REPRODUCTIVE MANAGEMENT OF CULTURED WHITE STURGEON

(*ACIPENSER TRANSMONTANUS*)

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Introduction

Aquaculture industry in California currently produces farmed sturgeon for the food market. Sturgeon mature at an older age compared to other fish, and growers rely on the seedstock obtained by spawning of wild-caught fish. They started rearing broodstock in the early 1980's, and the increasing numbers of ripe females become available for spawning. Gamete maturation, fertilization and early development were investigated in depth in wild chondrosteans (review of Dettlaff et al., 1993), but the knowledge of reproductive development in captive broodstock is limited (Le Menn and Pelissero, 1991; Fujii et al., 1991; Moberg and Doroshov, 1992). We provide brief summary of reproductive cycle in domestic white sturgeon and management procedures used by farmers. Data were obtained in collaborative White Sturgeon Broodstock Development Program, sponsored by the Aquaculture and Fisheries Program (UC Davis), six sturgeon production farms, and California Department of Fish and Game. Project was supported by the U.S. Department of Agriculture and Department of Commerce.

Reproductive cycle

All broodstocks are first generation wild fish originating from the Sacramento, Columbia, and Fraser Rivers. They are raised in tanks in well water at temperature 16-20° C and fed salmonid diets. Gonadal development and plasma hormonal profile are monitored by repeated annual biopsy of marked individuals, histology, and radioimmunoassays. Information is based on pooled stock of several farms with similar environmental and husbandry regimes.

The external sexual dimorphism is not expressed in white sturgeon at any stage of maturity. Anatomical gonadal sex differentiation occurs at age 1-2 years. Males complete first spermatogenesis at age 4 years (range 3-5) and exhibit annual cycles. Females mature at 8 years (range 6-11) and have biennial cycles. The endogenous growth of the oocyte and follicular differentiation are completed by age 4-5 years, but the onset of vitellogenesis may be delayed for several years. Such previtellogenic females, implanted slow-release pellets containing estradiol and/or LHRH-A, exhibit vitellogenin secretion but no uptake of yolk precursor by the oocyte (Moberg et al., 1991). White sturgeon vitellogenin and VG mRNA were purified (Kroll and Doroshov, 1991; Bidwell et al., 1991), and diagnostic techniques to monitor plasma vitellogenin level are now available (Linares-Casenave et al., this volume). First vitellogenesis starts in the summer and is completed in late fall of the next calendar year. During 16-18 months of vitellogenesis the oocyte diameter increases from 0.5 to 3.5 mm (Fig. 1). All females that were spawned twice exhibit biennial intervals between first and second spawning, similar with cultured siberian sturgeon, *Acipenser baeri* (Williot et al., 1991) and fertile hybrid *Huso huso* x *Acipenser ruthenus* (Burtzev, 1983). Final ovarian maturation extends in domestic females from February to June. During the last 3 months of the ovarian cycle sturgeon eggs undergo significant cytoarchitectural changes (Fig. 1). Water temperature at or above spawning optima (15° C) induces ovarian atresia, and a cooler temperature 10-12 °C is required to complete normal ovarian development (Webb et al., this volume).

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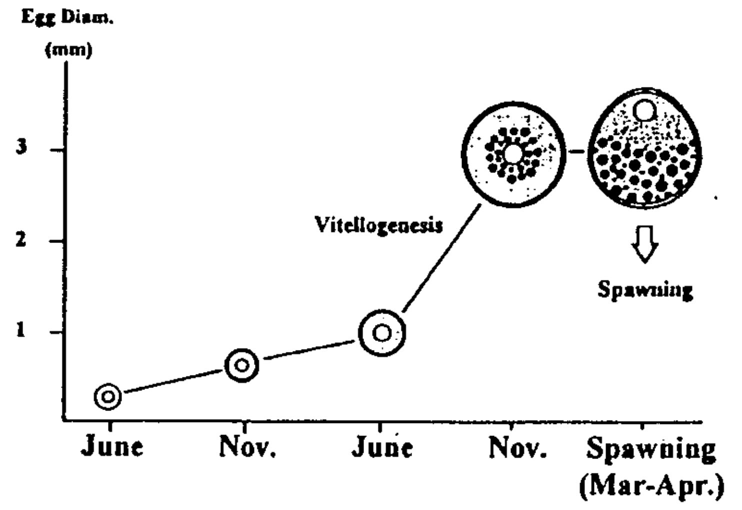

Figure 1. Scheme of egg development and growth in domestic white sturgeon

Gonadal cycle of sturgeon is controlled by a gonadotropin-releasing hormone (Sherwood et al., 1991) and pituitary gonadotropin (Kuznetsov et al., 1983). Recently, Moberg et al. (1991) described two gonadotropins, GTH-1 and GTH-2, and developed radioimmunoassays for these hormones in white sturgeon. In domestic fish plasma concentrations of GTH-1 are elevated during vitellogenesis and spermatogenesis, whereas the GTH-2 exhibits significant increase at ovulation (from 1-2 to 20-40 ng/ml). Plasma concentrations of androgen and estrogen increase during vitellogenesis and decrease during final ovarian maturation, with testosterone at high level through almost entire ovarian cycle (Fig. 2). Progestogen 17α,20β-Dihydroxy-4-pregnen-3-one was found in high concentration in ovulating female (3-10 ng/ml). However, the potential role of this hormone as a putative maturation-inducing substance is not established.

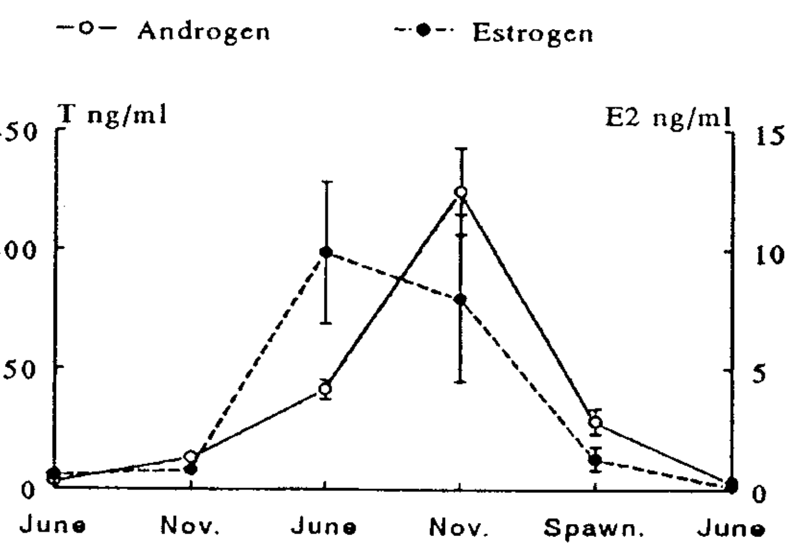

Figure 2. Plasma concentrations of sex steroids during the ovarian cycle ($\bar{x}$ ± s.d., n=9).

Broodstock management

Although puberty is significantly accelerated in cultured sturgeon compared with wild prototype, the absence of external sexual dimorphism, asynchronous onset of vitellogenesis, and temperature-sensitive final gonadal maturation make broodstock management difficult and labor-intensive. The following procedures are currently used in sturgeon farms to monitor reproductive development and to achieve satisfactory spawning performance. They include sexing, assessing stage of female maturity, and induced spawning.

Sexing. The broodfish are sexed when they are recruited from the production stock raised for the food market (age 3-4 years, body weight 10-15 kg). Sexing is performed by visual examination of gonads through an abdominal incision 2-3 cm long. Minor surgery is conducted on anesthetized fish placed on stretcher. The gonad is gently rolled towards the incision with Codman Allies forceps, exposing the lateral side of the organ. The ovary has ovigerous folds open to the coelom, pink or yellow in color. Testes are white and are enclosed in tunica. The incision is closed with a single stitch, using monofilament suture. An experienced farm crew can sex 100-150 fish in one day. Unselected stock has sex ratio 1:1, but in groups previously selected for large body size the female proportion may increase to 75 percent. The mortality after sexing is less than 2 percent. Males do not require any further sampling.

Ovarian maturity. The female stock is biopsied annually, usually in the late fall. The ovigerous folds of maturing females are greatly enlarged, and the ovarian follicles are readily observed through small abdominal incision. Three distinct stages are recognized by visual examination: 1) previtellogenic, with small translucent eggs; 2) mid-vitellogenic, with medium size white or grey eggs; 3) ripe, with large and black eggs, spawnable next spring. Small number of eggs (30-40) is sampled from ripe females, and this group is transferred in the ambient cool or chilled recirculated water with temperature 10-12° C. Sampled eggs are boiled, bisected and measured for oocyte polarization index (distance between germinal vesicle and animal pole cortex divided by egg diameter) using digital image-analyzer. In the fall polarization index ranges 0.17-0.35, decreasing to 0.05 - 0.15 before spawning. Time of final ovarian maturation is estimated by ranking fish with different polarization index.

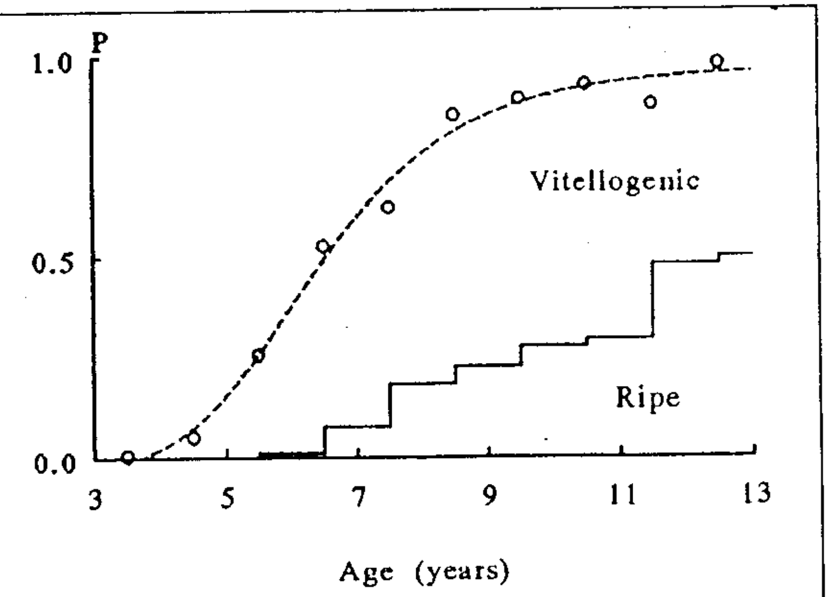

Figure 3. Proportions of vitellogenic (symbols and dotted line) and ripe (staircase line) females in domestic stock of three sturgeon farms (N=355).

Monitoring ovarian development in large female stock revealed that the recruitment of stock into first vitellogenesis occurs in age interval 4-9 years, and that in first spawning at age 6-11 years (Fig. 3). From the age 12 years practically all females are vitellogenic, with approximately 50 percent of ripe fish each year. Preliminary data suggest that female maturation is size-dependent. Body weight of domestic females at first spawning is 35 ± 17 kg (x ± s.d., n=70) and is similar to that of wild-caught females spawned by farmers during the last 3 years (36 ± 12 kg, n=38).

Induced spawning. Females, ranked by their polarization index in fall, are sampled once again in spring by catheterizing eggs through abdominal incision. Thirty follicles are incubated in Ringer solution (260 mOsm/kg, pH 7.5; Dettlaff et al., 1993) with 5 mg/ml of commercial progesterone, for 16 hours at 16 ± 0.5° C. The response is measured by rate of germinal vesicle breakdown (GVBD). Fish with GVBD 70-100 percent are induced to spawn within 1-2 weeks after sampling. Although the in vitro egg maturation response is closely associated with germinal vesicle position (Fig. 4), the egg maturation assay appears to be more accurate in predicting spawning response, compared to polarization index. The eggs of some females exhibit advanced germinal vesicle position but do not respond with meiosis, indicating "overripeness". In the other females, high rate of GVBD and ovulatory response with high egg fertility were observed at relatively high oocyte polarization index.

Ovulation and spermiation of white sturgeon are induced by the mammalian GnRH analog (LHRH-A) or common carp pituitary extracts. The latter is preferred by breeders because of shorter latency and accurate timing of ovulatory response. The LHRH-A is often used as a primer in ovulation induction. Well proportioned males with a high condition factor, chosen for spawning, are held at the low temperature (12-14° C) during the entire spawning season. They produces 60-120 ml of milt (collected by the catheter from urogenital duct) in 24 hours after single injection with 1-1.5 mg/kg common carp pituitary extract. The temperature is temporarily raised to 16° C after injection and lowered again to 12-14° C after spermiation, allowing for repeated collection of milt from the same male every 8-14 days. Fresh semen is stored in plastic bags with oxygen in a refrigerator and scored for density and motility. Females are injected twice: first, with 10 mg/kg LHRH-A in the evening hours, followed by the second injection of 4-5 mg/kg carp pituitary 12 hours later. The latency time is 23 ± 1 h (x ± s.d., n=70) after the second injection at water temperature 15-16° C. Ova are removed by aseptic caesarean surgery with

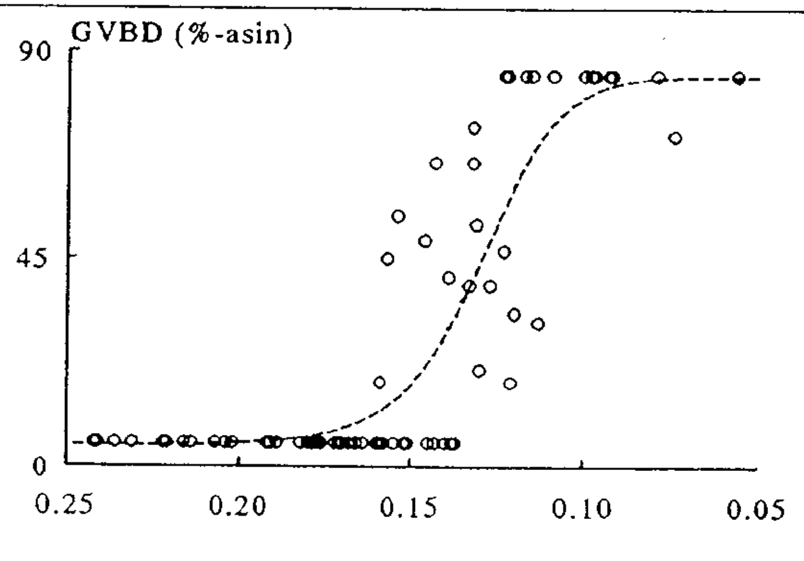

Figure 4. Relationship between *in vitro* GVBD response and oocyte polarization index in ripe females. Dotted line is logistic regression ($P < 0.05$, $R^2 = 0.786$)

anesthesia. The incision 10-12 cm long is closed by the internal and external stitches (PDS suture, Ethicon), fish is injected antibiotics and held in separate tank. Primary healing occurs within one month after surgery and complete healing in 3-4 months. The mortality is not encountered when surgery and post-operative procedures are properly followed. Spent females start vitellogenesis in the same year and mature in 2 years. The egg insemination and incubation procedures follow those described by Conte et al.(1988). The average fecundity of domestic females at first spawning is 130 thousand eggs (range 60-200). Fertilization success (4-8 cells embryo) ranges 70-95 percent. However, hatchability of eggs from domestic females is more variable, compared with wild-caught fish. In some progenies high embryonic mortality occurs at gastrulation and early neurulation, indicating inadequate egg quality. One domestic female produces in average 50 thousands viable larvae at first spawning.

Conclusion

Broodstock development and breeding of sturgeon require long-term commitment from commercial fish breeder. It will be beneficial to develop less invasive diagnostic techniques for reproductive management and spawning of sturgeon, in order to reduce the effect of handling and sampling stress on reproductive performance. The broodstock husbandry, including development of sturgeon broodstock-tailored diets, deserves greater attention to improve the quality of eggs for breeding and caviar production. Establishing sturgeon breeding program is equally important at present time, considering large size of fish, iteroparity, and limited broodstock numbers. Commercial sturgeon breeders of the West Coast made significant progress in artificial reproduction of white sturgeon. Some of the greatest benefits stemmed from their experience are understanding chondrostean reproductive cycle and captive breeding techniques. that can be used for conservation and environmental protection of the wild stocks.

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