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Effect of Ovatide Hormone in Oocyte Maturation in Sexually Immature *Puntius sophore* a Freshwater Cyprinid

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Abstract- The present investigations were conducted to evaluate the effect of ovatide treatment on the reproduction in *Puntius sophore.* Hormone was injected intramuscularly @ 4mg/kg and 0.5mg/kg body weight fortnightly for 90 days in two phases. Phase I was initiated at stage I (Resting stage) and Phase II at stage II(pre-maturing stage) of ovarian maturation. Results reveals that hormonal treatment induce ovarian maturation, stimulate ovulation, and expressed an advancement in time of spawning. It was revealed that spawning could be advanced by four months if treatment occur at stage I and by three months if treatment occur at stage II of the reproductive cycle.

Index Terms- Induced breeding, oocyte maturation, ovatide, *Puntius sophore*

I. Introduction

nduced breeding is generally carried out mainly with wild or Icaptive brood fish obtained from natural habitat during the spawning season. Induced breeding become an important tool in seed production before the actual time of breeding period. These studies aim to enhance year round availability of fish larvae rather than a seasonally restricted supply. Various workers (Sunderaraj *et.al* ,1966; Sinha, 1971**;** Lam, 1982; Koul and Rishi, 1986; Gandotra and Gupta,2003; Malhora *et.al*, 1991; Szabo, 2001; Rokade *et.al*, 2006; Rath *et.al*, 2007; Money and Thomas, 2008) have used different types of hormones (e,g) fish pituitary extract, LH-RH, mammalian gonadotropin and summach to control and regulate development in fishes.

The present studies reveal the effect of ovatide hormone on reproduction in *Puntius sophore* a freshwater cyprinid fish at different stages of maturation.

II. Material and Methods

Live specimens of *Puntius sophore* were collected from Gho-Manhasan stream in Jammu, Jammu & Kashmir, India, and kept for acclimatization.

Acclimatization: The fishes were acclimatized in the small cemented tanks under natural temperature and light conditions for atleast 15-20 days before the start of experiment.

They were given adequate amount of 40% protien/ (rice bran, diet cake and fish meal) during the period of whole experiment. To study the effect of ovatide on immature gonads of *Puntius sophore* ranging between 4.5-6.5cm size, the fishes were divided into two groups each comprising 20 fishes. i). Hormone injected group and ii). Control.

III. Experimental Design

In each set 20 fishes each (duplicate), dose D1 of ovatide and distilled water was given @ 3mg/kg and 0.5mg/kg body weight in groups fortnightly.

The hormone treatments were conducted in two phases, Phase I and Phase II. Phase I lasted for 45 days and was initiated at stage I of maturation. Phase II was initiated in the month of March, when the oocytes were at stage II. Three to four specimens were sacrificed after fifteen days, their ovaries were removed, weighed and tissue sections were also processed for histological examination of ovary.

Preparation of tissue for microscopic studies: Gonads fixed in Bouine's solution after thorough washing were dehydrated, cleared and finally embedded in parafin wax (melting point 52-54c). 5-7u thick sections of ovaries were stained using Mallory's triple and Haemotoxyline eosine stain.

Microtomy : From the stained sections diameter and percentage frequency of different stages of oocytes was worked out. Oocytes diameter was measured with the help of occular micrometer standrised against stage micrometer. For histological detail sections were photographed with the help of photo micrographic camera PM-6.

Procurement of hormone: Hormone used for present work has been procured from HEMMO, PHARMA, A, Wis of Tashan Textile. Mills Pvt. Ltd. (543, KALBADEVI ROAD, TALAO, MUMBAI, 400002) INDIA. Under brand name of Ovatide where it is commercially manufctureed under scientific conditions by using synthetic GnRH analogue and dopamine antagonist dissolved in a mixture of aqueous and organic solvent.

Prparation of injection solution : Different doses of the hormone solution to be injected as per the scheduled viz 0.2 mg, 0.3mg,0.4mg and 0.5mg/ kg body weight were prepare at the time of injection by diluting the concentrated ovatide in distilled water.

Morphological and histological examination of the gonads will be made as per following steps:

- Ovaries of the fish were carefully removed, excessive moisture was blotted and quickly weighed on an electronic balance.

- Ovaries were then fixed in bouin's fixative (freshly prepared from picric acid 75%, formalin 20% and acetic acid 5%) for 24 hours.
- They were then washed, dehydrated and embedded in paraffin wax .
- Transverse sections of ovaries were stained in haematoxylin-Eosin stain.
- Different phases of gonadal cycle were identified by observing the prepared slides under microscope.

Stage of gonadal development was assessed by calculating the gonadosomatic index

GSI =weight of gonads/ weight of fish ×100

- Detailed examination of the histology of the ovaries.
- Measuring the mean diameter of different oocyte stage by taking the average of the horizontal and vertical diameters of oocyte.
- Calculating percentage distribution of different stage oocytes.
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IV. Results

Phase I

When hormone treatment was initiated at stage I (Table 1) of oogensis in January, average oocyte diameter was 0.36±0.03µm. After 15 days of ovatide treatment 78.71±0.04% oocytes were at stage I and 21.27±1.02% at stage II, with an average oocyte diameter of 0.32±0.03µm. After 30 days of ovatide treatment 61.51±0.08% oocytes were at stage I, 14.28±1.37% at stage II and 13.57±1.21% at stage III as compare to control group which were 2.85±1.48% at stage II. After 45 days of ovatide treatment 22.31±1.21% oocytes were at stage III and 12.81±1.04% were at stage IV with an average oocyte diameter of 1.24±0.03µm of stage IV oocytes.Where as control group showed only 12.13±1.12% stage II and 2.51±1.02% stage III oocytes.

Except for a slight increase in size, both quantitatively as well as qualitatively, the control fish did not demonstrate any distinct change in maturation over the 45 days trial which showed 2.51±1.02% stage III oocytes only.

Phase II

When hormone treatment was initiated at stage II (Table1) of oogensis in March , average oocyte diameter was 0.62±0.03µm. After 15 days of ovatide treatment stage III oocytes 14.13±0.08%. After 30 days of ovatide treated group showed 10.57±1.21% stage III oocytesand 4.35±0.08% stage IV oocytes, where as control showed 5.81±1.2% stage III oocytes. After 45 days of ovatide treated group showed 12.31±1.21% stage III and 7.81±1.04% stage IV oocytes with an average stage IV oocyte diameter of 1.24±0.03µm, control group showed only 6.51±1.02% stage III oocytes. Investigations of male *Puntius sophore* revealed similar results. Males of *Puntius sophore* treated at stage II showed evidences of milt release under gentle pressure by the 40th day of ovatide treatment.

V. Discussion

In nature breeding period of *Puntius sophore* is between August and October, with a prolonged maturation period of 7-9 months. Present studies revealed that full maturation can be shortened to 55 days using ovatide treatment. The spawning period can be brought forward apprximately 4-4.5 months using ovatide hormone. Spawning occured at 80th day (Mid-April), in fishes treated with ovatide at stage II of maturation. In male *Puntius sophore* the ovatide treatment results in the male fishes spawn several days in advance of female. This difference may be due to the hypothalmio-pituitary gonadal complex in males being more sensitive to hormonal treatment than in females. The similar views has been previously proposed by Correan *et.al*,1976; ; Malhotra *et.al* ,1991; Sharma, 1992 and Gupta & Gandotra,2003.

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Table 1. Oocyte percentage and oocyte diameter during Phase I on ovatide treatment.
PHASE-I

	PERCENTAL OCCURENCE OF OOCYTES					OOCYTE DIAMETER			
DAYS	SET	STAGE I	II	III	IV	I	II	III	IV
0	WILD	100±0.00˚˚	-	-	-	0.36±0.03	-	-	-
15	C˚	97.21±0.02	1.27±0.08	-	-	0.28±0.03	0.62±0.03	-	-
	OT	78.71±0.04	21.27±1.02	-	-	0.32±0.03	0.66±0.03	-	-
30	C	96.45±1.2	2.85±1.48	-	-	0.32±0.03	0.66±0.03	-	-
	OT	61.51±0.08	14.28±1.37	13.57±1.21	-	0.34±0.03	0.66±0.03	1±0.03	-
45	C	85.35±1.08	12.13±1.12	2.51±1.02	-	0.3±0.06	0.56±0.09	1.04±0.06	1.14±0.06
	OT	40.71±1.37	25.36±1.02	22.31±1.21	12.81±1.04	0.36±0.06	0.42±0.36	0.36±0.62	1.24±0.03

Table 2. Oocyte percentage and oocyte diameter during Phase II on ovatide treatment.
PHASE-II

	PERCENTAL OCCURENCE OF OOCYTES					OOCYTE DIAMETER			
DAYS	SET	STAGE I	II	III	IV	I	II	III	IV
0	WILD	62.31±1.21˚˚	38.12±0.02	-	-	0.36±0.03	-	-	-
15	C˚	61.21±0.02	38.27±0.08	-	-	0.28±0.03	0.62±0.03	-	-
	OT	55.71±0.04	32.27±1.02	14.13±0.08	-	0.32±0.03	0.66±0.03	0.66±0.09	-
30	C	54.45±1.2	39.85±1.48	5.81±1.2	-	0.32±0.03	0.66±0.03	0.62±0.03	-
	OT	41.51±0.08	45.28±1.37	10.57±1.21	4.35±0.08	0.34±0.03	0.66±0.03	1.02±0.03	1.14±0.06
45	C	53.35±1.08	40.13±1.12	6.51±1.02	-	0.3±0.06	0.56±0.09	1.04±0.06	-
	OT	33.71±1.37	48.36±1.02	12.31±1.21	7.81±1.04	0.36±0.06	0.42±0.36	1.06±0.62	1.24±0.03

˚C= Control, OT= Ovatide treated.
˚˚M+SD = Mean + Standard deviation.

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