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The synthesis and fate of glycodelin in human ovary during folliculogenesis

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The ontogeny of glycodelin in human ovarian follicles during folliculogenesis was studied. Glycodelin immunoreactivity began to be detected in the granulosa cells and thecal cells of late secondary follicles. Immunoreactivity was also found in both the luteinized granulosa cells and cumulus cells obtained from women undergoing the assisted reproduction treatment. However, only the luteinized granulosa cells, and not the cumulus cells, expressed glycodelin mRNA. Results also showed that the cumulus cells took up radiolabelled glycodelin and partially deglycosylated some of it. Glycodelin (and a partially deglycolsylated form of glycoldelin) appeared to complex with two cytoplasmic or membrane components of the cumulus cells. The data also demonstrated that ZIF-1, a glycoprotein isolated from human follicular fluid, was immunologically similar to glycodelin. In conclusion, we suggest that glycodelin is synthesized in the granulosa cells of ovarian follicles at late secondary follicle stage. It then may be released into the follicular fluid from where it is taken up and partially modified by the cumulus cells.

Key words: cumulus cells/follicle/glycodelin/granulosa cells/ovary

Introduction

The control of oocyte development is complex and not well understood. Follicular fluid is a body fluid within the ovarian follicle containing inorganic salts, carbohydrates, mucopolysaccharides, lipids, proteins, steroids, peptide hormones and growth factors (Edwards, 1974; Gosden *et al*., 1988; Lenton *et al*., 1988). It is suggested that the follicular fluid plays important roles in the follicle growth, ovulation and maturation of the oocyte. Recent studies also indicate that the follicular fluid may be involved in fertilization (Lambert *et al*., 1992; Ravnik *et al*., 1992; De-Jonge *et al*., 1993; Yao *et al*., 1999, 2000) and in early embryo development (Fakih and Vijayakumar, 1990; Hemmings *et al*., 1994).

Glycodelin, formerly known as placental protein 14 (PP14), and progesterone-associated endometrial protein (PAEP), was originally extracted from human term placenta (Bohn *et al*., 1982). However, glycodelin is not synthesized in the placenta (Julkunen 1986; Julkunen *et al*., 1986b). It is present in amniotic fluid (Joshi *et al*., 1980), endometrium (Julkunen *et al*., 1986b), ovary (Kamarainen *et al*., 1996), oviduct (Julkunen *et al*., 1986c), maternal serum (Bolton *et al*., 1983) and haematopoietic cells of bone marrow (Kamaraiinen *et al*., 1994). Glycodelin-A is a human amniotic fluid-derived glycoprotein with contraceptive and immunosuppresive activities (Okamoto *et al*., 1991; Oehninger *et al*., 1995). Glycodelin-S, a differentially glycosylated isoform of glycodelin-A, is found in seminal plasma. In male tissues, it is localized to epithelial cells of the seminal vesicle, and ampulla of vas deferens (Julkunen *et al*., 1984; Morris *et al*., 1996; Koistinen *et al*., 1997). The biological significance of glycodelin is uncertain. It has been suggested that it may facilitate embryonic invasion via its immunomodulatory and immunosuppressive effects in the maternal endometrium around the time of implantation (Pockley *et al*., 1988; Okamoto *et al*., 1991).

A previous study reported the presence of glycodelin immunoreactivity in human ovarian tissue (Kamarainen *et al*., 1996). However, little is known about its fate during folliculogenesis. Glycodelin-A inhibits spermatozoa–zona pellucida binding (Oehninger *et al*., 1995). Although glycodelin immunoreactivity is known to be present in human follicular fluid, it has never been isolated from such fluid (Chryssikopoulos *et al*., 1996). Our recent findings show that human follicular fluid also inhibits the binding of human spermatozoa to the zona pellucida (Yao *et al*. 1996; Qiao *et al*., 1998). This inhibitory activity has been attributed to the presence of two glycoproteins, named as ZIF-1 and ZIF-2, in the follicular fluid (Yao *et al*., 1998). The biochemical characteristics of ZIF-1 are similar to glycodelin (Yao *et al*., 1998).

In order to obtain clues on the function of glycodelin in ovarian follicle, the first objective of this study was to investigate the fate of glycodelin during folliculogenesis using an immunohistochemical technique. The second objective was to investigate the site of biosynthesis of glycodelin by determining its mRNA expression in granulosa cells and cumulus cells. The third objective was to compare the immunoreactivity of ZIF-1 with glycodelin-A using an antibody against glycodelin. The final objective was to study one of the possible routes for uptake of glycodelin-A by the cumulus cells.

Materials and methods

Tissue samples

The University Ethics Committee, University of Hong Kong, approved the protocol for this study. Human granulosa cells and cumulus cells were obtained during ultrasound-guided oocyte retrieval from four women undergoing assisted reproduction treatment in Queen Mary Hospital, Hong Kong. Both the granulosa and cumulus cells were gently washed with phosphate-buffered saline (PBS) and were then either formalin-fixed for immunohistochemical analyses or stored at –70°C for RT–PCR. Human follicular fluid was also obtained during oocyte retrieval, as described previously (Yao *et al*., 1996).

For positive control tissue, normal secretory endometrium was donated with consent from patients attending for subfertility treatment. These women underwent assisted reproduction treatment but had no embryo transfers performed for various reasons such as failure of fertilization. The endometrial tissues were formalin-fixed, embedded in paraffin wax and cut as 6 μm sections for immunohistochemical analyses. Part of the secretory endometrium was stored at –70°C for mRNA isolation.

Normal human ovarian tissue sections (n = 13) were archival material from Tangdu Hospital, Xian, China, obtained from women of reproductive age undergoing gynaecological surgery for cervical carcinoma with reasons other than ovarian disease.

Purification of glycodelin-A and ZIF-1

Glycodelin-A was purified from amniotic fluid by affinity chromatography using a monoclonal anti-glycodelin (clone F43-7F9) sepharose column as described elsewhere (Riittinen *et al*., 1991). Briefly, amniotic fluid, containing 0.1% (v/v) Triton X-100, was loaded onto the affinity column. The column was washed with 50 mmol/l Tris-HCl buffered saline (TBS, pH 7.7), with 1 mol/l NaCl, containing 1% isopropanol and with 10 mmol/l ammonium acetate. Glycodelin-A was eluted with 0.1% trifluoroacetic acid and dialysed against 100 mmol/l sodium phosphate, pH 7.2. It was then further purified by a Superose-12 column (Pharmacia, Sweden) using PBS at a flow rate of 30 μl/min.

ZIF-1 was isolated from human follicular fluid. The isolation protocol was described previously (Yao *et al*., 1998). Briefly, ZIF-1 was isolated using concanavalin A affinity chromatography followed by ion-exchange chromatography (Mono Q) and gel filtration.

RT–PCR analysis of glycodelin

RNA was extracted from endometrium, peripheral blood leukocytes, granulosa cells and cumulus cells using the mRNA purification kit according to the manufacturer's instructions (Amersham Pharmacia Biotech, Buckinghamshire, UK). The extracted RNA was reverse transcribed into cDNA using the First-strand cDNA synthesis kit as specified by the manufacturer (Amersham). Random hexamers were used to generate cDNA copies of the messages. For the RT–PCR reaction, sense (code 415) and antisense (code 416) 18 bp oligonucleotide primers (Koistinen *et al*., 1997) were used for the amplification of glycodelin-A mRNA. The 20 bp oligonucleotide control primer sequences for amplifying the GAPDH gene were: 5′-CACCATCTTCCAGGAGCGAG-3′ and 5′-TCACGCCACAGTTTCCCGGA-3′. Genset Singapore Biotech Pte Ltd (Singapore) synthesized all the oligonucleotides. The PCR reactions for glycodelin or GAPDH were performed in a PTC-200 (MJ Research Inc., Watertown, MA, USA) at 94°C for 45 s, 64°C for 45 s, and 72°C for 45 s for a total of 30 cycles. An agarose gel (2.0%) was run to determine the size of the amplified fragment. The amplified products were visualized by staining with ethidium bromide. RNA from normal secretory endometrium was used as a positive control. A segment of the GAPDH cDNA was amplified to ensure that no degradation of RNA had occurred. In addition, a negative control (containing no products of the RT reaction) was included in each experiment to ensure that the amplified fragments were not due to contamination in any of the reagents. The identity of the amplified product was confirmed by nucleic acid sequencing using a dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA) and ABI 310 genetic analyser (Applied Biosystems).

Immunohistochemical analysis of glycodelin

The standard avidin-biotin peroxidase complex staining method was adopted. Briefly, paraffin wax-embedded sections of ovarian tissue, granulosa cells and cumulus cells were deparaffinized in xylene and rehydrated through descending concentrations of ethanol. Subsequently, sections were incubated in freshly prepared 3% hydrogen peroxide in absolute methanol for 30 min to block endogenous peroxidase activity. To enhance immunoreactivity, the paraffin-embedded tissue sections were subjected to microwave antigen retrieval. The sections were then incubated with 10% donkey serum in TBS followed by rabbit anti-glycodelin (5 μg immunoglobulin G/ml) (Mandelin *et al*., 2001) in a humidified chamber at 4°C overnight. Negative controls were incubated with 10% donkey serum or with the anti-glycodelin antiserum pre-absorbed with follicular fluid. The sections were further incubated with biotinylated mouse anti-rabbit antibody in a humidified chamber for 30 min at 37°C. After washing away the unattached secondary antibody, the sections were incubated with freshly prepared avidin-biotinylated horseradish peroxidase complex. Finally, the chromogenic reaction was carried out with 3,3′-diaminobenzidine tetrahydrochloride and the sections were counterstained with Mayer's haematoxylin.

Iodination of glycodelin-A

A total of 50 μg of purified glycodelin-A in 20 μl 0.05 mol/l PBS (pH 7.4) was mixed thoroughly with 2 mCi of carrier-free sodium ^{125}I (20 μl; Amersham) and 100 μg of freshly prepared chloramine T in 20 μl PBS in a small conical vial. Sodium metabisulphite (300 μg) in 50 μl PBS was used to stop the reaction after 60 s. Free ^{125}I was removed by passing the mixture through a 10 ml disposable desalting column. The first radioactive peak containing iodinated glycodelin-A was collected.

Glycodelin-A uptake by cumulus cells

Hyaluronidase-dispersed cumulus cells (0.2×10^6 cells) from patients admitted for ICSI treatment were incubated with ^{125}I-glycodelin-A (100 ng/ml) at 37°C in HEPES-buffered Earles balanced salt solution (EBSS). ^{125}I-glycodelin-A was also incubated with immortalized oviductal epithelial cells (Lee *et al*., 2001) and human fibroblasts isolated from human oviductal tissue by limiting dilution. After 5 h, the cells were centrifuged at 500 *g* for 5 min, and the supernatant was removed. The cells were then washed twice with HEPES-buffered EBSS. A gamma counter (Beckman-Coulter, Fullerton, CA, USA)

was used to determine the radioactivity associated with the cell pellet and the supernatant. The cells were subsequently lysed by freezing and thawing as described previously (Ling *et al*., 1996). The lysates and the supernatant were analysed by SDS- or native-gel electrophoresis, and the radioactive bands were visualized by exposing the gel to BIOMAX film (Kodak, NY, USA).

PNGase F digestion

An N-Glycosidase F Deglycosylation Kit (Roche Diagnositics GmbH, Mannheim, Germany) was used to deglycosylate glycodelin-A after incubation with the cumulus cells. Cumulus cell lysate (5 µl) obtained after incubation of the cells with ^{125}I-glycodelin-A or the remaining supernatant after incubation with 10 µl of the reaction buffer that came with the kit and 10 µl (12 units) of reconstituted N-glycosidase F for 2 h at 37°C. The deglycosylated samples were analysed by native-gel electrophoresis and/or SDS–PAGE. The radioactive bands were visualized by exposing the gel to BIOMAX film (Kodak).

Specificity of glycodelin-A uptake by the cumulus cells

Competitive binding analysis was used to investigate the specificity of cumulus cell uptake of glycodelin-A. Briefly, the binding of ^{125}I-glycodelin-A (35 fmol/assay) to the cumulus cells (0.05×10^6/assay) was determined in the presence of increasing concentrations (0.35, 3.5, 35, 350 and 3500 fmol/assay) of unlabelled glycodelin-A, β-lactoglobulin A (Sigma Chemical Co. Ltd, St Louis, MO, USA) or retinol binding protein (Sigma) at 37°C. Cell-bound radioactivity was determined after washing the treated spermatozoa twice with EBSS.

Imunoblotting analyses of glycodelin-A and ZIF-1

To determine whether ZIF-1 was a glycodelin-like protein, ZIF-1 (200 ng) and glycodelin-A (200 ng) were first denatured and resolved by 12.5% SDS-polyacrylamide gel. Subsequently, the gel was blotted on a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). Western blotting analysis was performed using rabbit anti-glycodelin (5 µg/ml) and horseradish peroxidase conjugated anti-rabbit immunoglobulin. The chromogenic reaction was developed by incubation with a freshly prepared solution of 3,3′-diaminobenzidine tetrahydrochoride.

Results

Localization of glycodelin in developing follicles

No glycodelin immunoreactivity was found in primordial, primary or early secondary follicles (Figure 1a,b). Glycodelin immunoreactivity appeared in the thecal cells and granulosa cells of late secondary follicles (Figure 1c), and in the follicular cells and fluid of Graafian follicles (Figure 1d). The glandular epithelial cells of secretory endometrium tissue used as a positive control also showed glycodelin immunoreactivity. No staining was observed in the sections incubated with either donkey serum alone or with glycodelin antiserum pre-absorbed with follicular fluid.

Glycodelin mRNA and protein expression in granulosa and cumulus cells

Glycodelin mRNA expression was observed in the secretory endometrium (positive control) as well as in the luteinized granulosa cells, but was absent in the cumulus cells (Figure 2). Amplification of GAPDH in the cumulus cells confirmed that the absence of a glycodelin signal in the cumulus cells was not due to RNA degradation. The absence of glycodelin gene expression from peripheral leukocytes demonstrated that the glycodelin mRNA amplified from granulosa cells did not originate from blood contamination. Interestingly, immunohistochemistry demonstrated the presence of glycodelin immunoreactivity in both the granulosa cells (Figure 1e) and the cumulus cells (Figure 1g). The intensities of immunoreactive signal in the granulosa and cumulus cells differed substantially from those of ovarian tissues described above, probably because the former were prepared from fresh specimens, whereas the latter were archival material. Therefore, the intensity of their immunoreactivities was not compared.

Uptake of ^{125}I-glycodelin by cumulus cells

Both Comassie Blue staining and gel autoradiography showed that the ^{125}I-glycodelin-A produced one single band. To characterize the interaction of glycodelin-A with cumulus cells, native gel and SDS–PAGE autoradiography was performed on cumulus cell lysates after incubation of the cells with ^{125}I-glycodelin-A at 37°C for 5 h. The experiment was repeated three times. A representative set of results is shown in Figure 3. In addition to the glycodelin-A band in the lysates of cumulus cells (lane 1), there were two extra radioactive bands. These two bands were absent in the supernatant of the incubation medium (lane 2). Both bands had molecular sizes larger than that of glycodelin-A, and disappeared when examined by SDS–PAGE (lane 5, Figure 4). With SDS–PAGE, the band corresponding to glycodelin-A was intensified and an additional band with a lower molecular size and intensity was apparent under the denaturing conditions used. Upon deglycosylation, only the band corresponding to deglycosylated glycodelin showed radioactivity in the supernatant as well as in the cell lysates (lanes 1 and 2, Figure 4).

As with the cumulus cells, the cell lysates of oviductal cells showed three radioactive bands by native gel analysis (Figure 3, lane 3). One of these bands corresponded to glycodelin-A. The positions of the other two radioactive bands were different from those of the bands in the cumulus cells. There was no radioactive band in the fibroblast cell lysates (Figure 3, lane 5), indicating that glycodelin is not taken up by fibroblasts.

Competitive binding assays for ^{125}I-glycodelin-A

Figure 5 shows the results of the competitive binding study of ^{125}I-glycodelin-A to the cumulus cells. Both retinol binding protein and β-lactoglobulin A did not affect the binding of ^{125}I-glycodelin-A to the cumulus cells, even when their concentrations were 100-fold higher than that of ^{125}I-glycodelin-A. On the other hand, cold glycodelin-A inhibited the binding of ^{125}I-glycodelin-A to the cumulus cells in a dose-dependent manner.

Western blot analysis of ZIF-1 and glycodelin-A

Strong glycodelin immunoreactivity was detected with both the positive control (lane 1, purified glycodelin-A) and with ZIF-1 (lane 2) in Western blot analysis (Figure 6). Both glycodelin-A and ZIF-1 showed one intense band at ~30 kDa.

Discussion

The first part of this study reports the expression pattern of glycodelin during folliculogenesis. The data support a previous

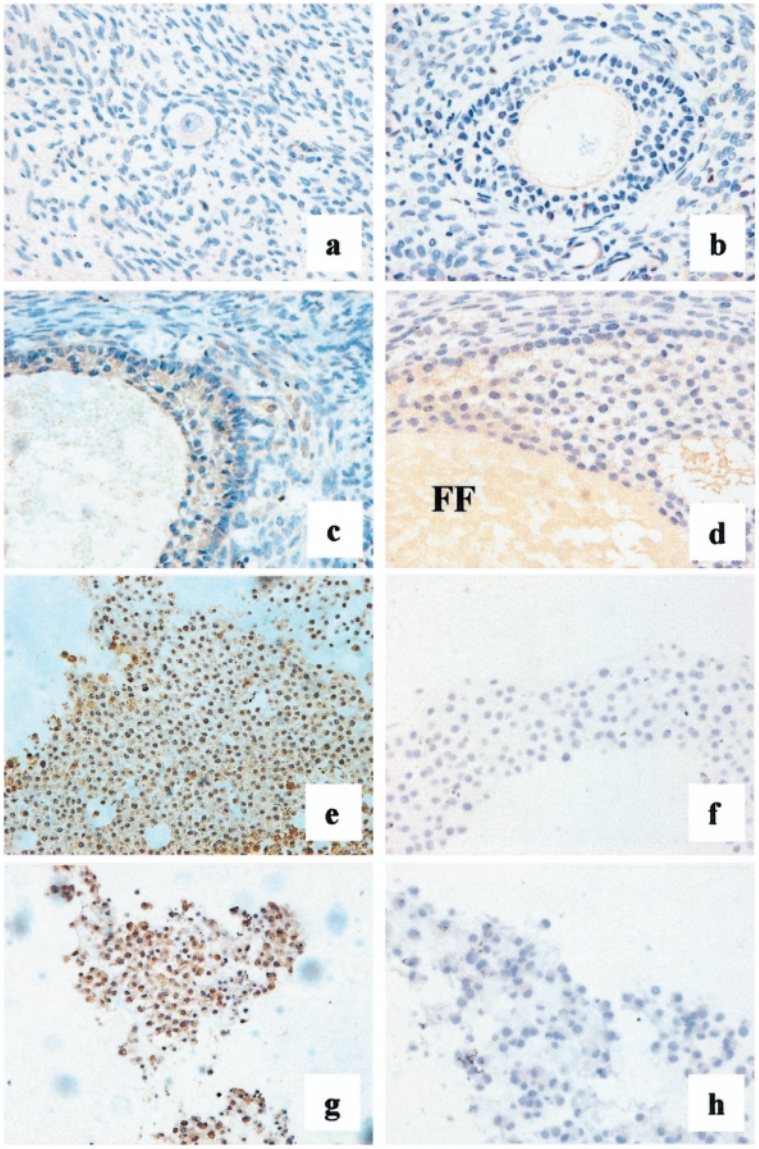

Figure 1. Immunohistochemical detection of glycodelin in the ovary. No glycodelin expression was found in (**a**) primodial follicles nor in (**b**) early secondary follicles. Glycodelin expression was detected in (**c**) late secondary follicles; (**d**) Graafian follicles and in (**e**) luteinized granulosa cells and (**g**) cumulus cells. Negative controls for (**f**) luteinized granulosa cells and (**h**) cumulus cells are also shown. FF = follicular fluid.

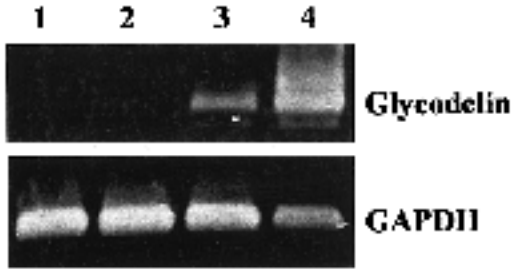

Figure 2. RT–PCR analysis of glycodelin (upper) and GAPDH (lower) expression in peripheral lymphocytes (lane 1), cumulus cells (lane 2), isolated luteinized granulosa cells (lane 3), and secretory endometrium (lane 4).

report indicating the presence of glycodelin in theca interna and granulosa cells of human follicles in the follicular phase (Kamarainen *et al*., 1996). The present study extends these findings in two ways. The first is to demonstrate that glycodelin immunoreactivity emerges in follicular cells at the late secondary stage of follicular growth. Based on observations that the glycodelin concentrations during menstrual phase are higher in women with higher progesterone concentrations (Joshi, 1983; Seppala *et al*., 1994), glycodelin secretion has long been associated with progesterone. However, there is controversy as to whether progesterone is directly involved in the synthesis and release of this glycoprotein. Serum progesterone peaks early in the luteal phase and is declining as glycodelin expression in the endometrium begins to increase (Julkunen *et al*., 1986a).

Recently, some studies have demonstrated that the profile of circulating relaxin is similar to that of glycodelin and that relaxin stimulates glycodelin secretion (Stewart *et al*., 1997; Tseng *et al*., 1999). Relaxin is a peptide hormone with well-known effects on pregnancy and parturition. Evidence

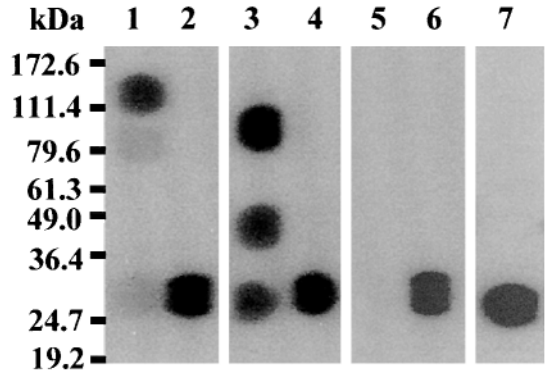

Figure 3. Detection of iodinated glycodelin-A complex formation in cumulus cells by 8% native gel autoradiography. Lane 1, lysates of cumulus cells; lane 2, supernatant of incubation mixture (for cumulus cells); lane 3, lysates of oviductal epithelial cells; lane 4, supernatant of incubation mixture (for oviductal epithelial cells); lane 5, lysates of fibroblasts; lane 6, supernatant of incubation mixture (for fibroblasts); lane 7, purified iodinated glycodelin-A.

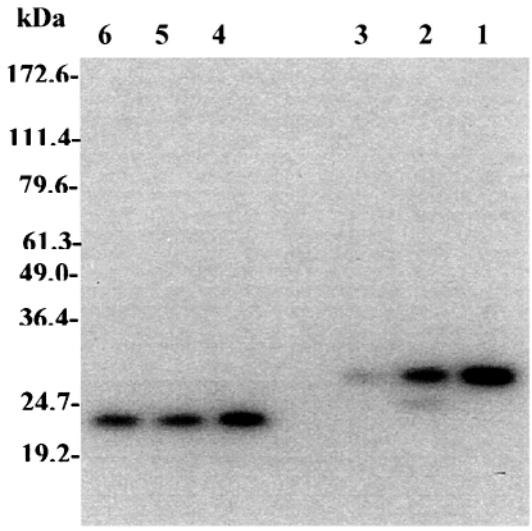

Figure 4. Detection of iodinated glycodelin-A in cumulus cells by 12% SDS–PAGE gel autoradiography. Lane 1, deglycosylated supernatant of incubation mixture; lane 2, deglycosylated lysates of cumulus cells; lane 3, purified iodinated deglycosylated glycodelin; lane 4, supernatant of incubation mixture; lane 5, lysates of cumulus cells; lane 6, purified iodinated glycodelin-A.

suggests that relaxin also affects folliculogenesis and the subsequent formation of the corpus luteum. Relaxin mRNA is present in the human ovary, corpus luteum, term placenta and decidua (Hudson *et al*., 1984; Ivell *et al*., 1989; Gunnersen *et al*., 1996). Immunoreactive human relaxin has also been detected in theca cells, in the corpus luteum (Stoelk *et al*., 1991) and in granulosa cells showing histological luteinization (Yki-Jarvinen *et al*., 1984). Thus, it is possible that relaxin stimulates the expression of glycodelin in the follicular cells during the follicular phase. In support of this is the expression of both relaxin and glycodelin in luteinized granulosa cells (Yki-Jarvinen *et al*., 1984; Kamarainen *et al*., 1996). The granulosa cells obtained from this study were also luteinized, as they were obtained from patients 36–38 h after HCG injection.

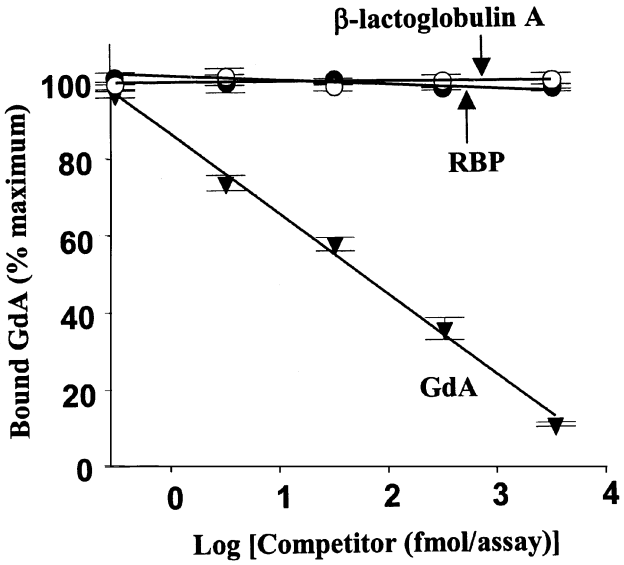

Figure 5. Competition between iodinated-glycodelin-A (GdA) and unlabelled glycodelin-A, β-lactoglobulin A and retinol binding protein (RBP). Each point represents the mean ± SEM of three experiments. In each experiment, all the determinations were done in duplicate.

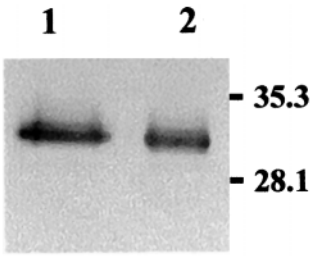

Figure 6. Immunoblot analysis of ZIF-1 and glycodelin-A using anti-glycodelin antibody. Lane 1, glycodelin-A; lane 2, ZIF-1.

The second new finding is the presence of glycodelin mRNA in granulosa cells, but not in cumulus cells. In contrast, glycodelin protein is detected by immunohistochemical analysis in both isolated luteinized granulosa and isolated cumulus cells. Kamarainen and coworkers could not demonstrate by in-situ hybridization the presence of mRNA of glycodelin in the normal ovary (Kamarainen *et al*., 1996). The discrepancy between their study and the present results is probably because RT–PCR used in this study is more sensitive. The present results suggest that granulosa cells synthesize glycodelin, which is then transported to the cumulus cells. It is well known that there are complex interactions between the oocyte, cumulus cells and granulosa cells, and differential responses of cumulus cells and granulosa cells to various treatments have been reported (Vanderhyden and Tonary, 1995; Armstrong *et al*., 1996).

Glycodelin protein could be transported from the granulosa cells to the cumulus cells by two possible routes. One is by means of gap junctions between these cells. The other is through the follicular fluid, i.e. the granulosa cells secrete glycodelin into the follicular fluid, and the cumulus cells take it up from the follicular fluid. This second route is more likely because of two observations. Firstly, immunoreactive

glycodelin has been quantified in human follicular fluid (Riittinen *et al*., 1989; Chryssikopoulos *et al*., 1996). Secondly, we have purified ZIF-1 from human follicular fluid, and ZIF-1 shares many characteristics with glycodelin. For instance, both have similar molecular sizes and inhibit spermatozoa–zona pellucida binding (Oehninger *et al*., 1995; Yao *et al*., 1998). Western blot analysis in the present study further showed that ZIF-1 reacts immunologically with anti-glycodelin. Based on these data, we suggest that ZIF-1 is at least a glycodelin-like molecule, although its exact identification needs to be confirmed.

The second route of transport of glycodelin was further investigated in this study by incubating cumulus cells with radioactively-labelled glycodelin-A. Our results show that the cumulus cells take up glycodelin-A from the surrounding culture medium. Immunohistochemical staining shows that the cytoplasm of cumulus cells contain glycodelin-like immunoreactivity. In the cumulus cells, the two bands with large molecular sizes in the native gel analysis in the glycodelin-A uptake experiment are likely to represent cytoplasmic components of the cumulus cells. This is because a mechanical method (freeze and thaw) without detergent was used to lyse the cells, so that the membrane components were less likely to have been solubilized in these lysates and appeared in the present native gel analysis. However, these data do not exclude the possibility that glycodelin is bound to the plasma membrane of the cumulus cells. The nature of these complexes is unknown. Under denaturing conditions, the complexes dissociate into glycodelin and a molecule with size smaller than that of glycodelin, but larger than that of completely deglycosylated glycodelin-A. The size of this small molecule is further reduced to that of completely degylcosylated glycodelin upon enzymatic deglycosylation, suggesting that this molecule is likely to be a partially deglycosylated form of glycodelin. These data are consistent with the hypothesis that cumulus cells take up glycodelin from the follicular fluid.

The uptake of glycodelin by the cumulus cells seems to be specific, as it is not affected by the two other members of the lipocalin family. Furthermore, this uptake is likely to be cell type specific. Glycodelin-A was taken up by the cumulus cells and by the oviductal cells, but not by the fibroblasts. Interestingly, the uptaken glycodelin appeared to form different complexes in different cell types. It is of interest to note that oviductal cells synthesize glycodelin (Maguiness *et al*., 1993; Laird *et al*., 1995; Saridogan *et al*., 1997), while the cumulus cells do not.

The mechanism by which glycodelin is taken up by cumulus cells is unknown. Glycodelin is a member of the lipocalin family of peptides. There are reports showing that a number of lipocalins are bound by specific cell-surface receptors and may be internalized by receptor mediated endocytosis (Senoo *et al*., 1990; Malaba *et al*., 1995). The present results indicated that cumulus cells partially deglycosylate glycodelin. Both glycodelin and its partial deglycosylated form appeared to be complexed with some cytoplasmic or membrane components. Whether these complexes would represent glycodelin–receptor complexes remains to be investigated. However, it is worth noting that in neutrophils, neutrophil lipocalin (NGAL) is attached to human neutrophil gelatinase via disulphide bridges (Kjeldsen *et al*., 1993).

In conclusion, this study provides data showing that glycodelin is synthesized by thecal and granulsoa cells. Glycodelin appears to be released into the follicular fluid and taken up and modified by cumulus cells. The physiological role of glycodelin in cumulus cells remains to be elucidated.

Acknowledgements

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