Late preovulatory synthesis of proteoglycans by the human oocyte and cumulus cells and their secretion into the oocyte-cumulus-complex extracellular matrices

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Summary. Light- and electron-microscope autoradiography using 3H-glucosamine and 3H-fucose as precursors was employed to investigate proteoglycan synthesis and secretion by late preovulatory human oocytes and cumulus cells. Both the oocyte and cumulus cells were found to be important cellular sources supplying proteoglycans to the oocyte-cumulus-complex extracellular matrices, i.e., the zona pellucida and the cumulus intercellular matrix. Both the oocyte and cumulus cells were shown to secrete labelled proteoglycans into the zona pellucida. Labelled proteoglycans were also detected in the cumulus intercellular matrix. Chase experiments revealed the labelled molecules to be relatively closely associated with both the zona pellucida and the cumulus intercellular matrix. Staining with chromic acid and phosphotungstic acid showed proteoglycan material to penetrate from the cumulus intercellular matrix into pores of the zona pellucida. This material is thought to be a structural equivalent of the newly synthesized proteoglycans secreted by cumulus cells and migrating into the zona pellucida (as detected by autoradiography). It is concluded that newly synthesized proteoglycans secreted by the oocyte and cumulus cells in the late preovulatory period are a component of the microenvironment in which fertilization takes place.

Introduction

The large antral follicles of mammals are rich in proteoglycans. It has been shown that granulosa cells are the main producers of follicular proteoglycans (Yanagishita and Hascall 1979), whose secretion is partly regulated by pituitary and local hormones (Yanagishita et al. 1981). The pattern of the secretion of proteoglycans depends on follicular maturity (Grimek et al. 1984) and is correlated with the oestrogen-to-progesterone ratio in the follicular fluid (Bellin and Ax 1984). Cumulus cells are modified granulosa cells whose preovulatory follicle-stimulating hormone (FSH)-dependent synthesis of hyaluronic acid has been shown, in mice, to be regulated by intrafollicular sulphated glycosaminoglycans (Eppig and Ward-Bailey 1984). There are some findings suggesting that glycosaminoglycan moieties of proteoglycans — particularly of those secreted by late preovulatory granulosa and cumulus cells — may play an important role in fertilization by exerting effects on sperm capacitation and acrosome reaction (for a review see Lenz et al., 1983), on the conversion of proacrosin into acrosin (Parrish et al. 1980) and on sperm-chromatin decondensation (Delgado et al. 1980) with the subsequent release of DNA-template restrictions (Delgado et al. 1984). The selective binding of labelled proteoglycans secreted by human preovulatory cumulus cells to human spermatozoa during capacitation in vitro has recently been directly demonstrated using autoradiography (Tesafík et al. 1984).

Follicular proteoglycans can gain access to the site of fertilization as components of follicular fluid and oocyte-cumulus complexes (OCCs) released at ovulation; thereafter, they are probably progressively substituted by oviducal-fluid components. The actual concentration of these molecules close to the ovulated oocyte, however, obviously depends on the continuing secretory activity of periovulatory cumulus cells and on the stability of the association between the secreted material and the extracellular matrices of the OCCs.

The aim of the present study was to examine the synthesis and secretion of proteoglycans by the human oocyte and cumulus cells shortly before and during ovulation, and to investigate the relationship of the proteoglycans secreted by these two cellular sources with the zona pellucida and cumulus intercellular matrix.

Materials and methods

Source and characterization of OCCs. Twenty OCCs were removed from 11 women by laparoscopically guided follicular aspiration (Tesafík et al. 1980) during a first-look laparoscopy. The patients had been selected for an in vitro fertilization programme conducted in the First Department of Obstetrics and Gynaecology at Brno. This form of intervention is recommended as the first diagnostic and potentially therapeutic approach in patients with unexplained infertility; the procedure makes it possible to evaluate the contribution of cervical, tubal and male factors to the patient's infertility, as well as allowing the responsiveness of her ovaries to gonadotropic stimulation to be tested (Pilka et al. 1983). When oocytes are recovered and the partner's sperm of acceptable quality is available, in vitro fertilization and embryo transfer are carried out during this stage of treatment. The material used in our study came from rare cases in which the partner had not been able to produce a semen sample or had severe asthenospermia, so that it was impos-
Fig. 1 and 2. Light-microscope autoradiographs of OCCs labelled for 2 h with $^3$H-glucosamine (1) and $^3$H-fucose (2). O, oocyte; CC, cumulus cell; ZP, zona pellucida; CM, cumulus intercellular matrix. × 1,000

Figs. 3 and 4. Electron-microscope autoradiographs of OCCs labelled for 2 h with $^3$H-glucosamine (3) and $^3$H-fucose (4) showing parts of the oocyte (3) and a cumulus cell (4). ZP, zona pellucida; LD lipid droplets. × 18,000

Fig. 5. Electron-microscope autoradiograph showing parts of a cumulus cell (CC) and the zona pellucida (ZP) of an OCC labelled with $^3$H-fucose for 2 h × 13,000

Fig. 6. Electron-microscope autoradiograph showing parts of the oocyte (O), the zona pellucida (ZP) and a cumulus cell (CC) of an OCC labelled with $^3$H-glucosamine for 2 h. × 10,000

Ovarian stimulation was performed by administering 100 mg clomiphene citrate (Gravosan, Spofa) on days 5–9 of the menstrual cycle, and ovulation was induced by a single injection of 6,000 IU human chorionic gonadotropin (hCG; Praedyn, Spofa). Follicular aspiration was performed 34–36 h after hCG treatment, i.e. on days 13–15 of the cycle, the time depending on the size of the leading follicle as evaluated by ultrasound. Fourteen OCCs from large antral follicles (4–6 ml follicular fluid) that fulfilled the criteria of healthy preovulatory follicles (Brailey et al. 1981) were selected for our study. All were characterized by complete cumulus expansion and a dispersed corona radiata.

Labelling of newly synthesized proteoglycans. Ham’s F-10 culture medium (Flow Laboratories) modified as previously described (Tesatik et al. 1984) was used for all cultures, washes and incubations. For proteoglycan labelling, this medium was enriched by the addition of either 7.4 MBq/ml D-[1-$^3$H]-glucosamine (Radiochemical Centre, Amersham; specific activity, 400 GBq/mmol) or 7.4 MBq/ml L-[6-$^3$H]-fucose (Amersham; specific activity, 900 GBq/mmol). Glucosamine was used to label the glycosaminoglycan chains of secreted proteoglycans, while fucose was used to label carbohydrate side chains other than glycosaminoglycans.

The first series of experiments involved 7 OCCs, 4 of which were labelled with $^3$H-fucose, while the other 3 were labelled with $^3$H-glucosamine. All incubations were carried out for 2 h in 0.1 ml