PrPSc accumulation in fetal cotyledons of scrapie-resistant lambs is influenced by fetus location in the uterus

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INTRODUCTION

Scrapie is a fatal, neurodegenerative disease of sheep and goats. It belongs to a group of diseases known as transmissible spongiform encephalopathies that are characterized by the accumulation of an abnormal isoform of a host-encoded protein, PrPc. The abnormally folded, partially proteinase K-resistant isofrom of PrPc is designated PrPSc and accumulates in the central and peripheral nervous system and the lymphoreticular system (Ikegami et al., 1991; Race et al., 1998). The placenta has also been shown to accumulate PrPSc (Andrèoletti et al., 2002a; Caplazi et al., 2004; Onodera et al., 1993; Race et al., 1998; Tuo et al., 2001, 2002). Infectivity of the placenta was shown in an oral dosing experiment carried out in sheep and goats (Pattison et al., 1972). PrPSc is thought to be transmitted from infected dams to susceptible lambs and cohort adult sheep during the perinatal period by exposure to infectious placental tissues and/or fluids, rather than in utero transmission (Andrèoletti et al., 2002b). Ovine susceptibility to scrapie is controlled by polymorphisms in the Prnp gene, encoding the PrP protein, at codons 136 (A or V), 154 (R or H) and 171 (R, Q or H) (Clouscard et al., 1995; Hunter et al., 1997b). Sheep with genotypes V136R154Q171/VRQ, ARQ/VRQ and ARQ/ARQ are the most susceptible to scrapie, whilst ARR/ARR and ARQ/ARQ sheep are considered to be resistant (Clouscard et al., 1995; Hunter et al., 1996, 1997b). Furthermore, in flocks where there is a high incidence of valine at codon 136 of the Prnp gene, sheep of genotype ARQ/ARQ are less susceptible or resistant to infection (Belt et al., 1995; Evoniuk et al., 2005; Goldmann et al., 1991; Hunter et al., 1991, 1994, 1997a; Laplanche et al., 1993).

Previous studies have reported on the role of fetal genotype in the control of PrPSc accumulation in the placenta. Accumulation of PrPSc has been demonstrated in 171 QQ placentomes of scrapie-infected ewes, but not in placentomes of infected ewes pregnant with 171 QR foetuses (Tuo et al., 2002). Furthermore, it has been shown that ARR/ARQ fetal cotyledons were negative for PrPSc accumulation when conceived by ARQ/VRQ dams (Andrèoletti et al., 2002b). Fetal cotyledons of genotype ARR/VRQ from ARQ/VRQ or ARR/VRQ dams were also shown to be negative for PrPSc accumulation. This study investigates how the spatial relationship of the fetuses to each other influences PrPSc accumulation in dams with multiple fetuses of different genotypes. Our results suggest that there can be a partial or incomplete anastomosis between the fetal blood supplies to
the cotyledons among fetuses on the same side of the uterine horn, resulting in PrPSc accumulation in cotyledons with resistant genotypes.

**METHODS**

**Animals and tissue collection.** Ewes used in this study were naturally infected Suffolk or Suffolk × Hampshire sheep between 2 and 4 years of age. The sheep were sourced from multiple flocks from across the United States. The scrapie status of the ewes was determined ante-mortem by immunohistochemistry (IHC) of the third-eyelid lymphoid tissue (O'Rourke et al., 2000). Post-mortem scrapie status was determined by IHC of the brainstem. Sheep with clinical signs of scrapie were euthanized humanely. All euthanized sheep were at 100 days or more gestation (full-term gestation is 144–150 days). For individual fetuses, between eight and ten placentomes (maternal caruncle plus fetal cotyledon) were collected from their respective placentae at necropsy. The blood supply from the fetal cotyledon was traced to the umbilicus of the fetus to ensure matching of the placentomes and fetuses. In addition, fetal cotyledons were genotyped and matched to the genotype of the fetus by genotyping a piece of fetal liver also collected at necropsy. Two placentomes were collected whole and stored in formalin until preparation for IHC. The remaining placentomes were gently separated manually into maternal caruncle and fetal cotyledon and stored at −20 °C until use. When necessary because of unexpected parturition, shed placentae were collected after parturition as soon as possible after they were passed. When spread out, the placentae duplicated the shape of the ewe’s uterus with two uterine horns. For shed placentae, between 10 and 15 cotyledons were collected from each section of the placenta corresponding to a uterine horn in order to obtain cotyledons from each fetus contained within the placenta. Cotyledons and lambs were genotyped to determine fetal positioning in utero. Cotyledons were stored at −20 °C until use. Animal care, handling and use in this study were approved by the Institutional Animal Care and Use Committee of Washington State University. The total number of dams and their respective fetuses used in the study are listed in Table 1.

**Tissue processing and Western blot analysis.** Samples were prepared and analysed by Western blot using a phosphotungstic acid precipitation method as described previously (Spraker et al., 2004) with the following exception: mAbs F99/97.6.1 (VMRD) and P4 (R-Biopharm AG) were used for detection of PrPSc. Cotyledon samples from known scrapie-infected and -uninfected sheep as determined by Western blot and IHC of brain tissue were run as positive and negative controls, respectively.

**IHC.** IHC was performed as described previously (O’Rourke et al., 2000; Tuo et al., 2002). Briefly, tissues were fixed in 10% neutral-buffered formalin for 2–7 days, treated with 96% formic acid for 1 h, rinsed with water for at least 10 min and fixed in formalin for an additional 24 h before embedding in paraffin. Tissue sections placed on glass slides were deparaffinized and stained by an automated system (Benchmark; Ventana Medical Systems). Primary mAbs F99/97.6.1 (recognizing the epitope QYQRES) and F89/160.1.5 (recognizing the epitope IHFG) were applied as a cocktail of each antibody at 5 μg ml−1. This was followed by the application of a biotinylated secondary antibody, streptavidin–horseradish peroxidase and detection with 3-αmino-9-ethylcarbazole–H2O2. Tissues from scrapie-infected and -uninfected sheep were used as controls with each run. Negative controls included isotype-matched irrelevant IgG and blocking of the mAbs with synthetic peptides to which the mAbs were raised. PrPSc immunoreactivity was visualized by using a bright-field microscope. Photomicrographs were taken with a microscope equipped with a digital camera.

**DNA isolation.** DNA was isolated from 600 μl aliquots of blood by using a PureGene kit (Gentra), following the manufacturer’s protocols. DNA was isolated from approximately 25 mg fetal cotyledon tissue by using a Qbiogene FastDNA kit (catalogue no. 6540-400) following the manufacturer’s protocols.

<table>
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<tr>
<th>Dam no.</th>
<th>Prnp genotype</th>
<th>Sibling fetal letter</th>
<th>Prnp genotype</th>
<th>Fetal sex</th>
<th>Uterine horn</th>
<th>PrPSc in cotyledon</th>
<th>Syr PCR product</th>
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**Table 1.** PrPSc accumulation and presence of Syr PCR product in fetal cotyledons according to dam genotype, fetal genotype and multiple births from dams with natural scrapie
Genotyping. Approximately 100 ng DNA was used as a template for amplification of the open reading frame of Prnp using the following primer pair: 5′-GCGGATTGATGTGACCA-3′ and 5′-TACAGGGCTGAGGTAC-3′, corresponding to nucleotide positions 22234–22252 and 23106–23125 of GenBank accession no. U67922. Reactions were performed in a final volume of 90 μl under the following conditions: 1× Qiagen PCR buffer, 2.5 mM MgCl₂ and 2.5 U Taq polymerase (Qiagen TaqPCR Core kit; catalogue no. 201225). Amplification was performed under a temperature regime of 95°C for 5 min, 62°C for 30 s and 72°C for 59 s for one cycle; 95°C for 30 s, 62°C for 30 s and 72°C for 59 s for 30 cycles; and 95°C for 30 s, 62°C for 30 s and 72°C for 7 min for one cycle. Amplified products were sequenced by standard dideoxynucleotide analysis (AmpliSeq Express) using primer pair 5′-GTGGTTCTTGGCC-3′ and 5′-AGGTGGTAGCC-3′, corresponding to nucleotide positions 22553–22573 and 22838–22860 of GenBank accession no. U67922. Genotypes are shown as the deduced amino acid sequences at codons 136 (alanine, A, or valine, V), 154 (arginine, R) and 171 (glutamine, Q, or arginine, R) of the diploid genotype.

Sry PCR. Previous studies have used the Sry gene as a DNA marker for sex identification in a variety of mammals (Griffiths & Tiwari, 1993; Szatkowska et al., 2004; Wilson & White, 1998). The Sry gene encodes a protein that has been identified as the testis-determining factor in mammals (Koopman et al., 1991). Sex identification is based on the presence of an amplified PCR product in a male and absence of product in a female.

Approximately 100 ng DNA was used as a template for the amplification of the Sry region of the Y chromosome (Payen & Cotinot, 1993; Wilson & White, 1998). The primer sequences used were 5′-CTGCTATTGATGTTGCCTGTA-3′ and 5′-CTGGATATTGTTGCTGCTGTA-3′, corresponding to nucleotide positions 2–20 and 58–78 of a published bovine Sry sequence (Payen & Cotinot, 1993). Reactions were performed in a final volume of 90 μl under the following conditions: 1× Qiagen PCR buffer, 2.5 mM MgCl₂ and 2.5 U Taq polymerase (Qiagen TaqPCR Core kit). Amplification was performed under a temperature regime of 94°C for 5 min, 60°C for 30 s and 72°C for 30 s for one cycle; 94°C for 15 s, 60°C for 30 s and 72°C for 30 s for 32 cycles; and 94°C for 15 s, 60°C for 30 s and 72°C for 5 min for one cycle. Sry PCR products of approximately 200 bp were analysed by gel electrophoresis on a 1.5% agarose gel stained with ethidium bromide. A second PCR product present both in males and females, the Prnp gene, was amplified and used as an internal control by using the protocol listed above for Prnp gene amplification. DNA isolated from the blood of the lambs was also used in Sry amplification reactions to test for freemartinism in the female lambs; freemartinism is a condition that occurs in twins of different sexes in which the placental membranes that connect the fetus to the dam are shared and the placental fluids are exchanged between the two fetuses. The exchange of fluid and blood between the two fetuses mixes the antigens responsible for carrying the unique sex characteristics of each fetus and the twins develop with some sex characteristics of both the male and female. The female fetus is usually most affected and is sterile and masculinized. Blood was also sent to a commercial laboratory (UC Davis Veterinary Genetics Laboratory, Davis, CA, USA) for freemartin testing.

PrPSc detection by ELISA. A commercial test developed for lymphoid tissue [Chronic Wasting Disease (CWD) Antigen Test kit; IDEXX HerdChek] was used for detection of PrPSc in cotyledon tissue. This test is used for the post-mortem detection of PrPSc in white-tailed deer retropharyngeal lymph-node tissue, but is also suitable for the detection of PrPSc in sheep cotyledons; the kit utilizes a PrPSc ligand immobilized on the surface of the CWD antigen capture plate and mAbs that recognize the ovine PrP protein. Briefly, 300 mg cotyledon tissue was homogenized twice in a FastPrep (Qbiogene) instrument (also known as a ribolyser) for 30 s at the maximum speed (6.5 m s⁻¹) in a disruption tube containing ceramic beads and 0.9 ml distilled, deionized water. The ELISA was then conducted according to the manufacturer’s protocols. Interpretation of samples analysed by ELISA was performed by comparing the A450 of the cotyledon samples with that of negative- and positive-control samples supplied with the kit (a reference wavelength of 620 nm was used). Samples are considered positive if the mean A450 values are greater than or equal to the cutoff value as calculated by the test manufacturer.

RESULTS

PrPSc detection in the placenta

PrPSc was detected by Western immunoblot, IHC and ELISA. When PrPSc was detected in a cotyledon belonging to a fetus, all other cotyledons tested from the fetus were also positive by all three tests (Table 1). Positive samples were determined by antibody binding to PrPSc and visualized as multiple bands on a Western immunoblot, as PrPSc plaques in fetal trophoblast cells by IHC or as absorbance readings above cutoff values in ELISA. Maternal caruncles were also collected at necropsy from dams 1, 2 and 5 and were found to be negative for PrPSc (data not shown). Dams 3, 4, 6 and 7 provided shed placenta, therefore only cotyledons were collected, as the caruncle is retained by the dam at parturition.

PrPSc accumulation in the placenta with multiple fetuses of genotype ARQ/ARR and ARQ/ARQ

The presence of PrPSc was detected in the cotyledons collected at necropsy corresponding to the fetus of genotype ARQ/ARR (fetuses 1B and 2B) and not in the cotyledons from the fetus of genotype ARQ/ARR (fetuses 1A and 2A) in twins from scrapie-positive dams of genotype ARQ/ARQ (dams 1 and 2) (Table 1). This supports previous findings of these fetal genotypes from similar dams (Andréoletti et al., 2002b; Tuo et al., 2002). PCR products for the male-specific Sry gene were found in cotyledons corresponding to the male fetus, but not in those corresponding to female fetuses (data not shown). Placentae with all female fetuses were also found to be negative for Sry gene PCR products (data not shown).

Cotyledons from the shed placenta of a scrapie-positive dam (dams 3) of genotype ARQ/ARQ that gave birth to triplets of genotypes ARQ/ARQ (lamb 3B) and ARQ/ARR (lambs 3A and 3C) were tested for PrPSc (Table 1, Fig. 1). Sequence analysis of the Prnp and Sry genes in the cotyledons from the tissue corresponding to each uterine horn demonstrated that lambs 3A (male ARQ/ARR) and 3B (female ARQ/ARR) shared one horn of the uterus and that lamb 3C (female ARQ/ARR) was alone in the other horn. ARQ/ARR cotyledons from lamb 3B (Fig. 1, lane 5) and ARQ/ARR cotyledons from lamb 3A (Fig. 1, lanes 4, 6 and 7) had accumulation of protease-resistant anti-PrP immunoreactive bands with a molecular mass consistent with
PrP\textsuperscript{Sc}. ARQ/ARR cotyledons from lamb 3C (Fig. 1, lane 8) showed no protease-resistant PrP immunoreactivity. Morphologically, PrP\textsuperscript{Sc} accumulated within trophoblast cells of the cotyledons of both lambs 3A and 3B (Fig. 2). No PrP\textsuperscript{Sc}-positive blood cells were observed in either lamb, indicating that the Western blot PrP\textsuperscript{Sc}-positive result in the genetically resistant lamb 3A was not due to blood cells originating in the genetically susceptible lamb 3B.

**PrP\textsuperscript{Sc} accumulation in the placenta with multiple fetuses of genotype ARQ/ARQ and VRQ/ARQ**

A scrapie-positive dam of genotype ARQ/VRQ (dam 4) delivered twin lambs. DNA sequencing of Prnp of the cotyledons from the shed placenta demonstrated that the female ARQ/ARQ fetus (lamb 4B) and the male ARQ/VRQ fetus (lamb 4A) developed in different uterine horns. PrP\textsuperscript{Sc} was detected in the cotyledons corresponding to the fetus of genotype ARQ/VRQ (lamb 4A) and not in the cotyledons from the fetus of genotype ARQ/ARQ (lamb 4B) (Table 1). PCR products for the male-specific Sry gene were found in cotyledons corresponding to the male fetus, but not in those corresponding to the female fetus.

Cotyledons collected at necropsy from a scrapie-positive dam (dam 5) of genotype ARQ/VRQ that was gestating quadruplet fetuses of genotypes ARQ/VRQ (lambs 5A, 5B and 5C) and ARQ/ARQ (lamb 5D) were sampled at necropsy and tested for PrP\textsuperscript{Sc}. PrP\textsuperscript{Sc} was detected in the cotyledons corresponding to the ARQ/VRQ fetuses (lambs 5A, 5B and 5C), but not in the cotyledons from the ARQ/ARQ fetus (lamb 5D) (Table 1). DNA sequencing of the cotyledons demonstrated that the male ARQ/VRQ fetuses (lambs 5A and 5B) developed in one uterine horn and that the female ARQ/VRQ and ARQ/ARQ fetuses (lambs 5C and 5D) developed in the other uterine horn. PCR products for the male-specific Sry gene were found in cotyledons corresponding to the male fetuses, but not in those corresponding to the female fetuses.

Cotyledons from the shed placenta of a scrapie-positive dam of genotype ARQ/VRQ (dam 6) that gave birth to triplets of genotypes ARQ/VRQ (lambs 6A and 6C) and ARQ/ARQ (lamb 6B) were tested for PrP\textsuperscript{Sc}. Sequence analysis of the Prnp and Sry genes in the cotyledons from the tissue corresponding to each uterine horn demonstrated that lambs 6A (male ARQ/VRQ) and 6B (female ARQ/ARQ) shared one horn of the uterus and that lamb 6C (male ARQ/VRQ) was alone in the other horn. PrP\textsuperscript{Sc} was detected in the cotyledons corresponding to the two ARQ/VRQ fetuses (lambs 6A and 6C), but PrP\textsuperscript{Sc} was also detected in the cotyledons from the ARQ/ARQ fetus (lamb 6B) (Table 1, Fig. 3). ARQ/VRQ cotyledons [lambs 6A (lanes 3, 4, 6 and 7)
Fig. 3. Western immunoblot of fetal cotyledons from genotypically susceptible (ARQ/VRQ) or resistant (ARQ/ARQ) fetuses from an ARQ/VRQ dam (dam 6; Table 1). Lane 1, negative control; lane 2, positive control; lanes 5 and 9, different cotyledons from lamb 6A (Table 1); lanes 3, 4, 6 and 7, different cotyledons from lamb 6B (Table 1); lane 8, lamb 6C (Table 1). The positions of molecular mass standards (in kDa) are indicated on the left.

and 6C (lane 8) and ARQ/ARQ cotyledons [lamb 6B (lanes 5 and 9)] had accumulation of protease-resistant anti-PrP immunoreactive bands with a molecular mass consistent with PrPSc (Fig. 3). A PCR product for the male-specific Sry gene was found in the female fetus (lamb 6B) that shared a uterine horn with the male fetus (lamb 6A). The male fetus (lamb 6C) that was alone in the other uterine horn also had a PCR product for the Sry gene (Table 1). Freemartin testing at a commercial laboratory was performed on blood from the three lambs and tested negative for freemartinism (data not shown).

A scrapie-positive dam of genotype ARQ/ARQ (dam 7) delivered twin lambs. DNA sequencing of the cotyledons from the shed placenta demonstrated that the male ARQ/ARQ fetus (lamb 7A) and the female ARQ/VRQ fetus (lamb 7B) developed in different uterine horns. PrPSc was detected in the cotyledons corresponding to the fetus of genotype ARQ/ARQ (lamb 7A) and not in the cotyledons from the fetus of genotype ARQ/VRQ (lamb 7B) (Table 1). PCR products for the male-specific Sry gene were found in cotyledons corresponding to the male fetus, but not in those corresponding to the female fetus.

DISCUSSION

This is the first study reporting results from births with multiple fetuses and the effect of fetal genotype on PrPSc accumulation in the case of births with multiple fetuses. We show that PrPSc can be detected in the cotyledons of some fetal genotypes considered resistant to scrapie when the fetus has shared the same uterine horn with a fetus of susceptible genotype where PrPSc has been detected. A PCR product specific for males was also found in the cotyledons from female fetuses that had shared the same uterine horn with a male fetus. Taken together, these results suggest a partial anastomosis between fetal cotyledon blood supplies in utero. This sharing of blood components between developing fetuses is a possible source of prion infection in scrapie-resistant fetuses.

We detected PrPSc in cotyledons of genotype ARQ/ARQ (lambs 1B and 2B) and not in cotyledons of genotype ARQ/ARR (lambs 1A and 2A) in twin births with one fetus of each genotype. This followed the expected pattern of PrPSc distribution with ARQ/ARQ as a susceptible genotype for PrPSc accumulation and ARQ/ARR as a resistant genotype. In a triplet birth involving animals of the same genotypes, however, PrPSc was detected in cotyledons from an ARQ/ARR fetus (lamb 3A) that shared the uterine horn with an ARQ/ARQ fetus (lamb 3B) positive for the presence of PrPSc. The cotyledons from the ARQ/ARQ female fetus had a male-specific PCR product for the Sry gene, suggesting exchange of blood cells between lambs 3A and 3B. However, the PrPSc positivity in the genetically resistant lamb 3A is probably not due to cross-contamination of blood cells from the genetically susceptible lamb 3B, as IHC localized PrPSc immunoreactivity in both fetuses to placental trophoblasts and not to blood cells. The third fetus in the opposite uterine horn of genotype ARQ/ARR was negative for the presence of PrPSc (lamb 3C). It is not known whether the genotypically resistant lambs from triplet births will go on to develop disease as adults when the cotyledons are positive for the presence of PrPSc in the shed placenta. Lambs 3A and 6B are being held at our facility under observation for signs of scrapie. To date, there have been only a few reports of scrapie-infected sheep carrying 171 arginine/arginine (RR) (Buschmann et al., 2004; Ikeda et al., 1995) and a few published reports of scrapie-infected sheep with 171 glutamine/arginine (QR) (Hunter et al., 1997a; Ikeda et al., 1995; Madec et al., 2004). It would be interesting to know whether these reports were from sheep that had shared the same uterine horn with a susceptible fetus in utero.

There have been several published reports of ARQ/ARQ sheep remaining scrapie-free in flocks that are heavily stocked with scrapie-carrying sheep (Belt et al., 1995; Goldmann et al., 1991; Hunter et al., 1991, 1997a; Laplanche et al., 1993). Andréolletti and colleagues reported that placentae from ewes of genotype ARQ/VRQ are positive for the presence of PrPSc when the fetus is of genotype ARQ/VRQ (Andréolletti et al., 2002b; Evoniuk et al., 2005). Our results confirm those of Andréolletti et al. (2002b) and Evoniuk et al. (2005) and additionally showed that the cotyledons from a twin lamb of genotype ARQ/ARQ (lamb 4B), born to the same ARQ/VRQ dam (dam 4), were negative for the presence of PrPSc. However, twins of genotypes ARQ/VRQ (lamb 7B) and ARQ/ARQ (lamb 7A) showed the opposite PrPSc distribution pattern when born to a ewe of genotype ARQ/ARQ (dam 7); the cotyledons from the ARQ/VRQ fetus were negative and the cotyledons from the ARQ/ARQ fetus were positive for the presence of PrPSc. 136 Valine in the fetus was protective for PrPSc accumulation when the dam did not carry 136 Valine, but was permissive for PrPSc accumulation when the dam also carried 136 Valine. Conversely, 136 Alanine in the fetus was protective for
PrP\textsuperscript{Sc} accumulation where the dam carried 136 valine, but was permissive for PrP\textsuperscript{Sc} accumulation when the dam did not carry 136 valine, except for dam 6, where there was potential exchange of blood cells between a susceptible ARQ/VRQ fetus and a resistant ARQ/ARQ fetus in the same uterine horn.

In a birth with triplets from an ARQ/VRQ dam (dam 6), the cotyledons from all of the fetuses were positive for the presence of PrP\textsuperscript{Sc}, including those from an ARQ/ARQ fetus (lamb 6B). Cotyledons with an ARQ/ARQ genotype were shown to be negative for the presence of PrP\textsuperscript{Sc} when conceived by an ARQ/VRQ dam (dam 4) in twin births from this study. The cotyledons from the female ARQ/ARQ triplet fetus (lamb 6B) showed a male-specific PCR product for the Sry gene, demonstrating a detectable amount of fetal blood exchange. A set of quadruplets from a necropsy at 120 days gestation had one fetus of genotype ARQ/ARQ (lamb 5D) and three fetuses of genotype ARQ/VRQ (lamb 5A, 5B and 5C). The cotyledons from all three of the ARQ/VRQ fetuses were positive for the presence of PrP\textsuperscript{Sc} and the cotyledons from the ARQ/ARQ fetus were negative for the presence of PrP\textsuperscript{Sc}. In this case, the fetuses that shared the same side of the uterine horn were of the same sex, there was no PCR product for the Sry gene detected in the cotyledons from the female fetuses.

The ovine placenta is of the syncytiotrophoblastic type and is composed of a flat apposition of chorionic epithelium (trophoblast) to uterine epithelium with a variable number of placentomes. The placentomes are composed of fetal chorionic villi (cotyledons) emmeshed with maternal endometrial crypts (caruncles). Binucleate trophoblast cells are common and unique to all ruminant placentae (Wooding et al., 1997). It was previously shown that PrP\textsuperscript{Sc} is found in uterine endometrial epithelium, trophoblast cells and multinucleate cells in maternal endometrial crypts (Tuo et al., 2002). The vasculature of each fetal cotyledon can be traced to individual fetuses’ umbilical cords. Based on the results of this study, we speculate that there is some sharing of the blood supply between cotyledons on the same side of the uterus in some multiple births. This would explain the presence of a male-specific PCR product in female cotyledon tissue, but does not necessarily explain the presence of PrP\textsuperscript{Sc} in cotyledons of genotypes previously shown to be resistant to PrP\textsuperscript{Sc} deposition. Although post-partum contamination of the cotyledons with the resistant genotype by maternal tissue or fetal tissue from the twin cannot be ruled out in Western blot, ELISA or the genetic analysis, the demonstration of similar patterns of intracellular accumulation of PrP\textsuperscript{Sc} in the fetal trophoblast cells of genetically susceptible and genetically resistant twins is compelling evidence that PrP\textsuperscript{Sc} can accumulate in the cotyledon of a genetically resistant fetus under these conditions. This suggests a fetal–fetal blood sharing among cotyledons, as opposed to a maternal–fetal blood sharing where the maternal genotype at 136 has been shown to influence the accumulation of PrP\textsuperscript{Sc} in fetal cotyledons.

It has been suggested that transmission at lambing may be reduced or eliminated if infected ewes conceive offspring with scrapie-resistant genotypes. These results show that PrP\textsuperscript{Sc} can be present in cotyledons of fetuses with genotypes thought to be resistant to scrapie when it occurs in multiple births with fetuses sharing the same uterine horn.

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