Iranian Journal of Fisheries Science 1 (1), 59-65 (1999)

In Vivo Oocyte Maturation and Ovulation in Females and Spermiation in Males of a Hybrid Sturgeon, bester

B. Mojazi Amiri [*1], M. Maebayashi[2], N. Omoto[2], S. Adachi[1] and K. Yamauchi[1]

1) Department of Biology, Faculty of Fisheries, Hokkaido University, Hakodate 041, Japan
2) Department of Research and Development, The Hokkaido Electric Power Co., Inc., Sapporo 004, Japan

Abstract: Bester, a hybrid sturgeon (*Huso huso* L. females × *Acipenser ruthenus* L. males), neither spermiate nor ovulate in the captivity. Thirteen-year-old adult male and female bester were injected with LH-RHa (0.1-0.3 mg/kg B. W.) intramuscularly and spawning status of the treated fish was checked 24-48 hours later. Additionally, changes in serum levels of DHP in both the responded and the non-responded individuals were monitored 0, 1, 3, 6, 9, 12, 24, 48 and 72 hours after the teatment.

Between 70-100 percent of the LR-RHa injected males individuals, and 11-40 percent of the females spawned 24-48 hours after the treatment; the rest did not respond to the injection. In the responded males, 3-9 hours after the treatment, serum levels of DHP increased; while in the females it occured first after 12-24 hours. In contrast, during the same period, serum levels of DHP remaind low in all non-responded individuals.

The present study indicated that using 0.1-1.3 mg/kg B. W. of LH-RHa can induce oocyte maturation and ovulation in the females and spermiation in the males cultured bester. The results suggested additionally DHP as an appropriated steroid that could be used in the final maturation stage of the gonads.

KEY WORDS: Bester, oocyte maturation, ovulation, spermiation, DHP,LH-RHa

Introduction

In the female bester, a cultured hybrid sturgeon (*Huso huso* L. females × *Acipenser ruthenus* L. males), the oocytes do not complete the final maturation under the culture conditions and undergo mass atresia after full migration of its germinal vesicle (GV) to the animal pole as in the males, spermiation does not occur in the same situation (Mojazi Amiri *et al.*, 1996a,b). Our *in vitro*

* Present Address: Dept. of Fisheries & Environmental Sciences, Faculty of Natural Recourses, University of Tehran, Karaj 31585-4314, Iran, email: bmamiri @ chamran.ut.ac.ir

experiments indicated that gonadal fragments of bester were able to synthesis a maturity steroid, 17α,20β-dihydroxy-4-pregnen-3-one (DHP), during the final maturation and the failure in spontaneous spermiation or ovulation was not due to the insufficient synthesis of DHP, but it may be due to the lack of precursors at the cholesterol cleavage enzyme (Mojazi Amiri *et al.*, 1998).

Hypophysation using sturgeon or carp pituitaries is the most usual method for artificial propagation of sturgeons in the world (Steffens *et al.*, 1990). Experiments showing the effects of synthetic gonadotropic compounds, e.g. GnRHa, on propagation of white sturgeon *Acipenser transmontanus* have been successfully conducted in USA (Doroshov *et al.*, 1985) . Goncharoc *et al.*, (1991) have carried out similar studies on other sturgeon species, i.e. stellate sturgeon *A. stellatus*, Russian sturgeon *A. guldenstaedti*, beluga *Huso huso* and sterlet *A. ruthenus* in the former USSR. In other countries such as Japan, where sturgeon or carp pituitaries are unreliable, it is more practical to artificially propagate bester using synthetic gonadotropin compunds such as LH-RHa (des Gly^{10} [$D\text{-}Ala^{6}$] LH-RH ethylamide). LH-RHa has been successfully employed to induced spawning in teleosts, namely Cyprinidae (Sokolowska *et al.*, 1985), and yellow perch *Perca flavescens* (Dabrowski *et al.*, 1994). In Russia, employing LH-RHa in propagation of sturgeon species is widespread (Barannikova *et al.*, 1989). Additionally, this method has been successfully used on sterlet *A. ruthenus* (Horvath *et al.*, 1986).

In our detailed study, *in vivo* induction of oocyte maturation and ovulation in females and spermiation in males using LH-RHa were conducted in order to provide an effective procedure for accelerating maturation and ovulation. Changes in serum levels of DHP, in both responded and non-responded individuals, were also monitored to give us a clue about DHP's role as a potential steroid in spermiation in the males or ovulation in the females.

Materials and Methods

During the study period from March 1993 to June 1993, three batches of thirteen-years old F_1 male and female bester with average weight of 8.1 kg and average length of 100.8 cm were injected intramuscularly with LH-RHa (0.1-0.3 mg/kg B. W.). In March, 16 females and 14 males, in April 10 females and 12 males, in July 9 females and 10 males were treated. Blood samples were collected from all treated fish 0, 1, 3, 6, 9, 12, 24, 48 and 72 houres after the treatment and serum levels of DHP were determined by radiommunoassay (RIA) as it has been

described by Young *et al.*, (1983). The treated females and males were kept in a circular tank under similar condition (water temperature, regime) according to our previous study (Mojazi Amiri *et al.*, 1996a,b). The spawning status of the treated fish were checked 24-48 hours after the injection and in the individuals responded to the treatment, eggs and sperm were collected with sperm-egg collector apparatus.

Statistical analysis

One way analysis of variance (ANOVA) followed by Duncan's multiple range was used to identify the significant differences in DHP levels as well as in the GVBD frequencies.

Results

After 24-48 hours of treatment with LR-RHa, 2 females and 9 males spawned in March, 4 females and 11 males in April and 1 females 10 males in July, while the rest did not responded to the injection. In the responded individuals, serum DHP increased gradually three hours after the treatment (0.3-0.5 ng/ml) to peak 9-12 hours later (1.5-2.5 ng/ml) in males (Fig. 1) and 12-24 hours later (1.5-

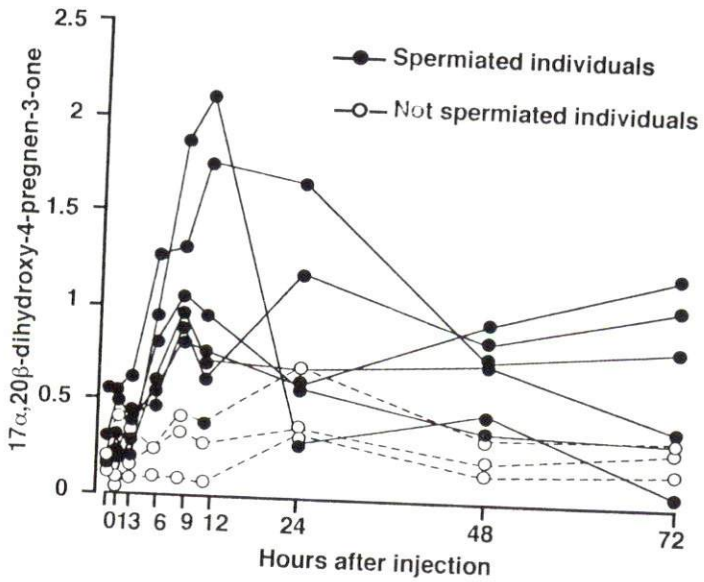

Fig. 1: Serum 17α,20β-dihydroxy-4-pregnen-3-one profiles with time following LH-RHa therapy in the males

3.5 ng/ml) in females (Fig. 2). In contrast, serum levels of DHP remained low in all non-responded individuals (0.5 ng/ml).

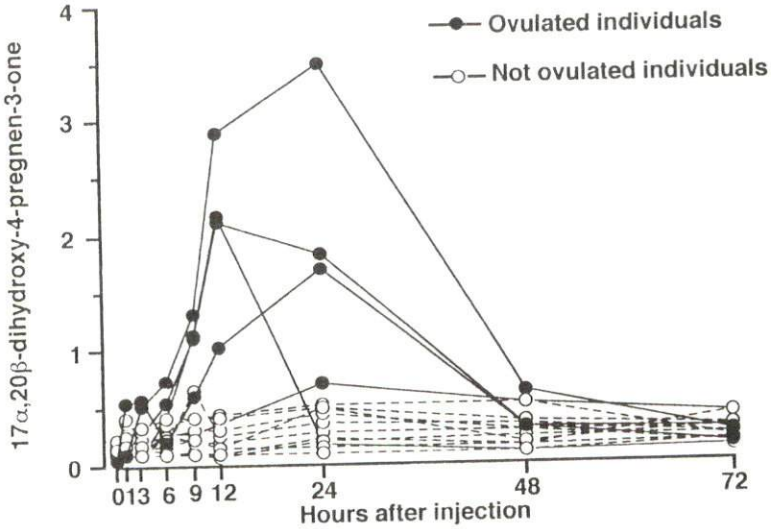

Fig. 2: Serum 17α,20β-dihydroxy-4-pregnen-3-one profiles with time following LH-RHa therapy in the females

Discussion

In the present experiment, *in vivo* induction of oocyte maturation and ovulation in females and spermiation in males using LH-RHa (0.1-0.3 mg/kg B.W.) was successfully performed. Several studies have reported successful induction of both maturation and ovulation in the female and spermiation in the male sturgeons using synthetic gonadotropin-releasing hormone (GnRH) (Doroshov and Lutes, 1984; Goncharov *et al.*, 1991). Reports on the using of LH-RHa were few, because of the doubt that GnRH is more effective than LH-RHa (Doroshov and Lutes, 1984). They have demonstrated that LH-RHa can also be used to induce gonadotropin secretion in the sturgeons as it has previously been used in some teleosts (Dabrowski *et al.*, 1994).

While 70-100 percent of LH-RHa injected males responded positively, the response of the females, which were treated with the same doses in the same month was low (11-40%). Our field observation revealed that when the fishes were manually stripped, 50 perecent of the intact male bester spermiated, while the same effort failed to induce ovulation in the females. This could also indicate

that there are fewer blocking factors affecting the spawning in the males than those influencing the females's spawning.

Serum levels of DHP elevated significantly ($P<0.01$) in the responded individuals; the concentration in the males rose 9-12 hours after the injection with LH-RHa, while in females it rose 12-24 hours after the treatment. Stripping in the males and spawning in the females occurred 24 and 48 hours after LH-RHa injection, respectively, revealing a time gap of 12-24 hours between occurrence of serum levels peak of DHP and spawning. Time gap between maturation and oviposition is dependent on hormonal or environmental factors (temperature, light, season) (Lam, T.J. , 1985 for review).

DHP is one of the most efficient steroids for *in vivo* induction of germinal vesicle breakdown (GVBD) in bester (Mojazi Amiri, *et al.*, 1996), and additionally unpublished data from our autoradiographic experiment using tritium (^3H) labelled 17α-hydroxy progesterone and pregnenolone in *in vivo* oocyte maturation showed a specified readioactive spot identified as DHP based on thin layer chromatography in the incubation wells where GVBD took place.

In conclusion, our study indicated that LH-RHa treatment (0.1-0.3 mg/kg of B.W.) could induce maturation and ovulation in female and spermiation in male cultured bester. These findings may also indicate that DHP is a relevent steroid in the final maturation of the gonads in bester.

Acknowledgement

The authors would like to thank Mr. K. Fuji, National Institute of Aquaculture, Mie, Japan, for providing the bester. The study was partly financed by grants from the Ministry of Education, Culture and Science of Japan.

References

Barannikova, I.A. ; Boev, A.A. ; Bokovskaya, O.S. ; Efimova, N.A. and Person, D.I. , 1989. Analysis of the maturation of the Russian sturgeon *Acipenser guldenstaedti*, under the influence of Luliberin in connection with its possible use in fish culture. J. Ichthyol, **29:** 124-133.

Dabrowski, K. ; Ciereszko, A. ; Ramseyer, L. ; Culver, D. and Kestemont, P. , 1994. Effects of hormonal treatment on induced spermiation and ovulation in the yellow perch *Perca flavescens*. Aquaculture. **120:** 171-180.

Doroshov, S.I. , 1985. Biology and culture of sturgeon (*Acipenseriformes*). *In* "Recent Advances in Aquaculture (Eds, Muir, J. F. and Roberts, R.J.), Vol. 11, pp. 251-274. Westview Press. Colorado.

Doroshov, S.I. and Lutes, P.B. , 1984. Preliminary data on the induction of ovulatory in white sturgeon Acipenser transmontanus Richrdson, Aquaculture , **38:** 221-227.

Goncharov, B.F. ; Lgumnova, L.V. : Polupan, I.S. ; Savelieva, E.A., 1991. Induced oocyte maturation, ovulation and spermiation in sturgeon (*Acipenseridae*) using synthetic analogue of gonadotropin-releasing hormone *in* "Acipenser" (Ed. Willot, P.), pp. 351-364, CEMAGREF publ., Bordeaux.

Horvath, L. and Orban, L. , 1995. Genome and gene manipulation in the common carp, Aquaculture, **129:** 157-181.

Lam, T.J. , 1985. Induced spawning in fish. *In*: "Reproduction and culture of milk fish" (Eds. Lee, C.D. and Liao, I.C.), pp. 14-56. Oceanic Institute and tankang marine laboratory, Hawaii and Taiwan.

Mojazi Amiri, B. ; Maebayashi, M. ; Hara, A. ; Adachi, S. and Yamauchi, K. 1996a. Ovarian development and serum sex steroid and vitellogenesis profiles in female cultured sturgeon hybrid, the bester. J Fish Biol. **48:** 1164-1178.

Mojazi Amiri, B. ; Maebayashi, M. ; Adachi, S. ; and Yamauchi, K. , 1996b. Testicular development and serum sex steroid profiles during the annual sexual cycle of male sturgeon hybrid, Bester. J. Fish. Biol. **48:**1039-1050.

Mojazi Amiri, B. ; Maebayashi, M. ; Adachi, S. ; Moberg, G.P. ; Doroshov, S.I. and Yamauchi, K. , 1998. *In vitro* steroidogenesis by testicular fragments and ovarian follicles in a hybrid sturgeon, bester. J. Fish Physiol. Biochem. (in press).

Steffens, W. ; Jahnichen, H. and Fredrich, F. , 1990. Possibilities of sturgeon culture in Central Europe. Aquaculture. **98:** 101-122.

Sokolowska, M. ; Peter, R.E. and Nahorniak, C.S. , 1985. The effects of different doses of piomozide and [D-Ala^6, Pro^9-N-ethylamid]-LHRH (LHRHa) on gonadotropin release and ovulation in female goldfish. Can. J. Zool. **63:** 1252-1256.

Young, G. ; Crim, L.W. ; Kagawa, H. ; Kambegawa, A., 1983. Plasma 17α,20β-dihydroxy-4-pregnen-3-one levels during sexual maturation of

amago salmon *Oncorhynchus rhodurus*: Correlation with plasma gonadotropin and *in vitro* production by ovarian follicles. Gen. Comp. Endocrinol. **51:** 96-105.