Disrupted sexual cycles in female grass carp (Ctenopharyngodon idella) raised under tropical conditions

F. Glasser

INRA-SCRIBE, campus de Beaulieu, 35 042 Rennes cedex, France.

Tel: (33) 2 23 48 50 02, fax: (33) 2 23 48 50 20, glasser@beaulieu.rennes.inra.fr

Abstract

In Côte d'Ivoire, grass carp (Ctenopharyngodon idella) has been integrated in the tilapia-based polyculture in order to increase pond productivity. In the tropical conditions prevailing there, female cycles seemed disrupted. We described oogenesis in these conditions using histological observations, monitoring of individual cycles with intraovarian biopsies, endocrinological monitoring (development of a specific ELISA for vitellogenin (Vg), measurement of plasmatic oestradiol-17β (E2) and testosterone) and comparison with grass carp raised in Poland. We stated that oogenesis was interrupted in all females at the migrating germinal vesicle stage, precluding ovulation or spawning without artificial induction. High rates of atypical post-vitellogenic oocytes (translucent, not filled with yolk granules) were observed in some females. Female individual cycles also displayed atypical features: cycles were sometimes (10% of females) blocked at the beginning of vitellogenesis, for females displaying abnormally low E2 (0.5 ng/ml) and Vg (27 µg/ml) levels compared to "normal" females (1.4 ng/ml and 223 µg/ml respectively). The duration of cycles was highly variable among females (a few days to several weeks). The sexual cycles were unsynchronised (all the ovarian stages could be found in all seasons) and the rate of females at end of vitellogenesis was low (<40% most of the year). These characteristics rise problems for artificial induction of spawning in small-scale hatcheries: it requires a large stock of broodfish and regular checking of female broodstock with intraovarian biopsies to detect responsive females.

Keywords: Ctenopharyngodon idella, vitellogenin, steroid, sexual cycle, temperature

1 Introduction

Small-scale aquaculture has developed in the rain forest areas of Côte d'Ivoire (West Africa), based on tilapia farming in earthen ponds with organic fertilisation, but is still confronted to agricultural by-products shortage. Grass carp (Ctenopharyngodon idella) was introduced in the polyculture in order to control weeds in ponds and increase pond productivity through supplemental feeding with terrestrial grass (Pennisetum purpureum). Grass carp is widely used worldwide for this purpose, and the growth performances are excellent in tropical areas. As its natural reproduction only occurs in large rivers with precise requirements of turbulence, current velocity, flow (Stanley et al., 1978), the only way to produce fry for fish farming is artificial reproduction. In the small Ivorian farms, this requires a good rate of mature brooders to induce spawning with reasonable chances of success. Preliminary observations in Côte d'Ivoire showed that a sufficient number of mature males could be found all the year round, whereas few responsive females were found. Reproduction in fish is under control of both external and internal factors (see for example reviews by Lam, 1983, and Bromage et al., 2001): environmental characteristics such as temperature, photoperiod, diet or social interactions are of great importance from gametogenesis to spawning, which are controlled by internal factors mainly involving the hypothalamus-hypophysis-gonad axis. This study was undertaken to describe female sexual cycles and characterise their dynamic around the year under "standard" Ivorian raising practices (small earthen pond, Pennisetum purpureum feeding). We used histological and endocrinological data, and comparison with fish raised in temperate zone (Poland).

2 Materials and Methods

2.1 Fish and structures

Grass carps were maintained in small earthen ponds (350 m²) in Gagnoa experimental station (Côte d'Ivoire, 5° N.). They were fed elephant grass (Pennisetum purpureum). Fish were individually marked by Alcyan blue on fins. For comparison purpose, grass carps from the Inland Fisheries Institute in Zabieniec (Poland) were maintained for two weeks before normal breeding period (early June) in tanks under controlled temperature conditions and natural photoperiod. One group (n=16) was maintained at 24±1°C and the other at 28±1°C. All fish manipulations were made under anaesthesia with 2-phenoxyethanol at 25 ppm.

2.2 Sexual cycle description

Oocyte samples (obtained from intraovarian biopsies or from ovaries after killing) were fixed with Bouin's fixative, dehydrated and paraffin sections were coloured according to Heidenhain's azan method. Oocyte stages were determined and correlated with oocyte diameter (calculated from perimeter measured by Optimas®).

2.3 Sexual cycle monitoring

Sexual development of females (n=15 to 50) was monitored for three years (1998-2000) in Ivory Coast, by intraovarian biopsy at intervals from 10 days to one month. Oocytes were put in NaCl 0.9% to avoid drying and 100 oocytes were measured using stereomicroscope. Ovarian stages were then determined, based on oocyte diameter distribution. Germinal vesicle position was determined on 50 post-vitellogenic oocytes cleared with Stockart's solution. Oocytes becoming translucent in less than 3 min. following Stockart's solution introduction were

considered as atretic. Blood samples were collected with heparinized syringes, then centrifuged and frozen at –20°C.

2.4 Vitellogenin assay

An enzyme-linked immunosorbent assay (ELISA) for grass carp vitellogenin (Vg) was developed according to Mourot and Le Bail (1995). Briefly, Vg synthesis was induced in male grass carp by oestradiol-17β (E2) intraperitoneal injections (5 mg/kg). Blood was collected using heparinized syringes in the presence of aprotinin, then centrifuged and frozen (-20°C). Vg was purified according to Tyler and Sumpter (1990): plasma was first passed through a gel filtration Sepharose-6B column in a 100 mM tris buffer. The vitellogenic fractions were identified by SDS-PAGE and concentrated before ion exchange chromatography (DEAE). The elution was performed with a NaCl gradient (range: 100 to 500 mM) in tris buffer. Vitellogenic fractions were identified by SDS-PAGE. Vg was stored in concentrated solution at –20°C. Specific antibodies were raised in rabbits by subcutaneous injections of pounded SDS-PAGE bands diluted in Freund's adjuvent. The specificity of antibody was assessed by immunohistochemistry, according to a method adapted from Sternberger et al. (1970). The ELISA method was based on competition between Vg coated in microplate wells and free Vg in the samples. Dilution tests were performed using serial dilutions of coated Vg and antibody. The best combination was determined as 100 ng/ml for the coating and 200 000 for antibody dilution. The assays were then performed according to Mourot and Le Bail (1995). Antibodies linked to coated Vg were revealed by peroxydase activity on o-phenylenediamine. Optical density was measured at 490 nm (Microplate autoreader EL 311, Bio-Tek Instruments).

2.5 Steroid assays

Plasmatic testosterone (T) and E2 levels were measured by radioimmunoassay adapted from Terqui et al. (1973), following cyclohexane/ethylacetate extraction. The radioactivity of immunoprecipitate was determined using scintillation fluid (Instafluor Packard) in a Tri-Carb 2100 TR counter (Packard).

2.6 Statistical analyses

Means were compared using one-way ANOVA and comparison of means with XXXX test. The accepted significance level was α=0.05. All data are presented as mean ± SEM.

3 Results

3.1 Ovarian stages

From histological observations, the diameters of the different oocyte stages were determined using Makeyeva and Yemel'yanova (1989) nomenclature (Table 1). Based on these data and the diameter histograms obtained from binocular observations, four ovarian stages were defined:

- previtellogenesis (PV): all diameters inferior to 400 µm, all oocytes in previtellogenesis or beginning of cytoplasmic vacuolisation (equivalent to stages 1 and 2 from Gupta, 1975).
- beginning of vitellogenesis (BV): most oocytes still in previtellogenic stage, apparition of vitellogenic oocytes (vacuolisation, beginning of yolk accumulation), less than 30% of all oocytes (stage 3 from Gupta, 1975).
- end of vitellogenesis (EV): bimodal repartition of oocyte diameters, with a mode in previtellogenic stages and a mode in late vitellogenic oocytes (diameter 700 to 1300 µm) corresponding to stage 4 from Gupta (1975).

- overripe (OR): late vitellogenic oocytes less numerous, large oocytes in atresia, and presence of oocytes of all stages (a new batch is ripening). This stage resembles stage 6 (partially spent ovary) from Gupta (1975), excepting that there are no extruded ova.

Gupta's stage 5 (ripe ovary) with partial ovulation was never observed. No GVBD was observed in grass carp ovaries: the most advanced stage was migrating germinal vesicle. All EV ovaries contained some atretic oocytes (< 15%).

Atypical late vitellogenic oocytes were observed in some females: they exhibited the same diameter as "normal" late vitellogenic oocytes, but were translucent when observed with stereomicroscope. In histological sections, the inner part was constituted by vesicles tainted in blue by Heidenhain's azan, contrary to the "normal" ones, full of red yolk granules. They didn't exhibit any feature of atretic follicles (membrane cleavage…). In these atypical oocytes, the yolk granules were restricted to a layer at the periphery of the oocyte. Immunohistochemistry with anti-Vg antibody showed a lesser affinity for these oocytes (see Figure 1).

No difference was observed in histological sections between normal vitellogenic females from Côte d'Ivoire and females from Poland.

3.2 Individual sexual cycles

The monitoring of ovarian stages of individual females performed during three years (1998-2000) reveals a great variety of cycle characteristics. In the majority of cases, the vitellogenesis lasted about 20 days. However, some females (around 10%) were blocked in BV stage for several weeks, and then went on with the vitellogenesis or went back to PV. Duration of the EV stage was also highly variable: some females stayed in this stage for several weeks, whereas other became overripe within a few days. Neither progression of vesicle migration nor evolution of post-vitellogenic oocyte diameters were observed in consecutive

biopsies of EV females. Females that were artificially induced to spawn with pituitary extract resumed vitellogenesis a few days after induction, and one female was successfully induced three times at one-month intervals.

3.3 Plasma hormone levels in relation to ovarian stages

Vitellogenin and steroids contents were determined for 140 blood samples representative of the different ovarian stages in Côte d'Ivoire, as well as for the 30 females from Poland. Table 2 displays analyses results.

Vg levels increased during vitellogenesis, from 7 to 742 µg/ml. There was no diminution associated with the OR stage. Vg levels of grass carp from Poland were lower, irrespective of temperature. There was a big difference between Vg levels of females blocked in BV (27 µg/ml) and females that would undergo a normal vitellogenesis (223 µg/ml).

E2 levels were low during PV, and then constantly high from BV to EV. Polish females levels were lower than Ivorian ones, irrespective of temperature conditioning. Here again, BV females displayed significantly lower levels when they were blocked (0.5 ng/ml vs. 1.4 ng/ml).

T levels were more homogenous among ovarian stages: the only significant differences laid between PV females in one hand and EV females from Ivory Coast and Poland raised at 28°C in the other hand.

In EV females, no relationship was found between plasma characteristics and germinal vesicle migration or atretic oocytes rate.

3.4 Population characteristics

Observation of females at different seasons of the year showed the absence of sexual synchronisation: all ovarian stages could be found all the year round. Most of time, females in EV are in small proportion (5 to 43%), but higher rates were observed at the end of the rainy season (October), reaching 68% in 2000. It was

impossible to identify a main recrudescence period, the higher number of vitellogenic females at the end of rainy season being due to the fact that less females became overripe during this season. In Poland, 90% of examined females, irrespective of thermal conditioning, were in EV when checked at the beginning of June.

4 Discussion

The oocyte stages described for cyprinids by Makeyeva and Yemel'yanova (1989) and for grass carp by Chen et al. (1969) were found in Côte d'Ivoire, and the associated diameters allowed to monitor individual cycles through intraovarian biopsies. The vitellogenesis was of the "group synchronous" type (de Vlamming, 1983), as evidenced by the bimodal pattern of oocyte diameter repartition. Presence of a small amount of atretic follicles in all EV females evoked a continuous turnover of postvitellogenic oocytes, as did the absence of progression in germinal vesicle migration in consecutive biopsies. Normal Ivorian females exhibited histological figures identical to females from Poland at the end of vitellogenesis. Steroid profiles displayed high variability among individuals in the same ovarian stage, as already observed by Manning and Kime (1984) in common carp, Cyprinus carpio. Mean E2 and T plasmatic levels were of the same order as found in goldfish (Razani et al., 1988) Gobio gobio (Rinchard et al., 1993) or common carp (Manning and Kime, 1984), but inferior to the goldfish levels in Kagawa et al. (1983). The plasmatic levels associated with the different ovarian stages were similar to the steroid profile in Gobio gobio (Rinchard et al., 1993), except a drop in E2 content at the end of vitellogenesis that we did not observe in our fish. Moreover, E2 levels of Ivorian females were superior to Polish females ones. At the same stage (spawning), short photoperiod (12L:12D) in goldfish at 24°C decrease E2 level compared to 16L:8D (Razani et al., 1988). The effect of

photoperiod was thus unlikely in our case. Manning and Kime (1984) observe that common carp plasmatic E2 level is lower at 24°C compared to 20°C, reflecting a more advanced ovary stage. This could explain the higher E2 level of Ivorian females: the oogenesis in Côte d'Ivoire was probably interrupted at a less advanced stage compared to Poland (although the different genetic origin of the two populations could also be responsible for this difference).

Atypical post-vitellogenic oocytes were observed in great number in some Ivorian females. These females were identical to normal females for endocrinology characteristics measured in our study, the only difference being a lesser affinity of oocyte content for anti-Vg antibody. The histological feature of these oocytes was totally different from atresia (no membrane fragmentation observed, no Vg at the oocyte centre). We don't know any description of similar pattern in other fish species. Histological figures evoked a vitellogenin incorporation problem, which we could not confirm by other means.

Female individual cycles displayed atypical features: cycles were sometimes blocked at the beginning of vitellogenesis; the duration of cycles was highly variable, some females underwent rapid regression, whereas other stayed at EV for several weeks. In grass carp, natural spawning occurs from 17.5 to 20°C, but optimal temperature for artificial reproduction seems to be 23-26°C (Bardach et al., 1972). This species originates in temperate areas (23 to 50°N), with annual photoperiod variations of 3 to 8 hours. Côte d'Ivoire climatic conditions must be near the upper thermal limit for reproduction (mean pond temperature between 26 and 31°C) and photoperiodic variations are very weak (less than 30 min). This could explain some of sexual cycle singularities observed in our study. Gonadal development of feral grass carp occurs in spring, under conditions of increasing photoperiod and temperature (Gorbach, 1972 in Shireman and Smith, 1983), and

spawning from March to July, according to thermal regime (Shireman and Smith, 1983). There don't seem to be a refractory period in Côte d'Ivoire, although it is often observed with this kind of sexual development. Two types of gonadotropins (FSH-like and LH-like) have been found in many fish species (Quérat, 1995), including cyprinids (Van der Kraak et al. (1992) in common carp, Yoshiura et al. (1997) in goldfish). In some species (like rainbow trout, Breton et al., 1998) vitellogenesis is under control of FSH whereas LH mainly controls final maturation and ovulation. In cyprinids, although no plasma FSH assay is available for the moment, their properties (Van der Kraak et al., 1992) and transcription profiles (Sohn et al., 1999) seem less clearly distinct. The lack of FSH assay precludes any complete hypothesis on the phenomena observed. Vitellogenesis interruption observed in some females was correlated with E2 and vitellogenin deficits. This could result from either gonadotropin depletion, loss of ovarian responsiveness to gonadotropin or inhibition of ovarian oestradiol synthesis capacities. High temperatures seem to increase gonadotropin (GtH) levels in goldfish (Gillet et al., 1977, 1981, Razani et al., 1988), but induce gonadal regression in this species at the beginning of vitellogenesis (Gillet et al., 1977, 1981, Razani and Hanyu, 1986, Razani et al., 1988). In Gobio gobio raised under constant conditions of temperature and photoperiod (20°C, 12L:12D) Kestemont (1990) observes a decrease in gonadotropin content. In common carp, Manning and Kime (1984) show a high variability between individuals, even at the same ovarian stage, concerning steroid production at different temperatures. However, they observe a lower oestradiol production for stage 3 (BV) at 32°C compared to 24 or 29°C. For the other stages, no effect of temperature on E2 production is noticed. At 24°C there is a correlation between plasmatic E2 level and ovarian in

vitro E2 secretion. Without FSH assay, it was impossible to precise the blocking stage.

The duration of female sexual cycles is highly variable. Kestemont (1990) report the same observation about Gobio gobio. Under constant temperature and photoperiod conditions (20°C, 12L:12D), gonad development is variable: some females accumulate yolk vesicles (but exhibit oocytes smaller than control), other regress. This is associated with a lower gonadotropin content. In goldfish, high temperatures rise GTH levels, but sometimes decrease Gonado-somatic index (GSI), perhaps by suppression of GTH daily cycles (Gillet et al., 1981), an hypothesis also evoked by Hontela and Peter (1978). The combination of high temperature and short photoperiod (25°C, 9L:15D) also cause ovary regression in *Notemigonus chrysoleucas* (de Vlamming, 1975). These conditions could explain the rapid regression of some females. However, some other stayed at EV stages and responsive to spawning induction for several weeks, whereas the responsive period in temperate hatcheries is very short (less than 2 weeks, Okoniewski, pers. comm.).

At the broodstock level, the main characteristics observed are the low rate of females completing vitellogenesis and the unsynchronisation of sexual cycles. In feral grass carp, there is a defined spawning season (Shireman and Smith, 1983) depending on temperature conditions. Even in Egypt, were temperature (13 to 29°C) and photoperiod seasonality is less marked than in their natural area (but yet far more marked than in Côte d'Ivoire), introduced grass carp exhibits a marked spawning season (April-June) (Zonneveld, 1984). Davies and Hanyu (1986) observe at high temperature (24°C) and short photoperiod (12L12D, inferior to the "critical" photoperiod for cyprinids they set at 13-14 hours) that common carp ovulations are less simultaneous and more partial than under

16L:8D. The constant high temperatures and "short" photoperiod were most probably responsible for the unsynchronisation of sexual cycles in Côte d'Ivoire.

5 Conclusion

Grass carp oogenesis was possible in earthen ponds under tropical conditions, but it was interrupted at the migrating germinal vesicle stage, what precludes any natural reproduction. Moreover, female individual cycles displayed atypical features in these conditions: the rate of females achieving complete vitellogenesis was quite low (less than 40% most of the year); the duration of cycles was highly variable; cycles were sometimes blocked at the beginning of vitellogenesis; atypical oocytes were observed in some females at the end of vitellogenesis. The low rate of post-vitellogenic females is a handicap for small-scale hatcheries: it requires a large stock of broodfish to find some responsive females for artificial reproduction. The unsynchronisation of sexual cycles rises the same problem, and requires frequent checkings of ovarian stage by intraovarian biopsies. However, this may also be an advantage in order to produce fry all the year round, provided post-vitellogenic females are responsive to induction whatever the season. It remains to be seen whether appropriate raising practices (other type of feeding, lower density…) could ensure higher maturity rates.

Acknowledgements. This study was part of a collaborative program between APDRA-CI (Association Pisciculture et Développement Rural en Afrique tropicale humide – Côte d'Ivoire, Daloa, Côte d'Ivoire) and INRA, financed by the French Ministry of Foreign Affairs. It was conducted in the experimental structures of APDRA-CI and with assistance of its technical staff. Chantal Cauty, at INRA-SCRIBE, provided valuable assistance in histology.

References

Bardach, J.E., Ryther, J.H., McLarney, W.O., 1972. Aquaculture : the farming and husbandry of freshwater and marine organisms. John Willey & Sons, New York, 868 pp.

Breton, B., Govoroun, M., Mikolajczyk, T., 1998. GTH I and GTH II secretion profiles during the reproductive cycle in female rainbow trout : relationship with pituitary responsiveness to GnRH-A stimulation. Gen. Comp. Endocrinol. 111, 38-50.

Bromage, N., Porter, M., Randall, C., 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture 197, 63-98.

Chen, F.Y., Chow, M., Sim, B.K., 1969. Induced spawning of the three major chinese carps in Malacca, Malaysia. Malaysian Agricultural Journal 47, 211-238.

Davies, P.R., Hanyu, I., 1986. Effect of temperature and photoperiod on sexual maturation and spawning of the common carp. I. Under conditions of high temperature. Aquaculture 51, 277-288.

De Vlaming, V.L., 1975. Effects of photoperiod and temperature on gonadal activity in the cyprinid teleost, Notemigonus crysoleucas. Biol. Bull. 148, 402-415.

De Vlamming, V.L., 1983. Oocyte development patterns and hormonal involvements among teleosts. In: Rankin, J.C., Pitcher, T.J., Duggan, R.T. (Eds.), Control processes in fish physiology. Croom Helm, London, 176-199.

Gillet, C., Billard, R., Breton, B., 1977. Effets de la température sur le taux de gonadotropine plasmatique et la spermatogénèse du poisson rouge Carassius auratus. Can. J. Zool. 55, 242-245.

Gillet, C., Billard, R., Breton, B., 1981. La reproduction du poisson rouge Carassius auratus élevé à 30°C. Effet de la photopériode, de l'alimentation et de l'oxygénation. Cahiers du laboratoire de Montereau 11, 49-56.

Gorbach, E.I., 1972. Fecundity of the grass carp (Ctenopharyngodon idella Val.) in the Amur basin. J. Ichthyol. 12, 616-625.

Gupta, S., 1975. The development of carp gonads in warm water aquaria. J. Fish Biol. 7, 775-782.

Hontela, A., Peter, R.E., 1978. Daily cycles in serum gonadotropin levels in the goldfish : effects of photoperiod, temperature, and sexual condition. Can. J. Zool. 56, 2430-2442.

Kagawa, H., Young, G., Nagahama, Y., 1983. Changes in plasma steroid hormone levels during gonadal maturation in female goldfish Carassius auratus. Bull. Jap. Soc. Scient. Fish. 49, 1783-1787.

Kestemont, P., 1990. Dynamic aspects of ovogenesis in an asynchronous fish, the Gudgeon Gobio gobio L. (Teleostei, Cyprinidae), under controlled temperature and photoperiod conditions. Aquat. Living Res. 3, 61-74.

Lam, T.J., 1983. Environmental influences on gonadal activity. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (Eds.), Fish Physiology, vol IX, part B. Academic Press, London, 65-116.

Makeyeva, A.P., Yemel'yanova, N.G., 1989. Periodization of oogenesis in cyprinids. J. Ichthyol. 29, 55-67.

Manning, N.J., Kime, D.E., 1984. Temperature regulation of ovarian steroid production in the common carp, Cyprinus carpio L., in vivo and in vitro. General and Comparative Endocrinology 56(3):376-388, 1984

Mourot, B., Le Bail, P.Y., 1995. Enzyme-linked immunosorbent assay (ELISA) for rainbow trout (Oncorhynchus mykiss) vitellogenin. J. Immunoassay 16, 365-377.

Quérat, B., 1995. Structural relationships between 'fish' and tetrapod gonadotropin. In: Goetz, F.W., Thomas, P. (Eds), Proc. 5th Int. Symp. Reproductive Physiology of Fish FishSymp 95, University of Texas, Austin, 7-9.

Razani, H., Hanyu, I., 1986. Annual reproductive cycle of 2-3 years old female goldfish and its artificial modification by manipulations of water temperature and photoperiod. Bull. Jap. Soc. Scient. Fish. 52, 965-969.

Razani, H., Hanyu, I., Aida, K., 1988. Environmental influences on ovarian activity and related hormones in yearling goldfish. Nippon Suisan Gakkaishi 54, 1505-1511.

Rinchard, J., Kestemont, P., Kuhn, E.R., Fostier, A., 1993. Seasonal changes in plasma levels of steroid hormones in an asynchronous fish the Gudgeon Gobio gobio L. (Teleostei, Cyprinidae). Gen. Comp. Endocrinol. 92, 168-178.

Shireman, J.V., Smith, C.R., 1983. Synopsis of biological data on the grass carp, Ctenopharyngodon idella (Cuvier and Valenciennes, 1844). FAO Fisheries Synopsis 135, Rome, 86 pp.

Sohn, Y.C., Yoshiura, Y., Kobayashi, M., Aida, K., 1999. Seasonal changes in mRNA levels of gonadotropin and thyrotropin subunits in the goldfish, Carassius auratus. Gen. Comp. Endocrinol. 113, 436-444.

Stanley, J.G., Miley, Jr., W.W., Sutton, D.L., 1978. Reproductive requirements and likelihood for naturalization of escaped grass carp in the United States. Trans. Am. Fish. Soc. 107, 119-128.

Sternberger, L.A., Hardy, Jr., P.H., Cuculis, J.J., Meyer, H.G., 1970. The unlabelled antibody enzyme method for immunohistochemistry: preparation and

properties of soluble antigen-antibody complex (horseradish-antihorseradish peroxidase) and its use in identification of spirochetes. J. Histochem. Cytochem. 18, 315-333.

Terqui, MM., Dray, F., Cotta, J., 1973. Variations de la concentration de l'oestradiol 17b dans le sang périphérique de la Brebis au cours du cycle oestral. C.R. Acad. Sc. Paris ser. D, 277, 1795-1798.

Tyler, C.R., Sumpter, J.P., 1990. The purification and partial characterisation of carp, Cyprinus carpio, vitellogenin. Fish. Physiol. Biochem. 8, 111-120.

Van der Kraak, G., Suzuki, K., Peter, R.E., Itoh, H., Kawauchi, H., 1992. Properties of common carp gonadotropin I and gonadotropin II. Gen. Comp. Endocrinol. 85, 217-229.

Yoshiura, Y., Kobayashi, M., Kato, Y., Aida, K., 1997. Molecular cloning of the cDNAs encoding two gonadotropin beta subunits (GTH-I beta and -II beta) from the goldfish, Carassius auratus. Gen. Comp. Endocrinol. 105, 379-389.

Zonneveld, N., 1984. The spawning season and the relation between temperature and stripping time of grass carp (Ctenopharyngodon idella Val.) in Egypt. Bamidgeh 36, 21-28.

Oocyte stage	Phase	Characteristics	Oocyte diameter (µm)
II (previtellogenesis)			40-200
III (vitellogenesis)	5	Beginning of cytoplasm vacuolisation	200-400
	6	Continuation of vacuole formation	400-500
	7	Intense accumulation of yolk granules	500-700
	8	Oocyte filled with yolk	700-1300

Table 1: Determination of grass carp oocyte stages according to Makeyeva and Yemel'yanova (1989) with the associated oocyte diameters.

1

2

Country	Ovarian stage[1]	Vg (µg/ml)[2]	E2 (ng/ml)[2]	T (ng/ml)[2]
Côte d'Ivoire	**PreVTG** *n=33*	7 ± 13^{a}	0.4 ± 0.3^{a}	1.4 ± 0.5^{a}
	Beginning of VTG (blocked) *n=17*	27 ± 28^{a}	0.5 ± 0.4^{a}	1.7 ± 0.6^{ab}
	Beginning of VTG (normal) *n=11*	223 ± 231^{b}	1.4 ± 1^{b}	2 ± 0.7^{abc}
	End of VTG *n=70*	742 ± 224^{c}	1.7 ± 1.1^{b}	3.6 ± 2.4^{c}
	Overripe *n=9*	778 ± 251^{c}	1.6 ± 1^{b}	2.1 ± 0.7^{abc}
Poland	**End of VTG (24°C)** *n=16*	522 ± 123^{d}	0.7 ± 0.5^{a}	2.4 ± 1.6^{abc}
	End of VTG (28°C) *n=14*	594 ± 213^{d}	0.6 ± 0.7^{a}	3.4 ± 1^{bc}

3 [1] VTG: vitellogenesis

4 [2] Same superscript indicates non-significant difference.

5

6

7 Table 2: Plasma characteristics of female grass carp in relation to ovarian stage, in

8 Côte d'Ivoire and Poland.

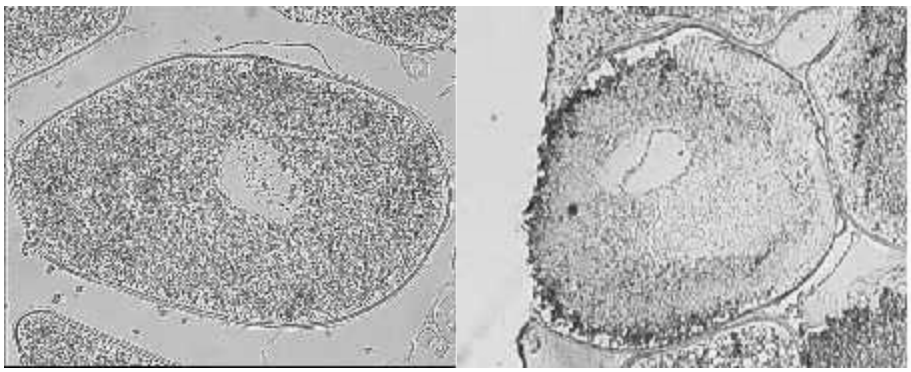

Figure 1: Normal (left) and atypic (right) grass carp oocytes after immunohistochemistry with anti-vitellogenin antibody.