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Published online 2014 March 19. **Brief Report**

Spawning Latency Period in Hormonal Induced Reproduction of Snow trout (*Schizothorax Zarudnyi* (Nikolskii, 1897))

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Background: The breeding performance is an important parameter to evaluate the breeding success in captivity conditions. The optimum dose of hormone in combination with latency period is desirable for getting best breeding performance in fish.
Objectives: The objective of the study was to find the spawning latency period in the hormonal induced reproduction of Snow trout with two inducers (Ovaprim and hCG) separately and in combination.
Materials and Methods: The fish spawners were randomly divided into five groups and treated with Ovaprim, Ovaprim and high dose of hCG, Ovaprim and low dose ofhCG, hCG and saline water as control.
Results: The results suggested that Ovaprim and high dose of hCG treatment lead to shorter latency time (40 hours and 40 minutes), but ovulation percent, percentage of live embryos in the eyed stage and ovulation synchronization were lower than treated groups with Ovaprim alone or Ovaprim and low dose of hCG. Females from the control and hCG groups did not spawn.
Conclusions: The highest hormonal stimulation effectiveness was recorded in the group in which one hormonal substance (Ovaprim) was applied. Therefore the accurate ovulation time of snow trout, *Schizothorax zarudnyi* was difficult to be predicted.

Keywords: hCG; Latency Period; Ovaprim; *Schizothorax Zarudnyi*; Snow Trout; Spawning

1. Background

Many fish populations worldwide have experienced a drastic reduction, largely due to the effects of the industry and habitat loss. One of the useful ways to replace declining natural stocks is through captive breeding or hatchery programs. Since 1997, population of snow trout *Schizothorax zarudnyi* has been decreasing. The original fish, snow trout *S. zarudnyi* is an endangered species of endemic fish of Lake Hamun; Sistan, Iran shows much promise on the grounds of its wide popularity and hardness in environmental conditions.

Large areas of Sistan & Baluchistan can be used as aquaculture areas. Local fish species were considered for their suitability in aquaculture and it was decided to select the snow trout *S. zarudnyi* as a possible candidate since it is delicious and has high nutritional value of cyprinid in general.

Prerequisites for being a successful aquaculture candidate are the ease in obtaining and raising fry or fingerlings, resistance against disease and other environmental conditions, simple culture methods and acceptable marketing qualities (1).

Environmental and hormonal manipulation of fish ovulation are important in the fish farming industry for two main reasons; to solve the problem of spawning asynchrony which necessitates frequent broodstock management (2, 3) and for accelerating or delaying gametogenesis in captive brood stocks, spawning may be scheduled to yield fry whenever needed (4). The use of exogenous hormones is an effective way to induced reproductive maturation and produce fertilized eggs (5). Originally, the culturists used carp pituitary (CP) which is still widely used, particularly for the major Indian carps, Chinese carps and the common carp *Cyprinus carpio* (4-6). Human chorionic gonadotropin (hCG) has been used to induce final maturation of oocytes as a tool of commercial aquaculture (5). The superactive luteinizing hormone-releasing hormone analogue (LHRHa) has been successfully used to induce final maturation and synchronize ovulation of many commercially cultured fish (7, 8). The use of different forms of gonadotropin releasing hormone

Implication for health policy/practice/research/medical education:
The study suggested that we cannot recommend a range of latency in *S. zarudnyi* by using ovaprim, which several reasons such as unknown age of wild broods, differences in broods weight, growth conditions, etc. are involved. It is necessary to obtain defined latency time for best breeding performance because the lower or higher doses reduce the egg output during breeding operation.

agonist (GnRHa), which stimulate secretion of endogenous gonadotropin hormone (GtH) (9, 10) Ovaprim and Ovatide are analogues of salmon gonadotropin releasing hormone (sGnRHa) with a dopamine blocker activity (11). The use of sGnRHa resulted in successful stimulation of ovulation in some of cyprinids (12, 13) and catfishes (14).

The breeding performance is an important parameter to evaluate the breeding success in captive condition depending on the type of hormone used and its potency, dose of hormone and maturity status of the fish (15). The success of induced breeding also depends on the latency period, which has been studied onseveral species (16, 17). Appropriate combinations of inducing agents and stripping time always yield maximum egg output during induced breeding (18). Improper coordination between these two will lead to breeding failure (15). In view of the above, we investigated the latency period of *S. zarudnyi* under controlled condition by induced breeding with Ovaprim and human chorionic gonadotropin (hCG).

Latency period is the time interval from the first injection to ovulation (19). The "latency period" greatly varies among species. For example, in salmonids this period extends to a few weeks (20), in Atlantic halibut *Hippoglossus hippoglossus* (Linnaeus, 1758) is 4 – 6 hours (21), in groupers of the genus Epinephelus is 1 – 2 hours (22), whereas in the white bass *Morone chrysops* (Rafinesque, 1820) it is only 30 minutes (23).

Systematically, *S. zarudnyi* belongs to teleostei class, cypriniformes order, cyprinidae family and schizothorax genus (24). The world distribution of *S. zarudnyi* is in semi temporal freshwater of western Asia (25). This is an endemic fish in Iran and mainly found in Sistan region (26).

Although, *S. zarudnyi* is believed to be "difficult to breed" in laboratory conditions, reports of success with the induced breeding of this species are available (27, 28). However, these studies are very restricted in analyzing the overall breeding performance.

2. Objectives

The effect of different hormonal injections and latency time combinations on ovulation has not been evaluated properly in the induced breeding of *S. zarudnyi*. The objective of the study was to find the spawning latency period in the hormonal induced reproduction of Snow trout by using two inducers (Ovaprim and hCG) separately and together.

3. Materials and Methods

The brood fish for experimentation were obtained from October to December 2010 from Chahnimeh reservoirs (Sistan province, Iran) using gillnets. Fish were transported to the hatchery of the Department of Sistan Fisheries in plastic containers with well-oxygenated water and proper conditioning. After transport, the fish were treated with solution of formaline (40 ppm) for 2 hours and were then placed in earth pond (0.35 ha area) until March.

Females and males Snow trout used in the experiments were 1328 ± 45 and 632 ± 17.6 g respectively. Age of the fish was not determined. At the time of reproductive season (March - April), when the water temperature was increasing, 44 females and 53 males fish were selected. Females with soft, distended belly and pink-red genital papilla and males, which released milt when subjected to gentle pressure on the abdomen area, were selected. Males and females fish were placed in separate concrete tanks of running water of 14-18 °C for 10 - 12 hours. Prior to injections, fish were anaesthetized in a water bath containing 0.05 - 0.07 mg.L $^{-1}$ clove solution.

Hormones used in this study were Human chronic gonadotropine –hCG (Pregnyl) provided by Daroupakhsh Co. for Pharmaceuticals and Chemicals Industries, Tehran, I.R. of Iran, under license of Organon, Oss Holland. Ovaprim contains the synthetic GnRH analog and domperidone dissolved in propylene glycol at 20 μg.mL $^{-1}$ and 10 mg.mL $^{-1}$, respectively obtained from Syndel Laboratories, Ltd., Vancouver, Canada.

After 24 hours of acclimation in 15-17°C water, the fishes were treated with hormonal injections. Fishes were divided into five groups Females in groups 1-4 received 1.5 mL Ovaprim .Kg $^{-1}$ B.W., Ovaprim + high dose of hCG (1.2 mL.kg $^{-1}$ + 5000 IU.Kg $^{-1}$), Ovaprim + low dose of hCG (1.5 mL.Kg $^{-1}$ + 1300 IU.Kg $^{-1}$), 2000 hCG mg.Kg $^{-1}$ respectively and the fifth group treated with NaCl 0.3 mg.kg $^{-1}$ as control group. Males in groups 1-4 received hormones synchronized to the 2nd female's injection as 0.3 mL Ovaprim.Kg $^{-1}$ B.W., Ovaprim + hCG (0.3 mL.kg $^{-1}$ + 1500 IU.Kg $^{-1}$), Ovaprim + hCG (1.5 mL.Kg $^{-1}$+ 200 IU.Kg $^{-1}$), 500 hCG IU.Kg $^{-1}$, respectively and the fifth group received NaCl 0.3 mg.Kg $^{-1}$ as control group. All injections were intraperitoneal at the base of the pectoral fin. Time intervals between respective injections were 24 hours, but this time between 3rd and 4th injections were 12 hours. Temperature during experiments was 15-17°C.

Fecundity rate was estimated by using volumetric technique. This technique is relying on simple proportionality to estimate the total fecundity from a specific number of eggs in a known volume of a subsample and a value for the total volume of the sample, and then calculate the total number of eggs in the ovary.

3.1. Statistical Analysis

The differences in latency period, survival of embryos to the eyed stage and survival of embryos from eyed stage to the larvae data were analyzed using one way analysis of variance (ANOVA) at minimum significant of $P < 0.05$. Regression analysis was performed to determine the correlation between latency time with body weight and latency time with ovulation.

4. Results

Results on the response to hormonal induction of ovulation, survival of embryos to the eyed stage, survival of embryos from eyed stage to the larvae, synchronization of ovulation and latency period for the different experiments are summarized in Table 1.

Successful ovulation was only obtained with Ovaprim. No female ovulated either in groups receiving hCG alone or control (groups 4 and 5).

5. Discussion

The latency period or response time is the time between the first hormonal injection and ovulation. This time is often related to the water temperature and the period, which decreases with an increase in temperature (29), but in this study we investigate the latency time at same temperature with different doses and types of hormonal injections.

Important differences were observed in latency time after the application of different spawning media (30). Differences in the latency time of tench (*Tinca tinca*) were observed in the case of different spawning agents (31). In our study, the latency periods between the hormonal stimulation and the ovulation with Ovaprim, Ovaprim + hCG (low dose) and Ovaprim + hCG (high dose) treatments were 60.15 ± 12.05 hours, 59 ± 8.38 hours, and 40.67 ± 6.35 hours, respectively (Table 2). In order to perform statistical analysis, the hour: minute format was transformed in only minute format, thus the maximum latency time was for Ovaprim as 3609 ± 723.05 minutes, while the minimum mount was 2440 ± 381.05 minutes for Ovaprim + hCG (high dose). The longtime of latency defined as lack of synchronization in achievement of readiness for spawning by the fish. The latency period of *S. zarudnyi* ranged from 34.5 - 71 hours at 16-17°C after administration of Ovaprim.

In case of stimulation with Ovaprim, we suggest application of the preceding injection of Ovopel (preparation containing a mammal analogue of GnRH and dopamine antagonist–metoclopramide) allowed shortening the time of ovulation to 48 hours in case of the dace (*Leuciscus leuciscus*) and 36 hours in case of the ide (*Leuciscus idus*) and synchronize it significantly (32). The shortest time between injections and ovulation was noted when Ovaprim with high dose of hCG was used as a spawning agent, almost 30% smaller in contrast to the fishes stimulated with Ovaprim singly or combination with low dose of hCG. In the case of ovaprim, females were responding in a longer period of time, but there was so much better ovulation (83.3%) results in comparison with females receiving Ovaprim and hCG, especially ovaprim and high dose of hCG (25%).

The differences in latency time in females treated with GnRHa, hCG and other hormonal preparations were previously reported in many species such as carp, and asian catfish *Pangasius hypophthalmus*. (33-35). It may be explained by the fact that GnRH release from the pituitary glands and the ovarian responses to the released hormones are sequential processes while in fishes injected with carp pituitary extract (C.P.E.) the ovarian response to the exogenous GtH was a single process. Probably hCG similar to C.P.E. acts on the gonads while GnRHa acts at a higher level of the reproductive axis. CPE usually involves a shorter latency time than Ovaprim, and this was noted in the case of cyprinids (35-37).

Another reason could be propylene glycol as a GnRHa + domperidone solvent cause lesser releasing of this compound in the blood circulation compared to hCG solution, which cause higher levels of latency period in GnRHa + domperidone treated fishes (10). In other words, it may be explained by the fact that GnRH release from the pituitary and the ovarian response to the released hormones is a sequential process while in fish injected with carp pituitary extract the ovarian response to the exogenous GtH was a single process (38). In snow trout, hCG has shortened the latency time, although this advantage cannot cover other hCG disadvantages in snow trout induced spawning.

The statistical analysis showed a Pearson correlation coefficient of 41 between latency time and fish body weight (group 1), meaning that there is a moderate positive relationship between the two variables (Figure 1). But, in groups 2 and 3, ovulation percentages were very low in comparison with group 1 (Table 1), so it is not necessary to discuss about their relationships.

Table 1 . Latency Period and Ovulation Percent of Snow Trout Treated with Different Hormones and Doses

Groups[c]	Ovulation. %		Working Fecundity, No. (%)	Mean Volume of Eggs/Fish, mL	Latency period	
	Full o.	Partial o.			(hh:mm)	Min, No. (%)
1	63.3	20	39531.25±7802.30 [a]	172.5±25.09 [a]	60:09	3609±723.05 [a]
2	-	25.0	18265.5±9704.69 [b]	246±28.21 [b]	40:40	2440±381.05 [b]
3	8	25	15682.33±5982.30 [b]	131.6±19.02 [b]	59:00	3540±503.05 [a]
4	-	0	-	-	-	-
5	-	0	-	-	-	-

[c] Groups designated by the same letter are not significantly different (P > 0.05).

Table 2. Latency Time Between Hormonal Injection and Ovulation of Snow Trout.

Fish Numbers	Date and Hour of Injection	Weight, g	Number of Injections	Time of Responding	Latency Time		Group, No.
	(D.M.YY hh:mm)			(D.M.YY hh:mm)	(hh:mm)	min	
1	07.03.10 08:30	1300	2	08.03.10 19:00	34:30	2070	1
2	07.03.10 08:30	1260	2	09.03.10 07:30	47:00	2820	1
3	07.03.10 08:30	1100	3	09.03.10 19:30	59:00	3540	1
4	07.03.10 08:30	1170	3	09.03.10 19:30	59:00	3540	1
5	07.03.10 08:30	1270	3	09.03.10 19:30	59:00	3540	1
6	07.03.10 08:30	1470	3	09.03.10 19:30	59:00	3540	1
7	07.03.10 08:30	2500	4	10.03.10 07:30	71:00	4260	1
8	07.03.10 08:30	1100	4	10.03.10 07:30	71:00	4260	1
9	07.03.10 08:30	1300	4	10.03.10 07:30	71:00	4260	1
10	07.03.10 08:30	1200	4	10.03.10 07:30	71:00	4260	1
2	11.03.10 10:00	1320	2	12.03.10 23:00	37:00	2220	2
4	11.03.10 10:00	1900	2	13.03.10 10:00	48:00	2880	2
8	11.03.10 10:00	1570	2	12.03.10 23:00	37:00	2220	2
1	12.03.10 08:00	1000	2	15.03.10 05.00	69:00	4140	3
3	12.03.10 08:00	1100	3	14.03.10 16.40	56:40	3400	3
4	12.03.10 08:00	1500	3	14.03.10 21.20	61:20	3680	3
5	12.03.10 08:00	1180	3	14.03.10 09.00	49:00	2940	3

The weak intensity R square of 0.05 meaning that approximately no percent of the variation in the ovulation latency time can be explained by the body weight in group 1 and the equation of the linear regression is y = 0.4112x+3046.9 + 3046.9 (Figure 1).

The statistical analysis shows a Pearson correlation coefficient of 0.02 between latency and ovulation of fishes meaning that there is a moderate negative relationship between the two variables, and a weak intensity R square of 0.36, meaning that approximately forty percent of the variation in the ovulation latency time can be explained by the latency time, the equation of the linear regression was y = -0.0234x +161.47 (Figure 2).

The study suggests that we cannot recommend a range of latency in *S. zarudnyi* by using Ovaprim; this is due to several reasons such as unknown wild broods, differences in broods weight, growth conditions, etc. It is necessary to find defined latency time for best breeding performance because the lower or higher doses would reduce the egg output during breeding operation. This information is of value for a commercial hatchery to get maximum quantity of egg during induced spawning of this snow trout. It seems that further studies on ovulation stimulation with Ovaprim can be recommended. The results would allow optimize the reproduction effects of this interesting species.

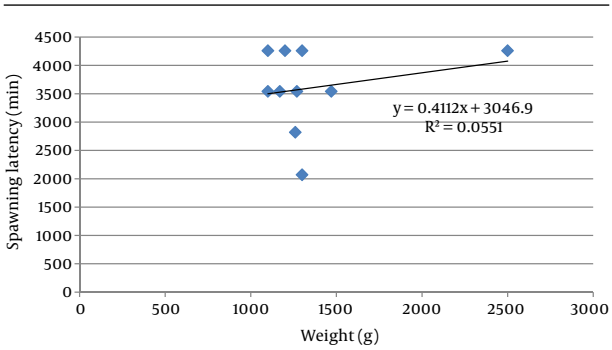

Figure 1. Scatter Plot for Relationship Between the Spawning Latency and Body Weight of Snow Trout (Group 1).

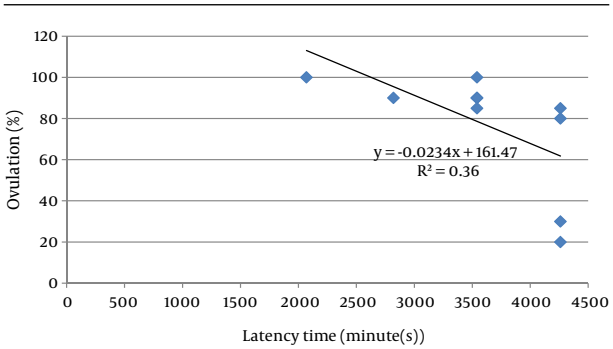

Figure 2. Scatter Plot of the Relationship Between the Latency Time and Ovulation Percent of Snow Trout (Group 1).

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Author's Contribution

All authors have participated equally.

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