Cell Tissue Res (1985) 240:199–208

Cell and Tissue Research

Functional aspects of the postovulatory follicle in the ovary of the African catfish, *Clarias gariepinus*, after induced ovulation

An ultrastructural and enzyme-histochemical study

R. van den Hurk and J. Peute

Zoological Laboratory, Section Comparative Endocrinology, State University of Utrecht, Utrecht, The Netherlands

Summary. In captive African catfish, *Clarias gariepinus*, ovulation was induced with human chorionic gonadotropin (HCG) 4 I.U./g body weight to study the function of postovulatory follicles (POFs). Ultrastructural and enzyme-histochemical data indicate that, apart from special theca cells, the granulosa of relative young POFs (i.e., from 16 h and 28 h after HCG-injection) is capable of producing steroids. Possible functions of the synthesized steroids are discussed. Histological comparison of POFs from stripped and from unstripped fish, as well as histochemical investigation of the contents of ovulated ova and granulosa of POFs at 48 h after HCG-injection, showed that the latter structure is involved in phagocytosis of the disintegrating ovulated eggs. The polysaccharide-lipid-protein material, initially taken up by heterophagolysosomes of the granulosa cells, subsequently undergoes fatty degeneration. The granulosa cells of the POFs showed strong acid phosphatase activity and abundant granular endoplasmic reticulum from 16 h after HCG-injection onward; heterophagolysosomes appeared at 32 h. These results indicate that after ovulation the phagocytotic function of the granulosa develops progressively. Autophagolysosomes, responsible for the final disintegration of POFs, become increasingly evident in the granulosa cells with increasing time after ovulation.

Key words: Ovary – Postovulatory follicles – Electron microscopy – Enzyme histochemistry – Teleosts, catfish

The function of the granulosa of intact and atretic follicles has been investigated in a large number of teleost species (for reviews, see Ball 1960; Lofts and Bern 1972; Guraya 1976; Saidapur 1978; Nagahama 1983). Relatively little information is available regarding its function after the discharge of the ovum from the follicle. In the majority of the oviparous teleosts investigated, postovulatory follicles (POFs) do not reorganize under the formation of corpora lutea, but instead collapse and become pyknotic, followed by rapid resorption (for review, see Guraya 1976). More recently, however, in various oviparous fish species steroidogenic activity has been demonstrated in theca and/or granulosa cells of POFs, using enzyme-histochemical and electron-microscopical techniques (for reviews, see Van den Hurk and Peute 1979; Fostier et al. 1983). Lam et al. (1978) suggested that in postspawning stickleback, *Gasterosteus aculeatus*, steroids produced by POFs might have a stimulatory effect on ovarian recrudescence and at the same time stimulate ovarian epithelium to secrete a fluid into the ovarian cavity for the maintanance of ovulated eggs. Recently, Van den Hurk and Lambert (1983) pointed to POFs as sources of steroid glucuronides in the zebrafish, *Brachydanio rerio*, which function as sex pheromones. Khoo (1975) observed a differentiation of cells of older POFs into oogonia in the goldfish, *Carassius auratus*. With regard to the POFs in the African catfish, *Clarias gariepinus*, Van den Hurk and Richter (1980) observed in the granulosa a weak activity of enzymes involved in steroidogenesis and a strong activity of these enzymes in the theca cells, shortly after induced ovulation by hypophysation. Richter and Van den Hurk (1982) also described the appearance of eosinophilic material in the granulosa cells of older POFs. They suggested that this material could be secreted into the ovarian cavity, where it would contribute to the disintegration and clearance of degenerated overripe eggs; however, conclusive evidence is lacking to date.

To obtain additional information concerning the possible function of the POF in captive *Clarias gariepinus*, this structure was studied at different times after artificially induced ovulation by means of histology, enzyme histochemistry and ultrastructural analysis. The effect of the absence or the presence of ovulated eggs on the functioning of POFs was studied histologically and enzyme-histochemically on stripped and unstripped fish, respectively.

Materials and methods

Sexually mature female African catfish, *Clarias gariepinus* (11 months old, approximately 200 g body weight and exposed to a simulated natural photoperiod, as characteristic for the season of the year in the Netherlands), were obtained from the Department of Fish Culture and Inland Fisheries, Agricultural University, Wageningen, The Netherlands. At the time of the present experiments the daily photoperiod was 12 L 12 D. Fish were kept individually in 150 l aquaria at 25° C for two days before the beginning of the experiments.

In the first experiment untreated fish and fish injected intramuscularly near the dorsal fin with 4.I.U. of human chorionic gonadotropin (HCG)/g body weight were used

Send offprint requests to: Dr. R. van den Hurk, Zoological Laboratory, State University of Utrecht, Padualaan 8, 3508 TB Utrecht, The Netherlands

Table 1. List of histological and enzyme-histochemical techniques used in experiments 1 (*I*) and 2 (*II*)

Reaction	Fixative	Method
Histology		
Mayer's haemalum and eosin[I,II]	Formol-calcium Frozen tissue	Burck 1981
Carbohydrates		
Best's carmine[II]	Carnoy	Chayen et al. 1969
Periodic-acid Schiff (PAS)[II]	Formol-calcium; Helly	Hotchkiss 1948, in Pearse 1968
Alcian blue, pH 2.5[II]	Formol-calcium; Helly	Pearse 1968
Proteins		
Dinitrofluorobenzene[II]	Formol-calcium; Helly	Burstone 1955 in Pearse 1968
Mercury bromophenol blue[II]	Formol-calcium; Helly	Bonhag 1955 in Pearse 1968
Lipids		
Sudan black B[II]	Frozen tissue	Chayen et al. 1969
Oil red O[II]	Frozen tissue	Lillie 1944 in Pearse 1968
Enzymes		
3β-Hydroxy-steroid dehydrogenase[I,II]	Frozen tissue	Van den Hurk 1973
Acid phosphatase[I]	Frozen tissue	Barka and Anderson 1963

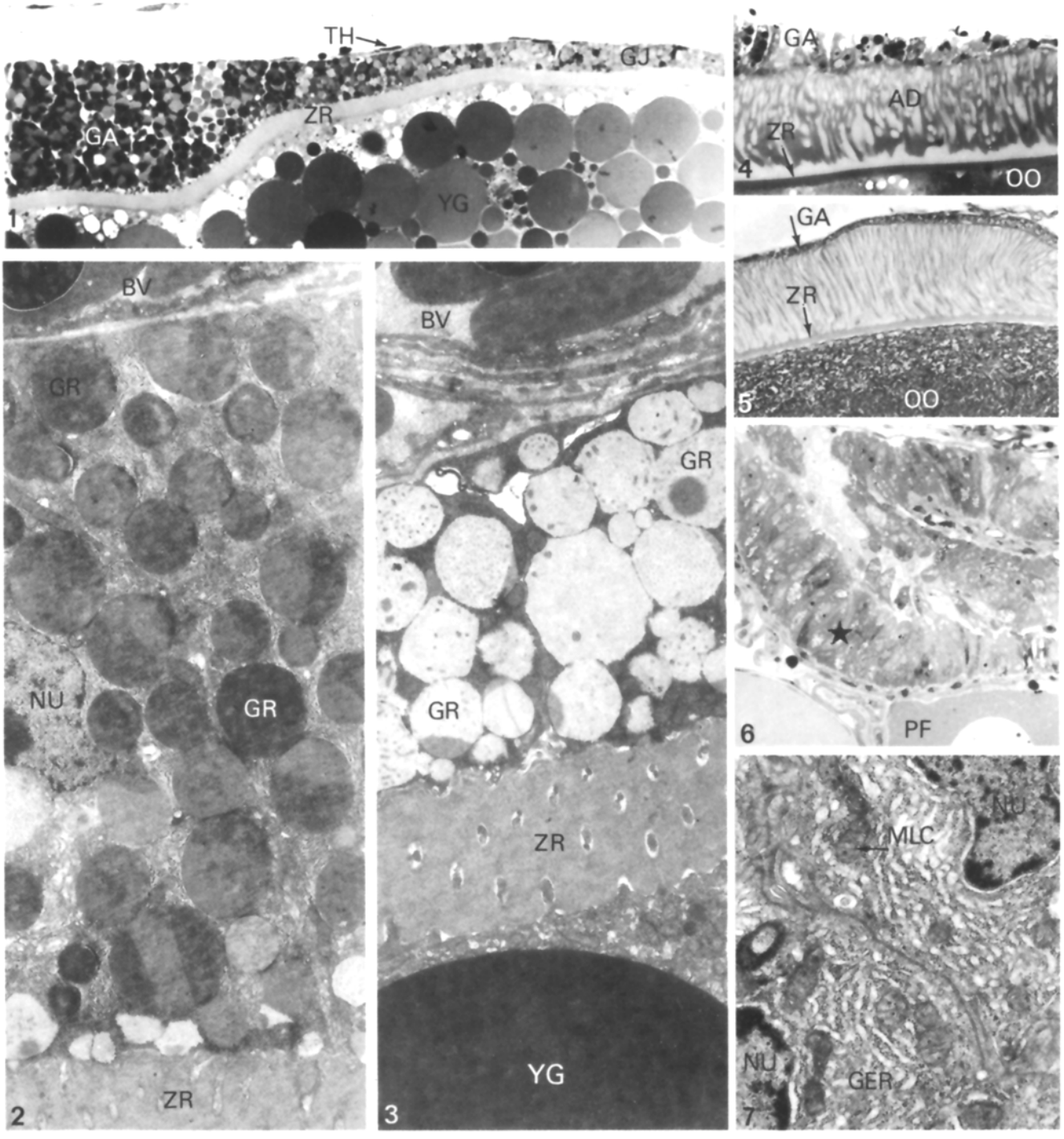
for histological, enzyme-histochemical and electron-microscopical examination of the ovaries. For electron-microscopical examination, central parts of the ovaries were fixed according to Peute et al. (1976). The tissues were dehydrated in graded ethanol and propylene oxide, followed by embedding in Epon. Thin sections were contrasted with uranyl acetate in 70% methanol and lead citrate and examined with a Zeiss EM-9 electron microscope. For light microscopy sections of 1-μm thickness were stained with a mixture of methylene blue and azure II (Richardson et al. 1960). In addition, central parts of gonads were fixed in formol-calcium, embedded in paraffin and cut into 5-μm thick sections, which were then stained with Mayer's haemalum and eosin (Burck 1981). Before embedding in paraffin, the tissues were not completely dehydrated by passing them quickly, i.e., 10 min per step, through a series of graded ethanols, since some residual water gave better histological results with yolk-laden eggs. The sections were prepared individually, under regularly moistening the cutting surface of the paraffin block with a lukewarm tissue. Enzyme-histochemical studies were made on 10-μm thick sections of central parts of ovaries quickly frozen at −20° C with CO_2. 3β-Hydroxy-steroid dehydrogenase (3β-HSD) and acid phosphatase were demonstrated according to the methods of Van den Hurk (1973, with epiandrosterone as a substrate) and Barka and Anderson (1963), respectively (Table 1). Control incubations were carried out in media without the substrate. The following criteria were used to score the intensity of the enzyme-histochemical reactions: − no reaction, + weak reaction, + + moderate reaction, + + + strong reaction.

A second experiment was carried out to compare the effects of stripping the ovulated eggs on the histology and enzyme histochemistry of the postovulatory follicle. Eight fish were injected with 4 I.U./g body weight HCG. Four of them were stripped three times, at 16, 18 and 20 h after injection, succesively, to ascertain the absence of ovulated eggs in the ovarian lumen at the time of decapitation; the other four fish were not stripped. Both at 48 h and at 120 h after ovulation four fish were decapitated, i.e., two stripped and two unstripped fish at each time; this was followed by removal of the gonads. Central parts of the ovaries were frozen or fixed in formol-calcium, or in Carnoy or Helly fluids. Helly-fixed material was embedded in 10% gelatin and then sectioned at 10 μm with a cryostat microtome at −20° C. Gonads fixed in formol-calcium or in Carnoy fluid were embedded in paraffin and sectioned at 5 μm. Unfixed ovaries were frozen with CO_2 and sectioned at 10 μm with a cryostat-microtome at −20° C. The histological and enzyme-histochemical techniques used are summarized in Table 1. Polysaccharides were demonstrated with the periodic acid Schiff (PAS) staining with and without previous acetylation and acetylation followed by deacetylation, and with Best's carmine method with and without treatment with 0.5% diastase (Chayen et al. 1969). The presence of acid mucopolysaccharides was tested with alcian blue 8GX at pH 2.5 (Pearse 1968). The mercury bromophenol-blue method and dinitrofluorbenzene staining (Pearse 1968) were applied for demonstrating proteins. Sudan black B (Chayen et al. 1968) and oil red O (Pearse 1968) were used to demonstrate lipids. Baker's pyridin extraction test was applied as a control for lipid stainings (Pearse 1968). 3β-HSD was demonstrated to investigate steroidogenic activity. For routine histological studies sections were stained with Mayer's haemalum and eosin.

Results

Experiment 1

Histology and ultrastructure. At the time of HCG-injection, the ovaries contain previtellogenic, vitellogenic as well as postvitellogenic follicles (PVFs). The latter follicles show completed vitellogenesis; their diameters measured 1000–1200 μm; the nuclei of the oocytes are located centrally. At the animal pole of the oocyte, i.e., the future attachment disk, granulosa cells of the PVFs are cylindrical and contain numerous granules of different stainability (Fig. 1) and electron density (Fig. 2; maximum diameter up to 3.3 μm). Lateral to this area and extending to the



Figs. 1–7. Abbreviations: *GA* part of granulosa forming attachment disk; *GJ* part of granulosa forming jelly coat; *ZR* zona radiata. *YG* yolk granule; *TH* theca layer; *NU* nucleus; *BV* blood vessel; *OO* ooplasm; *AD* attachment disk; *PF* previtellogenic follicle; *GR* granules

Fig. 1. Part of a postvitellogenic follicle (PVF). × 560

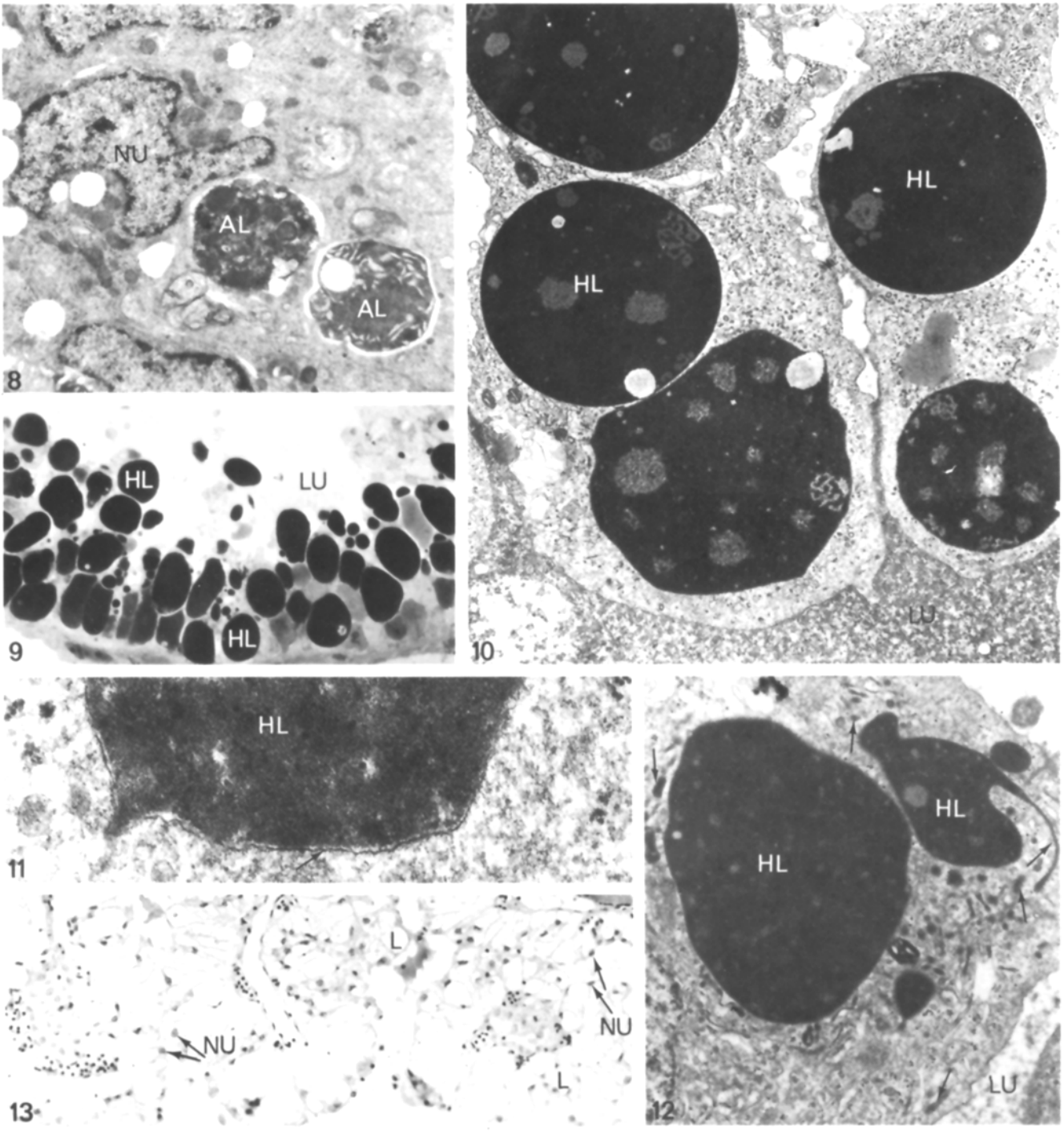
Fig. 2. Part of a cylindric granulosa cell of a PVF containing electron-dense granules. × 5400

Fig. 3. Part of a cubic granulosa cell of a PVF containing electron-lucent granules. × 5400

Figs. 4, 5. Part of a maturing follicle. Note flattening of granulosa during formation of attachment disk. × 224

Fig. 6. Part of a postovulatory follicle (POF) at 16 h after HCG-injection. Note the hypertrophied granulosa (*). × 560

Fig. 7. Part of two granulosa cells of a POF. Note mitochondria with lamellar cristae (*MLC*) and *GER*. × 1400



Figs. 8–13. Abbreviations: *AL* secondary autophagolysosome; *HL* heterophagolysosome; *NU* nucleus; *LU* lumen; *L* lipid accumulation

Fig. 8. Part of a granulosa cell of a POF at 16 h after HCG-injection containing secondary autophagolysosomes. × 6200

Figs. 9, 10. Part of the granulosa of a POF at 48 h after HCG-injection displaying heterophagolysosomes. Fig. 9: × 350; Fig. 10: × 6200

Fig. 11. Part of a heterophagolysosome showing its surrounding membrane (*arrow*). × 40600

Fig. 12. Part of a granulosa cell of a POF at 36 h after HCG-injection showing heterophagolysosomes and an associated network of channels and vesicles (*arrows*). × 17500

Fig. 13. Part of a POF at 120 h after HCG-injection. The granulosa cells are filled with lipid accumulations; their nuclei have shifted to the periphery. × 224

vegetative pole the granulosa cells gradually acquire a cubical appearance with granules of slight electron density and a weak stainability (Figs. 1, 3; maximum diameter up to 3.6 μm). All granulosa cells have a centrally located and polymorphic nucleus, which is situated amidst the granules. They furthermore contain granular endoplasmic reticulum (GER) and a few small mitochondria with lamellar cristae.

At 10 h after HCG injection, the ovaries contain maturing follicles (MFs), in addition to previtellogenic and vitellogenic follicles. At the animal site of the oocyte in MFs the surrounding granulosa cells have formed an attachment disk between the granulosa and the zona radiata. During the formation of the disk the appearance of the granulosa cells changes from cylindrical, still containing granules (Fig. 4), to flattened, without granular inclusions (Fig. 5). In addition, some GER and mitochondria with lamellar cristae are present. The electron density of the content of the granules corresponds to that of the attachment disk. At the lateral and vegetative sites the granulosa cells have formed a jelly coat, with the same electron density as the granules inside the cells. The morphology of these cells corresponds to that of the granulosa at the animal site, but their granules are less electron dense.

At 16 h after HCG-injection, ova have been ejected from their follicular envelope and are now stored in the ovarian cavity. The resulting postovulatory follicles (POFs) have a hypertrophied granulosa (Fig. 6), the cells having a few microvilli, abundant GER, many dictyosomes and mitochondria with lamellar cristae (Fig. 7). Depending on the investigated follicle, smooth endoplasmic reticulum (SER) is generally absent, or present in only small amounts. Lipid droplets are distributed throughout the granulosa cells but mainly at their basal site, with a round nucleus in juxtaposition (diameter: 3.5–4 μm). In each POF, some of the granulosa cells contain large lysosomal bodies (Fig. 8), with remnants of GER and mitochondria. These bodies are termed secondary autophagolysosomes as defined by De Duve and Wattiaux (1966).

At 28 h after HCG-injection, the ultrastructure of the granulosa of POFs has changed slightly compared to 16 h, i.e., the number of lysosomal bodies and the amount of GER have increased. At 32, 36, 48 and 72 h, the granulosa cells of the POFs contain an increasing number of small and larger spherical structures (Fig. 9) with a mainly strong electron-dense inhomogeneous content (Fig. 10) and surrounded by a membrane (Fig. 11). According to De Duve and Wattiaux (1966) such structures may be termed heterophagolysosomes. At the light-microscopical level these structures are eosinophilic spheres (ES). They appear to be larger with increasing time after ovulation. At the apical site of the cells a network of narrow channels with associated vesicles is present, the electron density of their contents corresponding to that of the lysosomes (Fig. 12). Frequently fusions are observed between vesicles, channels and heterophagolysosomes. The granulosa cells furthermore contain GER, a few dictyosomes, small mitochondria and some SER; the nucleus has a diameter of 2–2.5 μm. At these stages, ovulated eggs desintegrate; egg fragments are found throughout the ovarian cavity and between the ovarian lamellae.

At 120 h after HCG-injection, the granulosa cells of the POFs are filled with lipid accumulations, whereas ES have disappeared; their nuclei (1.5–2 μm) have shifted to the periphery (Fig. 13).

Table 2. 3β-HSD and acid phosphatase (*APT*) activity in the granules and special theca cells (*STC*) of vitellogenic (VF), postvitellogenic (*PVF*), mature (*MF*), and postovulatory (*POF*) follicles. Times indicate hours after injection with HCG. Fish were unstripped

Site	3β-HSD	APT
Granulosa VF, PVF	−	−/+
Granulosa MF	+/++	+
Granulosa POF 16 h	++	+++
Granulosa POF 28 h	+	+++
Granulosa POF 32 h	−/+	+++
Granulosa POF 36–120 h	−	+++
STC VF, PVF, MF, POF 16–72 h	+++	−
STC POF 120 h	−	−

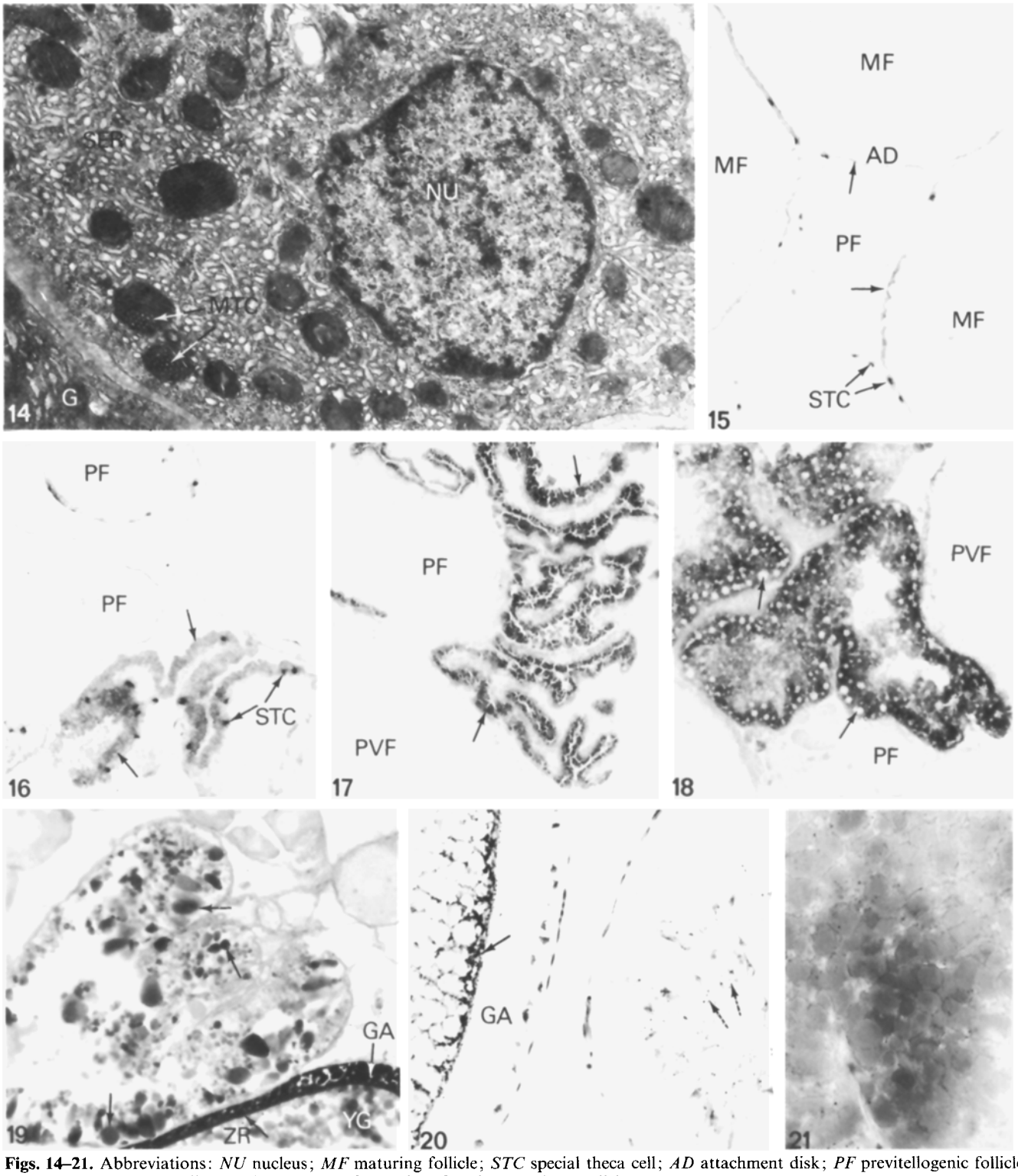
− no reaction; + weak reaction; ++ moderate reaction; +++ strong reaction

PVFs, MFs and POFs have a thin theca layer, generally consisting of fibroblasts and a few so-called special theca cells (Van den Hurk and Peute 1979; Fostier 1983). The latter are characterized by several large mitochondria with tubular cristae, numerous cisternae of SER, a small amount of GER and a few dictyosomes (Fig. 14). In POFs from 120 h after HCG-injection such special theca cells are not observed.

Enzyme histochemistry. As has previously been demonstrated (Van den Hurk and Richter 1980), initial controls show a strong 3β-HSD activity in the special theca cells of the vitellogenic follicles and the PVFs (Table 2) and also in a small number of the interstitial cells. Acid phosphatase activity is absent or weakly present in the granulosa cells of vitellogenic and postvitellogenic follicles (Table 2). At 10 h after injection with HCG, besides special theca cells and interstitial cells, also the granulosa cells of MFs show a weak to moderate 3β-HSD activity (Fig. 15); the latter sites display a weak acid phosphatase activity. From 16 h–120 h after HCG-injection the 3β-HSD activity in the granulosa gradually diminishes from moderate (16 h; Fig. 16) to weak (28 h) and nearly absent (32 h) to completely absent (from 36 h onwards). In the same period (16–72 h) the special theca cells of the POFs generally have a strong 3β-HSD activity, but at 120 h, this activity has disappeared. At 16 h and 28 h, acid phosphatase activity is strong and distributed throughout the cytoplasm of the granulosa cells of the POFs (Fig. 17). From 32 to 120 h, a strong acid phosphatase activity is located in the cytoplasmic regions between the spherical eosinophilic inclusions (ES; Fig. 18) or large lipid droplets in the granulosa of POFs.

Experiment 2

Effect of stripping on postovulatory follicles (POFs). At 48 h after HCG-injection, the granulosa of POFs from stripped fish show a weak 3β-HSD (Table 3) activity. The granulosa is built up of cubical to cylindrical cells, with relatively large nuclei (diameter: 3–4 μm). POFs of unstripped fish at 48 h after HCG-injection lack 3β-HSD activity in their granulosa. The granulosa cells of these POFs furthermore have small nuclei (diameter: 2–2.5 μm) and contain eosinophilic spheres (ES). The special theca cells of the POFs



Figs. 14–21. Abbreviations: *NU* nucleus; *MF* maturing follicle; *STC* special theca cell; *AD* attachment disk; *PF* previtellogenic follicle; *ZR* zona radiata; *YG* yolk granule; *GA* part of granulosa forming attachment disk

Fig. 14. Special theca cell containing mitochondria with tubular cristae (*MTC*) and abundant SER. ×14000

Fig. 15. 3β-HSD activity in the ovary at 10 h after HCG-injection. Note the weak to moderate enzyme activity in the granulosa (*arrows*) of MFs and the strong activity in the STC. ×88

Fig. 16. 3β-HSD activity in the ovary at 16 h after HCG-injection. Apart from a strong activity in STCs, a moderate activity is visible in the granulosa of a POF (*arrow*). ×88

Fig. 17. Strong acid phosphatase activity in the granulosa of a POF (*arrows*) at 16 h after HCG-injection. ×88

show a strong 3β-HSD activity, both in stripped and unstripped fish.

At 120 h, the granulosa cells of both stripped and unstripped fish have small nuclei (diameter: 2–2.5, and 1.5–2 μm, respectively), and lack 3β-HSD activity (Table 3). Contrary to unstripped fish, stripped animals still have POFs with strongly 3β-HSD-positive special theca cells. ES and abundant sudanophilic lipid material, as present in POFs of unstripped fish, are absent in those of stripped catfish.

Histochemistry of eosinophilic spheres (ES) in granulosa cells of POFs. The ES in the granulosa of POFs of unstripped fish at 48 h after HCG-injection give a distinct reaction with periodic acid-Schiff's reagent (Table 4, Fig. 19). This reaction is abolished in acetylated sections and reappears after deacetylation, which confirms the polysaccharide nature of the spherical structures. They furthermore stain weakly with alcian blue, suggesting the presence of acidic polysaccharides. They also react with mercury bromophenol blue and dinitrofluorobenzene, as well as with Sudan black B and oil red O, when pyridine extraction is omitted. These results are indicative for the presence of proteins and neutral fats, respectively. The ES thus contain a complex of acidic polysaccharides, lipids and proteins. Only the smaller ES in addition stain with Best's carmine (Fig. 20). This staining is abolished after pretreatment with diastase, which is indicative of the presence of glycogen.

At 120 h after HCG-injection, the granulosa cells of the POFs are filled with extremely large and rounded masses of sudanophilic (Fig. 21) and oil red O-positive material. These lipid masses do not stain with PAS, Best's carmine, alcian blue, dinitrofluorobenzene or mercury bromophenol blue, which points to the absence of carbohydrates and proteins.

Histochemistry of ovulated eggs and postvitellogenic follicles (PVFs). Ovulated ova and non-ovulated oocytes in PVFs contain yolk granules that moderately stain with PAS (Table 4; Fig. 19). The reaction disappears and reappears after acetylation and deacetylation, respectively. The yolk granules furthermore stain with dinitrofluorobenzene and mercury bromophenol blue; they also show a weak affinity for Sudan black B and oil red O, when pyridine extraction is omitted, and do not stain with alcian blue. Yolk granules thus consist of a polysaccharide-lipid-protein complex. Glycogen deposits are present between the yolk granules (Fig. 20), since at these sites a positive reaction with Best's carmine has disappeared after diastase treatment. The zona radiata strongly stains with PAS, without previous acetylation and after deacetylation, and with dinitrofluorobenzene and mercury bromophenol blue. Polysaccharides and proteins thus are the major components of the zona radiata.

Table 3. Effects of stripping on postovulatory follicles of the African catfish

Characteristics	Time after HCG injection			
	Stripped fish		Unstripped fish	
	48 h	120 h	48 h	120 h
Diameter nuclei GC (μm)	3–4	2–2.5	2–2.5	1.5–2
Eosinophilic spheres GC	–	–	many	scarce
Sudanophilic material GC	–	–	–	much
3β-HSD GC	+	–	–	–
3β-HSD STC	+++	+++	+++	–

GC granulosa cells; *STC* special theca cells; – negative or absent; + weak; +++ strong

Table 4. Histochemistry of ovulated ova, postvitellogenic follicles (PVFs) and eosinophilic spheres (ES) in the granulosa of postovulatory follicles

Reaction	ES 48 h	PVFs/Ovulated ova						
		YG	OO	ZR	GA	AD	GJ	JC
PAS	+/++	+/++	+	+++	+++	+++	–	–
Best's carmine	+ or –[a]	–	+++	–	–	–	–	–
Alcian blue	+	–	–	–	–	–	++	++
DNFB/MBB	++	++	+	+++	+++	+++	+	+
Sudan black/Oil red O	+	+	–	–	–	–	–	–

DNFB dinitrofluorobenzene; *MBB* mercury bromophenol blue; *YG* yolk granules; *OO* ooplasm; *ZR* zona radiata; *GA* granules in granulosa of PVFs contributing to formation of attachment disk; *AD* attachment disk; *GJ* granules in granulosa of PVFs contributing to formation of jelly coat; *JC* jelly coat; – no reaction; + weak reaction; ++ moderate reaction; +++ strong reaction

[a] Only the smaller structures show a positive reaction

Fig. 18. Strong acid phosphatase activity in the granulosa of POFs at 36 h after HCG-injection. Note absence of reaction in the spherical inclusions (*arrows*). ×88

Fig. 19. Part of an ovary at 48 h after HCG-injection; PAS-staining. Positive reaction is present in the spherical inclusions (*arrows*) of a POF, the zona radiata, yolk granules, and granules in the granulosa of a PVF that contribute to an attachment disk. ×88

Fig. 20. Part of an ovary at 48 h after HCG-injection. Glycogen depositions are present in the ooplasm of a PVF (*solid arrow*) and in the smaller spherical inclusions of a POF (*dotted arrows*). ×560

Fig. 21. Sudan black staining of the spherical lipid accumulations in a POF at 120 h after HCG-injection. ×224

The attachment disk of ovulated ova and the granules of PVFs that contribute to the formation of this disk are PAS-positive without previous acetylation and, after deacetylation, alcian blue negative; they stain intensely with dinitrofluorobenzene and mercury bromophenol blue. These histochemical characteristics point to the presence of polysaccharides and proteins. The jelly coat of ovulated ova and the granules in the granulosa of PVFs that contribute to the formation of this coat are PAS negative, alcian blue positive and stain weakly with dinitrofluorobenzene and mercury bromophenol blue. These features are indicative for the presence of acidic mucopolysaccharides and a low protein content.

Discussion

The present ultrastructural data demonstrate that, in *Clarias gariepinus*, large numbers of granules are released by the granulosa of maturing follicles to form an attachment disk and jelly coat around the zona radiata of oocytes. These observations confirm earlier histological data (Richter and Van den Hurk 1982). The low protein content in the granules forming the jelly coat might explain their slight electron density.

In various fish species steroidogenic activity has been demonstrated in theca and/or granulosa cells of different follicular stages, using enzyme-histochemical and electron-microscopic techniques (for review, see Fostier et al. 1983). In a previous enzyme-histochemical study on *Clarias gariepinus*, treated with carp pituitary suspension, Van den Hurk and Richter (1980) demonstrated the presence of enzymes involved in steroid formation. For example, 3β-HSD was observed in theca cells of various stages of follicles and in granulosa cells of maturing and postovulatory follicles. These findings are confirmed by the enzyme-histochemical results of the present study, in which egg maturation and ovulation was induced with HCG. In this study, distinct ultrastructural features for steroid biosynthesis, such as mitochondria with tubular cristae and SER, are indeed found in theca cells, but are absent (mitochondria with tubular cristae) or scarcely present (SER) in granulosa cells. However, in addition to some SER, the granulosa cells of young POFs, i.e., from 16 h and 28 h after HCG-injection, contain other features generally related to steroidogenic activity, such as many lipid droplets and numerous Golgi elements (Fawcett 1975). The absence of mitochondria with tubular cristae in these granulosa cells might be due to the relative low steroidogenic potency of the granulosa, as has been shown by the weak 3β-HSD activity, or to the lack of certain enzyme systems that are involved in the synthesis of steroids (Van den Hurk et al. 1982b). The presence or absence of mitochondria with tubular cristae in theca cells and granulosa cells might thus point to a different steroidogenic potency of these cells.

Such a difference has recently been demonstrated biochemically for the Amago salmon, *Oncorhynchus rhodurus*, (Nagahama 1983). According to this author the theca cells of maturing follicles produce 17α-hydroxy progesterone, which serves as a precursor for the synthesis of 17α,20β-dihydroxy-4-pregnen-3-one in the granulosa. Lambert and van den Hurk (1982) demonstrated a considerable production of 17α-hydroxy progesterone and 17α,20β-dihydroxy-4-pregnen-3-one by the catfish ovary, just prior to ovulation. Thus, the appearance of 3β-HSD in the granulosa of maturing follicles, as demonstrated in the present study, apparently reflects the synthesis of these progestagens. There is evidence for the involvement of these progesterone derivatives in inducing in vitro oocyte maturation in various teleost species (Osanai et al. 1973; Jalabert 1976; Iwamatsu 1978; Goetz and Bergman 1978). 17α,20β-dihydroxy-4-pregnen-3-one also promotes oocyte maturation in *Clarias gariepinus*, when injected intramuscularly (Richter, unpublished).

Lambert and Van den Hurk (1982) furthermore demonstrated the formation of testosterone-glucuronide in postovulatory ovaries. At that stage, stroma cells and granulosa cells of POFs show 3β-HSD activity. Van den Hurk (unpublished results) recently demonstrated in the granulosa of POFs uridine-diphosphateglucose dehydrogenase, a key enzyme in the formation of glucuronids. The enzyme-histochemical findings thus strongly point to the granulosa of POFs as the source of steroid-glucuronide synthesis. The steroid conjugates may be either of importance in the biological inactivation of steroids (Kime 1980) or be functional as sex pheromones in fish (Colombo et al. 1982; Van den Hurk et al. 1982a; Van den Hurk and Lambert 1983).

Apart from a function in steroid formation by POFs, the presence of abundant GER in the hypertrophied granulosa of POFs, together with the presence of strong acid phosphatase activity also point to a high production of proteinaceous substances involved in phagocytotic processes. These processes become evident in the granulosa of POFs from 32 h after HCG-injection, when ovulated eggs in the ovarian cavity disintegrate. At that time eosinophilic spheres, so-called heterophagolysosomes (De Duve and Wattiaux 1966), appear in the granulosa cells. They are connected via channels with vesicles at the apical site of the granulosa cells. Most probably their contents are derived from broken-down ovulated eggs, since the granulosa cells of POFs in stripped fish, i.e., in the absence of ovulated eggs, lack these heterophagolysosomes. Furthermore, the histochemical composition of oocytes and unstripped eggs, especially that of yolk granules, appear to be similar to that of the lysosomal contents, indicating a close functional relation between the disappearance of the oocytes and the appearance of the lysosomes. A similar phagocytosis of oocyte material has frequently been observed in the granulosa of atretic follicles in various fish species (Bretschneider and Duyvené de Wit 1947; Barr 1963; Braekevelt and McMillan 1967; Lambert 1970; Van den Hurk and Peute 1979; Lang 1981a), including *Clarias gariepinus* (Richter and Van den Hurk 1982). Lang (1981b) found an accumulation of large globules containing proteins and cholesterol-related material in the granulosa of POFs in *Perca fluviatilis*. She, however, ascribed her findings to active synthetic processes of the granulosa cells.

The polysaccharide-lipid-protein complex, initially taken up by the heterophagolysosomes from approximately 32 h after HCG-injection onwards, appears to be transformed into lipids at 120 h. This demonstrates a fatty degeneration of the phagocytotic material by the granulosa of the POFs. A similar mechanism has also been described for atretic follicles and finally results in the appearance of pigment granules (Guraya 1976; Saidapur 1978). In natural populations of African catfish, a phagocytotic phenomenon displayed by the granulosa of POFs has never been observed (Viveen and Van den Hurk, unpublished results). Such a function becomes obviously manifest under abnor-

mal circumstances, such as captivity, when oviposition does not take place spontaneously.

Some of the granulosa cells of the investigated POFs contained large lysosomal bodies with remnants of cell organelles. These bodies are autophagolysosomes as defined by De Duve and Wattiaux (1966). They take part in the degradation of the contents of the granulosa cells and become more and more evident with increasing time after ovulation. Such autophagolysosomes were also observed in some granulosa cells of POFs of *Perca fluviatilis* (Lang 1981b) and contribute to the final degradation of POFs. A complete degradation of POFs has not been observed in the present material. Therefore, ovaries from more than 120 h after HCG-injection have to be studied.

Contrary to unstripped catfish at 48 h after HCG-injection, ovaries of stripped fish contain POFs with relatively large and active nuclei and a marked 3β-HSD activity in their granulosa cells. These results point to a release of compounds by disintegrating ovulated, but unstripped eggs inhibiting the production of steroids. This phenomenon might be necessary to stop the production of sex pheromones by the granulosa cells of the POFs in order to avoid a potential spawning, since fertilization of overripe eggs may lead to birth of deformed fry. Fertilization of eggs from 20 h after HCG-injection onwards leads to low percentages of hatched eggs (Eding et al. 1982) and high percentages of deformed fry (Richter, unpublished results). Compounds released from overripe eggs might also stimulate the phagocytotic process in the granulosa of POFs, resulting in the degradation of unstripped eggs.

The strong 3β-HSD activity in the granulosa of relatively old POFs of stripped fish does not seem to be of importance for reproduction, since ova are absent. The ovaries of these stripped fish produce large amounts of steroid-glucuronids (Schoonen and Lambert, unpublished results). Unlike the situation in unstripped fish, the steroid production in the POFs of stripped fish possibly reflects inactivation of synthesized steroids, as proposed by Kime (1980), rather than production of sex pheromones, as suggested by Lambert and Van den Hurk (1982).

In conclusion, the granulosa of young postovulatory follicles (16 and 28 h after HCG-injection) of captive African catfish is able to produce steroids, whereas that of older POFs (32–120 h after HCG-injection) is involved in phagocytosis of the disintegrating ovulated eggs. The material taken up undergoes fatty degeneration.

Acknowledgements. The authors are indebted to Mr. V. de Nijs for carrying out the main part of the practical research. They are grateful to Prof. Dr. P.G.W.J. van Oordt for critically reviewing the manuscript. Thanks are also due to Ing. E.H. Eding (Agricultural University, Wageningen, The Netherlands) for taking care of the experimental circumstances of the fish, Mrs. A.M.E.R. de Lange for typing the manuscript, and the members of the light-optical registration department.

References

Ball JN (1960) Reproduction in female bony fishes. Symp Zool Soc London 1:105–135

Barka T, Anderson P (1963) Histochemical method for acid phosphatase using hexazonium pararosaline as coupling. J Histochem Cytochem 10:741–753

Barr WA (1963) The endocrine control of the sexual cycle in the plaice, *Pleuronectes platessa* (L.). I. Cyclical changes in the normal ovary. Gen Comp Endocrinol 3:197–204

Braekevelt CR, McMillan DB (1967) Cyclic changes in the ovary of the stickleback, *Eucalia inconstans* (Kirtland). J Morphol 123:373–396

Bretschneider LH, Duyvené-De Wit JJ (1947) Sexual endocrinology of non-mammalian vertebrates. Elsevier, Amsterdam, p 146

Burck H-C (1981) Histologische Technik, Leitfaden für die Herstellung mikroskopischer Präparate in Unterricht und Praxis. Thieme, Stuttgart, New York

Chayen J, Bitenski L, Butcher R, Poulter L (1969) Histochemistry, a guide to practical histochemistry. Oliver, Boyd, Edinburgh

Colombo L, Belvedere PC, Marconata A, Bentivegna F (1982) Pheromones in teleost fish. In: Richter CJJ, Goos HJTh (comps) Proc Int Symp Repr Phys Fish, Pudoc, Wageningen, pp 84–94

De Duve C, Wattiaux R (1966) Functions of lysosomes. Ann Rev Physiol 28:435–492

Eding EH, Janssen JAL, Kleine-Staarman GHJ, Richter CJJ (1982) Effects of human chorionic gonadotropin (HCG) on maturation and ovulation of oocytes in the catfish *Clarias lazera* (C & V). In: Richter CJJ, Goos HJTh (comps) Proc Int Symp Repr Phys Fish, Pudoc, Wageningen, p 195

Fawcett DW (1975) Ultrastructure and function of the Sertoli cell. In: Handbook of physiology. Sect 7, Vol V, Am Physiol Soc, Washington

Fostier A, Jalabert B, Billard R, Breton B, Zohar Y (1983) The gonadal steroids. In: Fish physiology. Vol IX Reproduction. Part A Endocrine tissues and hormones, pp 277–372

Goetz FW, Bergman HL (1978) The effects of steroids on final maturation and ovulation of oocytes from brook trout (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). Biol Reprod 18:293–298

Guraya SS (1976) Recent advances in the morphology, histochemistry, and biochemistry of steroid-synthesizing cellular sites in the non-mammalian vertebrate ovary. Int Rev Cytol 44:365–409

Iwamatsu T (1978) Studies on oocyte maturation of the medaka *Oryzias latipes*. V. On the structure of steroids that induce maturation in vitro. J Exp Zool 204:401–408

Jalabert B (1976) In vitro oocyte maturation and ovulation in rainbow trout *Salmo gairdneri*, northern pike *Esox lucius*, and goldfish *Carassius auratus*. J Fish Res Board Can 33:974–988

Khoo KH (1975) The corpus luteum of goldfish (*Carassius auratus* L.) and its functions. Can J Zool 53:1306–1323

Kime DE (1980) Androgen biosynthesis by testis of the goldfish, *Carassius auratus*, in vitro: The effect of temperature on the formation of steroid glucuronides. Gen Comp Endocrinol 41:164–172

Lam TJ, Nagahama Y, Chan K, Hoar WS (1978) Overripe eggs and postovulatory corpora lutea in the threespine stickleback, *Gasterosteus aculeatus* L., form trachurus. Can J Zool 56:2029–2036

Lambert JGD (1970) The ovary of the guppy, *Poecilia reticulata*. The atretic follicles, a corpus atreticum or a corpus luteum praeovulationis. Z Zellforsch 107:54–67

Lambert JGD, Van den Hurk R (1982) Steroidogenesis in the ovaries of the African catfish, *Clarias lazera*, before and after an HCG induced ovulation. In: Richter CJJ, Goos HJTh (comps) Proc Int Symp Repr Phys Fish, Pudoc, Wageningen, pp 99–102

Lang I (1981a) Electron microscopic and histochemical investigations of the atretic oocyte of *Perca fluviatilis* L. (Teleostei). Cell Tissue Res 220:201–212

Lang I (1981b) Electron microscopic and histochemical study of the postovulatory follicles of *Perca fluviatilis* L. (Teleostei). Gen Comp Endocrinol 45:219–233

Lofts B, Bern HA (1972) The functional morphology of steroidogenic tissues. In: Steroids in non-mammalian vertebrates. Academic Press, New York, London, pp 37–125

Nagahama Y (1983) The functional morphology of teleost gonads. In: Hoar WS, Randall DJ, Donaldson EM (eds) Fish physiology. Vol IX Reproduction. Part A: Endocrine tissues and hormones. Academic Press, pp 223–276

Osanai K, Hirai S, Sato R (1973) In vitro induction of oocyte maturation in the chum salmon. Zool Mag 82:68–71

Pearse AGE (1968) Histochemistry, theoretical and applied. Vol 1, Churchill Livingstone Ltd., London

Peute J, De Bruyn MGA, Seldenrijk R, Van Oordt PGWJ (1976) Cytophysiology and innervation of gonadotropic cells in the pituitary of the black molly (*Poecilia latipinna*). Cell Tissue Res 174:35–54

Richardson KC, Jarett L, Finke EM (1960) Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol 35:313–323

Richter CJJ, Van den Hurk R (1982) Effect of 11-desoxycorticosterone acetate and carp pituitary suspension on follicle maturation in the ovaries of the African catfish, *Clarias lazera* (C & V). Aquaculture 29:53–66

Saidapur SK (1978) Follicular atresia in the ovaries of non-mammalian vertebrates. Int Rev Cytol 54:225–244

Van den Hurk (1973) The localization of steroidogenesis in the testes of oviparous and viviparous teleosts. Proc Kon Ned Acad Wet Ser C 76:270–279

Van den Hurk R, Lambert JGD (1983) Ovarian steroid glucuronides function as sex pheromones for male zebrafish, *Brachydanio rerio*. Can J Zool 61:2381–2387

Van den Hurk R, Peute J (1979) Cyclic changes in the ovary of the rainbow trout, *Salmo gairdneri*, with special reference to sites of steroidogenesis. Cell Tissue Res 199:289–306

Van den Hurk R, Richter CJJ (1980) Histochemical evidence for granulosa steroids in follicle maturation in the African catfish, *Clarias lazera*. Cell Tissue Res 211:345–348

Van den Hurk R, 't Hart LA, Lambert JGD, Van Oordt PGWJ (1982a) On the regulation of sexual behaviour of male zebrafish, *Brachydanio rerio*. Gen Comp Endocrinol 46:403 (Abstr.)

Van den Hurk R, Lambert JGD, Peute J (1982b) Steroidogenesis in the gonads of rainbow trout fry, *Salmo gairdneri*, before and after the onset of gonadal sex differentiation. Reprod Nutr Dev 22:413–425

Accepted November 26, 1984