Comparative Medicine
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Vol 70, No 6
December 2020
Pages 510–519

Original Research

Cotton Rat Placenta Anatomy and Fc Receptor Expression and Their Roles in Maternal Antibody Transfer

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Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis and viral pneumonia in infants and young children worldwide. Currently no vaccine is available to prevent RSV infection, but virus-neutralizing monoclonal antibodies can be given prophylactically, emphasizing the protective potential of antibodies. One concept of RSV vaccinology is mothers' immunization to induce high antibody titers, leading to passive transfer of high levels of maternal antibody to the fetus through the placenta and to the neonate through colostrum. Cotton rats are an excellent small animal model for RSV infection and have been used to test maternal immunization. To mechanistically understand antibody transfer in the cotton rat model, we characterized the cotton rat placenta and Fc receptor localization. Placentas from cotton rats at midgestation (approximately day 14) and at late gestation (approximately day 25) and neonatal (younger than 1 wk) gastrointestinal tracts were collected for light microscopy, immunohistochemistry, and transmission electron microscopy. The cotton rat placenta is hemotrichorial and has 5 distinct layers: decidua, junctional zone, labyrinth, chorionic plate, and yolk sac. Consistent with the transfer of maternal antibodies, the majority of the Fc receptors are present in the yolk sac endoderm and fetal capillary endothelium of the chorionic plate, involving 10% of the cells within the labyrinth. In addition, Fc receptors are present on duodenal and jejunal enterocytes in cotton rats, similar to humans, mice, and rats. These findings provide the structural basis for the pre- and postnatal transfer of maternal antibodies described in cotton rats.

Abbreviations: FcRn, fetal Fc receptor; PAS, periodic acid–Schiff–diastase; rER, rough endoplasmic reticulum; RSV, respiratory syncytial virus; TEM, transmission electron microscopy; uNK cells, uterine natural killer cells

DOI: 10.30802/AALAS-CM-20-000040

Cotton rats (*Sigmodon hispidus*) have been used to study the pathogenesis of human respiratory syncytial virus (RSV) infection.[6,15,29] This species is considered the best small animal model for RSV pathogenesis, because RSV replicates similarly in cotton rats as in infants and induces an adaptive immune response, as is seen in infants with moderately severe RSV infection.[4,5,33,38] Cotton rats have been used to accurately predict the efficacy of the only prophylactic treatment for RSV, a monoclonal antibody against the fusion protein.[5,17,31] In addition, cotton rats are the 'gold standard' for RSV vaccine development.[6,15,29,39]

A proposed method to protect infants from RSV-induced severe respiratory disease is to vaccinate pregnant mothers to increase the level of maternal antibodies transferred to fetuses and infants.[2] Human gestation has a duration of approximately 270 d, with midgestation corresponding to approximately day 135 and late gestation to approximately day 225. The human placenta is hemomonochorial in that trophoblasts are in direct contact with the maternal blood supply. A single continuous layer of trophoblasts separates fetal capillaries from the maternal blood.[23,34] Maternal antibodies are predominantly transferred in utero through the placenta. Maternal IgG is endocytosed by syncytiotrophoblasts, where IgG_1 and IgG_4 bind to the neonatal Fc receptor (FnRn) at an acidic pH in early endosomes.[11,25] The endosomal IgG-FcRn complex undergoes transcytosis and fuses with the basal membrane where IgG disassociates from the FcRn. The FcRn is then recycled back to the maternal blood surface of the syncytiotrophoblasts.[11,25,28,30] After birth, the FcRn in the intestine binds maternal IgG in the milk at the acidic apical surface of the epithelium and transports it in an endosome to the basolateral surface, where the maternal IgG disassociates and enters the neonatal blood stream.[20,21]

In cotton rats, gestation is 28 d in duration, with midgestation at approximately day 15 and late gestation at about day 25. The average litter size is 6 pups, with a range of 4 to 8 pups. Reportedly, maternal antibodies to RSV are transferred to cotton rat pups both prenatally and postnatally.[32] Maternal immunization with RSV vaccine candidates in cotton rats has demonstrated that maternal antibodies are transferred to pups and confer protection, but this protection is only short-lived.[2] In contrast, maternal antibodies after measles immunization provide long-term protection in cotton rats.[28] Further investigation of the mechanisms of prenatal and postnatal antibody transfer in cotton rats is necessary to understand these differences and evaluate RSV vaccine candidates. As a basis for these physiologic studies, the placental anatomy and the expression of FcRn in cotton rat must be characterized. In this study, we report the histologic and ultrastructural characterization of the cotton rat placenta

Received: 24 May 2020. Revision requested: 20 Jul 2020. Accepted: 24 Aug 2020.
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at mid- and late gestation and the localization of FcRn in the placenta and neonatal intestine.

Materials and Methods

Animals. Inbred cotton rats were bred and maintained in breeding pairs, as previously described.[15,16] Cotton rats were free from mouse and rat parvovirus, minute virus of mice and rats, parvovirus NS1, mouse hepatitis virus, murine norovirus, Theiler murine encephalomyelitis virus, murine and rat rotavirus, Sendai virus, pneumonia virus of mice, reovirus, *Mycoplasma pulmonis*, lymphocytic choriomeningitis virus, adenovirus types 1 and 2, ectromelia virus, mouse pneumonitis virus, polyoma virus, mouse cytomegalovirus, Hantaan virus, *Encephalitozoon cuniculi*, *Filabacterium rodentium*, mouse thymic virus, Prospect Hill virus, lactase dehydrogenase-elevating virus, Toolan H1 virus, Kilham rat virus, rat sialodacryoadenitis virus, rat theilovirus, and *Pneumoncystis carinii* according to quarterly health monitoring of immunocompetent CD1 sentinel mice and rats exposed to 100% pooled dirty bedding from cotton rats at each cage change. All experimental procedures received IACUC approval through Ohio State University, and cotton rats were cared for in accordance with *the Guide for the Care and Use of Laboratory Animals*.[18]

Animals were housed as previously described[16] in standard polycarbonate cages (Tecniplast, Exton, PA), with breeding cotton rats and preweaned litters on aspen bedding in the AAALAC-accredited University Laboratory Animal Resources vivarium of Ohio State University. Cotton rats were maintained in an environment with a temperature of 20 ± 2 °C, 30% to 70% relative humidity, a 12:12-h light:dark cycle, and ad libitum access to chlorinated reverse-osmosis–purified water and standard rodent diet (Teklad 2019s, Envigo, St Louis, MO). Cages were sanitized by using a rack washer, and cleaning in the room was completed by using Opticide (Micro-Scientific Industries, Gurnee, IL) or SporKlenz (Steris, St Louis, MO) disinfectant.

Pathology. Female cotton rats were euthanized via carbon dioxide inhalation. Complete reproductive tracts, gastrointestinal tracts, and lungs were sampled from a single nongravid female cotton rat and from gravid female cotton rats at gestational days 15 and 25. All tissues, including 3 to 5 individual placentas from each gravid cotton rat, were fixed in 10% neutral buffered formalin. All tissues were routinely processed, embedded in paraffin wax, and sections (4 µm) were stained routinely with hematoxylin and eosin. Light microscopic (model CX41, Olympus, B and B Microscopes, Pittsburgh, PA) evaluation was performed by a veterinary anatomic pathologist in training (MM) and a board-certified veterinary pathologist (KMDL). For each cotton rat, 6 to 10 sections of placenta were evaluated.

Histochemistry and immunohistochemistry. Sections of formalin-fixed, paraffin-embedded, individual placentas were stained with Periodic acid–Schiff–diastase (PAS) and as various immunohistochemical stains including: von Willebrand factor (factor VIII-related antigen; dilution, 1:1000; polyclonal rabbit antihuman; catalog no. A0082; Dako, Carpinteria, CA); cytokeratin (1:50; monoclonal antihuman; M3515; Dako); vimentin (1:250; monoclonal antirabbit; 2862; Epitomics, Burlingame, CA); CD68 (1:50; monoclonal antimouse; MCA341R; Serotec, Kidlington, United Kingdom); NK1.1 (1:50; monoclonal antimouse; 553165; BD Biosciences, San Jose, CA); FCRN (1:50; polyclonal goat antimouse IgG, AF6775; R and D Systems, Minneapolis, MN); and FCGRT/FCRN (1:500; polyclonal rabbit antihuman IgG; ab98201; Abcam, Cambridge, MA). Neonatal gastrointestinal tracts were stained with FCGRT/FCRN. Transverse sections of individual, gestational day 15 placentas were cryopreserved by using optimal cutting temperature compound (Sakura Finetek, Torrance, CA) and stained with ALP (1:400; polyclonal rabbit antirat, ab95462, Abcam), human chorionic gonadotropin (1:450; polyclonal rabbit antihuman, A0231, Dako), and placental lactogen (1:50; polyclonal rabbit antihuman, ab15554, Abcam).

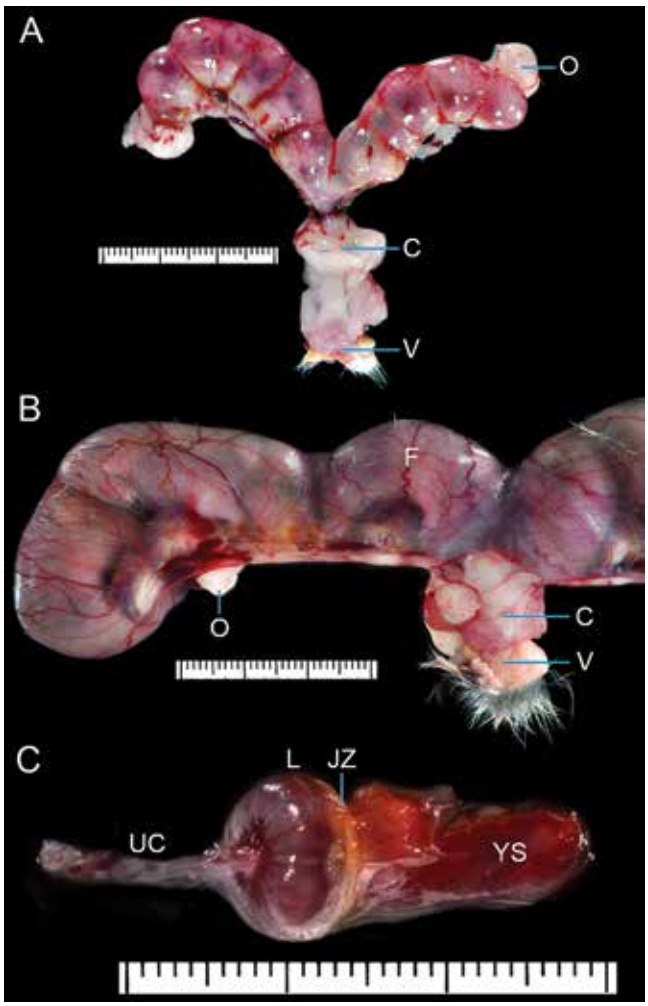

Figure 1. Gross images of cotton rat uterus and placenta. (A) Midgestation (GD15) gravid reproductive tract. The cotton rat female tubular genitalia is characterized by a single vaginal cavity, single muscular and bulbous cervix, a single uterine body, and 2 uterine horns (bicornuate), each with a serpiginous oviduct and associated ovary. (B) Late gestation (GD25) reproductive tract. Each gravid uterine horn contains multiple fetuses each within their separate yolk sac, and attached to the uterus via the umbilical cord and a discoid placenta. (C) Single discoid placentation site (fetal membranes) from a late gestation (GD25) fetus. The umbilicus is a single strand of tortuous vessels and connective tissue that enters the fetus. The surface of the discoid placenta toward the fetus where the umbilicus enters the placenta, has a dark red zone, which represents the highly vascularized labyrinth. The adjacent yellow band represents the junctional zone. The yolk sac is a thin-soft membrane that envelops the fetus. The yolk sac was iatrogenically ruptured and reflected backward to evaluate the other layers grossly. Ovaries (O), cervix (C), single fetus (F), vagina (V), umbilical cord (UC), labyrinth (L), junctional zone (JZ and arrow), yolk sac that is inverted manually (YS).

Transmission electron microscopy (TEM). Sections (1 mm^3) of individual, gestational day 15 placentas were fixed in 3% buffered glutaraldehyde and postfixed with 1% osmium tetroxide, serially dehydrated, infiltrated in an acetone–epoxy plastic, and then embedded in a plastic mold. Ultrathin sections (55 to 60

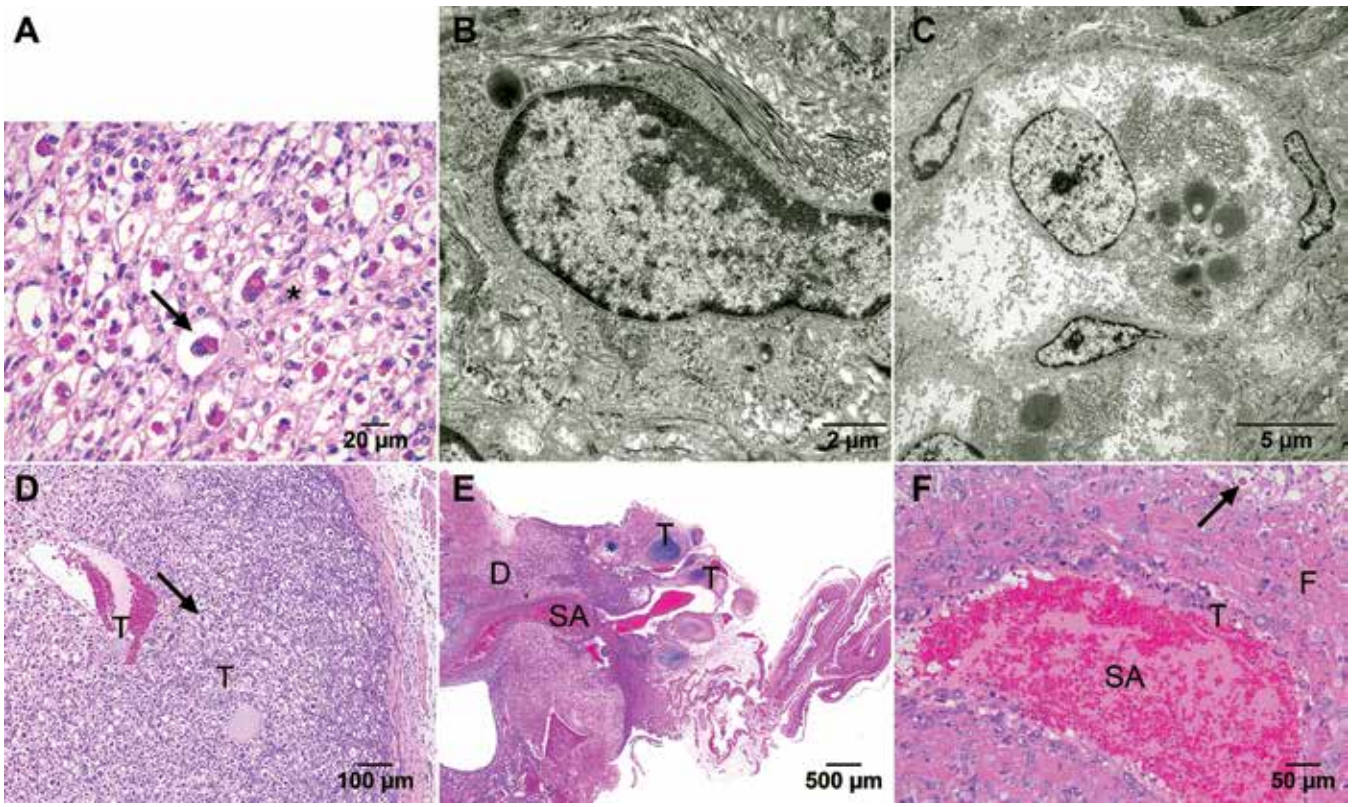

Figure 2. Macroscopic view of cotton rat placenta. (A) Gestational day 15. (B) Gestational day 25. CP, chorionic plate; D, decidua; JZ, junctional zone; L, labyrinth; M, myometrium; TGC, trophoblast giant cell; VYS, visceral yolk sac. Hematoxylin and eosin staining, bar scale represents 500 µm.

µm) of the plastic blocks were cut using an ultramicrotome, placed on copper grids, and stained with uranyl acetate and lead citrate. Sections were then examined under a transmission electron microscope (JEM-1400, JOEL USA, Peabody, MA), and images were captured by using an SIS Veleta 2K camera (Olympus Soft Imaging Solutions, Muenster, Germany).

Results

Placental anatomy. The macroscopic anatomy of the female reproductive tract of cotton rats is similar to that in other rodents and is described in further detail in Figure 1 A through C. The cotton rat female tubular genitalia is characterized by a single vaginal cavity, single muscular and bulbous cervix, a single uterine body, and two uterine horns (bicornuate), each with a serpiginous oviduct and associated ovary. Each gravid uterine horn contains multiple fetuses each within their separate yolk sac and attached to the uterus via the umbilical cord and a discoid placenta. The umbilicus is a single strand of tortuous vessels and connective tissue that enters the fetus. The surface of the discoid placenta towards the fetus where the umbilicus enters the placenta, has a dark red zone, which represents the highly vascularized labyrinth. The adjacent yellow band represents the junctional zone. The yolk sac is a thin soft membrane that envelops the fetus. The cotton rat placenta is histologically classified into 5 distinct layers: decidua, junctional zone, labyrinth, chorionic plate, and yolk sac (Figure 2 A and B), according to comparable human, mouse, and rat placental anatomy.[1,7,9,12,13,34] The fetal component comprises the yolk sac, chorionic plate, labyrinth, and junctional zone, as determined by comparing the morphology and immunolabelling of cotton rat cells with those of human and other rodent placentas. Likewise, the maternal component comprises the decidua.

Decidua and uterus. The decidua is a mixture of decidualized stromal cells and uterine natural killer (uNK) cells. The decidualized stromal cells were vimentin-positive (data not shown) spindle cells with abundant, lacey, vacuolated, variably PAS-positive cytoplasm (data not shown) and a single, round to oval nucleus (Figure 3 A). In addition, cotton rat uNK cells were variably immunoreactive for the macrophage marker CD68 (data not shown); however, immunostaining for the decidual marker ALP was unsuccessful. TEM showed that the decidualized stromal cells had abundant cytoplasmic glycogen, several profiles of rough endoplasmic reticulum (rER) and mitochondria, and few lysosomes (Figure 3 B). Decidualized stromal cells formed tight junctions to adjacent stromal cells, with dissecting interstitial collagen.

uNK cells were characterized histologically by a clear halo (representing artifactual separation); a single, round nucleus; and abundant, PAS-positive, brightly eosinophilic cytoplasmic granules (Figure 3 A). The cells did not stain with antibodies against vimentin, cytokeratin, CD68, or NK1.1. TEM revealed occasional tight junctions with other uNK and interweaving collagen fibrils between the uNK and decidualized stromal cells. In addition, cotton rat uNK cells had abundant rER, few mitochondria, electron-lucent granular cytoplasmic substance, and fewer than 6 electron-dense, granular vesicles consistent with PAS-positive cytoplasmic granules (Figure 3 C). Approximately half of the cells within the decidua of the midgestation cotton rat placenta were uNK cells, with the remaining cells corresponding to decidualized stromal cells (Figures 2 A and 3 A). Conversely, in late-gestation decidua, one fourth of the cells were uNK, and the remaining cells were densely compacted decidualized stromal cells with abundant eosinophilic cytoplasm (Figure 2 B).

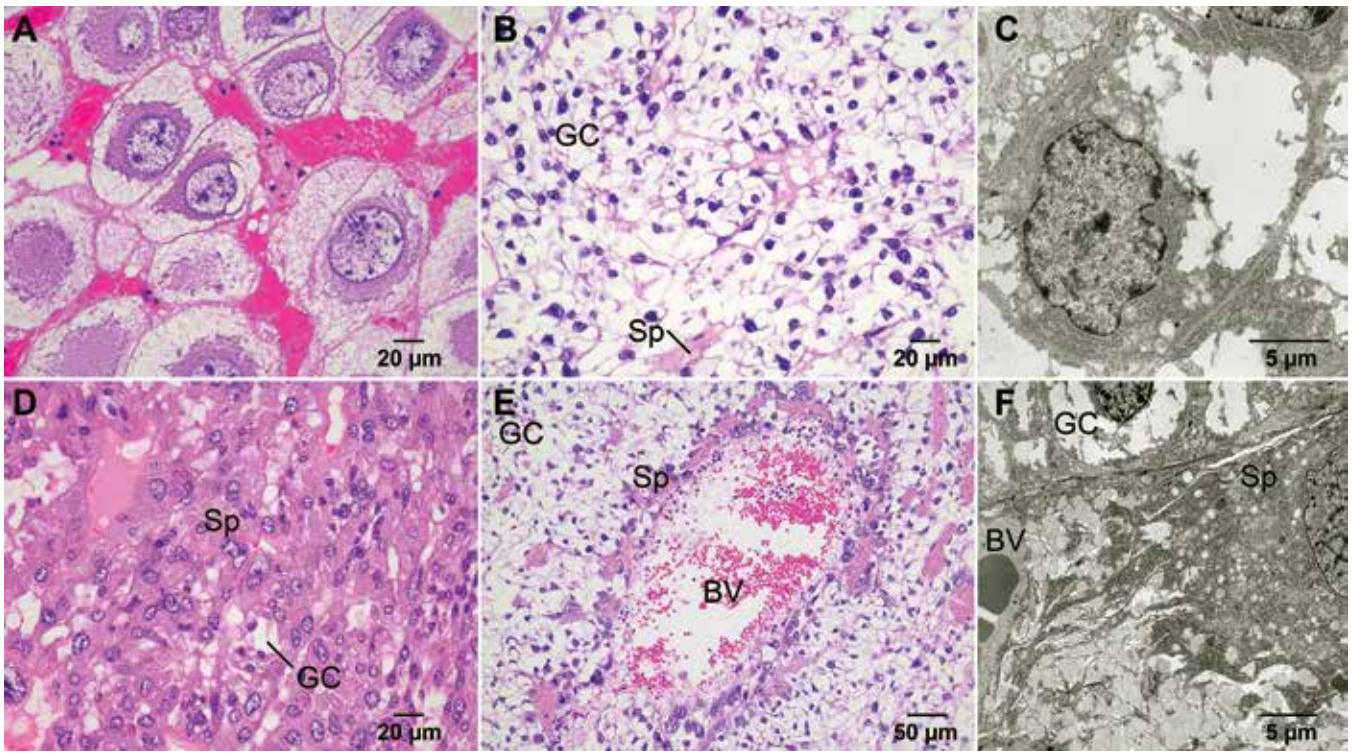

Figure 3. Histologic and ultrastructural characteristics of the cotton rat decidua. (A) Light microscopy (hematoxylin and eosin staining), gestational day 15. TEM, gestational day 15, (B) decidual cell; (C) uterine NK cell. Light microscopy, gestational day 25, (D) decidua with invasive trophoblasts; (E) decidua with a tortuous and remodeled spiral artery and exiting trophoblasts in uterine vessels; (F) decidua with trophoblast remodeled spiral artery. *, decidualized stromal cell; arrow, uterine natural killer cell; D, decidua; F, fibrinoid matrix F; SA, spiral artery; T, trophoblast.

Some sections of mid- and late-gestation cotton-rat placentas contained a thin, densely packed band of decidualized stromal cells, with few uNK between the junctional zone and the overlying layer of decidua (which contained higher numbers of uNK). In mid- and late-gestation cotton rats, trophoblasts invaded into the decidual interstitium adjacent to vessels, between uNK and decidualized stromal cells, in the myometrium, and occasionally in vessels lined by endothelium exiting the uterine serosa (Figure 3 D and E). In the spiral arteries traversing through the decidua, trophoblasts invaded and lined arteries and was accompanied by a fibrinoid exudate that expanded the vessel walls (Figure 3 F).

Junctional zone. Beneath the decidual layer is the junctional zone, also known as the basal zone or trophospongium. This layer comprised spongiotrophoblasts, glycogen cells, and trophoblast giant cells, with all 3 cell types demonstrating cytokeratin immunoreactivity (data not shown). Between decidua and glycogen cells (spongiotrophoblasts) was a layer of trophoblast giant cells. Histologically, these cells had marked anisocytosis and anisokaryosis, were 80 to 150 µm in diameter, and were bathed in maternal blood in their sinusoidal form (Figure 4 A). The outer rim of cytoplasm was finely vacuolated and lacey, with a more densely eosinophilic perinuclear cytoplasm.

During midgestation, the predominant cell type within the junctional zone of the cotton rat placenta was the glycogen cell, which had a single, round nucleus and abundant clear lacey cytoplasm, with scant perinuclear eosinophilic cytoplasm (Figures 2 A, 4 B, and 4 E); binucleated cells were occasionally present. The cell membrane and scant perinuclear cytoplasm were PAS positive, with the remaining cytoplasm lightly PAS positive (data not shown). TEM demonstrated that the perinuclear cytoplasm of the glycogen cells comprised rER and mitochondria. The remaining cytoplasm consisted of electron-lucent, finely granular substance with papillary projections (Figure 4 C).

The late-gestation placenta had dramatically fewer glycogen cells than the midgestation organ, and the predominant cell type was the spongiotrophoblast (Figures 2 B and 4 D). In both stages of gestation, the spongiotrophoblasts had moderate amounts of eosinophilic, lacey, PAS-positive (data not shown) cytoplasm that formed stellate-like cytoplasmic projections (Figures 4 B, 4 D and 4 E). These cells had a single round-to-elongated nucleus, with moderate anisokaryosis, stippled chromatin, and frequent binucleated or multinucleated cells (syncytia). In midgestation, spongiotrophoblasts were scattered between glycogen cells and highly concentrated around maternal blood vessels (Figure 4 E). In contrast, spongiotrophoblasts formed sheets of cells with interspersed glycogen cells in late gestation; however, the spongiotrophoblasts are still highly associated with the maternal blood supply (Figure 4 D). The endothelium of the traversing maternal vasculature was discontinuous, and spongiotrophoblasts were in direct contact with maternal blood (Figure 4 E). TEM further demonstrated this organization, in addition to the rER-rich cytoplasm of these cells that contained dozens of electron-dense vesicles and mitochondria (Figure 4 F). Protruding into the lumens of the maternal vessels were hundreds of cytoplasmic papillary projections. Tight junctions adhered spongiotrophoblasts to neighboring glycogen cells. Immunohistochemically, all junctional zone cells were negative for ALP, placental lactogen, and human chorionic gonadotropin.

Labyrinth. The maternal–fetal interface in cotton rats is the labyrinth, which is composed of maternal sinusoids, cytotrophoblasts, syncytiotrophoblasts, and fetal capillaries. Late-gestation placental labyrinths were larger than those at midgestation (Figure 2 A and B). Cotton rats have a hemotrichorial placentation,

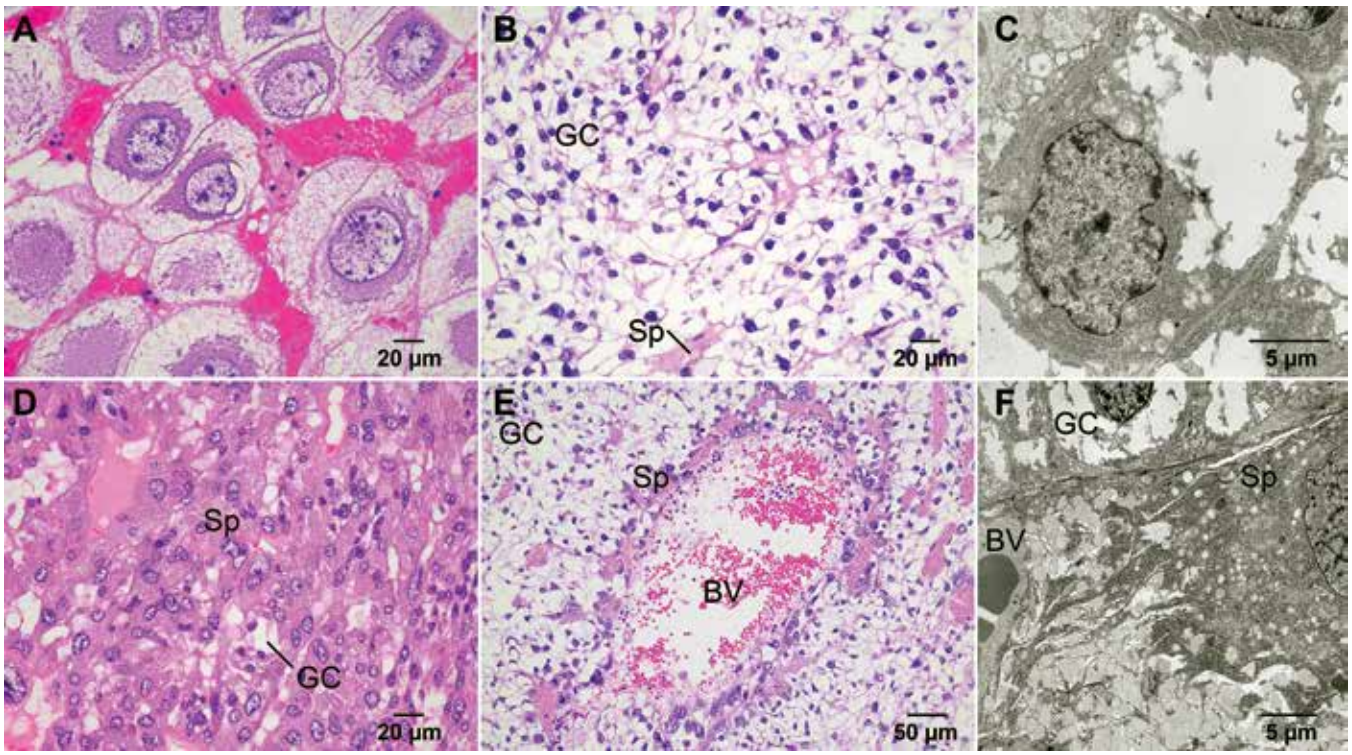

Figure 4. Histologic and ultrastructural characteristics of cotton rat trophoblast giant cells and the junctional zone. (A) H and E of GD15 trophoblast giant cells (TGC). (B) H and E of GD15 junction zone glycogen cells. (C) TEM of GD15 glycogen cell. (D) GD25 junctional zone with a predominance of spongiotrophoblasts. (E) GD15 maternal blood vessel with surrounding spongiotrophoblasts. (F) TEM of GD15 spongiotrophoblast adjacent to maternal blood vessel. Glycogen cells (GC), spongiotrophoblast (SP), maternal blood vessel (BV).

in which 3 layers of trophoblasts (cytotrophoblast, syncytiotrophoblast I [SI] and II [SII]) separate the maternal and fetal blood supplies and which was best visualized by using TEM (Figure 5 A through C).

Maternal sinusoids in cotton rats contain maternal anucleated RBC, easily distinguishing them from nucleated fetal red blood cells. Maternal sinusoids are supplied by centrally located arterioles present in the junctional zone and lined by cytotrophoblasts.; in other rodents, these cells are also known as cellular trophoblasts or mononuclear trophoblasts. In cotton rats, these cytotrophoblasts were mononuclear and had marked karyomegaly, with coarsely stippled chromatin, prominent nucleoli, and moderate amounts of eosinophilic cytoplasm (Figure 5 A). Large nuclei of cytotrophoblasts often bulged into the maternal sinusoid. Ultrastructurally, direct contact between cytotrophoblasts and maternal sinusoids was appreciated, as well as numerous villous projections extending into the sinusoid from the cytotrophoblast (Figure 5 D). Also present were numerous profiles of rER and smooth endoplasmic reticulum, few mitochondria, and several vesicles. Occasionally the cytotrophoblast layer became thin, with only scant overlying cytoplasm and villous projections separating the maternal sinusoid from the syncytiotrophoblast layer (Figure 5 C). Tight junctions and desmosomes were present between the cytotrophoblast and the underlying syncytiotrophoblast layer I. Both the cytotrophoblasts and the syncytiotrophoblasts are immunoreactive for cytokeratin, allowing for identification and distinction from the vimentin-positive fetal capillary endothelium (Figure 5 F and G).

Histologically, syncytiotrophoblast layers I and II were indistinct and often resembled multinucleated spindle-like cells with marked anisokaryosis and abundant, lacey, vacuolated, eosinophilic cytoplasm (Figure 5 A). Unexpectedly, both were cytokeratin negative (Figure 5 F). TEM further emphasized the multinucleated nature of syncytiotrophoblasts. Syncytiotrophoblast layer II, subjacent to the cytotrophoblasts, contained slightly osmophilic vesicles, several mitochondria and rER, and numerous cytoplasmic filaments. Syncytiotrophoblast layers II and SI had numerous interlacing cytoplasmic projections and were separated by gap junctions and infrequent tight junctions (Figure 5 E). Syncytiotrophoblast layer I, which lies between syncytiotrophoblast layer II and the fetal endothelium, also had numerous, slightly osmophilic vesicles (consistent with lipid), and few mitochondria and rER. Syncytiotrophoblast layer I and fetal endothelium shared a basal lamina and were closely adhered to one another. The fetal endothelium had numerous rER, frequent mitochondria, and infrequent cytoplasmic fibrils, consistent with elastin or smooth muscle actin. In addition, the endothelium contained several cytoplasmic villous projections into the fetal capillary lumen. Within the capillary lumens were nucleated fetal RBC, which were appreciated histologically and by TEM.

Yolk sac. The yolk sac of cotton rats has 2 components, the parietal yolk sac and visceral yolk sac. The visceral yolk sac attaches to the chorionic plate, wraps around the labyrinth, and transitions into the parietal yolk sac overlying the Reichert membrane at the trophoblast giant cells and labyrinth, forming the yolk sac between the labyrinth–parietal yolk endoderm and visceral yolk endoderm. The parietal yolk sac is a single, cuboidal cell layer overlying the Reichert membrane (Figure 6 A and B). Histologically, the parietal yolk sac endoderm had scant, eosinophilic cytoplasm and a single, polygonal nucleus. The cotton rat Reichert membrane is a homogenous brightly eosinophilic band of variable thickness. During midgestation, it was intermittently indiscernible and 2 to 4 µm in thickness (Figure 6 A); in late gestation, membrane thickness ranged from 6 to 40 µm (Figure 6 B). Under TEM, the membrane composition

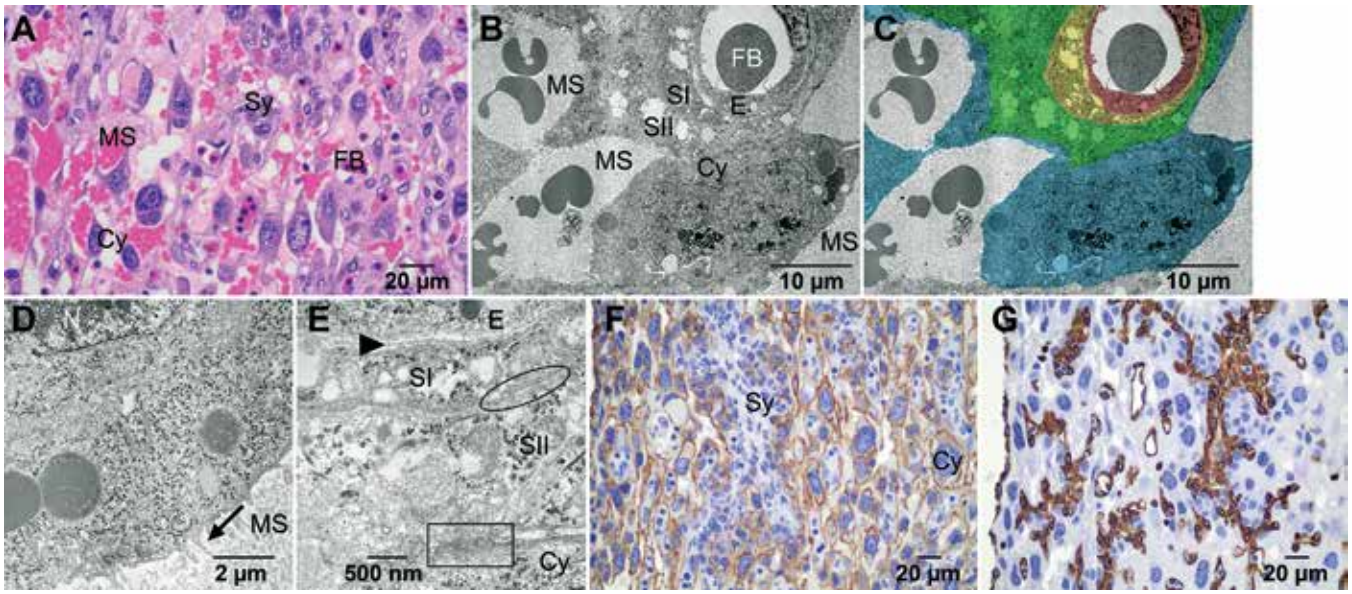

Figure 5. Histologic and ultrastructural characteristics of cotton rat labyrinth. (A) TEM of GD15 labyrinth. (B) TEM of GD15 labyrinth. (C) TEM of GD15 labyrinth with coloring of the different layers between the maternal and fetal blood supplies. (D) TEM of cytotrophoblast. (E) TEM of cellular junctions between layers of labyrinth. (F) Cytokeratin of GD15 labyrinth. (G) Vimentin staining of GD15 labyrinth. Maternal sinusoid (MS), cytotrophoblast (CY, blue), syncytiotrophoblast I (SI, yellow), syncytiotrophoblast II (SII, green), syncytiotrophoblast (SY), fetal capillary endothelium (E, red), fetal red blood cell (FB), nucleus (N), villous projections (arrow), tight junction (square), basement lamina (arrowhead), gap junction (oval).

was heterogeneous, with finely to coarsely granular and fibrillar material arranged parallel to the membrane surface (Figure 6 C). TEM also demonstrated that the parietal yolk endoderm was comprised of abundant smooth ER and rER, with few electron-lucent vesicles. On the labyrinthine side of the Reichert membrane were multiple maternal blood sinusoids lined by a single, mononuclear, cytokeratin-positive, fenestrated trophoblast similar to cytotrophoblasts.

The visceral yolk sac of cotton rats was villous, with projections oriented toward the labyrinth (Figure 6 A and B). The visceral yolk sac contained connective tissue, multiple endothelium-lined small-caliber vessels and a single columnar cell layer of yolk sac endoderm. The endoderm had abundant, vacuolated, eosinophilic cytoplasm with multiple, apical, brightly eosinophilic, cytoplasmic droplets (Figure 6 D). Large pools of fetal nucleated red blood cells within vessels are prominent during midgestation (Figure 6 A), with fewer smaller pools of nonnucleated red blood cells in late gestation. During late gestation, the endoderm is enlarged with more frequent eosinophilic droplets. Under TEM, the visceral yolk sac endoderm is columnar to pseudostratified with apical, villous, cytoplasmic projections and several, apical, deeply and moderately osmophilic vesicles (Figure 6 E). Also present in the cytoplasm are abundant smooth ER, rER, mitochondria, and basally oriented electron-lucent vesicles. The visceral yolk sac endoderm adheres to adjacent endodermal cells through desmosomes and adherens junctions. The endoderm overlies a thin basement membrane with underlying nonfenestrated endothelium covering the fetal capillary, which contains nucleated fetal red blood cells. Between the endothelium and the outer serosal squamous cell is a band of collagen fibrils.

Chorionic plate, umbilicus, and amnion. The chorionic plate of cotton rats is a band of connective tissue and endothelial-lined fetal vessels, associated with cuboidal epithelium on the surface toward the labyrinth (Figure 6 F). The fetal vessels and associated connective tissue stroma extended into the labyrinth and then branched into the fetal capillaries in the labyrinth. The umbilical artery and vein supply the chorionic plate vessels, and the connective tissue within the umbilical cord was continuous with the connective tissue comprising the chorionic plate. Near the junction of the umbilicus and chorionic plate is the attachment site of the amnion. The amnion encircles the fetus and is formed of 2 layers of flattened epithelium, with scant clear space and intervening connective tissue.

Placental circulation. In the placentae of cotton rats, the maternal source of oxygenated blood and maternal antibodies originates with a large artery extending from a large-caliber perimetrial artery into the myometrium. The artery then extends in a serpiginous pattern into the decidua and junctional zone, where fibrin and remodeling by trophoblasts are evident (Figure 7 A) . The artery extends into the labyrinth, where it forms large pools or sinuses lined by cytotrophoblasts beneath the Reichert membrane (Figures 6 A and 7 A). The large sinuses then branch into smaller maternal sinusoids, as previously described. The returning deoxygenated blood from the labyrinth filters into larger sinuses lined by spongiotrophoblasts in the junctional zone (Figure 7 B and D). Two large spongiotrophoblast-lined veins are present on the lateral sides of the serpiginous artery within the junctional zone. The venous sinuses transition into endothelium-lined venules and veins in the decidua and exit the myometrium and perimetrium as a large-caliber vein (Figure 7 E). The umbilical artery transports the deoxygenated fetal blood into small-caliber vessels of the chorionic plate that branch into the labyrinth and form fetal capillaries associated with syncytiotrophoblasts (Figure 7 C). Fetal capillaries then exit into chorionic plate venules, which empty into the umbilical cord vein. Also branching from the umbilicus are arterioles and venules that supply the capillary system of the visceral yolk sac (Figure 7 C).

FcRn expression. We tested 2 antibodies for labeling the neonatal Fc receptor (FcRn) in cotton rats, one against mouse FcRn (AF6775; R and D Systems) and the other against human FcRn (ab98201; Abcam). Although the antimouse FcRn antibody failed to label the cotton rat FcRn in the placenta, we were able to optimize the antihuman FcRn antibody for localizing cotton rat FcRn (Figure 7 F through I). The immunoreactivity of FcRn

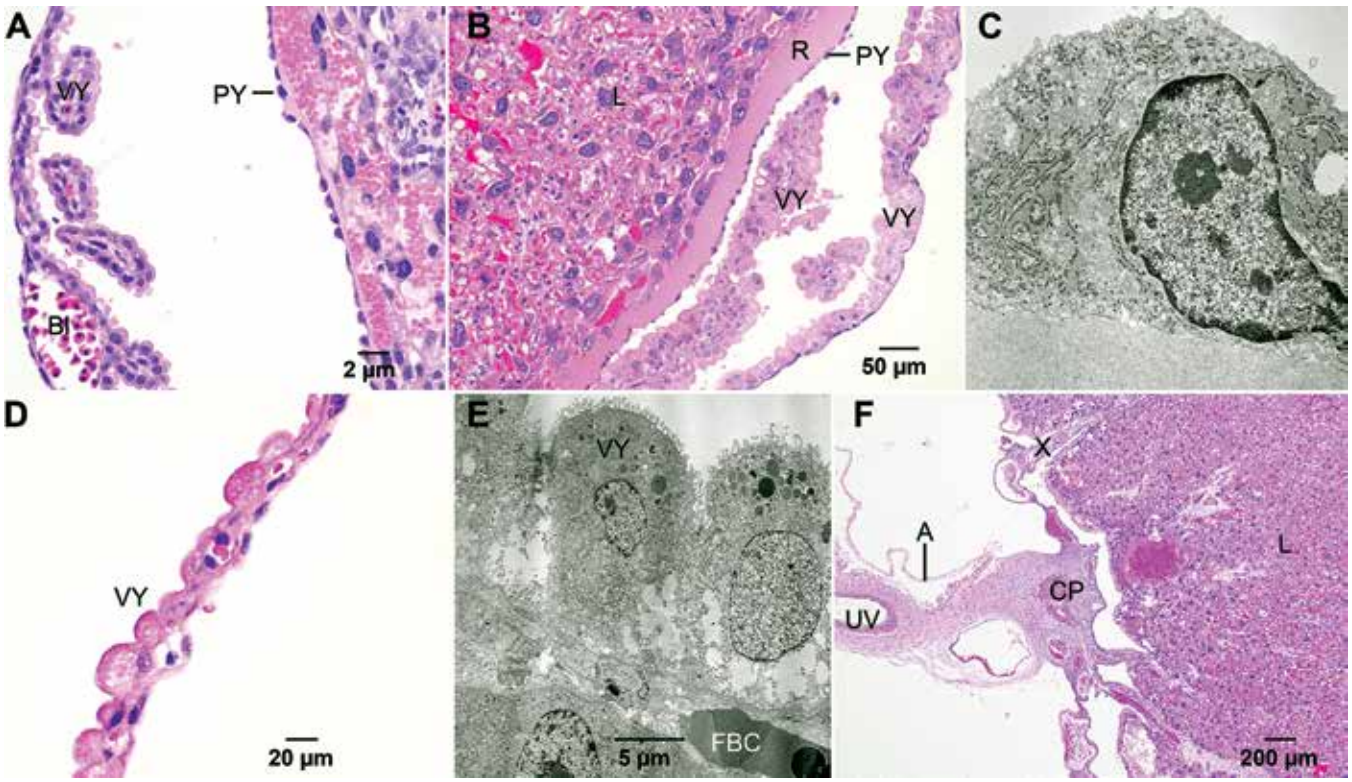

Figure 6. Histologic and ultrastructural characteristics of the cotton rat yolk sac. (A) H and E of GD15 labyrinth, parietal and visceral yolk sac. (B) H and E of 25D labyrinth and yolk sac. (C) TEM of GD15 parietal yolk sac and Reichert membrane. (D) H and E of GD25 parietal yolk sac. (E) TEM of visceral yolk sac. (F) H and E of GD25 umbilicus, amnion, chorionic plate and labyrinth. Labyrinth (L), Reichert membrane (R), Visceral yolk endoderm (VY), Parietal yolk endoderm (PY), fetal red blood cell (FBC), blood island (BI), Umbilical vein (UV), Chorionic plate (CP), Amnion (A), infiltrating fetal capillaries into the labyrinth (X).

was moderate to strong in the endothelium of small-caliber venuoles and vein within the chorionic plate and extended from the chorionic plate into the labyrinth (Figure 7 F). The umbilical cord vein and artery endothelium exhibited similar staining properties. In addition, scattered positive, round-to-spindle cells interpreted as fetal macrophages were noted in the chorionic plate and umbilical cord connective tissue (Figure 7 F). Similarly, stained cells were present in the labyrinth stroma, which may represent macrophages analogous to Hofbauer cells in humans (Figure 7 G). Deeper within the labyrinth, some (but not all) fetal capillary endothelial cells exhibited faint membranous staining. Finally, flat spindle cells interpreted as syncytiotrophoblasts that lined blood-filled sinuses containing anucleated red blood cells exhibited variable but moderate cytoplasmic and membranous staining (Figure 7 G).

The main site of FcRn expression in the cotton rat placenta was the visceral yolk sac endoderm, with strong cytoplasmic, especially apical, staining (Figure 7 H). The PAS-positive (data not shown), histologically eosinophilic apical granules within the endoderm may represent the endosomes in which FcRn are located. In addition, the capillary endothelium within the visceral yolk sac exhibited faint membranous staining.

Using immunohistochemistry, we also examined FcRn expression in gastrointestinal tracts (oral cavity to anus) from 6-d-old cotton rats. FcRn expression was limited to the small intestine and was characterized by intense diffuse cytoplasmic staining in duodenal and jejunal enterocytes (Figure 7 I).

Discussion

Cotton rats are used as an animal model to test RSV vaccines. To better understand the transfer of maternal antibodies after maternal immunization, we investigated the histologic and ultrastructural anatomy of the cotton rat placenta, as well as neonatal Fc receptor localization.

The placenta of cotton rats is hemotrichorial, with 3 trophoblast layers separating the maternal blood from the fetal capillary endothelium. This arrangement is different from the hemomonochorial human placenta[7,14] but is similar to that of rats and mice. Compared with the organ in mice and rats, the cotton rat placenta has a continuous and thicker Reichert membrane and lacks metrial glands and an inverted yolk sac. All 3 rodent species have arterial canals lined by spongiotrophoblasts in the junctional zone and similar arterial and venous branching and blood flow.[10,26] However, the invasiveness and function of the trophoblasts differ between species, and these differences are key to understanding why cotton rats show trophoblast deportation to lungs similar to the situation in humans (but not in mice or rats). In cotton rats, decidual arteries are remodeled by trophoblasts, whereas the arteries in mice and rats are remodeled by uNK cells. In humans, trophoblasts can invade up to the inner third of the myometrium, whereas in mice and rats, trophoblast invasion is more superficial and does not extend beyond the decidua.[7,14] Comparable to those in humans, cotton rat trophoblasts invade throughout the decidua, myometrium, and uterine veins. Furthermore, extravillous trophoblasts in humans express a fibrinoid matrix that aids in the remodeling of the spiral arteries to allow optimal blood flow to the human placenta.[1,7] Likewise, the spiral arteries in the cotton rat decidua and junctional zone are lined by trophoblasts that produce a fibrinoid matrix (Figure 3 F). In this study, we confirmed a previous report that cotton rat trophoblasts are found in the lungs of pregnant cotton rats as pulmonary emboli (data not shown),

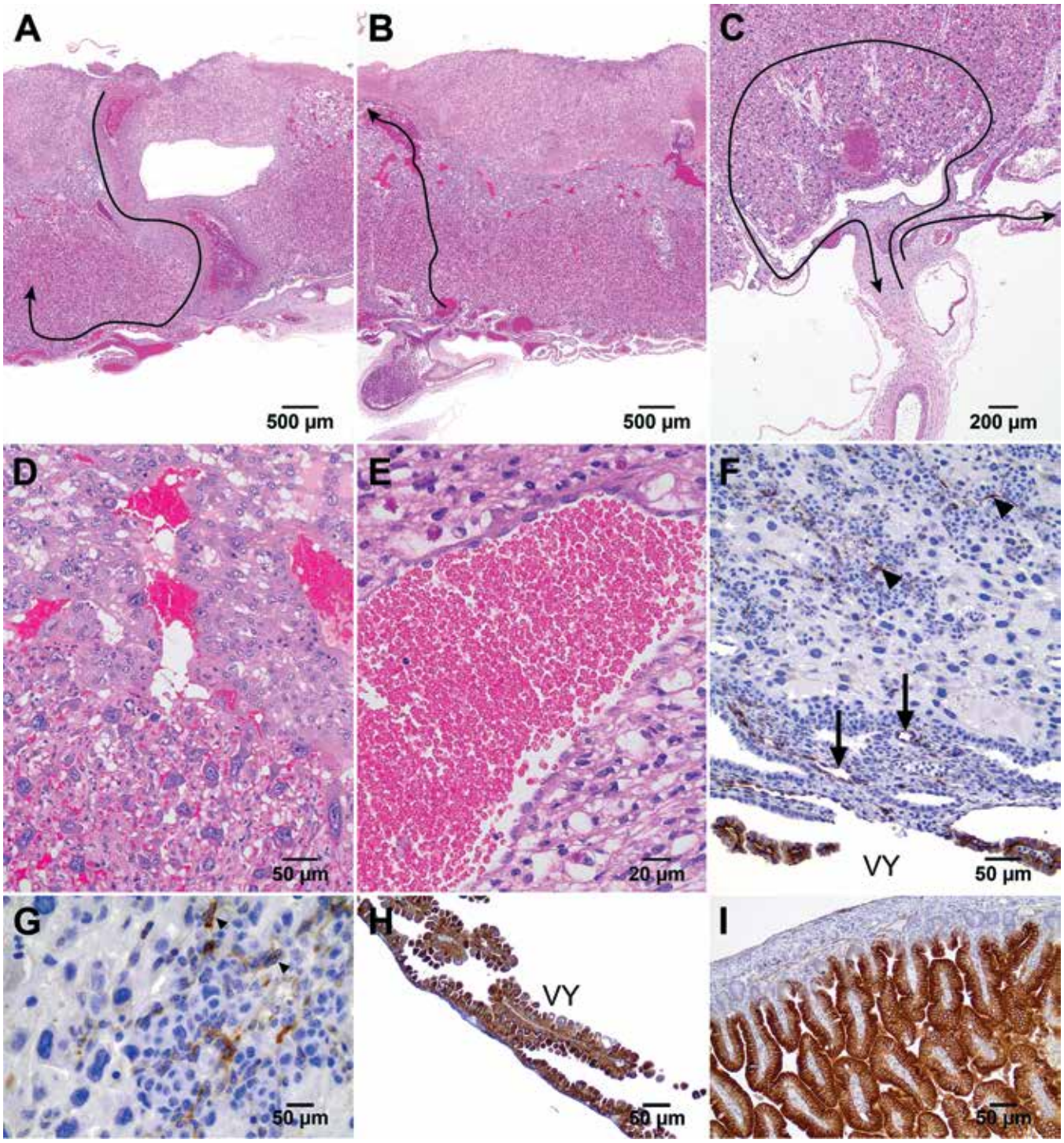

Figure 7. Cotton rat placental blood flow and FcRn expression. (A) H and E with illustration of maternal arterial blood flow. (B) H and E with illustration of maternal venous blood flow. (C) H and E with illustration of fetal circulation from the umbilical artery to the chorionic plate to the labyrinth with the return of fetal blood to the umbilical vein (circular arrow), and from the umbilical artery to the chorionic plate to the yolk sac (vitelline) capillaries (curvilinear arrow). (D) H and E of GD25 maternal venous flow from the labyrinth into the junctional zone. (E) H and E of GD15 decidua and endothelial lined spiral vein. (F) Antihuman FCGRT/FCRN IHC of GD15 labyrinth and chorionic plate. (G) Antihuman FCGRT/FCRN IHC of GD15 labyrinth. (H) Antihuman FCGRT/FCRN IHC of GD15 visceral yolk sac. (I) Antihuman FCGRT/FCRN IHC of neonatal cotton rat duodenum. Visceral yolk sac endoderm (VY), positive staining fetal capillary endothelium (arrow), positive staining presumptive Hoffbauer cells (*), positive staining syncytiotrophoblasts (arrowhead).

similar to women with normal pregnancies and gestational pathologies.[24] Our current findings further support the use of cotton rats to study the role of trophoblast deportation in gestational pathologies of humans.[1,34]

Early studies of maternal immunization with RSV vaccine candidates have demonstrated that maternal antibodies provide protection, albeit short-lived.[2] This situation is in contrast to measles immunization and indicates a need to mechanistically address the role of maternal antibodies. The current study characterizes the locations of Fc receptors in cotton rats. The primary modes of maternal antibody transfer in cotton rats are through the placenta prenatally and the small intestine via colostrum

postnatally;[32] FcRn expression in cotton rats is consistent with these modes of transfer. Similar to that in mice, rats, and rabbits, the primary site of Fc receptor expression in the cotton rat placenta is the visceral yolk sac endoderm.[3,8,21,26,35,37] The PAS-positive, electron-dense apical vesicles of the yolk sac corresponded to positive FcRn staining and likely represent FcRn-containing endosomes responsible for prenatal immunoglobulin transport.[21,26,35] However, as in humans, FcRn is also expressed on the syncytiotrophoblasts of the maternal sinuses of the labyrinth and on the endothelium of the fetal capillaries in cotton rats, and these cells may contribute to maternal antibody transfer.

Furthermore, consistent with reports in mice, rats, and pigs, cotton rats have FcRn within small intestinal enterocytes.[3,20,36] Postnatal maternal antibody transfer is estimated to account for half of maternal antibody transfer in cotton rats.[32] Human fetal intestinal epithelium has similarly been documented to have FcRn expression apically, which may function in the transportation of maternal IgG during amniotic fluid ingestion.[20,36] In humans, Fcγ receptors are reported to be expressed in Hofbauer cells in the villar stroma of placentas, in fetal endothelium, and in syncytiotrophoblasts. Their function is not fully understood, but they are proposed to bind immune complexes to prevent their transport to the fetus.[19,25,27,30,37,38] Because this removal of antibodies might influence the composition of the transferred maternal antibodies, a study of these receptors (upon availability of reagents) in cotton rats may provide new insights.

In conclusion, our study provides a complete characterization of the cotton rat placenta to aid future studies that investigate the efficacy and safety of RSV-specific maternal antibody transfer in utero. In cotton rats, neonatal Fc receptors are expressed in the placenta and intestine, consistent with maternal antibody transfer as characterized in other rodents and humans. However, the functions of neonatal Fc receptors at these various locations remain to be determined. In addition, further studies are required to characterize completely the Fc receptors and other Fcγ receptors, visualize their interactions with maternal antibodies, and analyze their functions in binding antibody-antigen complexes and maternal antibody transfer.

Acknowledgments

We acknowledge the Comparative Pathology and Mouse Phenotyping Shared Resource (CPMPSR) of the Ohio State University Comprehensive Cancer Center for excellent necropsy (Julie Rectenwald), histotechnological (Anne Saulsbery), immunohistochemical (Florinda Jaynes), and electron microscopy (Alan Fletcher) support, as well as the College of Medicine, Department of Pathology, Transmission Electron Microscopy Laboratory (Edward Calomeni), and College of Veterinary Medicine Medical Illustrator (Tim Vojt). This work was supported by the Ohio State University Comprehensive Cancer Center and the National Institutes of Health under grant number P30 CA016058, and the Genentech Fellowship under grant number GRT00044407.

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