

Effect of cortisol on female freshwater fish *Notopterus notopterus*

D.S. Shankar and R.S. Kulkarni

Fish Endocrinology Research Unit, Department of Zoology, Gulbarga University, Gulbarga-585 106, India

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Abstract: In the present study, effect of cortisol on the ovary of the freshwater fish *Notopterus notopterus* was studied during four phases of the reproductive cycle. The hormone was injected 60µg/fish for ten days. Cortisol in fish is known to increase the metabolic activity. After the hormone treatment the ovarian somatic index and the hepatosomatic index increases during non breeding phase. The young oocytes transferred into vitellogenic oocytes in all the phases after cortisol treatment. The cortisol induced increased ovarian activity may be due to increase in the metabolic activity through the involvement of hepatic cells specially during non-breeding period providing support for energy production for reproductive activity in the freshwater fish *N. notopterus*.

Key words: *Notopterus*, Cortisol, Oocytes, Liver.

Introduction

Cortisol is quantitatively the most important steroid found in teleost plasma in different species (Matty, 1985). The hormone exhibits changes in its level with respect to season, time of the day and physiological state of the animal (Lange and Hanke, 1988). In teleost fishes corticosterone and cortisol are hormones produced from adrenocortical tissue, in which cortisol seems to be more abundant (Hanke, 1984). This hormone is generally considered to be a metabolic hormone in fish and several studies have implicated that the hormone is involved in the regulation of hepatic intermediary metabolism (Freeman and Idler, 1973; Chan and Woo, 1978; Lidman *et al.*, 1979; Leach and Taylor, 1982; Davis *et al.*, 1985; Foster and Moon, 1966; Vijayan *et al.*, 1991, 1993). One way that has been used to study the physiological effects of cortisol are elevated during pre-ovulation period in teleost fishes and may play a number of important regulatory roles during this time including its action in females as regulation of final oocyte maturation (Jalabert, 1976; Cook *et al.*, 1980), ovulation (Hirose and Ishida, 1974), spawning behaviour (Bry, 1985) and the mobilisation of energy stores required for reproduction (Greenberg and Wingfield, 1987). During other times however, cortisol can have deleterious effects on reproductive function, such as reducing normal sex steroid production, pituitary gonadotropin gamete quality, and subsequently larval quality (Carragher *et al.*, 1989; Carragher and Sumpter, 1990; Compbell *et al.*, 1992; 1994). Mommsen *et al.* (1999) have reviewed the physiological role of cortisol in growth and reproduction in fish. Glucocorticoides are known to suppress reproductive functions, forming part of the mechanism involved in delaying reproduction at times of stress and this phenomenon appears to be an adaptive response to divert metabolic building blocks away from biosynthetic pathways. Ding *et al.* (1994) demonstrated that exogenous cortisol in fishes increases the hepatic transcription of the vitellogenin gene, without a concomitant enhancement of liver or plasma vitellogenin in treated fishes. In semelparous pacific salmon, (genus, *Oncorhynchus*) hypercortisolism may be the cause of

“programmed death” after spawning (Dickhoff, 1989; Stein Behrens and Sapolsky, 1992). It is not correctly understood how cortisol can have such diametric effects on reproduction in fish, although one possibility is that cortisol target tissue sensitivity may be altered by physiological changes that accompany reproductive maturation. Additional information on the factors that control the mature and immature cortisol rise in fish may help to explain this paradox and increase our understanding of the reproductive functions of cortisol.

It is clear that even though the literature is extensive with respect to cortisol and metabolism, we are a long way from a complete understanding of the role of this complex hormone in fish specially with regard to its role in reproduction and such studies are restricted to only some Indian fishes. Hence, in the present investigation an attempt is made to understand the effect of cortisol on the responses of ovary and liver in female fish *Notopterus notopterus* during different phases of the reproductive cycle.

Materials and Methods

Hormone treatment: In the month of January-February (preparatory phase), April-May (prespawning phase), August-September (spawning phase) and November-December (post spawning/immature stage), 50 fishes, *N. notopterus* having 100-120g body weight and 24-26cm in length were collected from over live fish stock and used after their acclimatization to lab conditions. The fishes were divided into two group. Group I served as control and injected with vehicle (olive oil) and Group II were injected with the hormone cortisol. Cortisol (11β: 17α: 21 trihydroxy-4-pregnene-3:20-dione) was obtained from S.D. Fine Chemicals Ltd., Biosar and injected intramuscular at dose of 60µg near the caudal region below the lateral line per day for 10 days.

After 10 days of treatment all the experimental and control fishes were sacrificed by decapitation in the respective phases and ovary, and liver tissues were dissected out carefully weighed and processed for histological studies.

Table – 1: Effect of cortisol on OSI and HSI in the female freshwater fish *N. notopterus* (Pallas) during four reproductive phases.

Group	Preparatory		Prespawning		Spawning		Post spawning	
	OSI	HSI	OSI	HSI	OSI	HSI	OSI	HSI
Control	2.334 ± 0.152	1.130 ± 0.038	3.67 ± 0.021	0.690 ± 0.018	1.160 ± 0.167	0.362 ± 0.018	0.418 ± 0.141	0.487 ± 0.101
60 µg treated	1.098 ± 0.065**	1.095 ± 0.020**	3.403 ± 0.074	0.575 ± 0.015**	0.518 ± 0.045**	0.558 ± 0.156	2.11 ± 0.275***	0.642 ± 0.186

Hormonal and vehicle injections were given every day for 10 days. The values are expressed in mg/50 gm net wt. of the tissue.

Note: All values are expressed as mean ± standard error (SE) n=6

* p<0.05, ** p<0.01, *** p<0.001, Differs from the values of the control treated with cortisol, respectively.

Before fixing the ovary in the fixatives for histological studies, the total ovarian, liver and body weights were taken for the determination of OSI and HSI by using following formula:

$$1. \text{ Ovarian somatic index (OSI)} = \frac{\text{Weight of the ovary}}{\text{Weight of the body}} \times 100$$

$$2. \text{ Hepatosomatic index (HSI)} = \frac{\text{Weight of the liver}}{\text{Weight of the body}} \times 100$$

In all the cases six observations were made and the results (data) were expressed as arithmetic means with their standard deviation, standard error (mean ± S.E.) and student "t" test were made as described by (Snedecore and Cochran, 1967; Fisher, 1963) the values were judged almost significant p<0.05, p<0.01 and highly significant p<0.001.

Results and Discussion

The ovarian somatic index (OSI) of control fish increases during preparatory phase reaching maximum during prespawning phase after which it reduces in spawning phase, and was minimum in post spawning phase (Table 1 and Fig. 1). In the cortisol treated fish, at a dose of 60 µg, the OSI reduced to 50% during preparatory phase, while it remained to normal levels during prespawning phase, the significant reduction in OSI was noticed during spawning phase. However, it increased slightly during post spawning phase. The results indicate an increase in the OSI during post spawning that may be due to the growth of the ovary.

The hepatosomatic Index (HSI) of control fish, increases during preparatory phase thus reduces during prespawning phase and was continued upto spawning phase after which increases in post spawning phase which was significant in preparatory phase. Cortisol administration during preparatory phase reduced HSI, while it increases in other phases, significant increase was noticed after 60µg cortisol treatment during post spawning phase (Table 1).

Histological studies of the ovary in a control fish during preparatory phase consist of oocytes belonging to 9 oogenetic stages, majority of them are at the stage of development transforming young oocytes into previtellogenic oocytes. The oocyte cytoplasm is filled with the vacuoles containing secretory material. The nucleus of the oocytes contain few to many nucleoli, the vitellogenic process has

initiated in most of the oocytes. The follicular cells are differentiated in these vitellogenic oocytes. In the cortisol treated fish, the ovary has increased in weight in comparison to control. The ovary consist of all the stages of oocytes and most of the oocytes are transforming into vitellogenic oocytes. The developed oocytes contain large amount of yolk. The follicular cells have increased in their number and are well differentiated in histological sections.

The ovary in a control fish during prespawning phase consists of all the nine oogenetic stages including some vitellogenic oocytes belonging to migratory nucleus. In the treated fish, the ovary consists of advanced stages of oocytes. The cytoplasm of the oocytes was filled with large number of vacuoles. The follicular layer has increased in the thickness and many cells are arranged above the vitelline membrane.

The ovary of early spawning phase contains oocytes belonging to earlier as well as advanced stages of oocytes (Fig. 1). In the treated fish, the young oocytes exhibit vitellogenic activity which is characterised by the presence of vacuoles in the oocytes (Fig. 2).

In the post spawning phase, the ovary consists of young oocytes which are mostly non vitellogenic some vitellogenic and some unspawned oocytes having intact germinal vesicle at the periphery. The treated ovary consists of vitellogenic tertiary stage, oocytes with large number of cortical alveolae in the cytoplasm (Fig. 2). The vitellogenic oocytes contain centrally placed nucleus containing large number of nucleoli. These results indicate that in all the phases younger oocytes have been initiated for vitellogenic process which is characterized by the appearance of vacuoles and increase in the cytoplasm with distinctive differentiation of follicular epithelium. In the posts spawning phase, although ovary consists of young oocytes in the control fish while cortisol treatment advances the growth of the oocytes.

The gonadal cycle of *N. notopterus* can be divided into four phases during one year period as preparatory phase (January to March), prespawning phase (April to June), spawning phase (August and September) and post spawning phase (November to December). The developing oocytes in the preparatory phase, developed oocytes in the prespawning, ripe oocytes in the spawning phase and regressed oocytes in post spawning phase have been noticed. Studies based on the gonadosomatic index and histological structure during different

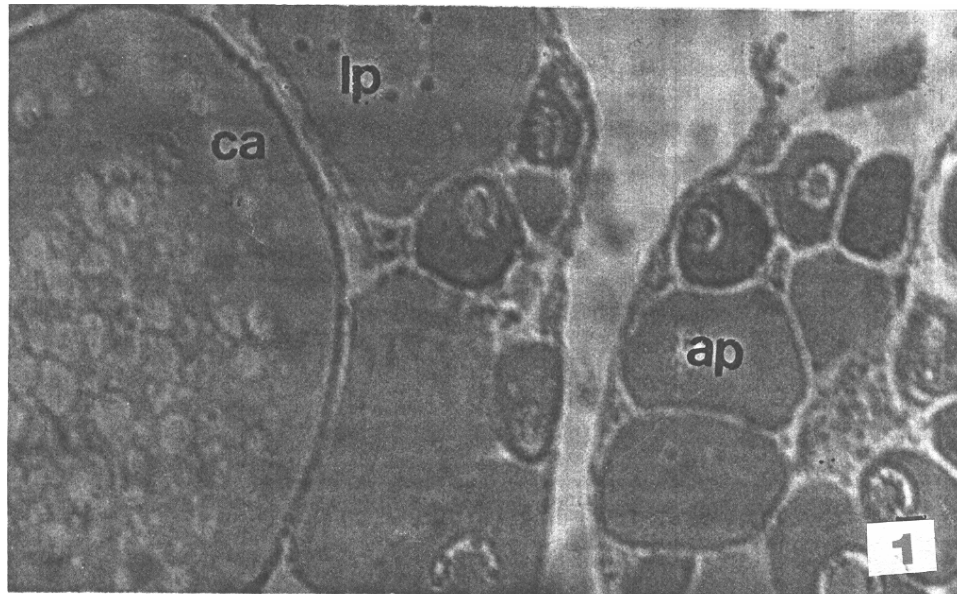


Fig. 1: Section of the ovary showing early perinucleolus, (ep) late perinucleolus (lp) and cortical alveolae (ca) oocyte during April (H & E X 375).

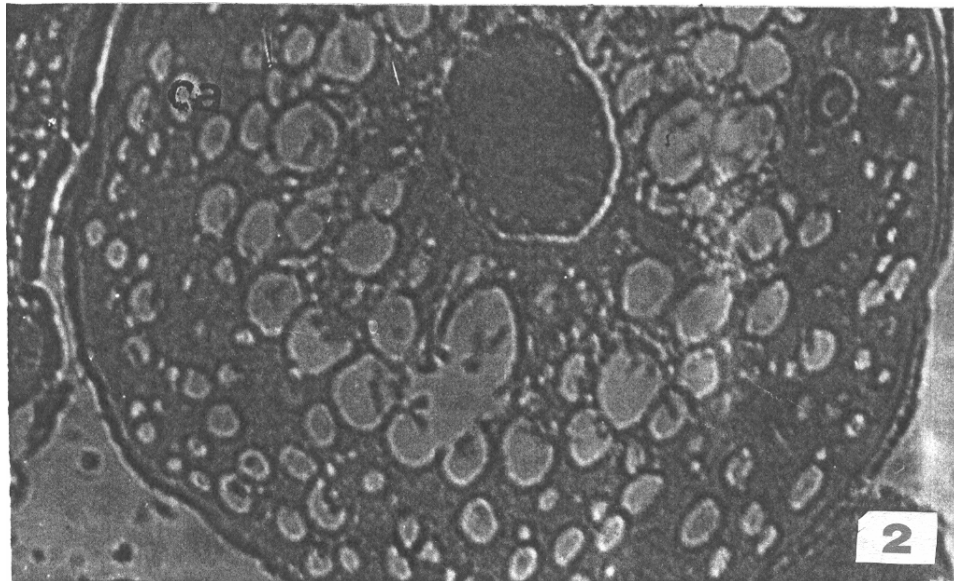


Fig. 2: Showing vitellogenic tertiary stage oocyte with large number of cortical alveolae (ca) in the cytoplasm of a fish injected with 60 μg cortisol (H & E X 375).

months, has been conducted in a variety of Indian teleosts (Malhotra, *et.al.*, 1989). Studies pertaining to corticosteroid action on the gonadal responses are limited (Burlakov, *et al.*, 1995). The cortisol is an important hormone produced from the fish inter renal gland and is identified as important metabolic hormone (Mommssen *et.al.*, 1999). The adrenal gland of teleost fish is located inside the head kidney which is called as inter renal and has been shown to be steroidogenic in variety of fish including Indian teleosts (Kulkarni and Sathyanesan, 1978; 1982).

In the present study at a dose level of 60 μg /fish, the fishes were comfortable and free from any stress. Although skin colour was changed from silvery brown to black at the time of administration, but it returned back to original colour after 30 minutes as adaptation. Studies pertaining to corticosteroid administration for inducing maturation in teleost fishes have been reported in very few species (Donaldson and Hunter, 1983). It has been also suggested that deoxycorticosteron and 11-deoxy cortisol may displace 17α , 20β progesterone bound to plasma protein, and cortisol and cortisone may lower oocyte

sensitivity to 17α , 20β - progesterone. Injection of cortisol or HCG increases both the water content and the sodium concentration of the ovary (Hirose and Ishida, 1974). Similar effects on ovarian water and sodium after injection of corticosteroids have been described in *Tilapia nilotica*. In this species cortisol at a dose level of 175mg/kg induced ovulation. On the other hand, higher or lower doses of cortisol or corticosterone were less effective (Babiker and Ibrahim, 1979). Sundararaj and Goswami (1966; 1972) have shown that cortisol acetate and cortisone were effective in the Indian catfish, *H. fossilis* at a dose of 25mg/fish for inducing ovulation.

The measurement of GSI is an important tool in determining gonadal state (Neelakantan *et.al.*, 1989) and this has been used in a variety of Indian fishes under normal and experimental conditions to show the state of gonads. In the present study, the OSI in four reproductive phases indicate that it increases in breeding period and reaches maximum during prespawning phase. Changes in OSI in response to cortisol administration during four different phases indicate that it decreases during preparatory phase and increases during post spawning phase. These results indicate that the reduction of GSI (OSI) during preparatory and prespawning phase in *N. notopterus* may be due to its involvement in oocyte maturation and spawning. Sundararaj and Gowswami (1971) and Sundararaj *et al.*, (1982) have observed greater concentration of corticosteroids compared to estrogens during spring and suggests that these steroids may have a role in oocyte maturation and ovulation.

The appearance of vacuoles containing secretory material in the cortisol injected fish may suggest that they are activated for vitellogenesis which is accompanied by intense metabolic activity (Nakano, 1969). According to most of the investigations on the oocytes growth in teleosts, cortical alveoli make their first appearance in the peripheral ooplasm from where their formation spreads towards inner ooplasm and finally aggregate in the cortical ooplasm to constitute conspicuous zone (Guraya, 1986). The first structures to appear within the oocytes cytoplasm during the gonadotrophin – dependent growth phase are the PAS positive cortical alveoli. The hormonal regulation of cortical alveoli formation is very rarely studied (Guraya, 1986). In the hypophysectomised gold fish, estradiol, estrone and estriol can induce the formation of yolk vesicles or cortical alveoli (Khoo, 1979). Similarly in the present study cortisol administration has caused favourable factors directly or indirectly in inducing vesicle formation in the oocytes leading to the growth and activation of vitellogenic activity in the young oocytes.

In the fish, *N. notopterus* initially, one or two nucleoli are present in the nucleus of young oocytes and as the oocytes grows and differentiates further, there is increase in the number of nucleoli in the vitellogenic oocytes. In the cortisol injected fish, the nucleoli number increased in the younger oocytes. It is reported that multiple nucleoli formation apparently reflect an amplification of genes (Vincent *et.al.*, 1969; Vlad, 1976; Monaco *et.al.*, 1980) and contributes abundant ribosomes to the

ooplasm of growing oocyte for supporting endovitellogenesis. Similarly increase in the nucleoli of the nucleus in young oocytes of *N. notopterus* after cortisol treatment may be related to the endovitellogenic activity (Fig. 2). However, exovitellogenesis also activated in vitellogenic oocytes through the involvement of liver after cortisol treatment in the present study.

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Correspondence to:

Dr. D.S. Shankar

Fish Endocrinology Research Lab

Dept. of Zoology, Gulbarga University

Gulbarga-585 106, (Karnataka), India

E-mail: Raghavendraskulkarni@rediffmail.com

ds_shankar@rediffmail.com

Tel.: +91-8472 – 445513 (O)

+91-8472 – 270479 (R)

Fax: +91 08472 445632 / 445450