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THE ROLE OF PINEAL GLAND IN SEASONAL CHANGES OF BLOOD ESTRADIOL LEVEL IN IMMATURE AND MATURE CARP FEMALES

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A B S T A C T. Effects of pinealectomy (Px) on the blood estradiol (E) level inmature and in mature carp females during the annual cycle were investigated. Significant differences ($p<0.01$) in the E levels between the Px fish and the control group were found only in mature females during the spring period before the spawning season. These results may indicate that the pineal gland can affect one of the phases of gonad maturation cycles in carp, probably through a stimulating influence of melatonin on E level in the final phase of vitellogenesis.

Key words: PINEAL GLAND, ESTRADIOL, OVARIAN MATURITY, CARP

INTRODUCTION

Apart from the changes of gonadotropin (GtH) concentration in blood during the annual cycle of development of carp ovary, as well as in other fish (Bieniarz *et al.* 1978, 1992) seasonal changes of steroid levels (including estradiol) have been described in inmature (Eleftheriou *et al.* 1968, Godoviz *et al.* 1984) and in mature fish (Kime, Dolben 1985, Galas, Bieniarz 1989).

The steroid hormones influence these processes during the early stages of gonad maturation (Pandey 1969), because at that time the gonadotropin level in blood and in pituitary gland is the lowest (De Vleming 1974, Billard, Breton 1978, Crim, Evans 1979). An initially low blood estradiol level in carp females, when ovaries are in maturity stages II and the beginning of III (according to Sakun and Bucka 1968), increases during the first vitellogenesis (fourth year of life) and during ovaries maturation (Fostier *et al.* 1983), i.e. till the stage IV of ovarian maturity, when vitellogenesis terminates.

In mature females (spawners), the estradiol (E) level drops down after spawning, then increases during vitellogenesis (Godoviz *et al.* 1984, Galas, Bieniarz 1989) and

drops again before spawning (Eleftheriou *et al.* 1968, Godoviz *et al.* 1984). The decrease of E concentration in blood of females before spawning results from a negative feed back in relation to GtH, the release of which induces oocyte maturation and ovulation (Fostier *et al.* 1983). Other studies have shown that pinealectomy carried out in mature carp females resulted in a delay of the germinal vesicle migration and its break down (GVBD) during the spring-summer period, hence a retardation of full maturity followed (Popek *et al.* 1991). Also, the pinealectomy in carp females during the spawning period did not produce any statistically significant changes in the dynamics of GtH2 release in relation to the females which underwent pseudo-operations (Popek et al. 1994). It was therefore interesting to examine the possible impact of pineal gland (melatonin) on the blood estradiol level in immature an in mature carp and to demonstrate the likely contribution of this organ (hormone) to the control of one of the gonadal cycle phases i.e. the vitellogenesis.

MATERIAL AND METHODS

The research was carried out on 18 two years old carps of average weight 600 g (group 1), and on 42 mature carp females aged 5 years, of average weight 3 kg (group 2). The fish were kept in traditional carp ponds during the entire period of experimentation. Before the pinealectomy, the fish were transferred to artifically aerated 2 m^3 thanks. In order to maintain the natural photoperiod and temperature both of these factors were electronically controlled according to their outdoor seasonal changes.

The fish were operated in summer when L:D equalled 16:8 h and temperature reached 20°C. In both groups, half of the fish underwent pinealectomy (Px), another half - a pseudo-operation, following the method described by Popek *et al.* (1994). Blood samples for the estimation of estradiol levels were taken from the caudal vein, always at noon, during the following periods: June (L:D = 16:8), September (L:D = 12:12), December (L:D = 8:16), and March (L:D = 12:12). After cessation of the experiment, the immature fish were killed and sex was determined, while in mature fish biopsy of gonads served the same purpose (Bieniarz, Epler 1967). Estradiol level was estimated by the method of Hotchiss *et al.* (1971). Single classification analysis of variance and Duncan's test were applied to the data on E levels and the results were presented in figures 1 and 2 showing means and ±SE.

RESULTS

GROUP 1 (IMMATURE FISH)

During summer (June), autumn (September) and winter (December), the E level fluctuated between 88 and 115 ng/ml, and there was no significant difference between the Px fish and those of the control group. In spring (March), in Px fish the E level equalled 2.4 (±1.1) ng/ml, while in the controls - 2.9 (±1.5) ng/ml. No statistically significant differences in the E levels between the first three seasons and between groups, including spring, were discovered (Fig. 1).

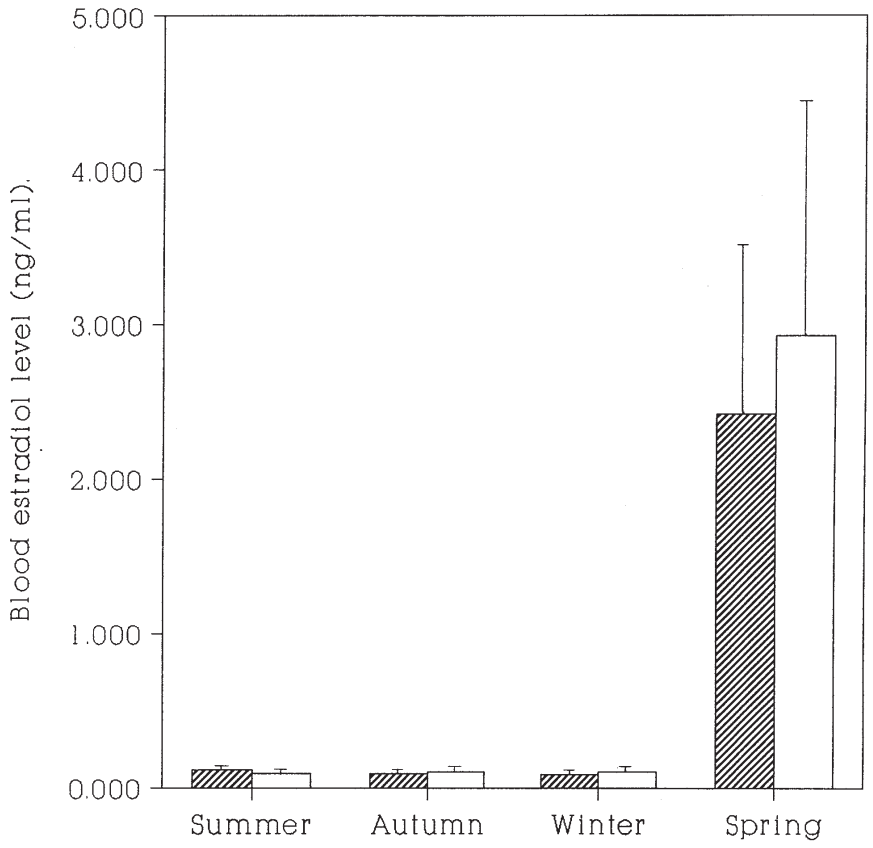

Fig. 1. Seasonal changes of estradiol level in blood of immature carp females (means ±SE). Hatched columns - fish without pineal gland, blank columns - controls after pseudo-operation

GROUP 2 (MATURE FISH)

During summer, autumn and winter, the E levels in fish without and those with the pineal gland varied between 0.9 ng/ml (winter) and 3.7 ng/ml (summer), and the differences between these two sets were statistically insignificant. In spring, however, the mean E level in Px females amounted to 6.5 (±2.1) ng/ml, but in the controls it was significantly ($p<0.01$) higher, viz. 11.5 (±2.9) ng/ml (Fig. 2).

DISCUSSION

Removal of the pineal gland from immature fish did not alter the basic annual profile of blood estradiol levels. In all seasons of the year (June, September, Decem-

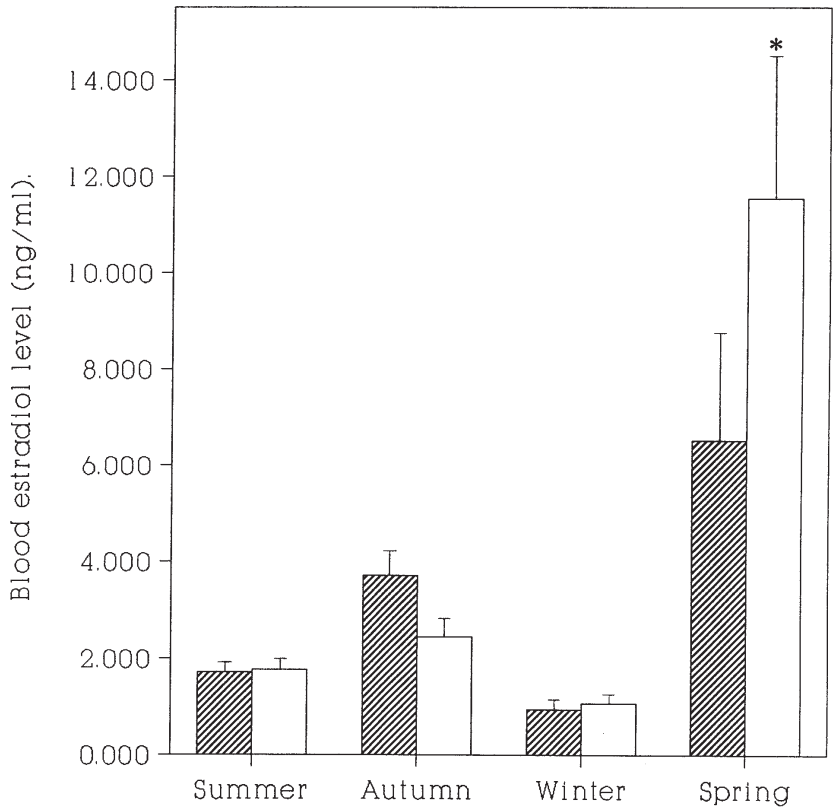

Fig. 2. Seasonal changes of estradiol level in blood of mature carp females (means ±SE). Columns as in Fig. 1. A star above column indicates significantly ($p<0.01$) greater value than all others.

ber, March) differences between E levels in the respective groups were statistically insignificant. A distinct increase of the level in spring (Fig. 1) in both groups, i.e. that with and without the pineal gland, resulted mainly from the increase of steroidogenesis in the females which entered the sexual maturity period. However, because of great variability of the E level (high SE), even these high levels did not differ significantly from the levels estimated in the other periods.

On the other hand, statistically significant shifts of E levels were evidenced in mature fish, viz. the pinealectomy resulted in a significant ($p<0.01$) reduction of E level in blood (Fig. 2). It is symptomatic that significant differences were recorded in spring only, when basic or complementary vitellogenesis took place (Bieniarz *et al.* 1977). Since the E level was lower in this period in Px fish by as many as 75% than in the control fish, it can be presumed that the phenomenon resulted from the lack of pineal gland only, as this gland is a link in the reproduction control in seasonally reproducing animals. Most likely lack of this gland during vitellogenesis disturbs the steroidogenic function of ovaries, what is manifested by a diminished estradiol secretion. However, a thorough interpretation of this phenomenon is not an easy task. An attempt to relate E levels (this paper) to the GtH2 levels in immature and maturing fish from their 17 to 52 month of life, estimated by Bieniarz *et al.* (1992), is also difficult. The source of this difficulty consists in that high E levels in maturing fish in spring do not concur with an increase of GtH2 levels, which in 2 and 3 year old fish vary between 10 and 30 ng/ml. Whereas in 4 year old fish, the high GtH2 level (170-235 ng/ml) was recorded in the initial period of vitellogenesis by the above cited authors, in later phases of vitellogenesis no elevated GtH2 level in blood was found - till the age of 52 months, when experiments terminated (Bieniarz *et al.* 1992). It is possible that pineal gland removal in mature fish can be related to a shift of secretion from GtH1, which is responsible mainly for vitellogenesis processes (Bieniarz *et al.* 1992) to GtH2 - which controls oocyte maturation and spawning. However, lack of appropriate data in literature makes the above conjecture impossible to prove.

Furthermore, any immediate effect of melatonin on the steroidogenesis in cells of the follicular theca, and thereby on oocytes maturation, was not demonstrated (*in vitro* culture of carp oocytes, Popek *et al.* 1996). It remains therefore to seek an intermediate path along which effects of pineal gland (melatonin) on the estradiol level in carp female blood take place, i.e. to look for a steroid that is directly involved in the trophoplasmatic growth and in the later processes of carp oocyte maturation.

REFERENCES

Bieniarz K., Epler P. 1976 – Preliminary results of the *in vivo* studies on ovarian resorption in carp, *Cyprinus carpio* L. – J. Fish. Biol. 8:449-451

Bieniarz K., Epler P. 1977 – Histochemical changes in the ovaries of mature female carp in summer time – Invest. Pesquera, 41:95-102

Bieniarz K., Epler P. 1992 – Advances in reproductive endocrinology of fishes – J. Physiol. Pharmac. 43:215-222

Bieniarz K., Epler P., Breton B., Luong Ngoc Thuy 1978 – The annual reproduction cycle in adult carp in Poland: ovarian state and serum gonadotropin level – Ann. Biol. Anim. Biocim. Biophys., 18:917-921

Bieniarz K., Weil C., Mikołajczyk T., Breton B., Bougoussa M., Billard R. 1992 – Maturational gonadotropic hormone (GtH) and gonadotropin releasing hormone (GnRH) changes during growth and sexual maturation of female carp (*Cyprinus carp* L.) – Pol. Arch. Hydrobiol. 39:231-241

Billard R., Breton B. 1978 – Rhythms of reproduction in teleost fish – In: Rhythmic Activity of Fishes (Ed.) J.E. Thorpe, Academic Press, New York 31-53

Crim L.W., Evans D.M. 1979 – Stimulation of pituitary gonadotropin testosterone in juvenile rainbow trout (*Salmo gairdneri*) – Gen. Comp. Endocrinol. 37:192-196

De Vlaming V.L. 1974 – Environmental and endocrine control of teleost reproduction – In: Control of Sex in Fishes (Ed.) C.B. Schreck, Virgina Polytechnic Institute and State University, Blacksburg, VA 13-83

Eleftheriou B.E., Norman R.L., Sammerfield R.S. 1968 – Plasma levels 1,3,5(10)-estratriene-3,17b diol and 3,17b-dihydroxy 1,3,5(10)-estatriene-16-one in the carp – Steroids, 11:89-92

Fostier A., Jalabert B., Billard R., Breton B., Zohar Y. 1983 – The gonadal steroids – In: Fish physiology (Ed.) W.S. Hoar, D.J. Randall, E. Donaldson, Academic Press, New York, 277-371

Galas J., Bieniarz K. 1989 – Seasonal changes of sex steroids in mature female and male carp (*Cyprinus carpio* L.) – Pol. Arch. Hydrobiol. 36:407-416

Godoviz P.L., Nikitina M.M., Rozen V.B., Winogradow V.K. 1984 – Endocrine functions of the carp (*Cyprinus carpio*) gonads in postembryonic ontogeny – Obsc. Biol. 2: 365-372

Hotchiss j., Atkison L.E., Knobil E. 1971 – Time course of serum estrogen and luteinizing hormone (LH) concentration during the menstrual cycle of the rhesus monkey – Endocrinology, 89: 177-183

Kime D.E., Dolben I.P. 1985 - Hormonal changes during induced ovulation of the carp *Cyprinus carpio* –Gen. Comp. Endocrinol. 58: 137-149

Pandey S. 1969 – Effects of hypophysectomy on the testes and secondary sex characters of the adult guppy – Can. J. Zool. 47: 775-781

Popek W., Bieniarz K., Epler P. 1991 – Role of pineal gland in sexual cycle in common carp – In: Chronobiology and chronomedicine (Ed.) J. Surowiak, M.H. Lewandowski, Verlag Peter Lang, Frankfurt, 99-1102

Popek W., Breton B., Piotrowski W., Bieniarz K., Epler P. 1994 – The role pineal gland in the control of a circadian pituitary gonadotropin release rhythmicity in mature female carp – Neuroendocrinol. Lett. 16: 183-193

Popek W., Epler P., Kime D.E., Sokołowska-Mikołajczyk M. 1996 – Melatonin does not affect steroidogenesis or maturation of carp oocyctes *in vitro* during the prespawning period. *In vivo* study – Pol. Arch. Hydrobiol. 43/3:379-385

Sakun O.F., Bucka H.A. 1968 – Determination of maturity stages and investigations of sexual cycles in fishes – Min. Ryb. Hoz. Murmansk, 1-46

STRESZCZENIE

UDZIAŁ SZYSZYNKI W SEZONOWYCH ZMIANACH POZIOMU ESTRADIOLU W KRWI NIEDOJRZAŁYCH I DOJRZAŁYCH SAMIC KARPIA

Do badań użyto niedojrzałych (2 lata) i dojrzałych (5 lat) samic karpia. W czerwcu (L:D=16:8) u połowy ryb z obu grup operacyjnie usunięto szyszynkę a u pozostałych wykonano operację pozorną. W tydzień po zabiegu, od wszystkich samic pobrano krew z żyły ogonowej w celu określenia stężenia estradiolu. Kolejne pobory (zawsze o tej samej porze doby) wykonano jesienią (L:D=12:12), zimą (L:D=8:16) oraz wiosną (L:D=12:12). Wykazano, że w porównaniu do grup kontrolnych, brak szyszynki spowodował statystycznie istotny ($p<0.01$) spadek stężenia estradiolu w krwi tylko u samic dojrzałych i tylko w okresie wiosennym, poprzedzającym sezon tarłowy.

Uzyskane dane mogą świadczyć, że szyszynka może oddziaływać na jeden z etapów cyklu dojrzewania gonad u karpi, prawdopodobnie poprzez stymulujący wpływ melatoniny na poziom estradiolu w końcowej fazie procesu witellogenezy.

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