Dynamics of extracellular matrix in ovarian follicles and corpora lutea of mice

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Abstract Despite the mouse being an important laboratory species, little is known about changes in its extracellular matrix (ECM) during follicle and corpora lutea formation and regression. Follicle development was induced in mice (29 days of age/experimental day 0) by injections of pregnant mare’s serum gonadotrophin on days 0 and 1 and ovulation was induced by injection of human chorionic gonadotrophin on day 2. Ovaries were collected for immunohistochemistry (n=10 per group) on days 0, 2 and 5. Another group was mated and ovaries were examined on day 11 (n=7). Collagen type IV α1 and α2, laminin α1, β1 and γ1 chains, nidogens 1 and 2 and perlecan were present in the follicular basal lamina of all developmental stages. Collagen type XVIII was only found in basal lamina of primordial, primary and some preantral follicles, whereas laminin α2 was only detected in some preantral and antral follicles. The focimatrix, a specialised matrix of the membrana granulosa, contained collagen type IV α1 and α2, laminin α1, β1 and γ1 chains, nidogens 1 and 2, perlecan and collagen type XVIII. In the corpora lutea, staining was restricted to capillary sub-endothelial basal laminae containing collagen type IV α1 and α2, laminin α1, β1 and γ1 chains, nidogens 1 and 2, perlecan and collagen type XVIII. Laminins α4 and α5 were not immunolocalised to any structure in the mouse ovary. The ECM composition of the mouse ovary has similarities to, but also major differences from, other species with respect to nidogens 1 and 2 and perlecan.

Keywords Follicle · Corpus luteum · Extracellular matrix · Collagen · Laminin · Perlecan · Nidogen · Mouse (CBAxC57BL/6F1)

Introduction

Basal laminae are specialised sheets of extracellular matrix (ECM; Paulsson 1992; Timpl and Brown 1996) that underlie epithelial and endothelial cells or envelop whole cells such as nerve or muscle cells. They separate cells from adjoining stroma, influence their behaviour and can selectively retard the passage of cells and molecules...
Basal laminas are composed of lattice-type networks of collagen type IV and laminin, which are stabilised by the binding of entactin/nidogen 1 or 2 (Schmeinskey et al. 2002) and probably also of fibulins and heparan sulphate proteoglycans, such as perlecans, to the collagen and laminin. Collagen type IV comprises three α chains of which there are six different chains of collagen type IV α (α1–α6, each encoded by a separate gene; Hay 1991) and various combinations of collagen type IV α chains can assemble to form unique isoforms in vivo (e.g. α1α1α2, α3α4α5; Sado et al. 1998). Laminins are composed of three different chains, viz. α, β and γ chains (Aumailley et al. 2005), of which there are five different α chains, three β chains and three γ chains (all encoded by separate genes). The combination of α, β and γ chains potentially gives rise to 45 (5×3×3) different molecular combinations, with alternative splicing resulting in more combinations (Aumailley et al. 2005). Collagen type XVIII, which has structural properties of both a collagen and proteoglycan, is also associated with the basal laminas, in particular the sub-endothelial basal laminas (Marneros and Olsen 2005). Basal laminas thus can vary considerably in composition, which might influence their physical and biological properties and have differential effects on cells.

In adult ovaries, considerable tissue remodelling occurs as follicles grow, ovulate or regress and when corpora lutea are formed or regress. These processes require the expansion of basal laminas within follicles and corpora lutea and their later degradation. Much of the research on basal lamina matrices has been conducted in bovine (for a review, see Irving-Rodgers et al. 2006c), with fewer studies in humans (Irving-Rodgers et al. 2006b, 2009b). Most of the focus in follicles has been on the follicular basal lamina underlying the membrana granulosa, the changes that it undergoes during follicular development and the two ultrastructural phenotypes that are related to granulosa cell undergoing transgenic animals and for genetic N-ethyl-N-nitrosourea mutagenesis screening (Papathanasiou and Goodnow 2005). As such, we need to increase our knowledge of the ovarian matrix in the murine ovary. Currently, much of the study of the matrix in mouse ovaries has focused on the cumulus oocyte complex (Camaioni et al. 1996; Rodgers et al. 2003; Dunning et al. 2007) or stromal matrix. However, studies have identified collagen type IV isotypes (Frojdman et al. 1998; Nakano et al. 2007) and the glycoprotein usherin, which contains four domains common to basal lamina and ECM (thrombospondin, laminin type IV, laminin epidermal-growth-factor-like and fibronectin type III) (Pearsall et al. 2002) in the murine ovary. Here, we expand our knowledge on the composition of basal lamina matrices in the murine ovary, in both non-pregnant and pregnant mice.

Materials and methods

Experimental animals

All animal experimental protocols were approved by both The University of Adelaide and The Institute of Medical & Veterinary Science (IMVS) Animal Ethics Committees and were conducted in accordance with the National Research Council (NRC) publication Guide for Care and Use of
Laboratory Animals (copyright 1996, National Academy of Science). Twenty 5-day-old female CBA×C57BL/6 F1 mice in the weight range of 10–12 g were obtained from the Laboratory Animal Services, The University of Adelaide, and housed in the IMVS Animal Care Facility with controlled light cycles (12 h light:12 h dark). Four days later, the mice were subjected to induction of ovulation with gonadotrophins. Pregnant mare’s serum gonadotrophin (PMSG; Intervet, Victoria, Australia) was administered at 5 IU intraperitoneally on days 0 and 1 and 5 IU human chorionic gonadotrophin (hCG; Organon, NSW, Australia) on day 2. Some groups were mated by caging females with 7-week-old fertile CBA males overnight (2 females with 1 male per cage). Vaginal plugs were checked 24 h later and the time that vaginal plugs appeared was noted. Mice (n=10 per group) were killed with carbon dioxide on days 0, 2, 5 and 11 relative to the first injection of PMSG. Both ovaries from each animal were harvested, one ovary being fixed in 4% paraformaldehyde (Merck, Victoria, Australia) in 0.1 M phosphate buffer (pH 7.4). These tissues were then washed several times in phosphate-buffered saline (PBS) and dehydrated in ascending series of ethanol from 70% to 100% (Leica TP1020, Automatic Tissue Processor; Leica, Nussloch, Germany) and embedded in paraffin blocks (Leica EG1140H, Paraffin Embedding Station) for subsequent haematoxylin-eosin staining. The other ovary from each animal was frozen, embedded in Optimal Cutting Temperature compound (Sakura Finetechnical, Tokyo, Japan) and stored at −20°C until use. Unfixed sections were dried under vacuum for 5 min followed either by fixation in 100% acetone, 10% neutral buffered formalin (BFS), 100% ethanol or left unfixed. Sections were then rinsed (3×5 min) in changes of hypertonic PBS (10 mM sodium/potassium phosphate with 0.274 M NaCl, 5 mM KCl, pH 7.2) before treatment with blocking solution (10% normal donkey serum [catalogue no. D-9663; Sigma] in antibody diluent containing 0.55 M sodium chloride and 10 mM sodium phosphate, pH 7.1) for 30 min at room temperature. Incubation with primary antibodies was carried out overnight at room temperature. The secondary antibodies used and the labelling conditions are summarised in Table 1. For dual localisations, sections were also treated with the nuclear stain 4',6'-diamidino-2-phenylindole dihydrochloride (DAPI) solution (Molecular Probes, Eugene, Ore., USA). Sections were mounted in mounting medium for fluorescence (catalogue no. S3023; Dako, Carpinteria, Calif., USA) and photographed with an Olympus BX50 microscope with an epifluorescence attachment and a Spot RT digital camera (Diagnostic Instruments, Sterling Heights, Mich., USA).

Results

Ovaries were examined at 29 days of age (day 0) before the onset of puberty and on day 2 after induction of follicle growth with PMSG (injections days 0 and 1). Ovulation was induced with hCG (day 2). One group of mice were not mated and their ovaries were examined on day 5, whereas the other group was mated and ovaries were examined on day 11 relative to first day of injection of PMSG (day 0). The ovaries at day 0 exhibited small antral follicles (between 200 and 400 μm); at day 2, they contained additional larger antral follicles (between 300 and 550 μm); on days 5 and 11, the ovaries also contained corpora lutea. The percentages of healthy antral follicles were (mean ± SEM): 51±8%, 87±4%, 51±10% and 62±7% on days 0, 2, 5 and 11, respectively. Day-5 animals (n=10) had 6±1 corpora lutea per ovary cross section. Fifteen animals examined following ovulation induction and mating were pregnant (75%) with 14±2 implantation sites per animal at day 11. Only the pregnant animals from this group were examined further. Table 2 shows the number of

Immunohistochemistry

Table 1 summarises the antibodies used for immunofluorescence and the relevant fixation conditions. Localisation was undertaken by using an indirect immunofluorescence method (Irving-Rodgers et al. 2002). Tissue sections (7 μm) were cut on a CM1800 Leica cryostat (Adel, Altona North, Vic, Australia), collected on superfrost glass slides and stored at −20°C until use. Unfixed sections were rinsed (3×5 min) in changes of hypertonic PBS (10 mM sodium/potassium phosphate with 0.274 M NaCl, 5 mM KCl, pH 7.2) before treatment with blocking solution (10% normal donkey serum [catalogue no. D-9663; Sigma] in antibody diluent containing 0.55 M sodium chloride and 10 mM sodium phosphate, pH 7.1) for 30 min at room temperature. Incubation with primary antibodies was carried out overnight at room temperature. The secondary antibodies used and the labelling conditions are summarised in Table 1. For dual localisations, sections were also treated with the nuclear stain 4',6'-diamidino-2-phenylindole dihydrochloride (DAPI) solution (Molecular Probes, Eugene, Ore., USA). Sections were mounted in mounting medium for fluorescence (catalogue no. S3023; Dako, Carpinteria, Calif., USA) and photographed with an Olympus BX50 microscope with an epifluorescence attachment and a Spot RT digital camera (Diagnostic Instruments, Sterling Heights, Mich., USA).

Histology

Paraffin sections (3 μm) were cut by using a CM1850 V2.2 Leica microtome (Leica Microsystems, Nussloch, Germany) and collected on Superfrost glass slides (HD Scientific, Australia). After 1 h incubation at 60°C, the slides were dewaxed in xylene (2×5 min) and in 100% ethanol (2×5 min) and rinsed in distilled water. The sections were stained with haematoxylin (catalogue no. GHS232; Sigma) for 5 min, rinsed in tap water and in acid alcohol for 5 s and subsequently stained in eosin (catalogue no. HT110132; Sigma) for 20 s. Afterwards, the slides were rinsed in tap water, in 70% ethanol for 1 min, in 100% ethanol (3×1 min) and in xylene (3×5 min) and mounted in Pertex (Medite, Burgdorf, Germany). The haematoxylin-eosin-stained sections were used for the classification of atresia, the estimation of average numbers of healthy antral follicles and corpora lutea on days 0, 2, 5 and 11 and the counting of implantation sites in the mated mice.
ovaries examined at each time point for each ECM molecule, either alone or in combination. The results are presented as a description of changes during follicular and luteal development.

**Table 1** Primary antibodies, secondary antibodies, and labelling and fixation conditions used for immunohistochemistry for each antigen. Secondary antibodies used were either biotin-SP-conjugated AffiniPure donkey anti-rat IgG (BD anti-rat, 1:100; catalogue no. 711-066-153) or anti-rabbit IgG (BD anti-rbt, 1:100; catalogue no. 711-066-152), followed by Cy3-conjugated streptavidin (SA-Cy3, 1:100; catalogue no. 016-084-084) or fluorescein (DTAF)-conjugated streptavidin (SA-DTAF, 1:100; catalogue no. 016-010-084), or Cy3-conjugated AffiniPure donkey anti-rabbit IgG (D anti-rbt-Cy3, 1:100; catalogue no. 711-166-152) or fluorescein isothiocyanate (FITC)-conjugated AffiniPure donkey anti-rabbit IgG (D anti-rbt-FITC, 1:100; catalogue no. 711-015-125); all secondary antibodies and conjugated streptavidins were from Jackson ImmunoResearch Laboratories (West Grove, Pa., USA).

| Antigen (species) | Host species | Code or clone number | Source or Reference | Dilution | Secondary antibody | Conjugates | Fixation |
|-------------------|--------------|----------------------|---------------------|----------|--------------------|------------|----------|
| Laminin α1 (mouse) | Rabbit | 317 | Durbeej et al. (1996) | 1:1000 | D anti-rbt-Cy3 | None | Unfixed |
| Laminin α2 (mouse) | Rat | 4H8-2 | Sigma Aldrich | 1:2000 | D anti-rbt-Cy3 | None | 100% acetone |
| Laminin α4 (mouse) | Rabbit | 377 | Sorokin et al. (2000) | 1:500 | BD anti-rat | SA-Cy3 | Unfixed |
| Laminin α5 (mouse) | Rabbit | 405 | Sorokin et al. (2000) | 1:500 | BD anti-rat | SA-Cy3 | Unfixed |
| Laminin β1 (mouse) | Rat | LT3 | Millipore | 1:50 | D anti-rbt-Cy3 | None | Unfixed |
| Laminin γ1 (mouse) | Rat | 3E10 | Sixt et al. (2001) | 1:2 | BD anti-rbt | SA-DTAF | Unfixed |
| Type IV collagens α1 - α6 (human) | Rabbit | 92462 | Erickson and Couchman (2001) | 1:200 | BD anti-rbt | SA-DTAF | 100% acetone |
| Type XVIII collagen (mouse) | Rabbit | 92462 | Erickson and Couchman (2001) | 1:200 | BD anti-rbt | SA-DTAF | 100% acetone |
| Nidogen-1 (mouse) | Rabbit | 914 | Dziadek et al. (1985b) | 1:200 | D anti-rbt-FITC | None | Unfixed |
| Nidogen-2 (mouse) | Rabbit | 1130 | Papaccio et al. (1998) | 1:800 | D anti-rbt-FITC | None | 10% BFS |
| Perlecain (mouse) | Rabbit | 906 | Dziadek et al. (1985a) | 1:100 | BD anti-rbt | SA-DTAF | Unfixed |
| PECAM-1/ CD31 | Rat | 390 | BD Phar-mingen | 1:500 | BD anti-rbt | SA-Cy3 | 10% ethanol |

Matrix in follicles

The follicular basal lamina contained collagen type IV α1 and α2 at all stages from primordial to large preovulatory stages (data not shown) confirming previous data (Nakano et al. 2007). Laminin α1 was similarly present at all stages (Fig. 1d, e) but laminin α2 was only detected in some follicles and only at the preantral and antral stages (Fig. 1a, b). Laminin α4 or α5 chains were not detected at any stage (Fig. 2a′, b′, b′′). Laminin α4 was detected in heart and skeletal muscle (Fig. 2c, c′) and laminin α5 was detected in kidney (Fig. 2d), indicating that the antisera reacted with mouse laminins α4 and α5. Laminin β1 (Fig. 1g, h, m, n) and γ1 chains (Fig. 1d, e) were present at all stages of follicular development. Both nidogens 1 and 2 were also present throughout development (Fig. 1j, k, m, n). Of the heparan sulphate proteoglycans examined, perlecain was present at all stages (Fig. 1a, b), whereas collagen type XVIII was present only in primordial and primary follicles and in some preantral but not in antral follicles (Fig. 1g, h).

Focimatrix was present in the preantral and antral follicles at days 0, 2, 5 and 11 and appeared as larger and fewer aggregates (approximately six in cross section) than those observed in the bovine ovary (Irving-Rodgers et al. 2004) or occurred as fibrous structures throughout whole
membrana granulosa. Nidogens 1 and 2 and perlecan (Figs. 1a, b, 3a, b) showed fibrous patterns of expression, whereas the aggregates contained collagen type IV α1 and α2 (data not shown; as observed previously by Nakano et al. [2007]), laminins α1 (Figs. 1d, 3c), β1 (Figs. 1g, h, m, n, 3d–f) and γ1 (Figs. 1d, 3c) chains and also nidogen 1 (Figs. 1j, k, 3e) and nidogen 2 (Figs. 1m, n, 3d) and perlecan (Figs. 1b, 3a). Focimatrix was not observed in all antral follicles (Table 3).

The focimatrix in some preantral follicles contained collagen type XVIII (Figs. 1g, h, 3d) and, surprisingly, even in follicles where the follicular basal lamina did not contain collagen type XVIII. However, focimatrix with collagen type XVIII was more abundant in follicles that were surrounded by a basal lamina positive for collagen type XVIII. In co-localisation studies, laminin α1 co-localised with laminin γ1 (Figs. 1d, 3c), nidogen 1 (Fig. 3e) and nidogen 2 (Figs. 1m, n, 3f) co-localised with aggregates of laminin β1 focimatrix, and laminin β1 co-localised with collagen type XVIII (Figs. 1g, h, 3d).

Thecal matrix in antral follicles contained laminin β1 (Fig. 1g, h, m, n) and γ1 (Fig. 1d, e) chains, perlecan (Fig. 1a–c), nidogen 1 (Fig. 1j, k) and nidogen 2 (Fig. 1m, n) but not collagen type IV α1 and α2 (data not shown), as observed previously (Nakano et al. 2007), laminin α1, α2, α4 or α5 chains, or collagen type XVIII. Thecal capillary sub-endothelial basal lamina contained the same components as the thecal matrix but additionally collagen type IV α1 and α2 (data not shown) as observed previously (Nakano et al. 2007).

Matrix in atretic follicles

The follicular basal lamina of atretic follicles showed the same composition as that of healthy follicles. It contained collagen type IV α1 and α2 (as shown previously by Nakano et al. 2007), laminin α1 (Fig. 1f), laminin α2 (Fig. 1c; but only in some large follicles), laminin β1 (Fig. 1i, o) and γ1 (Fig. 1f) chains, nidogen 1 (Fig. 1i) and nidogen 2 (Fig. 1o) and perlecan (Fig. 1c), whereas laminin α4 and α5 and collagen type XVIII (Fig. 1i) were not present. Atretic follicles also possessed focimatrix containing laminin α1 (Fig. 1f), β1 (Fig. 1i, o) and γ1 (Fig. 1f) chains, nidogens 1 and 2, perlecan (Fig. 1c) and collagen type XVIII (Fig. 1i).

Matrix in corpora lutea

No parenchymal matrix, as observed in human (Irving-Rodgers et al. 2006b) or bovine (Irving-Rodgers et al. 2004) corpora lutea, was detected and no basal laminae surrounding individual luteal cells were observed. However, luteal capillary sub-endothelial basal lamina was readily observed and contained collagen type IV α1 and α2 (data not shown; as observed previously by Nakano et al. 2007), laminin α1 (Fig. 1e), β1 (Fig. 1h, m) and γ1 (Fig. 1e) chains, nidogen 1 (Fig. 1k) and nidogen 2 (Fig. 1m), perlecan (Fig. 1b) and collagen type XVIII (Fig. 1h) but not laminin α2 (Fig. 1b), α4 or α5 (data not shown) chains. Figure 4 illustrates the co-localisation of some of these ECM components with the endothelial cell marker PECAM-1 (CD31). No differences were observed between luteal capillary sub-endothelial basal lamina composition in corpora lutea from experimental days 5 and 11 (estimated to be days 2 or 9 post-ovulation).

Matrix in other ovary compartments

The epithelial basal lamina of the ovarian surface contained collagen type IV α1 and α2 (data not shown; as observed previously by Nakano et al. 2007), laminin β1 (Fig. 1g) and γ1 (Fig. 1d, e) chains, nidogen 1 (Fig. 1j, k) and nidogen 2 (Fig. 1m), and perlecan (Fig. 1a, b) but no laminin α1 (Fig. 1d, e), α2 (Fig. 1a, b), α4 or α5 chains or collagen type XVIII (Fig. 1g). Arteriole sub-endothelial basal laminas contained collagen type IV α1 (data not shown). Some of these basal laminas contained laminin β1 (Fig. 1g, h), laminin γ1 (Fig. 1e–g), nidogen 1 (Fig. 1k, l) and nidogen 2 (Fig. 1m, n), perlecan (Fig. 1b) and collagen type XVIII (Fig. 1g, h), but none contained laminin α1, α2, α4 or α5 chains. The basal lamina of arteriole smooth muscle contained collagen type IV α1 and α2; some of these basal laminas contained laminin α2, laminin β1, laminin γ1 (Fig. 1f), nidogen 1 and 2, perlecan and collagen type XVIII (Fig. 1h) but none contained laminin α1, α4 or α5.

Discussion

We provide new information on the localisation of basal lamina components, such as the laminins, nidogens, and the heparan sulphate proteoglycans, perlecan and collagen type XVIII in mouse ovaries, including those associated with the follicular basal lamina, focimatrix, thecal matrix, corpora lutea, vasculature and surface epithelium. Some developmental changes in mouse matrices are common to other species; however, distinct species differences exist, with mouse ovaries clearly having differences in their ECM composition.

The composition of the basal laminas in the follicles of the mouse (Nakano et al. 2007; current study), rat (Frojdman et al. 1998) and bovine (Rodgers et al. 1998; van Wezel et al. 1998; McArthur et al. 2000) have now been examined. As the follicular basal lamina expands in surface area, it changes in composition. In both mouse and bovine, collagen type IV α1–α6 are present in primordial
from preantral stages onwards (McArthur et al. 2000). However, the stage at which collagen type IV α3–α6 decline differs between the two species: at the primary stage in mice (Nakano et al. 2007) and at the early antral stage in bovine (Rodgers et al. 1998). This species difference in the precise timing of the loss of collagen type IV α3–α6 might reflect the finding that mouse follicles reach ovulatory stages much quicker than bovine follicles as they are considerably smaller with fewer granulosa cells at ovulation. Rat follicular basal lamina similarly contain collagen type IV α1 and α2 throughout follicular development (Frojdman et al. 1998). They also have collagen type IV α3 at early stages but exhibit low expression of α4 and α5 from the secondary stage of development (Frojdman et al. 1998).

Laminin α1 is present in the mouse follicular basal lamina at all stages of follicle growth but laminin α2 has only been detected in some follicles, and only at the preantral and antral stages. This same pattern has been observed in the bovine with no apparent explanation of the differences between laminin-α2-positive and laminin-α2-negative follicles (van Wezel et al. 1998), except that it might reflect differences in the theca of these follicles, as laminin α2 is generally of stromal origin (Vuolteenaho et al. 1994; Sorokin et al. 1997; Lefebvre et al. 1999). Laminin α4 or α5 chains have not been detected at any stage in the mouse. Laminin β1 and γ1 chains are present at all stages of follicular development in the mouse, whereas laminin γ1 is also found during follicular growth in bovine but laminin β1 only appears transiently during development at the preantral stage (van Wezel et al. 1998). Both nidogen 1 and nidogen 2 have been observed throughout folliculogenesis in the mouse, but they only appear in the bovine at the preantral and antral stages (McArthur et al. 2000; Irving-Rodgers and Rodgers 2006). Of the heparan sulphate proteoglycans, perlecan is present at all stages, whereas collagen type XVIII has been detected only in murine primordial and primary follicles and in some preantral but not in antral follicles. This differs from the bovine in which collagen type XVIII occurs at all stages but is not detectable once the follicle is degenerated (Irving-Rodgers et al. 2006a; Irving-Rodgers and Rodgers 2006) and perlecan is present from preantral stages onwards (McArthur et al. 2000).

The focimatrix in the mouse clearly differs from that seen in other species. It is found in the preantral and antral follicles of days 0, 2, 5 and 11 as larger and fewer aggregates (approximately six in cross sections of a follicle) than those observed in the bovine ovary (Irving-Rodgers et al. 2004) or as fibrous structures throughout the membrana granulosa. Nidogen 1 and 2 and perlecan are fibrous, whereas the aggregates contain collagen type IV α1 and α2, as observed previously (Nakano et al. 2007) and laminins α1, β1 and γ1 chains. The focimatrix in some preantral follicles contain collagen type XVIII, including follicles in which the follicular basal lamina is negative for collagen type XVIII. However, focimatrix with collagen type XVIII is more abundant in follicles that have collagen type XVIII in their follicular basal lamina. The focimatrix has not been observed in all antral follicles. Bovine follicles contain all the same components, except that they exhibit laminin β2 and not β1, but the aggregate is more homogeneous and only appears once follicles are larger than 5 mm diameter, increasing in amount until ovulation when it is degraded (Irving-Rodgers et al. 2004). The reason that the focimatrix is so variable in mouse ovaries is unknown at this stage.

Throughout the theca interna, basal lamina components have been identified that are not in association with any conventional basal lamina; this has been termed the thecal matrix. In the mouse, the theca matrix contains laminin β1 and γ1, as in bovine follicles, which contain laminin γ1 (van Wezel et al. 1998), but unlike in the bovine, it also contains perlecan and nidogens 1 and 2. Collagen type IV α1 and α2 are also not present in the thecal matrix of the mouse; this is consistent with the previously observation by Nakano et al. (2007) who have found no collagen type IV
α1–α6. In mouse, Berkholtz et al. (2006) have illustrated collagen type IV in the theca, although the resolution is not sufficient to distinguish sub-endothelial collagen from thecal matrix.

On comparing bovine and murine follicles, major differences can generally be seen in the expression patterns of nidogens 1 and 2 and perlecan. In the bovine, they appear at the preantral stage and remain present during the antral stage (McArthur et al. 2000; Irving-Rodgers and Rodgers 2006), whereas in mouse, they are present at all stages. In the theca, these molecules are present as aggregates, just as the collagens and laminins are in mouse, but nidogens 1 and 2 and perlecan have a fibrous pattern within the murine membrana granulosa. The thecal matrix of mice also contains nidogens 1 and 2 if they are all expressed, and in both species, this is the case. However, the reason that these molecules differ so considerably between mice and bovine is not clear at this stage. We assume that the arrangement of nidogens 1 and 2 and perlecan in mice is the true expression pattern of these components in this species. The more restricted staining pattern in the bovine ovary might be caused by the use of primary antibodies with specificity against mouse, which may react with these components to a more limited extent in the bovine ovary than in the mouse ovary. Alternatively, this could reflect a difference in the cross-linking of these molecules to other less-soluble molecules in the ovary in mice, rather than there being an inherent major difference in the mouse.

The sub-endothelial basal laminas of the mouse thecal capillary contain laminin β1 and γ1, perlecan, nidogens 1 and 2 and collagen type IV α1 and α2. Collagen type IV α1 and α2 have also been demonstrated in the blood
vessels in the theca of rat (Frojdman et al. 1998) and cow, in addition to laminin β1 and β2 (Rodgers et al. 1998; van Wezel et al. 1998). On interpreting the results from Huet et al. (1997), the sub-endothelial basal lamina of the ovine thecal capillary clearly contains laminin, collagen type IV and a heparan sulphate proteoglycan (Huet et al. 1997).

Basal lamina matrix components have now been identified in the corpora lutea of bovine (Irving-Rodgers et al. 2004), human (Irving-Rodgers et al. 2006b) and murine (Nakano et al. 2007; present results) ovaries. In general, two different types of basal lamina matrix occur in the corpora lutea: sub-endothelial basal lamina and an interstitial matrix located as aggregates at irregular intervals between the non-vascular cells (Irving-Rodgers et al. 2004, 2006b). The earlier speculation about the presence of a basal lamina associated with luteal cells (Deane et al. 1966; Deane et al. 1966).

**Table 3** Number of preantral and antral follicles examined and mean percentage ± SEM of follicles with focimatrix identified by immunostaining of cross sections for various extracellular matrix molecules

| Matrix molecule       | Number of examined follicles | Percentage of positive follicles |
|-----------------------|------------------------------|---------------------------------|
| Laminin α1            | 94                           | 70.1±7.6                        |
| Laminin β1            | 260                          | 40.8±6.8                        |
| Laminin γ1            | 94                           | 71.5±9.7                        |
| Nidogen-1             | 260                          | 58.3±5.9                        |
| Nidogen-2             | 120                          | 36.7±7.0                        |
| Perlecan              | 230                          | 38.7±2.8                        |
| Collagen type XVIII    | 267                          | 23.8±3.9                        |
Farin et al. 1986; O’Shea 1987; Kenny et al. 1989) has previously been discussed in detail (Irving-Rodgers et al. 2006c). The current study adds further evidence that no such basal lamina exists. In the mouse basal lamina, matrix components are all associated with the sub-endothelial basal lamina, which are positive for collagen type IV $\alpha_1$ and $\alpha_2$, as observed previously (Nakano et al. 2007), and laminin $\alpha_1$, $\beta_1$ and $\gamma_1$ chains, nidogens 1 and 2, perlecan and collagen type XVIII but not laminin $\alpha_2$, $\alpha_4$, or $\alpha_5$. In addition, no differences have been observed between the younger cyclic corpora lutea and the older pregnancy corpora lutea. The human luteal sub-endothelial basal laminae contain collagen type IV $\alpha_1$ and laminins $\alpha_5$, $\beta_2$ and $\gamma_1$ (Irving-Rodgers et al. 2006b). In the human, laminin $\alpha_4$ and $\beta_1$ chains occur in the sub-endothelial basal lamina only at mid-luteal to regressing stages and no laminin $\alpha_1$ and $\alpha_3$ chains are present (Irving-Rodgers et al. 2006b).

Endostatin is a proteolytic fragment of collagen type XVIII that can potently inhibit angiogenesis and induces tumour regression in mice (Boehm et al. 1997) and human (Herbst 2002). The presence of collagen type XVIII in the follicular basal lamina early in follicular development but not at the later antral stages correlates inversely with vascularisation of the thecal layers, suggesting that endostatin is produced or released from the follicular basal lamina during follicular development. However, collagen type XVIII null mice are fertile thereby suggesting that collagen type XVIII is not absolutely necessary for follicular development.

Thus, in summary, whereas the structure and composition of basal lamina matrices in mouse is generally similar...
to those in other species, the patterns of nidogens 1 and 2 and perlecan are different in the follicular basal lamina, foci matrix and thecal matrix. This may reflect a difference in the cross-linking of these molecules to other less-soluble molecules in the ovary in mice, rather than representing an inherent major difference in the mouse.

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