Comparison of the Effects of Gonadotropic Preparations of the Carp and Stellate Sturgeon Pituitaries on \textit{in vivo} and \textit{in vitro} Oocyte Maturation in the Siberian Sturgeon \textit{Acipenser baeri} Brandt

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Abstract—Injections of 2.5 mg/kg of stellate sturgeon (\textit{Acipenser stellatus} Pall.) pituitary extract and 5 mg/kg of carp (\textit{Cyprinus carpio} L.) pituitary extract in Siberian sturgeon (\textit{Acipenser baeri} Brandt) females did not reveal significant differences in the effects of these preparations. There were no differences in the percentage of females that responded by ovulation, duration of the period from injection to ovulation, rate of ovulation, or quality of mature eggs as estimated by the percentage of fertilization or percentage of normal embryos at the small yolk plug stage. Thus, an insufficient efficiency in the artificial reproduction of the Siberian sturgeon grown in captivity is not related to the use of the carp pituitary preparation as a stimulus. Estimation of the ratio of specific activities of the pituitary extracts and purified gonadotropins of the stellate sturgeon and carp by \textit{in vitro} oocyte maturation has shown that it varies within wide limits as a function of the medium composition and physiological state of follicles. Hence, the ratio of activities of the gonadotropins of different species as determined by \textit{in vitro} maturation of sturgeon oocytes may markedly differ from that upon injection of these preparations in breeders.

Key words: sturgeon, \textit{Acipenser baeri}, artificial reproduction, oocyte maturation, ovulation, egg quality, \textit{in vitro}, \textit{in vivo}, gonadotropins, biological activity, culture medium.

Three hormonal preparations are now used for artificial reproduction of sturgeon: sturgeon and carp pituitaries and a synthetic analog of the gonadotropin-releasing hormone. The use of sturgeon pituitaries as stimuli was proposed and widely used in the USSR (for review, see Barannikova, 1987). In countries where sturgeon pituitaries were unavailable, carp pituitaries were used. Positive results were obtained on the Siberian sturgeon \textit{Acipenser baeri} (Williot and Rouault, 1982; Williot and Brun, 1982), white sturgeon \textit{A. transmontanus} (Doroshov \textit{et al}., 1983; Doroshov and Lutes, 1984), shortnosed sturgeon \textit{A. brevirostrum} (Smith \textit{et al}., 1985), and Atlantic sturgeon \textit{A. oxyrhynchus} (Parauka \textit{et al}., 1991). Analogs of the gonadotropin-releasing hormone also proved to be effective (Doroshov and Lutes, 1984; Goncharov, 1984; Arlati \textit{et al}., 1988; Fujii \textit{et al}., 1991; Goncharov \textit{et al}., 1991). However, since different sturgeons differ in their sensitivity to these preparations (Goncharov \textit{et al}., 1991), the use of pituitaries has not yet been eliminated from the agenda.

The use of any of these hormonal preparations rarely leads to high quality eggs in females. In this study, we tried to establish the extent to which insufficient efficiency of artificial reproduction in the Siberian sturgeon grown in captivity may be related to the use of pituitaries of a remote species (carp) or their inadequate dose.

The efficiency of sturgeon and carp pituitaries for artificial reproduction of the white sturgeon has already been compared and was proven to be similar (Doroshov and Lutes, 1984). However, since those authors used a small number of females, they considered their results preliminary and suggested that the results be interpreted with caution.

In addition to comparing the effects of the stellate sturgeon and carp pituitaries \textit{in vivo}, we also wanted to determine the ratio of the activities of these preparations in order to establish whether insufficient efficiency of Siberian sturgeon artificial reproduction is related to the use of inadequate carp pituitary doses.

In all publications describing the stimulation of the maturation of sturgeons gametes by carp pituitaries, doses of homologous pituitaries were indicated (in mg of acetonized pituitary powder per kg fish mass) that exceeded almost two- or three-fold those of homologous pituitaries commonly used (Williot and Rouault,
shown that this is the minimum diameter at which the oocyte diameter exceeded the small yolk plug stage (stage 17) (Dettlaff et al., 1998). The oocyte polarization index (OPI) was determined on the oocytes fixed by boiling (15 oocytes for each female) (Kazanski et al., 1978). This characteristic was used later in the distribution of females by experimental groups. Males were also chosen that had a spermatozoa-containing liquid fraction in testis samples.

The components of culture media were purchased from Sigma (USA), and the commercial preparation of carp pituitary was obtained from Argent Cy, while the stellate sturgeon (Acipenser stellatus) pituitaries were taken from adult fish migrating to the Volga River for spawning. In in vitro experiments, stellate sturgeon gonadotropin was used that was purified as described elsewhere (Burzawa-Gerard et al., 1975) with modifications. The purified carp gonadotropin and stock powder of acetonized pituitaries, used for its purification, were kindly provided by E. Burzawa-Gerard.

Experiments on artificial reproduction. Five experiments were conducted from March 16 to April 29. Ten females and 10–15 males were chosen at random from the previously selected fishes on the morning of the experiment and were transferred from the external cement basins to plastic basins under the roof supplied by the same river water. Shortly after noon, the females were divided in two groups of similar OPIs, which was determined during the preliminary selection.

At the same time, the females were operated on, and several dozen follicles were taken. For morphometry, 30 follicles were boiled, the envelopes were removed using sharp forceps, and two diameters and the shortest distance from the germinal vesicle surface to the oocyte’s surface were measured under a dissection microscope to exactly 0.03 mm. Since the oocytes rarely represent ideal spheres, the diameter was calculated as the mean of two distances: the distance between the poles and the distance perpendicular to it. The OPI was calculated as the ratio (%) of the shortest distance from the germinal vesicle surface to the oocyte’s surface to the mean oocyte’s diameter.

Soon after the removal of the follicles, the females were injected with a suspension of pituitary powder of stellate sturgeon (2.5 mg/kg body mass) or carp (5 mg/kg). After the injection, the females were transferred into individual basins, 2 m in diameter, supplied with running water at a constant temperature of 15°C. The males were injected with a suspension of carp pituitaries at 2 mg/kg of their body mass and placed in two basins. The egg removal, de-adhesion, artificial insemination, and incubation were performed as described elsewhere (Goncharov et al., 1999). The osomotic index was calculated as a percentage ratio of the mass of eggs to the fish mass. The percentage of fertilization (stage 5–7) and the percentage of normal embryos at the small yolk plug stage (stage 17) (Dettlaff et al., 1993) were determined by examining the developing embryos and the corresponding accounts of samples (200–300 embryos) under the dissection microscope.