Proc. Estonian Acad. Sci. Biol., 1996, 45, 1/2, 15—18
https://doi.org/10.3176/biol.1996.1/2.02

NUCLEAR TRANSFORMATIONS DURING THE OOCYTE FINAL MATURATION IN THE SPINED LOACH *COBITIS TAENIA*

Toomas SAAT

Tartu Ülikooli zooloogia ja hüdrobioloogia instituut (Institute of Zoology and Hydrobiology, University of Tartu), Vanemuise 46, EE-2400 Tartu, Eesti (Estonia)

Presented by A. Järvekülg

Received 4 December 1995, revised version received 5 January 1996, accepted 15 January 1996

Abstract. Nuclear and cytoplasmic transformations of the spined loach oocytes were studied during their in vitro maturation. By the moment of ovulation, the oocyte nucleus reached metaphase II, and ovulated oocytes were capable of activation. In vitro culture conditions supported the normal progression of the oocyte final maturation.

Key words: oocyte maturation, meiosis, in vitro, *Cobitis*.

INTRODUCTION

Spined loaches of the genus *Cobitis* are widely distributed in Eurasian fresh waters. Hybridization of sympatric species has yielded in several triploid and tetraploid biotypes, which are represented only or almost only by females (Васильев, 1985; Vasil'ev et al., 1989; Kim & Lee, 1990).

The diploid—triploid—tetraploid complex of spined loaches inhabiting the Moskva River (Russia) consists of two diploid species, *Cobitis taenia* L. (somatic chromosome number $2n=48$) and *Cobitis granoei* Rendahl ($2n=50$), one all-female triploid ($3n=74$), and two tetraploid biotypes ($4n=98$ and $4n=99$). Males are common among diploid fish but can be found more or less regularly also among tetraploids (Васильев, 1985; Vasil'ev et al., 1989). The mode of reproduction of triploids is gynogenesis (Саат, 1991a). This mode of clonal reproduction is the result of modified oogenesis (production of unreduced eggs) and merospermy (the spermatozoon entering the egg does not transform into a male pronucleus).

Diploid species of the complex are represenleted by females and males and they probably exhibit normal meiosis during their gametogenesis. To prove it and to compare this process in diploids and polyploids, we investigated the progression of nuclear transformations during the oocyte final (meiotic) maturation in different representatives of the complex. In this paper, a description of nuclear and cytoplasmic alterations of *C. taenia* oocytes during their in vitro maturation is presented.

MATERIAL AND METHODS

Fish were collected on spawning grounds (Moskva River near Zvenigorod, Russia) and transported alive to the laboratory. Ploidy of fish was confirmed by measuring the erythrocyte size (Васильев, 1985) and by flow-cytometry, measuring the amount of DNA in erythrocyte nuclei (Tambets, 1990).

Prior to in vitro experiments, females were injected with 10—20 IU of human chorionic gonadotropin for 6—10 h. Such "priming" enhances the in vitro maturation of loach oocytes (Саат, 1990). Excised gonads were placed in medium M199. Small fragments of gonad containing 2—10 full-grown oocytes were separated, pooled, and transferred into sterile plastic Petri dishes with M199 supplemented with 1 $\mu g \cdot ml^{-1}$ of 17α-hydroxyprogesterone. Experiments were run at 21—22 °C. Further details of experiments have been described previously (Саат, 1990).

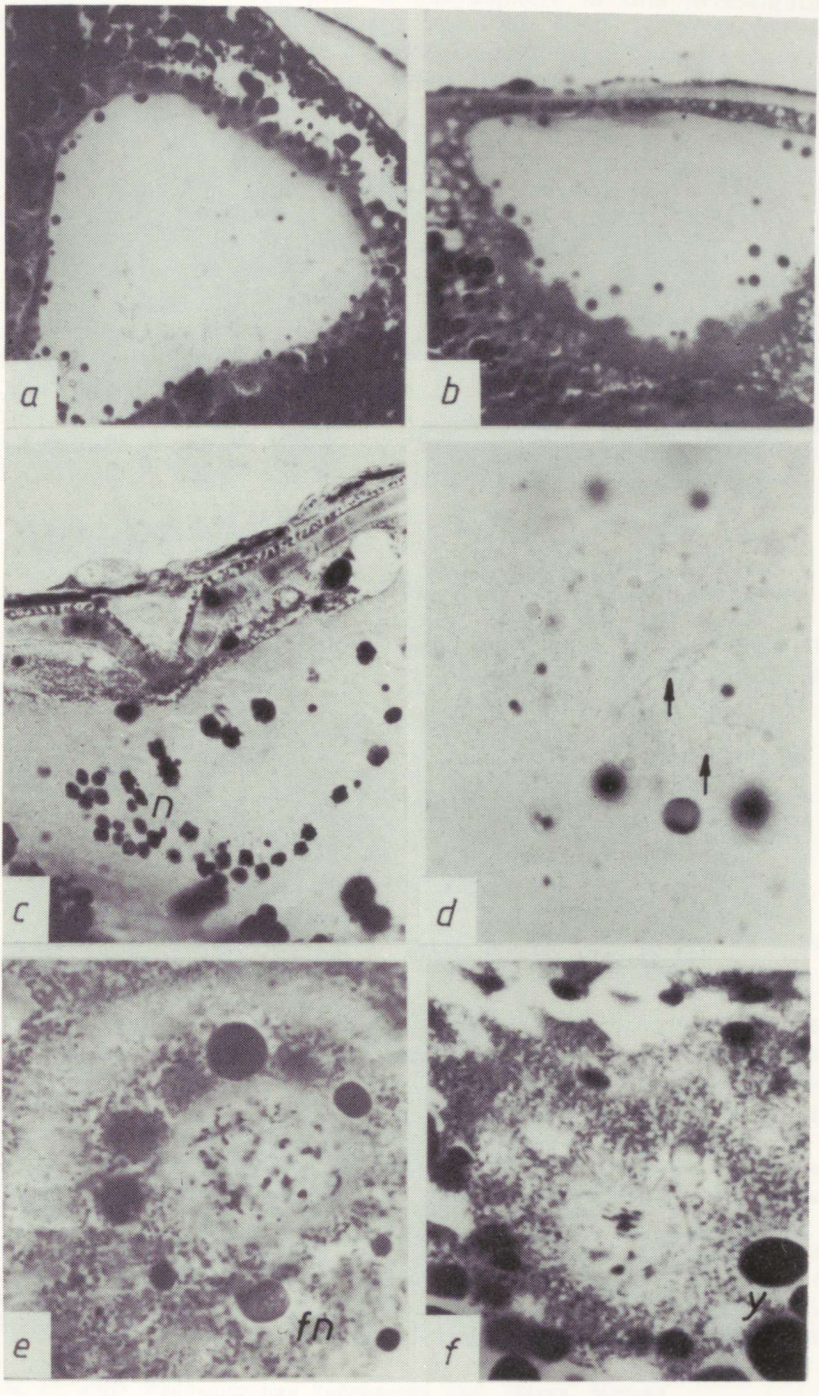
Samples of oocytes were taken every 1—2 h (until ovulation) and preserved in Bouin's or Sanfelice's fixative (Ромейс, 1953). Oocytes of two females were selected for further investigation; they were embedded in paraffin, serially sectioned at 8 μm, stained with Heidenhein's iron hematoxyline, and investigated in light microscope. Criteria of maturation stages (including the meiotic divisions) are according to Saat (1993).

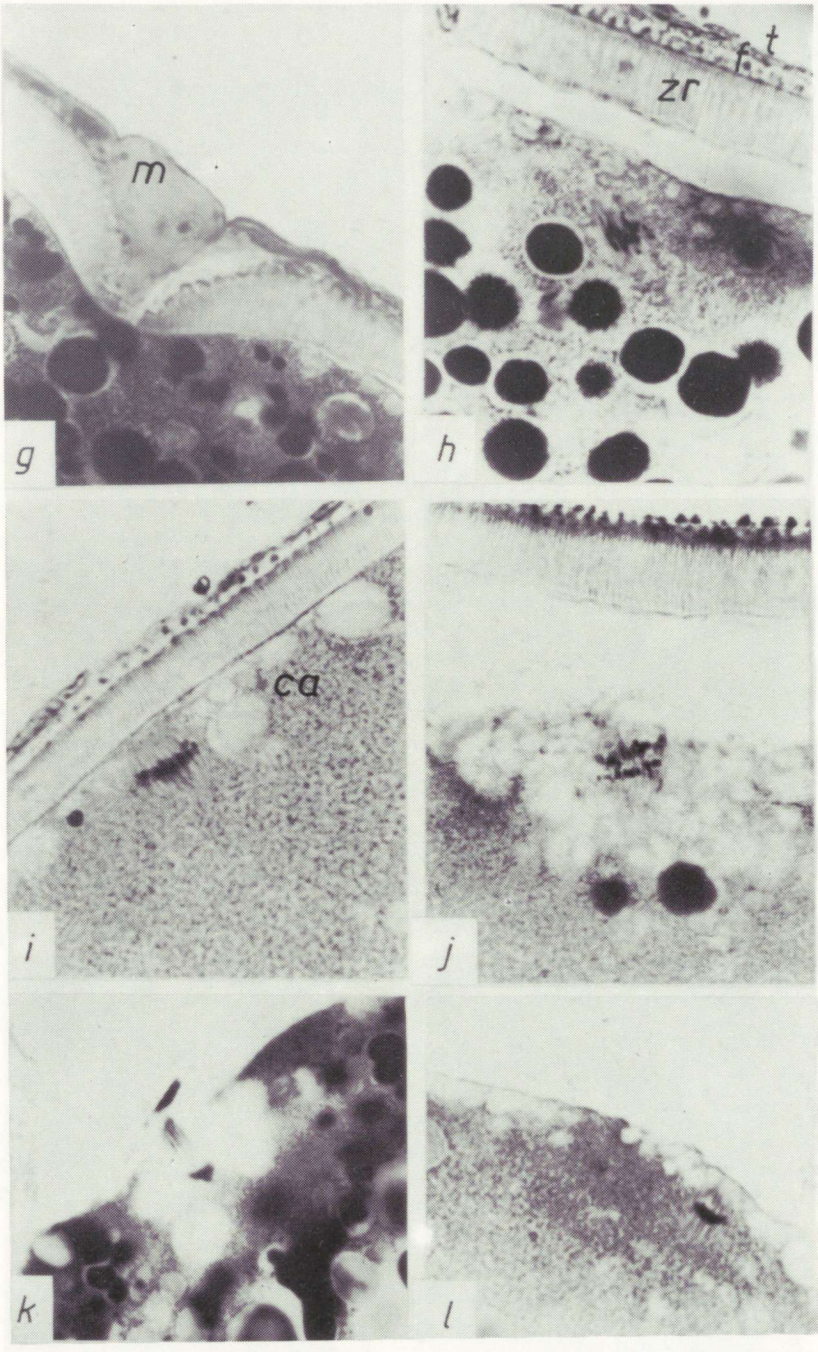
RESULTS AND DISCUSSION

By the beginning of the in vitro incubation of the oocyte, the germinal vesicle (GV) had, due to in vivo priming, remarkably shifted to the animal pole of oocyte (maturation stage 2, the GV migration; Fig., *a*). One or two hours later, the GV had completed its migration and was situated immediately beneath the micropyle (stage 3; Fig., *b*). Thereafter, about 1 h later, the GV begins to break down; nucleoli approach one another and fuse (stage 4; Fig., *c*). At this time, shortening bivalents with one or a few chiasmata can be detected in the region surrounded by fusing nucleoli (Fig., *d*). At the next stage, nucleoli in teleost eggs fuse into a compact capsule of karyosphere; shortened chromosomes (bivalents) can be found in a hollow of the capsule (stage 5, capsule of karyosphere) (cf. Saat, 1993). The typical capsule of karyosphere was not found in the spined loach oocytes. All these transformations take place during the meiotic prophase.

About 0.5—1 h later, the capsule breaks down and chromosomes are released into cytoplasm; this is prometaphase of the first meiotic division (stage 6). At the beginning, remnants of both the nuclear envelope and the capsule of karyosphere surround the chromosomes (Fig., *e*). Meiotic spindle forms in the vicinity of micropyle (Fig., *f*, *g*). The spindle migrates towards the animal pole (Fig., *h*). By the beginning of metaphase I (stage 7), about 1 h later, the spindle has reached oolemma not far (usually <100 μm) from micropyle (Fig., *i*). Metaphase I lasts about 2 h. The next two stages, anaphase I (stage 8; Fig., *j*) and telophase I (stage 9; Fig., *k*), are shorter, lasting together about 1 h. The next stage, prometaphase II (stage 10), is very short (Saat, 1993) and was not detected in this material. Mature egg is arrested at metaphase II (Fig., *l*); the completion of meiosis takes place, as in other fishes, after activation (normally by a spermatozoon) of the spawned egg.

Oocyte maturation in *Cobitis taenia*: *a*, migrating GV (stage 2); *b*, the GV migration completed (stage 3); *c*, beginning of GV breakdown and fusion of nucleoli (stage 4); *d*, bivalents (arrows) (stage 4); *e*, early prometaphase I (stage 6); *f*, prometaphase I, formation of the meiotic spindle (stage 6); *g*, micropyle; *h*, migrating meiotic spindle, late prometaphase I (stage 6); *i*, metaphase I (stage 7); *j*, anaphase I (stage 8); *k*, teleophase I (stage 9); *l*, metaphase II (stage 11). Stages according to Saat (1993). Bar, 40 μm (*a*—*c*), 10 μm (*d*—*l*). *ca*, cortical alveolus; *f*, follicular cells; *fn*, fused nucleoli (a fragment of the capsule of karyosphere); *m*, micropylar cell; *n*, nucleoli; *t*, theca; *y*, yolk granule; *zr*, *zona radiata*.


a
b
n
c
d
fn
e
y
f


m
g
t
zr
h
ca
i
j
k
l

The nuclear transformations of *C. taenia* oocytes are typical of teleosts; they include two normal meiotic divisions. We could not detect characteristic of teleosts compact capsules of karyosphere in this investigation. However, this is probably associated with too long intervals between sample fixations. Typical capsule in teleost eggs can be found during 1—2 τ_0 (Saat, 1993). The τ_0 value for spined loach is 33 min at 21 °C, and 30 min at 22 °C (Саат, 1991b), and the duration of this stage is probably less than 1 h. The duration of the period from prometaphase I to metaphase II in the spined loach was 4—4.5 h, or approximately 8—9 τ_0, which corresponds to that in other fish species (Saat, 1993).

Also, we could not detect any abnormalities in the oocyte cytoplasmic maturation. The latter includes the concentration of yolk-free cytoplasm in the animal pole region and arrangement of cortical alveoli immediately beneath the oolemma (Saat, 1993). Mature ovulated eggs were normally activated when placed in fresh water.

Obviously, the in vitro culture conditions applied in this investigation support the normal final maturation of the spined loach oocytes. It should be stressed that abnormalities (both in nuclear and cytoplasmic events) are very common, when not proper in vitro culture conditions are applied (Саат, 1982; Saat & Veersalu, 1990).

ACKNOWLEDGEMENTS

I thank Dr. V. P. Vasil'ev (Moscow) for his help in ploidy determinations. This research was supported by the Estonian Science Foundation (grant 420).

REFERENCES

Kim, I.-S., Lee, J.-H. 1990. Diploid—triploid hybrid complex of the spined loach *Cobitis sinensis* and *C. longicorpus* (Pisces, Cobitidae). — The Korean Journal of Ichthyology, **2**, 2, 203—210.

Saat, T. 1993. The morphology and chronology of oocyte final maturation and fertilization in fish. — In: Walther, B. T. and Fyhn, H. J. (eds.). Physiological and Biochemical Aspects of Fish Development. University of Bergen, Norway, 71—85.

Saat, T., Veersalu, A. 1990. Changes in the oocyte structure in the carp, *Cyprinus carpio*, during *in vitro* maturation. — Journal of Ichthyology, **30**, 1, 17—27.

Tambets, M. 1990. Uued diploidsed-polüploidsed kompleksid hingul (*Cobitis*, Teleostei). Ploidsuse ja sigimisviiside määramine. Diploma paper, Institute of Zoology and Hydrobiology, University of Tartu.

Vasil'ev, V. P., Vasil'eva, K. D., Osinov, A. G. 1989. Evolution of a diploid—triploid—tetraploid complex in fishes of the genus *Cobitis* (Pisces, Cobitidae). — In: Dawley, R. M. and Bogart, J. P. (eds.). Evolution and Ecology of Unisexual Vertebrates. Bull. 466, New York State Museum, Albany, New York, USA, 153—169.

Васильев В. П. 1985. Эволюционная кариология рыб. Наука, Москва.

Ромейс Б. 1953. Микроскопическая техника. ИЛ., Москва.

Саат Т. В. 1982. Хронология созревания ооцитов вьюна и становление у них способности к развитию. — Онтогенез, **13**, 3, 257—265.

Саат Т. В. 1990. Созревание *in vitro* ооцитов у диплоидных и полиплоидных щиповок (Cobitidae). — Онтогенез, **21**, 4, 416—421.

Саат Т. В. 1991a. Размножение диплоидных и полиплоидных щиповок (*Cobitis*, Teleostei). Созревание ооцитов и оплодотворение у триплоидной формы. — Онтогенез, **22**, 5, 533—541.

Саат Т. В. 1991b. Продолжительность митотического цикла в период синхронных делений дробления (τ_0) и скорость развития при разных температурах у потомства диплоидных и полиплоидных щиповок (*Cobitidae*). — Онтогенез, **22**, 1. 103—106.

TUUMA TRANSFORMATSIOONID HINGU *COBITIS TAENIA* L. OOTSÜÜTIDE KÜPSEMISEL *IN VITRO*

Toomas SAAT

On uuritud *in vitro* küpsevate hingu ootsüütide meioosi käiku. Meioos kulges kaladele tüüpiliselt: ootsüüdid ovuleerusid metafaasis II. Ovuleerunud munad olid aktiveerumisvõimelised. See viitab ka tsütoplasmaatiliste muutuste normaalsele kulgemisele *in vitro*.

ЯДЕРНЫЕ ПРЕОБРАЗОВАНИЯ ПРИ СОЗРЕВАНИИ *IN VITRO* ООЦИТОВ ЩИПОВКИ *COBITIS TAENIA* L.

Тоомас САТ

Были изучены ядерные и цитоплазматические преобразования при созревании *in vitro* ооцитов одной из двух диплоидных форм диплоидно—полиплоидного комплекса щиповок из р. Москвы. К моменту овуляции ядро ооцита достигало стадии метафазы II мейоза и ооциты были способны к активации. Морфология и хронология ядерных преобразований у щиповки сходны с таковыми у других видов рыб.