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

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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 treatment.

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 occurred first after 12-24 hours. In contrast, during the same period, serum levels of DHP remained 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

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experiments indicated that gonadal fragments of baster 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. gueldenstaedti, 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 compounds such as LH-RHa (des Gly\(^{10}\) [D-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 baster 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 hours after the treatment and serum levels of DHP were determined by radiomunoassay (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-

![Graph image](Image)

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 percent 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 baster (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 radioactive 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 baster. These findings may also indicate that DHP is a relevent steroid in the final maturation of the gonads in baster.

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