to latent period for ovulation.

Oocyte stage at injections*			Ovulating Agent**	Temp. (C)	Ovulated/ injected	Latent period (hr)	
Prime	Interval (hr)	Resolve				Resolve	Total
I	24, 24***	II, III	P	13–14	0/2	both died	
II	24, 24***	III, IV	P	13–14	2/2	18–19	66
III	24	—	P	13–14	1/1	25	49
IV	22	—	P	17–18	1/1	18	40
III	24	—	L	13–14	1/3	15	39
III	12	IV	L	17–18	6/6	24–26	36–38
III–IV	12	V	L	15–16	8/9	24–28	36–40
III-IV	22	—	L	18–19	5/5	19	31
IV	22	—	L	17–18	2/2	13.5	35

* See Figure 1.
** P = fresh-frozen paddlefish pituitaries; L = LH-RHa.
*** Second resolving injection.

the GV shifts further to approximately ½–⅔ the distance to the pole (Fig. 1, Stage III) and there is a more uniform dark pigmentation, rather than the ring-like pattern seen earlier. However, Stage-II and Stage-III oocytes are difficult to differentiate without direct reference to the GV position. As GVM continues, a clear area appears at the animal pole (Fig. 1, Stage IV). Consequently, at Stage IV, a relatively broad band of pigment occupies the middle two-thirds of the animal hemisphere and the GV is now located at the polar edge of the pigment band. Appearance of the clear area at the animal pole is indicative of Germinal Vesicle Breakdown (GVB), and subsequently completion of the first meiotic division and ovulation. The clear area of the oocyte contracts slightly prior to ovulation, and the GV is no longer visible in gross cross-section (Fig. 1, Stage V). The ovulated egg (Fig. 1, Stage VI) has a distinct elevated protuberance at the animal pole/micropylar area, and the pigment is slightly more diffuse than that of earlier stages.

Application to Propagation

Direct observation of oocyte GV position, or the changing pattern of pigmentation can be used to stage oocytes of paddlefish. Position of the GV was used as an indication of maturational activity, once GVM had been initiated. However, because the GV is centrally located for most of the prespawning period these characteristics are useful as indicators of approximate time to ovulation only after some displacement has occurred.

Oocytes from females collected early in the spring-time spawning cycle were typically in Stage I or II. Some females that were collected when spring-time ambient temperatures were approaching the spawning range, and that had oocytes with a centrally located GV (Stage I), were not ovulated even after multiple injections (Table 2). Oocyte maturation (GVM) was stimulated by two resolving injections but these fish died before ovulation occurred. However, when GVM was proceeding (Stage II), responsiveness to induction was more predictable. Twenty four of 28 females with oocytes at Stage III or IV were induced to ovulate within 31 to 49 hr after the priming injection. Two factors, temperature and inducing agent, appeared to influence the timing of ovulation.

Because temperature affects maturational rate of oocytes in fishes, incorporation of a temperature component could improve predictive power of time to ovulation of hormonally injected paddlefish. Only general inferences can be made from the present data. Higher temperatures within the normal spawning range did result in shorter times to ovulation (Table 2). Temperature influence on developmental rate for the Russian sturgeon, *Acipenser guldenstadti,* is described by Ginsberg and Dettlaff (21). Staging under different temperature regimes may provide more insight into establishing a GVM rate and improve the usefulness; however, the inducing agent must also be considered.

In our study, the latent period for females injected with LH-RHa was generally shorter than that of females injected with pituitary extract. Further, the response to LH-RHa stimulation appeared to be less temperature sen-

sitive than that of pituitary induction. Semmens (18) also reported shorter latent periods for paddlefish injected with LH-RHa compared to pituitary stimulation. The reciprocal response would be expected based on the pathways and modes of action of the 2 agents. Rottmann and Shireman (22) reported that latent time to ovulation of Chinese carps was longer for LH-RHa than for pituitary induction.

Conclusions

Environmental factors influence the progress of gamete maturation. Intraovarian samples can provide a window to the process and indicate the status of the maturational trajectory. Whether a female is responding to an inducing agent, or will need additional stimulus to ovulate are pertinent questions during artificial propagation. The position of the GV, or in the case of paddlefish, the ova pigment pattern, can be used to provide more information during induction, or can be used as tools to examine more detailed relationships. In the latter context, a GVM-rate/temperature relationship would be useful in the artificial propagation of paddlefish. With this information, other factors, such as a more thorough comparison of the efficacy of inducing agents could be examined. Applicability of staging techniques may provide useful tools in facilitating artificial propagation of related fishes threatened with extirpation such as the Chinese Paddlefish, *Psephurus gladius*.

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