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Developing follicles of the spotted ray *Torpedo marmorata* express different glycoside residues in relation to granulosa differentiation and vitelline envelope formation

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Summary. Lectins constitute a class of proteins/glycoproteins that specifically bind to terminal glycoside residues. The present investigation aimed to identify lectin-binding sites in developing follicles of *Torpedo marmorata*. Using eleven lectins (WGA, GSI-A4, GSI-B4, PSA, UEA-I, PNA, MPA, Con-A, DBA, LCA, BPA, SBA), we demonstrated that the biochemical nature and the distribution of carbohydrate residues significantly change during oogenesis in the granulosa cells and the vitelline envelope. In fact, a progressive appearance of surface glycoproteins bearing terminated ß-GlcNAc O-linked side chains was observed in the granulosa during the differentiation of pyriform-like cells from the small ones via intermediate cells simultaneously with a significant reduction of the D-Gal chains present in their nucleus. Glycoproteins bearing ß-GlcNAc O-linked side chains were first evident on the surface of small cells in contact with the oocyte, then on the intermediate ones, and finally on pyriform-like cells. The distribution pattern of such glycoproteins over the differentiated granulosa cells remained unchanged during the subsequent stages of the oocyte growth so granulosa cells preserved the same sugar distribution pattern. Furthermore, a progressive loss of D-Gal residues was evident in the nucleus of granulosa cells. In fact, staining for D-Gal was intense in the nucleus of small follicle cells and progressively reduced till disappearing in differentiated pyriform-like cells. Conversely, the small follicle cells located under the basal lamina were devoid of ß-GlcNAc residues, and the nuclear content in D-Gal remained unchanged. This finding strongly suggests that surface glycoproteins containing ß-GlcNAc residues, and the nuclear content in D-Gal might be related to the differentiation of pyriform-like cells.

The present investigation also demonstrates that the content of the sugar residues of the vitelline envelope (VE) changes during oocyte growth, suggesting that pyriform-like cells may contribute to its formation.

Key words: Lectin, Pyriform-like cells, Granulosa, Vitelline envelope, Cartilaginous fish

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Introduction

The follicular epithelium (granulosa) surrounding the oocyte in *Torpedo marmorata* is multi-layered and polymorphic for the presence of three kinds of follicle cell: small, intermediate and pyriform-like. The last ones are very peculiar since they are connected to the oocyte via an intercellular bridge (Prisco et al., 2002a). Such an organization has also been described in another species of cartilaginous fish, *Raja asterias*, where it has been demonstrated that pyriform-like cells differentiate from small cells once they have formed the intercellular bridge (Andreuccetti et al., 1999). Pyriform-like cells act as nurse cells, by supplying the oocyte with different materials via the intercellular bridge (Andreuccetti et al., 1999). A similar organization is present in squamate reptiles as well (Ghiara et al., 1968; Neaves, 1971; Taddei, 1972; Andreuccetti et al., 1978; Hubert 1985; Klosterman, 1987; Andreuccetti, 1992; Motta et al., 1995, 1996) in this order we have demonstrated that large pyriform cells differentiate from small cells via intermediate cells (Andreuccetti et al., 1978; Filosa et al., 1979), and supply the oocyte with RNA, mitochondria, ribosomes, vesicles, and DNA via the intercellular bridges (Motta et al., 1995, 2001). Therefore, the ovarian follicles of cartilaginous fish and squamate reptiles represent a peculiar highly integrated system, where granulosa cells significantly contribute to oocyte growth. This may represent an excellent example of convergent evolution, probably arising from the fact that both taxa are specialized in the production of yolky eggs (Andreuccetti et al., 1999).

More recently, several investigations on growing follicles of *Torpedo marmorata* have indicated that such a species is a good non-mammalian experimental model for studying oocyte growth and the mechanisms related

to follicologenesis. In fact, oogonia and prefollicular oocytes are still evident at birth (Prisco et al., 2001), and follicles at different stages of growth are present in adult specimens (Prisco et al., 2002a,b).

To better understand the characteristics of ovarian follicles in cartilaginous fish, we studied the distribution of glycoside residues by using a lectin panel, and demonstrated that it significantly changes during follicle development in relation to the stage of follicular differentiation.

Materials and methods

Specimens of adult females (5) of the spotted ray, *Torpedo marmorata*, were collected in the Gulf of Naples and maintained in seawater tanks in our Department. They were a kind gift of the "A. Dorhn" Zoological Station of Naples. The experiments were admitted by institutional committees (Ministero della Salute) and organized to minimize the number of animals used.

The specimens were anaesthetized with MS-222 (42 mg/l) prior to sacrifice. Ovaries were dissected and fixed *in toto* or in small pieces. All samples were processed for light microscopy. The ovarian follicles were isolated using a dissecting microscope and fixed in 4% paraformaldehyde, 0.1% glutaraldehyde, in 0.1 M sodium cacodylate buffer pH 7.4, for 2.5 hrs at room temperature; then they were washed for 3x10 min in buffer, treated with 0.2 M NH_4Cl for 1 hr to aminidate free aldehyde groups, and washed again for 3x10 min in buffer. The follicles were then dehydrated in ethanol and embedded in Embed 812 (Polysciences, Milan, Italy). Semithin sections (1 mm thick) were cut with a diamond knife, mounted on slides and dried at 45 °C overnight. The sections were deplasticized on slides with potassium methoxide (modified from Maxwell, 1978), washed in running water for 10 min, 2x5 min in BDW and in 0.01 M phosphate buffered saline (PBS), pH 7.4, for 10 min. The slides were then incubated for 20 min in the dark with fluorescein-labeled (FITC) lectins (Table 1) at 15-60 μg/ml in the appropriate buffer (see below), washed twice in PBS, once in PBS plus 0.05% para-phenylendiamine and mounted with 90% glycerol in PBS plus 0.05% para-phenylendiamine. The buffers used were: PBS (WGA, UEA-I, DBA, BPA, PNA, LCA, SBA, Sigma, Milan, Italy); PBS supplemented with 0.5 mM $CaCl_2$ (GS-I-A_4 and B_4, EY, Florence, Italy); tris-buffered saline (TBS) 0.05 M, pH 7.4, supplemented with 1 mM $MnCl_2$ and $CaCl_2$ (ConA); and TBS (PSA). Table 1 schematizes the lectins and the working conditions used in the present study. The controls to test sugar specificity were performed by pre-incubating the lectins with the appropriate hapten sugar (0.2 M) (purchased from Sigma Chemical Co.) for 1 hr prior to section treatment.

To test whether the selected lectins bound glycolipids or glycoproteins, deplasticized sections were treated with chloroform/methanol (2:1 vol/vol) for 10 min at room temperature, and extensively rinsed in methanol and water before lectin labeling (Suzuki et al., 1993). ß-elimination for the removal of O-glycosidically-linked oligosaccharide side chains was also tested before labeling with two selected lectins, MPA and Con A, which bind terminated sugars respectively in O-linked and N-linked glycoconjugates; for this purpose, deplasticized sections were incubated in 0.1 N NaOH for 2-24 hrs at room temperature (modified from Egea et al., 1989) and extensively rinsed in PBS. For control, parallel sections were treated in the same way but without NaOH before lectin labeling. Sections were observed under a fluorescence microscope and photographed using Kodak Ektachrome 160 or 320 T with x40 and x63-immersion objectives, and their labeling was defined as positive or negative by the same observer.

Results

In *Torpedo marmorata*, the granulosa undergoes

Table 1. Lectins, buffers, concentrations used, carbohydrate specificity and competing sugars.

LECTIN	BUFFER	CONCENTRATION (μg/ml]	SPECIFICITY	COMPETING SUGARS
Arachis hypogaea (PNA)	PBS	40	ßGal1,3GalNac>aandbGal	D-Gal
Bauhinia purpurea (BPA)	PBS	40	GalNAc>Gal	D-GalNAc
Canavalia ensiformis (ConA)	TBS-Ca	30	αMan>αGlc>αGlcNAc	Methyl–α-Man
Dolichos biflorus (DBA)	PBS	40	αGalNAc1,3 GalNAc>GalNAc	αD-GalNAc
Griffonia simplicifolia I-A4 (GS-I-A4)	PBS-Ca	30	αGalNAc>>αGal	D-GalNAc
Griffonia simplicifolia I-B4 (GS-I-B4)	PBS-Ca	30	αGal	D-Gal
Pisum sativum (PSA)	TBS	30	αMan>αGlc=αGlcNAc	Methyl-α-Man
Triticum vulgare (WGA)	PBS	15-30	ßGlcNAc1,4bGlcNAc>GlcNAc>Neu5Ac	D-GlcNAc
Ulex europaeus I (UEA-I)	PBS	30-60	αL-Fuc	L-Fuc
Lens culinaris (LCA)	PBS	30	αMan>αGlc=αGlcNAc	Methyl-α-Man
Maclura pomifera (MPA)	PBS	40	GalNac>DGal	D-GalNAc

From Goldstein and Poretz, 1986: Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; Fuc, fucose; Man, mannose; Metil-α-Man, methil-alfa-mannoside; Neu5Ac, sialic acid.

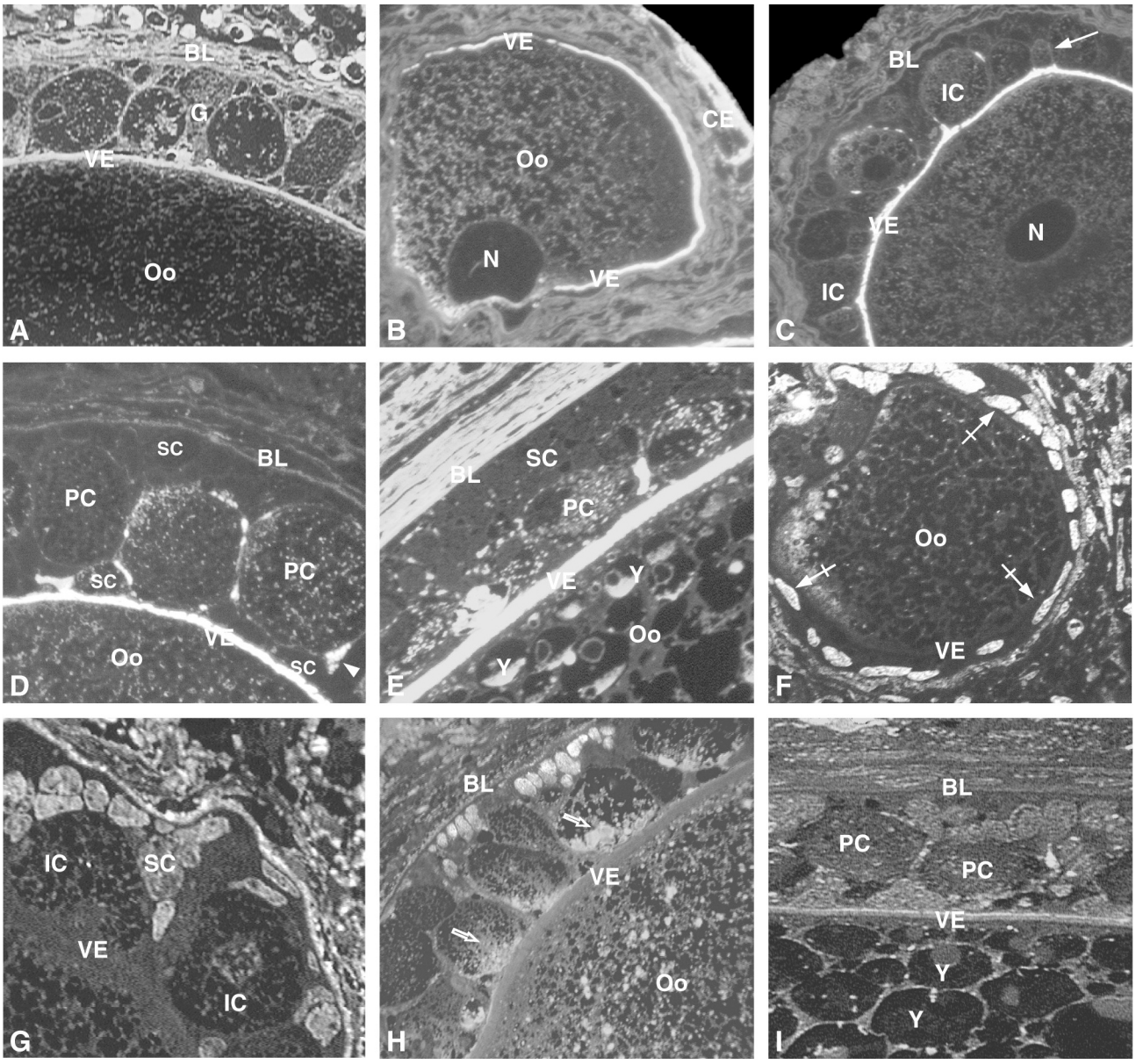

Fig. 1. Deplasticized 1 μm-thick sections of samples stained with ConA **(A)**, WGA **(B-E)**, MPA and DBA **(F-I)** lectins.**A.** Follicle 1000 mm in diameter: lectin staining is evident on the granulosa (G), the vitelline envelope (VE), the oocyte (Oo) and the basal lamina (BL). **B..** Early diplotene oocyte 60 mm in diameter surrounded by a monolayer of small cells. Lectin staining is evident only at the level of VE and on celomatic epithelium (CE). **C.** Follicle 100 μm in diameter containing small and intermediate cells. Note that the surface of small (arrow) and intermediate cells (IC), as well as the VE, is stained with WGA. **D.** Follicle 200 μm in diameter. The surface of small (SC) and pyriform-like cells (PC) in contact with the Oo is labeled, differently from SC located under the BL. Very deep staining is evident on the VE, the intercellular spaces among the G (arrowheads) as well as on the cytoplasmic vacuoles present within granulosa cells and oocyte. **E.** Follicle 3000 μm in diameter. SC, PC in contact with the Oo and the VE show WGA binding sites, present also on the oocyte cortex where yolk globules (Y) are localized. No labeling is evident on the SC located underneath the BL. **F:** Primary follicle surrounded by a single layer of squamous cells. Binding sites for DBA (data not shown) and MPA are located within the nuclei of granulosa cells (arrows with bar) and outside the follicle. No binding site is evident at the level of the VE. **G:** Follicle 200 mm in diameter showing SC and IC. Note that the nuclei of SC are deeply labeled by MPA, differently from the nuclei of IC, which show a weak reaction. Still no labeling is evident at the level of the VE, inside the cytoplasm of G and within the oocyte. **H.** Follicle 1000 mm in diameter. The distribution of binding sites for lectin at the level of G is comparable to that of Fig.1G. Furthermore, one can observe the presence of labelled cytoplasmic vacuoles (arrow) within G and the Oo, as well as the presence of weak labeling on the VE next to the G**. I.** Follicle 3000 mm in diameter. Binding sites for MPA lectin are now evident at the level of the PC, the Oo and on the VE. BL: basal lamina; CE= coelomic epithelium; G: granulosa; IC: intermediate cells; N: nucleus; Oo: oocyte; PC: pyriform-like cells; SC: small cells; VE: vitelline envelope; Y: yolk globules. Bars: 20 μm.

significant changes during oocyte growth. In primary follicles (up to 100 μm in diameter) it is formed by a single layer of small cells. Later in follicles 100-300 mm in diameter, the small cells fuse their plasma membrane with that of the oocyte and differentiate in pyriform-like cells via intermediate cells. As a consequence, the granulosa progressively becomes polymorfic and multilayered. During the following stages of previtellogenesis (follicles up to 3000 μm in diameter) and vitellogenesis (follicles >3000 μm in diameter) the organization of the granulosa remains unchanged (for details see Prisco et al., 2002a,b).

Binding sites for LCA, ConA, GSI-A4, GSI-B4, UEA-I, PNA were always evident on the various constituents of the ovarian follicles (Fig. 1A), whereas those for WGA, MPA and DBA showed a peculiar pattern that markedly changed during oocyte growth. In primary follicles, binding sites for WGA were present on the VE, while small follicle cells and the oocyte were completely unlabeled (Fig.1B). At the level of the granulosa, binding sites for WGA first appeared in follicles 100-150 μm in diameter containing small and intermediate cells (Fig.1C); in these follicles, WGA labeling was also evident close to the vitelline envelope, on the surface of the small cells located near the oocyte surface, and on the intermediate cells (Fig.1C). As the oocyte grew and the pyriform-like cells appeared (follicles 200-300 mm in diameter), WGA labeling became visible on the pyriform-like cell surface, within large vacuoles located inside both intermediate and pyriform cells, and in the oocyte (Fig.1D). It was also frequently observed on the intercellular spaces, between the intermediate and the pyriform-like cells, which were in continuity with the perivitelline space where the vitelline envelope was depositing (Fig.1D). WGA labeling remained unchanged on the granulosa cells and the vitelline envelope during the subsequent stages of oocyte growth. (Fig.1E).

The following results indicate that, simultaneously with the appearance of the glycoside residues for WGA on the surface and the cytoplasm of intermediate and pyriform-like cells, the nucleus of granulosa cells progressively changed in D-Gal content. Indeed it appeared highly stained with MPA and DBA in small follicles (up to 150 μm) (Fig.1F). In larger ones (follicles 150-200 μm in diameter), such a labeling progressively

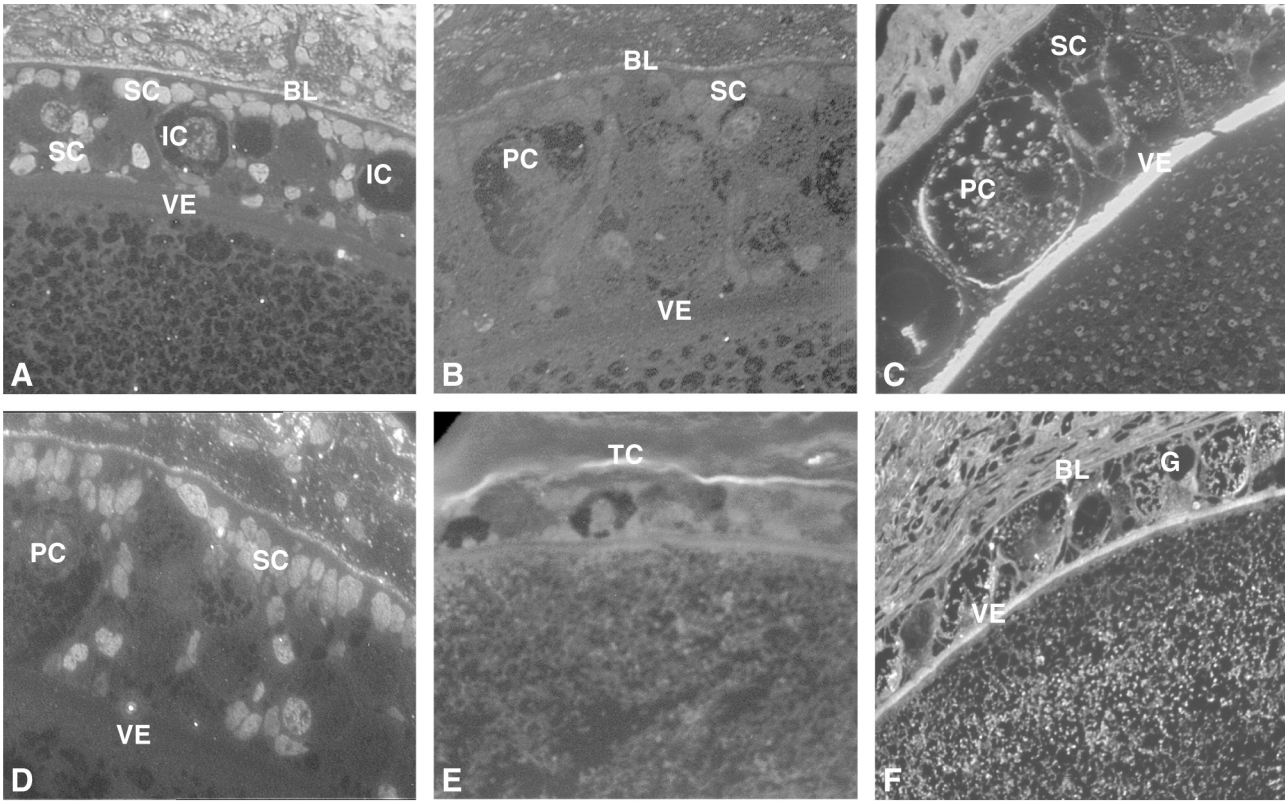

Fig. 2. A: Labeling is not modified after the preincubation of samples with GlcNAc, one of the two hapten sugars for MPA. **B:** No labeling is evident after preincubation of the samples with the two hapten sugars for MPA, GlcNAc and D-Gal. **C, D:** No modification of labeling with MPA (C) and WGA (D) is evident after the extraction of lipids with chloroform/methanol (compare Fig. 2C,D with Fig. 1C ,G). **E:** Follicle 1000 mm in diameter labeled with MPA and processed for b-elimination. A very weak MPA labeling persists only on the theca layer (TC). **F:** Follicle 1000 mm in diameter labeled with ConA. ConA labeling remains unchanged after ß-elimination. X Legend: BL= basal lamina; G= granulosa cells; IC= intermediate cells; PC= pyriform cells; SC= small cells; VE= vitelline envelope. Bars= 50 μm.

reduced, so that it was intense in the nucleus of small cells and weaker in that of the intermediate ones (Fig 1G). In fully differentiated pyriform-like cells, pale or no staining for both lectins was evident in their nuclei (Fig. 1H; see also Fig. 2A). Furthermore, MPA and DBA labeling was present on the vitelline envelope of follicles containing differentiated pyriform-like cells. It is noteworthy that, in these follicles, MPA and DBA staining appeared first on pyriform-like cells (Fig.1H), and only later on the vitelline envelope (Fig. 1I). The positive reaction for both lectins persisted during the vitellogenic stage of growth (Fig. 1I). Interestingly, the surface of small cells located underneath the basal lamina were not labeled by WGA (Fig. 1C-E), and their nuclei were labeled by MPA and DBA (Fig. 1G,H; see also Fig. 2A).

MPA labeling persisted when the lectin was previously incubated with GalNAc (Fig. 2A) or D-Gal (data not shown), the corresponding competing sugars for MPA lectin; on the contrary labeling was no more evident when MPA lectin was previously incubated with both competing sugars, GalNAc and D-Gal (Fig. 2B). No modification of the binding pattern for lectins was observed when the sections were treated for lipid extraction prior to the labeling with WGA (Fig. 2C) and MPA (Fig.2D). Labeling with MPA on the granulosa, the vitelline envelope and the oocyte was abolished by ß-elimination (Fig. 2E); conversely, no modification of labeling with ConA was observed after ß-elimination (Fig. 2F), indicating that N-linked sugars (Cummings et al., 1989) are not affected under the present experimental conditions.

Discussion

Our results demonstrate that in *Torpedo marmorata* the follicular epithelium and the vitelline envelope undergo relevant modifications in their composition in glycoside residues. The granulosa cells engaged in the differentiation of pyriform-like cells are the only ones to show glycoprotein bearing terminal O-linked side chains of D-GlcNAc on their surface. Evidence was obtained by carrying out experiments of lipid extraction and ß-elimination, and labeling the follicular epithelium with a panel of FICT-lectins during different stages of oocyte growth. Labeling for some lectins (ConA, GSI-A4, GSI-B4 UEA-I, PNA, PSA, BPA, LCA) was always evident and remained unchanged, whereas WGA labeling on the follicular epithelium markedly changed during the different stages of oocyte growth. In particular, the latter was absent from small cells, and progressively appeared first on the surface of small cells facing the oocyte and the oocyte itself, and then on the surface of intermediate and pyriform-like cells. Conversely, the small cells located under the basal lamina were always negative. The appearance of glycoproteins bearing terminal ß-GlcNAc on the surface of follicle cells involved in the differentiation of pyriform-like cells supports the view that, as in reptiles (Andreuccetti et al., 2001; Uliano et al., 2001), surface glycoproteins play a key role in the differentiation of pyriform-like cells, probably by attending the contact and/or fusion between the oocyte and the follicle cell membrane, as well as in the maintenance of the differentiated state of pyriform-like cells. One can postulate that, as in mammals (Vanderhyden et al., 1990), the oocyte may induce the expression of a specific lectin receptor on the surface of small cells and their following differentiation into pyriform cells (Andreuccetti et al., 2001). Significantly, the small cells located both under the basal lamina and among the pyriform-like cells but not in contact with the oocyte surface were never labeled with WGA lectin.

During the differentiation of small cells into pyriform-like cells, the nuclear content also changed significantly in sugar residues. The nuclei of the differentiating small cells were characterized by a high content of glycoprotein bearing terminal glycoside residues of D-GalNAc O-linked that progressively reduced, to disappear in the nuclei of pyriform-like cells. It is well documented that nuclear glycosaminoglycans play a role in the control of the cell cycle (Fedarko et al., 1989; Ishihara and Conrad, 1989; Hiscock et al., 1994). The present observations suggest that the change in sugar residues of glycoproteins bearing terminal D-GalNAc in the nucleus of differentiating follicle cells may play an important role in the control of the cellular cycle of small follicle cells. One can hypothesize, in particular, that the progressive losts of glycoproteins bearing D-GalNAc might induce a re-programming of small cells, so that they lose the ability to divide and acquire that to differentiate into pyriform-like cells. Unlike other follicle cells, the small cells located underneath the basal lamina maintain their nuclear content in glycoproteins bearing Gal residues and do not differentiate into pyriform-like cells.

The present results also demonstrate that the sugar composition of the vitelline envelope changes as the oocyte grows. Unlike other carbohydrate residues (Gal, GlcNAc, Man, Fuc), which are always present in the entire thickness of the vitelline envelope whatever the stage of oocyte growth, ß-GalNAc residues are expressed only after the appearance of pyriform-like cells in the granulosa. In this regard, it is noteworthy that such carbohydrate residues are evident at first in the cytoplasm of differentiating pyriform-like cells and progressively appear in the vitelline envelope. This suggests that glycoproteins bearing GalNAc sugar residues might be synthesized within the granulosa cells and then transferred into the perivitelline space, contributing to vitelline envelope organization, as observed in non-mammalian (Andreuccetti and Carrera, 1987; Andreuccetti et al., 1999) and mammalian vertebrates (Gwatkin et al, 1979; Wolgemuth et al., 1982; Tesoriero, 1984; Parillo and Verini Supplizi, 1999). Finally, the present investigation strongly suggests that a component of the vitelline envelope may be synthesized outside the ovary, as in bony fish (Bonsignorio et al., 1996). In fact, in primary smallest

follicles surrounded by a squamous follicular epithelium, the vitelline envelope showed a deep positive reaction to WGA, that was absent in the follicular epithelium and in the oocyte.

In conclusion, the present data corroborate our previous data (Andreuccetti et al., 2001; Uliano et al., 2001) that, unlike what happens in mammals, (Shalgi et al, 1991, Aviles et al., 2000), in non mammalian vertebrates, follicle cells express a large amount of sugar residues (see also Sarasquete et al., 2002), with a distribution remarkably changing in relation to the differentiation of the follicular epithelium and the vitelline envelope.

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References

Andreuccetti P. (1992). An ultrastructural study of differentiation of pyriform cells and their contribution to the oocyte growth in representative squamata. J. Morphol. 212, 1-11.

Andreuccetti P., Taddei C. and Filosa S. (1978). Intercellular bridges between follicles cells and oocyte during the differentiation of follicular epithelium in Lacerta sicula Raf. J. Cell Sci. 33, 341-350.

Andreuccetti P. and Carrera M. (1987). The differentiation of the zona pellucida (VE) in the lizard *Tarentola mauritanica*. Devel. Growth Differ. 29,113-122.

Andreuccetti P., Iodice M., Prisco M. and Gualtieri R. (1999). Intercellular bridges between granulosa cells and the oocyte in the elasmobranch Raja asterias. Anat. Rec. 255, 180-187.

Andreuccetti P., Famularo C., Gualtieri R. and Prisco M. (2001). The pyriform cell differentiation in Podarcis sicula is accompanied by the appearance of surface glycoproteins bearing α-GALNAC terminated chains. Anat. Rec. 263, 1-9.

Aviles M., Okinaga T., Shur B.D. and Ballesta J. (2000). Differential expression of glycoside residues in mammalian zona pellucida. Mol. Rep. Dev. 57, 296-308.

Bonsignorio D., Perego L., Del Giacco L. and Cotelli F. (1996). Structure and macromolecular composition of the zebrafish egg chorion. Zygote 4, 101-108

Cummings R.D., Merkle R.K. and Stults N.L. (1989). Separation and analysis of glycoprotein oligosaccharides. Methods Cell Biol. 32, 141-183

Egea G., Goldstein I.J. and Roth J. (1989). Light and electron microscopic detection of (3-Gal-ß,l-4,GlucNAc-pl) sequences in asparagin-linked oligosaccharides with the *Datura stramonium* lectin. Histochemistry 92, 515-522.

Fedarko N.S., Ishihara M. and Conrad H.E. (1989). Control of cell division in hepatoma cells by exogenous heparan sulfate proteoglycan. J. Cell Physiol. 139, 287-294.

Filosa S., Taddei C. and Andreuccetti P. (1979). The differentiation and proliferation of follicle cells during oocyte growth in Lacerta sicula. J. Embryol. Exp. Morphol. 15, 297-316.

Ghiara G., Limatola E. and Filosa S. (1968). Ultrastructural aspect of nutritive process in growing oocytes of lizard. D: S: Bocciarelli (ed.): Electron Microscopy. Vol. 2 Rome: Tip. Poliglotta, 331-332.

Goldstein I.J. and Poretz R.D. (1986). Isolation, physicochemical characterization, and carbohydrate-binding specificity of lectins. In: The Lectins: Properties, Functions and Applications in Biology and Medecine. Liener I.E., Sharon N. and Goldstein I.J. (eds). Academic Press. Orlando. pp 335-347.

Gwatkin R.B.L., Meyenhofer M. and Williams D.T. (1979). Localization of zona antigen in bovine ovary sections by fluorescent antibody. Gamete Res. 2, 201-206.

Hiscock D.R.R., Yanagishita M. and Hascall V.C. (1994). Nuclear localization of glycosaminoglycans in rat ovarian granulosa cells. J. Biol. Chem. 269, 4539-4546.

Hubert J. (1985). Origin and development of oocytes. In: Biology of the Reptilia. Gans, Billet and Maderson (eds). Development A. Vol. 14. NY; J.Wiley & Sons, pp. 41-74.

Ishihara M. and Conrad H.E. (1989). Correlations between heparan sulfate metabolism and hepatoma growth. J. Cell Physiol. 138, 467-476

Klosterman L. (1987). Ultrastructural and quantitative dynamics of the granulosa of ovarian follicles of the lizard *Gerrhonotus coeruleus* (Fam. Anguidae). J. Morphol. 192, 125-144.

Maxwell M.H. (1978). Two rapid and simple methods used for the removal of resins from 1.0 μm thick epoxy sections. J. Microsc. 112, 253-255.

Motta C.M., Castriota Scanderberg M., Filosa S. and Andreuccetti P. (1995). Role of pyriform cells during the growth of oocytes in the lizard Podarcis sicula. J. Exp. Zool, 273, 247-256.

Motta C.M., Filosa S. and Andreuccetti P. (1996). Regression of the epithelium in late previtellogenic follicles of *Podarcis sicula*: a case of apoptosis. J. Exp. Zool. 276, 233-241.

Motta C.M., Tammaro S., Cicale A., Indolfi P., Iodice C., Spagnuolo M.S. and Filosa S. (2001). Storage in the yolk platelets of low MW DNA produced by the regressing follicle cells. Mol. Reprod. Dev. 59, 422-430

Neaves W.B. (1971). Intercellular bridges between follicle cells and the oocyte in the lizard *Anolis carolinensis*. Anat. Rec. 170, 285-302.

Parillo F. and Verini Supplizi A. (1999). Glycohistochemical investigation of canine and feline zonae pellucidae of preantral and antral oocytes. Acta Histochem. 101, 127-146.

Prisco M., Ricchiari L. and Andreuccetti P. (2001). An ultrastructural study of germ cells during ovarian differentiation in *Torpedo marmorata*. Anat. Rec. 263, 239-247.

Prisco M., Ricchiari L. and Andreuccetti P. (2002a). Ultrastructural studies on developing follicles of the spotted ray *Torpedo marmorata*. Mol. Reprod. Dev. 61, 78-86

Prisco M., Romano M., Ricchiari L., Limatola E. and Andreuccetti P. (2002b). An ultrastructural study on the vitellogenesis in the spotted ray *Torpedo marmorata*. Gen. Comp. Endocrinol. In press.

Sarasquete C., Cardenas S., Gonzalez de Canales M.L. and Pascual E. (2002). Oogenesis in the bluefin tuna, *Thunnus thynnus* L.: A histological and histochemical study. Histol. Histopathol. 17, 775-788

Shalgi R., Maymon R., Bar-Shira B., Amihai D. and Skutelsky E. (1991). Distribution of lectins receptors sites in the zona pellucida of follicolar and ovulated rat oocytes. Mol. Reprod. Dev. 29, 365-372.

Suzuki E., Sano A., Kuriki T. and Miki T. (1993). Separation and determination of phospholipids in plasma employing thin-layer chromatographic plate with concentration zone or solid phase

extraction. Biol. Pharm. Bull. 16, 77-80

Taddei C. (1972). Significance of pyriform cells in ovarian follicle of *Lacerta sicula.* Exp. Cell Res. 72, 562-566.

Tesoriero J.V. (1984). Comparative cytochemistry of the developing ovarian follicles of the dog, rabbit and mouse: origin of the zona pellucida. Gamete Res. 10, 301-318.

Uliano R., Ricchiari L., Prisco M. and Andreuccetti P. (2001). Surface glycoproteins bearing α-GalNAc terminated chains accompanie pyriform cell differentiation in lizards. J. Exp. Zool. 290, 769-776.

Vanderhyden B.C., Caron P.J., Buccione R. and Eppig J.J. (1990). Developmental pattern of the secretion of cumulus expansion-enabling factor by mouse oocyte and the role of oocytes in promoting granulosa cell differentiation. Dev. Biol. 140, 307-317

Wolgemuth D.J., Celenza J. and Dunbar B.S. (1982). Localization and development stage-specific appearance of zona pellucida antigens in the rabbit ovary. J. Histochem. Cytochem. 30, 607.

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