



EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the risk of transmission of classical scrapie via in vivo derived embryo transfer in ovine animals

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SCIENTIFIC OPINION

Scientific Opinion on the risk of transmission of classical scrapie via *in vivo* derived embryo transfer in ovine animals¹

EFSA Panel on Biological Hazards^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The risk of transmission of classical scrapie via the transfer of *in vivo* derived embryo in ovines was assessed, taking into account the scientific information made available since the last EFSA opinion on this topic (2010) (see <http://www.efsa.europa.eu/en/efsajournal/pub/1429.htm>). The potential impact of PrP genotype of the embryo and/or of the ram and donor ewe on this risk was also assessed. The new data made available over the last three years further reinforce the view that classical scrapie could be vertically transmitted in sheep. Since the possibility of such vertical transmission was already considered in the previous opinion, its conclusions and recommendations relating to the risk of classical scrapie transmission via embryo transfer remain valid. In ovines, the susceptibility to classical scrapie infection in sheep is strongly influenced by certain polymorphisms of the *PrP* gene. Under natural exposure conditions, animals that are heterozygous or homozygous A₁₃₆R₁₅₄R₁₇₁ display respectively a low or negligible risk of being infected. The genetic control of the susceptibility to classical scrapie is also likely to impact on the risk of transmitting the disease via embryo transfer. Irrespective of the embryo's genotype, embryos derived from rams and dams carrying at least one ARR allele would significantly decrease this risk (compared to an embryo from parents of unknown genotypes). The use of homozygous ARR embryos would provide the highest level of safety regarding the risk of transmitting classical scrapie through embryo transfer (*in vivo* derived embryos). The use of heterozygous ARR embryos would ensure a higher level of safety compared to Q₁₇₁/Q₁₇₁ embryos. Finally, it was concluded that, providing the OIE recommendations and procedures relating to embryo transfer are adhered to, the risk of transmitting classical scrapie due to the transfer of homozygous or heterozygous ovine ARR embryos can be considered negligible.

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KEY WORDS

Classical scrapie, ovine, sheep, embryo transfer, transmission risk

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SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ) was asked to deliver a scientific opinion on the risk of transmission of classical scrapie via *in vivo* derived embryo transfer in ovine. The Panel was requested to provide an updated scientific opinion on the risk of transmission of classical scrapie via *in vivo* derived embryo transfer in ovine animals, taking into account any new scientific information made available since its last assessment on this issue in 2010⁴. In addition, the Panel was requested to elaborate on this risk of transmission according to the PrP genotype of the embryo and/or of the ram and donor ewe.

The Panel reviewed the scientific literature published since the adoption of the previous Opinion covering this issue in 2010 and concluded that relevant findings available further reinforce the view that classical scrapie in small ruminants could be vertically transmitted. Since the possibility of vertical transmission was already considered in the previous opinion, these new findings do not make it necessary to revise the conclusions related to the risk of classical scrapie transmission via embryo transfer.

With regard to the effect of genotype, the susceptibility to classical scrapie infection in sheep is strongly influenced by certain polymorphisms of the *PrP* gene. Under natural exposure conditions, animals that are heterozygous or homozygous A₁₃₆R₁₅₄R₁₇₁ show respectively a low or negligible risk of being infected by classical scrapie. This genetic control of the susceptibility to classical scrapie infection directly influences the risk of transmitting the disease via embryo transfer in that, irrespective of the embryo's genotype, the use of embryos derived from rams and dams carrying at least one ARR allele would significantly decrease the risk of transmitting classical scrapie via embryo transfer (by comparison to an embryo from parents of unknown genotypes). Furthermore, the use of homozygous ARR embryos would provide the highest possible level of safety with regard to minimising the risk of transmitting classical scrapie through embryo transfer (*in vivo* derived embryos). The use of heterozygous ARR embryos would ensure a higher level of safety by comparison with homozygous Q₁₇₁ (A/V₁₃₆, R/H₁₅₄) embryos.

Providing that the OIE recommendations and procedures relating to embryo transfer are adhered to, the risk of transmitting classical scrapie by the implantation of homozygous or heterozygous ARR ovine embryos can be considered negligible.

The Panel indicated that the recommendations relating to the risk of transmitting classical scrapie via embryo transfer that were formulated in the 2010 opinion remain valid. In particular, the presence of infectivity in ovine embryos collected from scrapie infected dams bearing susceptible genotypes needs to be assessed before a definitive assessment of the risk of transmitting classical scrapie via the use of embryos bearing a susceptible genotype can be made.

⁴ EFSA Panel on Biological Hazards (BIOHAZ), 2010. Scientific Opinion on Risk of transmission of TSEs via semen and embryo transfer in small ruminants (sheep and goats). EFSA Journal, 8(1):1429(1), 39 pp.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In an opinion published in January 2010⁵ EFSA has concluded that the risk of TSE transmission associated with semen and embryos collected from Classical Scrapie or BSE incubating sheep and goats ranges from negligible to low, the data being insufficient to conclude that such a risk is negligible.

Stringent conditions have therefore been maintained in the EU TSE legislation⁶ regarding the trade and import of ovine embryos.

However, the Health and Scientific Advisory Committee of the International Embryo Transfer Society (IETS) came to the conclusion, in January 2010 as well, that classical scrapie in sheep should be categorized as category 1. The IETS category 1 lists the diseases or agents for which it has been proven that the risk of transmission with embryos is negligible.

As a consequence of this IETS categorisation, the OIE has amended in 2010 the articles 4.7.14 and 14.9.1 of the Terrestrial Animal Health Code, respectively on the risk of disease transmission via *in vivo* derived embryos and on scrapie. *In vivo* derived sheep embryos handled in with Chapter 4.7 (recommendations for the collection and processing of *in vivo* derived embryos from livestock and horses), have been added to the list of "safe commodities" for trade.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested to provide an updated scientific opinion on the risk of transmission of classical scrapie via *in vivo* derived embryo transfer in ovine animals, taking into account any new scientific information made available since its last assessment on this issue.

Additionally, EFSA is requested to elaborate on this risk of transmission according to the PrP genotype of the embryo and/or of the ram and donor ewe.

⁵ Scientific Opinion on Risk of transmission of TSEs via semen and embryo transfer in small ruminants (sheep and goats), EFSA Journal 2010; 8(1):1429 [39 pp.]

⁶ Regulation No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies (OJ L 147, 31.5.2001, p. 1)

ASSESSMENT

1. Introduction

1.1. Approach to the assessment

In order to address the terms of reference received from the European Commission (EC), the Panel on Biological Hazards (BIOHAZ) reviewed the new scientific data that has been made available since the publication of the earlier Scientific Opinion on the risk of transmission of TSEs via semen and embryo transfer in small ruminants (EFSA Panel on Biological Hazards, 2010).

In addition, in order to clarify the difference between the IETS and EFSA 2010 opinion on the qualification of the risk of transmission of classical scrapie via embryos, experts from IETS were invited to present and discuss the scientific data they used for their assessment. The discussion was held during the first meeting of the *ad hoc* working group set up by the BIOHAZ Panel to assist with the drafting of the Opinion.

This document uses standard terminology regarding the different genotypes conferring variable susceptibility to classical scrapie, as described previously (EFSA Panel on Biological Hazards, 2005). Briefly, the sheep prion protein gene encodes a protein-coding open reading frame of 254 amino-acid codons. Common polymorphisms at codons 136 (V or A), 154 (H or R) and 171 (R or Q) define alleles (represented by the single letter amino-acid code in the order 136:154:171; eg. ARQ: alanine at codon 136, arginine at codon 154 and glutamine at codon 171) that are linked to survival time of sheep exposed to classical scrapie.

2. Literature review of new knowledge published since 2010

A review of the scientific literature was conducted using Web of KnowledgeSM, including articles published from 2009 to December 2012.

The search string used was:

(scrapie OR TSE OR prion OR "slow virus" OR PrP*) AND (ewe OR ram OR sheep OR lamb OR ovine) AND (embryo OR "germ cell" OR semen OR foetus OR fetus OR placenta)

This search resulted in 215 articles identified. From these, two were finally considered relevant for this mandate. The results reported in these publications are discussed below.

2.1. Garza et al., (2011). Detection of PrP^{res} in genetically susceptible fetuses from sheep with natural scrapie

Three A₁₃₆R₁₅₄Q₁₇₁/ARQ genotype pregnant sheep displaying clinical signs of scrapie were selected in three different flocks. After culling a number of tissues were collected in the ewes (nervous and lymphoid tissues), and in the six foetuses (brain, spleen, ileocecal valve and retropharyngeal lymph node). In addition, placentomes and amniotic liquid corresponding to each foetus were collected. A sampling procedure intended to prevent potential contamination of the foetus by the infected dams was applied.

Abnormal PrP (PrP^{Sc}) was detected in the central nervous system and in the lymphoid tissues of the three ewes confirming their infected status. PrP^{Sc} was also detected in the placentomes of all six fetuses. In contrast, foetus' tissues and amniotic fluids were all negative for PrP^{Sc}.

Prions are primarily composed of multimers of a misfolded form (PrP^{Sc}) of the host-encoded prion protein (PrP^C). They propagate by recruiting and converting PrP^C into PrP^{Sc} and fragmentation of PrP^{Sc} multimers is thought to provide new PrP^{Sc} seeds for the conversion reaction. The protein misfolding cyclic amplification (PMCA) technology is aimed at replicating this phenomenon *in vitro*, allowing amplification of minute amounts of prions (Saborio et al., 2001). It facilitates the combining of a PrP^C-

containing substrate with previously undetectable amounts of PrP^{Sc} by repetitive cycles of incubation and sonication leading to amplification of abnormal PrP^{Sc}.

Tissues collected from each foetus and amniotic fluids samples were used to seed PMCA reactions that used brain homogenates from mice expressing the ovine PrP as a substrate. After PMCA amplification PrP^{Sc} could be detected in at least one sample collected from each foetus, including lymph nodes and central nervous system (CNS).

According to the authors these results suggest that '*in utero transmission of scrapie could be possible.*'

The results reported by Garza et al. using tissues collected in foetus are not fully consistent with the current view about scrapie infection in lambs. Indeed, according to a large number of data, in the majority of cases the infection would be the consequence of perinatal oral exposure. This statement relies on:

- the absence of PrP^{Sc} detection in susceptible foetuses in late gestation even when the placenta was PrP^{Sc} positive, (Andreoletti et al., 2002).
- the PrP^{Sc} dissemination scheme observed in the tissues of lambs during the first months after birth (involving first the ileal peyer's patches before progressively spreading to lymphoid organs and more later to CNS) which contradicts the idea of a dissemination of the agent to a large panel of tissue *in utero*.

In the Garza study, although precautions were taken during the collection of the samples at necropsy, cross-contamination between infected dam's tissues and foetus cannot be fully ruled out. No control foetus (foetus collected from a non scrapie infected sheep, or foetus with heterozygous ARR genotype in the uterus of an infected ewe) was included in the study. Such controls are extremely important when considering the capacity of prion amplification by PMCA and raises some concerns when making a final interpretation of the results.

Nevertheless, if valid, the results presented in this publication would support the possibility of vertical transmission of classical scrapie in sheep.

The existence of potential vertical transmission of classical scrapie in small ruminants (which is supported by other evidence) was already taken into account in the 2010 EFSA opinion.

2.2. Rubenstein et al., (2012) PrP^{Sc} detection and infectivity in semen from scrapie-infected sheep

In this study, semen from four highly susceptible genotype scrapie infected rams (three ARQ/VRQ and one VRQ/VRQ) and non-infected controls belonging to an endemically infected flock were selected.

Semen samples from four of these rams (three -affected ARQ/VRQ and one infected VRQ/VRQ at preclinical stage of the disease) were inoculated intra-cerebrally in mice that over-express the ovine PrP (*TgSShpPrP* mice). In all four cases, western blotting demonstrated the accumulation of PrP^{Sc} in the brain in a proportion of the inoculated mice. Unfortunately, the experimental design (low number of inoculated mice, killing of some mice before clinical onset, lack of titration) precludes the estimation of the infectious titre in the tested semen samples. Presence of infectivity in the semen could have different consequences for embryo exposure to classical scrapie agent depending on the nature of the infectious components. Whereas infectivity present in seminal plasma would probably have no impact on embryo, its association with the spermatozoid could lead to its contamination during the fusion between the germ-cells. The results of this study do not indicate the nature of the infectious components in the semen (seminal plasma versus spermatozoid), which preclude an assessment of the final risk that the presence of infectivity in ram semen might represent for the embryos.

In parallel, semen from the same scrapie-infected and three apparently healthy rams was used to seed serial PMCA amplification using TgSShpPrP mice brain homogenate as substrate. PrP^{Sc} was detected in a proportion of the PMCA reactions seeded with samples from infected rams using an enhanced sensitivity immunoassay (Surround optical fiber immunoassay, SOFIA (Chang et al., 2009)).

These results contrast with those reported by Sarradin et al., (2008). In this study, the semen collected from three classical scrapie infected rams (seminal plasma and spermatozoa) was tested by bioassay in transgenic mice (Tg338) over-expressing the ovine VRQ prion protein. None of the mice inoculated with 20 µl of semen (containing about 10⁸ spermatozoa, one quarter of the quantity used in insemination) developed scrapie in the time frame of the experiment (up to 749 days post inoculation).

When considered together, these results indicate that infectivity (probably at a limited level) can be present in the semen of a ram infected with classical scrapie. However they do not demonstrate that semen from infected rams is able to transmit classical scrapie to the ewes or to the embryo.

In conclusion, the new results published since the adoption of the previous BIOHAZ Panel Opinion covering this issue in January 2010 further reinforce the view that classical scrapie in sheep could be vertically transmitted. Since this possibility of vertical transmission was already considered in the earlier opinion, these new results do not make it necessary to revise the conclusions relating to the risk of classical scrapie transmission via embryo transfer.

3. Assessment by the International Embryo Transfer Society (IETS)

The scientific data used by the Health and Scientific Advisory Committee (HASAC) when assessing the risk of transmission of classical scrapie via *in vivo* derived embryo transfer is described in a research update published in January 2012⁷. The IETS experts' assessment was based on the information published in several scientific studies, which were all considered in the EFSA 2010 opinion. In their interpretation, IETS experts considered that together these publications demonstrated the absence of scrapie transmission in 215 lambs born after the transfer (in healthy recipient ewes) of embryos collected from classical scrapie infected ewes. In IETS' opinion these results support the contention that the risk of transmission of classical scrapie via embryo transfer is negligible.

According to the EFSA 2010 opinion each of these studies has its specific intrinsic experimental limits (see Appendix B of that opinion) and several of them significantly differ in their design (duration of the lamb monitoring after birth, the scrapie agent involved, PrP genotype of the animals), which preclude merging all the results for conducting a global assessment.

BIOHAZ and WG experts considered that when assessing the risk of transmitting classical scrapie via embryo transfer in infected ewes bearing susceptible genotypes this risk should be assessed on the basis of the value of each study considered individually. As stated in the 2010 opinion: '*The more significant experiment concluded that scrapie transmission risk via the transferred pre-implantation embryos could be as high as 9.1% (CI 95%) (Low et al., 2009)*⁸'. This risk cannot be considered to be negligible.

Data considered by both the experts of the IETS and the BIOHAZ panel in its 2010 opinion for assessing the risk of classical scrapie transmission via embryo transfer are identical. The differences in the conclusion of the two assessments lay in a dissimilar interpretation of the limits of the experimental data supporting the absence of classical scrapie transmission risk associated with embryo transfer.

⁷ http://www.iets.org/pdf/Research_subcommittee_bibliographic_analysis_2012.pdf, page 63

⁸ This potential risk is inferred from embryos derived from fully susceptible genotype ewes that were infected with classical scrapie

4. PrP gene polymorphism and classical scrapie transmission risk

4.1. PrP genotype and susceptibility to classical scrapie

Scrapie susceptibility in sheep is strongly associated with polymorphisms of the *PRNP* gene which encodes PrP protein (EFSA Panel on Biological Hazards, 2006). It has been studied both in controlled experimental challenges of sheep with different strains of scrapie and in naturally occurring outbreaks of scrapie on farms throughout Europe. Experimental and natural scrapie have sufficient similarity to allow general conclusions to be reached about *PRNP* genetics from comparison of both types of study. In sheep, the major polymorphisms associated with susceptibility or resistance are at codons 136 (A or V), 154 (R or H) and 171 (R, Q or H) (Clouscard et al., 1995; Hunter et al., 1996). Depending on *PRNP* genotype there is a complete range of scrapie susceptibility from the highly susceptible VRQ/VRQ animals to the slightly less susceptible ARQ/VRQ and ARQ/ARQ genotype animals and on to the more resistant sheep which include those homozygous or heterozygous for AHQ and heterozygous for ARR animals. ARR/ARR sheep are considered to have high (but not absolute) resistance to scrapie. This statement is based on a number of experimental challenge studies in sheep bearing a range of different PrP genotypes and on thousands of observations carried out in naturally infected flocks in both Europe and North America (EFSA Panel on Biological Hazards, 2006; Hunter et al., 1996; Hunter et al., 1997; Groschup et al., 2007).

Under natural exposure conditions, the risk of scrapie infection in heterozygous ARR animals is strongly reduced in comparison with animals bearing a susceptible genotype (Q_{171}/Q_{171})⁹. In homozygous ARR animals this risk can be considered negligible (Baylis et al., 2004; Elsen et al., 1999).

The risk for an embryo to be exposed to classical scrapie infectivity is directly dependent on the infectious status of the dam (ovocyte / uterine environment) and more accessory of the ram (semen). Further details on how these risks were estimated can be found in Appendix A. It can therefore be considered that the potential risk of an embryo to be exposed to classical scrapie agent is correlated to the genotype of its parents. Table 1 summarizes the risk for an embryo to be exposed *in utero* to classical scrapie agent during the pre-implantatory period according to both the ram and dam genotypes that would be raised in an infected flock.

Table 1: Risk for an embryo to be exposed *in utero* to classical scrapie agent during the pre-implantatory period according to polymorphism of the PrP in both the ram and dam genotypes. By convention $A_{136}R_{154}R_{171}$ allele is named R_{171} , and ARQ, VRQ, and AHQ alleles are named Q_{171} in this table.

		Ram genotype		
		Q/Q_{171}	R/Q_{171}	R/R_{171}
Dam genotype	Q/Q_{171}	high	high	high
	R/Q_{171}	low	low	low
	R/R_{171}	low	negligible	negligible

4.2. Embryo's PrP genotype and classical scrapie agent accumulation in the gravid uterus

In susceptible genotype animals infected with classical scrapie there are consistent results supporting a lack of detectable PrP^{Sc} in the non-gravid uterus and in uterine regions which are not involved in placentomes during pregnancy (Andreoletti et al., 2002; Tuo et al., 2002). Data related to TSE infectivity in ovary and (non-gravid) uterus wall are very limited (Hourigan et al., 1979; Hourigan, 1988). However, they indicate a probable absence or low infectivity titre in these tissues.

⁹ By convention Q_{171} refers to ARQ, VRQ, and AHQ alleles.

In susceptible genotype sheep infected with classical scrapie the main structure accumulating the TSE agent in the gravid uterus is the foetus's component of the placentome (trophoblast).

However, the accumulation of abnormal PrP in the trophoblastic epithelium placenta is also tightly controlled by the foetus genotype (Andreoletti et al., 2002; Tuo et al., 2002).

In VRQ/VRQ ewes inseminated with ARR/VRQ ram semen, a clear segregation was observed according to the foetus genotype; while all the placentae of ARR/VRQ foetuses were negative, foetuses' VRQ/VRQ placentae were consistently positive. In ewes that were pregnant with several foetus both positive (VRQ/VRQ foetus) and negative placentae (ARR/VRQ foetus) were found in the uterus depending only on the foetus genotype (Andreoletti et al., 2002; Lacoux et al., 2007). However, a third study has reported some PrP^{Sc} in placental tissue related to resistant foetuses when the same uterine horn holds a susceptible sibling (Alverson et al., 2006).

These data support the view that the capacity of foetus/embryo's structures to replicate/accumulate classical scrapie agent following its *in utero* exposure is influenced by its genotype; the expression of at least one ARR allele by the foetus being able to strongly limit the accumulation of abnormal PrP in the tissues derived from an exposed foetus.

Since the risk of an embryo to be exposed to classical scrapie is correlated with its parents' genotypes (see 4.1), the theoretical risk for an embryo to carry infectivity or to be infected with classical scrapie will be strongly impacted by the combination of its parents' and its own genotype (see Table 2). See Appendix A for further details.

Table 2: Theoretical risk for an embryo to carry infectivity or to be infected with classical scrapie infectivity according to the combination of its parents and its own genotype. As above, A₁₃₆R₁₅₄R₁₇₁ allele is named R₁₇₁ and ARQ, VRQ, and AHQ alleles are named Q₁₇₁ in this table.

Dam genotype	Ram genotype	Risk of <i>in utero</i> exposure to classical scrapie agent	Embryo's genotype	Capacity of embryo's derived tissue to replicate classical scrapie	Resulting theoretical risk of classical scrapie presence in the embryo
Q/Q ₁₇₁	Q/Q ₁₇₁	high	Q/Q ₁₇₁	high	high
	R/Q ₁₇₁	high	Q/Q ₁₇₁	high	high
	R/R ₁₇₁		R/Q ₁₇₁	low	low
R/Q ₁₇₁	Q/Q ₁₇₁	low	Q/Q ₁₇₁	high	low
	R/Q ₁₇₁		R/Q ₁₇₁	low	low
	R/R ₁₇₁	low	Q/Q ₁₇₁	high	low
			R/Q ₁₇₁	low	low
		low	R/R ₁₇₁	negligible	negligible
R/R ₁₇₁	Q/Q ₁₇₁	low	R/Q ₁₇₁	low	low
	R/Q ₁₇₁	negligible	R/Q ₁₇₁	low	negligible
	R/R ₁₇₁		R/R ₁₇₁	negligible	negligible
	R/R ₁₇₁	negligible	R/R ₁₇₁	negligible	negligible

The risks presented in Table 2 are purely theoretical and assume the possibility of embryo infection following *in utero* exposure. Experimental data have demonstrated that the final risk of transmitting scrapie via the transfer of embryos bearing a susceptible genotype (Q/Q₁₇₁) that have been collected in scrapie infected ewes bearing a fully susceptible genotype range from low to negligible.

According to the IETS manual, the OIE recommends that only healthy ewes are used as embryo donors. It further describes procedures that reduce the risk of transmitting infectious diseases by embryo transfer (washing of embryos, source of hormones, surgical procedure, etc). As acknowledged in the EFSA 2010 Opinion, compliance with these recommendations would lead to a reduction of the potential risk of transmission of classical scrapie.

In conclusion, in sheep, the susceptibility to classical scrapie infection is strongly influenced by the polymorphisms of the *PrP* gene. Under natural exposure conditions animals that are heterozygous or homozygous ARR show respectively a low or negligible risk of being infected by classical scrapie. This genetic control of the susceptibility to classical scrapie infection directly influences the risk of transmitting the disease via embryo transfer:

- Irrespective of the embryo's genotype, the use of embryos derived from rams and dams carrying at least one ARR allele would significantly decrease the risk of transmitting classical scrapie via embryo transfer (by comparison to an embryo from parents of unknown genotypes).
- The use of homozygous ARR embryos would provide the highest possible level of safety with regard to the risk of transmitting classical scrapie through embryo transfer (*in vivo* derived embryos).
- The use of heterozygous ARR embryos would ensure a higher level of safety by comparison with Q₁₇₁/Q₁₇₁ embryos.
- The risk of transmitting classical scrapie by implantation of a Q₁₇₁/Q₁₇₁ embryo collected from a dam with an unknown classical scrapie status cannot be considered negligible.

Furthermore, providing that the OIE recommendations and procedures related to embryo transfer are adhered to, the risk of transmitting classical scrapie by the implantation of homozygous or heterozygous ovine ARR embryos can be considered negligible.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- Since the 2010 opinion only two relevant studies have been published. None of them suggest a need to revise the previous conclusion adopted by the BIOHAZ Panel in January 2010 *i.e.* '*Based on the data currently available the risk of TSE transmission associated with embryos collected from Classical scrapie incubating ewes and she-goats ranges from negligible to low. However, data are insufficient to conclude that such a risk is negligible.*'
- In sheep the susceptibility to classical scrapie infection is strongly influenced by the polymorphisms of the *PrP* gene. Under natural exposure conditions, animals that are heterozygous or homozygous ARR show respectively a low or negligible risk of being infected by classical scrapie.
- This genetic control of the susceptibility to classical scrapie infection directly influences the risk of transmitting the disease via embryo transfer:
 - Irrespective of the embryo's genotype, the use of embryos derived from rams and dams carrying at least one ARR allele would significantly decrease the risk of transmitting classical scrapie via embryo transfer (by comparison to an embryo from parents of unknown genotypes).
 - The use of homozygous ARR embryos would provide the highest possible level of safety with regard to the risk of transmitting classical scrapie through embryo transfer (*in vivo* derived embryos).
 - The use of heterozygous ARR embryos would ensure a higher level of safety by comparison with Q₁₇₁/Q₁₇₁¹⁰ embryos.
 - The risk of transmitting classical scrapie by implantation of a Q₁₇₁/Q₁₇₁ embryo collected from a dam with an unknown classical scrapie status cannot be considered negligible.
- Providing that the OIE recommendations and procedures relating to embryo transfer are adhered to, the risk of transmitting classical scrapie by the implantation of homozygous or heterozygous ovine ARR embryos can be considered negligible.

RECOMMENDATIONS

- The recommendations relating to the risk of transmitting classical scrapie via embryo transfer that were formulated in the 2010 opinion remain valid.
- In particular, the presence of infectivity in ovine embryos collected from scrapie infected dams bearing susceptible genotypes needs to be assessed before a definitive assessment of the risk of transmitting classical scrapie via the use of embryos bearing a susceptible genotype can be made.

¹⁰

By convention Q₁₇₁ refers to ARQ, VRQ, and AHQ alleles.

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APPENDICES

A. THEORETICAL RISK FOR AN EMBRYO TO CARRY INFECTIVITY OR TO BE INFECTED WITH CLASSICAL SCRAPIE INFECTIVITY ACCORDING TO THE COMBINATION OF ITS PARENTS AND ITS OWN GENOTYPE

The classical scrapie infectivity that might be carried by a sheep embryo is dependent on several factors:

- the infectious status of the dam (ovocyte / uterine environment), which will depend on the dam's genotype.
- the infectious status of the ram (semen), also influenced by the ram's genotype, and,
- the embryo's own genotype.

These elements are combined, according to the different genotypes, in the decision tree in the figure below. In this tree, the following assumptions are made:

- the risk of exposure to scrapie agent for an ovocyte/embryo *in utero* in the dam depends mainly of the dam genotype (and related infectious status) and only marginally of the ram genotype and infections status (potential infectious spermatozoid that would enter in the ovocyte). In other words, the dam infectious risk has a greater influence than the ram infectious risk on the risk of exposure to scrapie for an ovocyte/embryo.
- the final risk of an embryo to support prion replication is dominantly depending on its genetic susceptibility. Thus, genetic susceptibility of the embryo will have a greater influence on the risk of an embryo to support prion replication than the risk of *in utero* exposure.

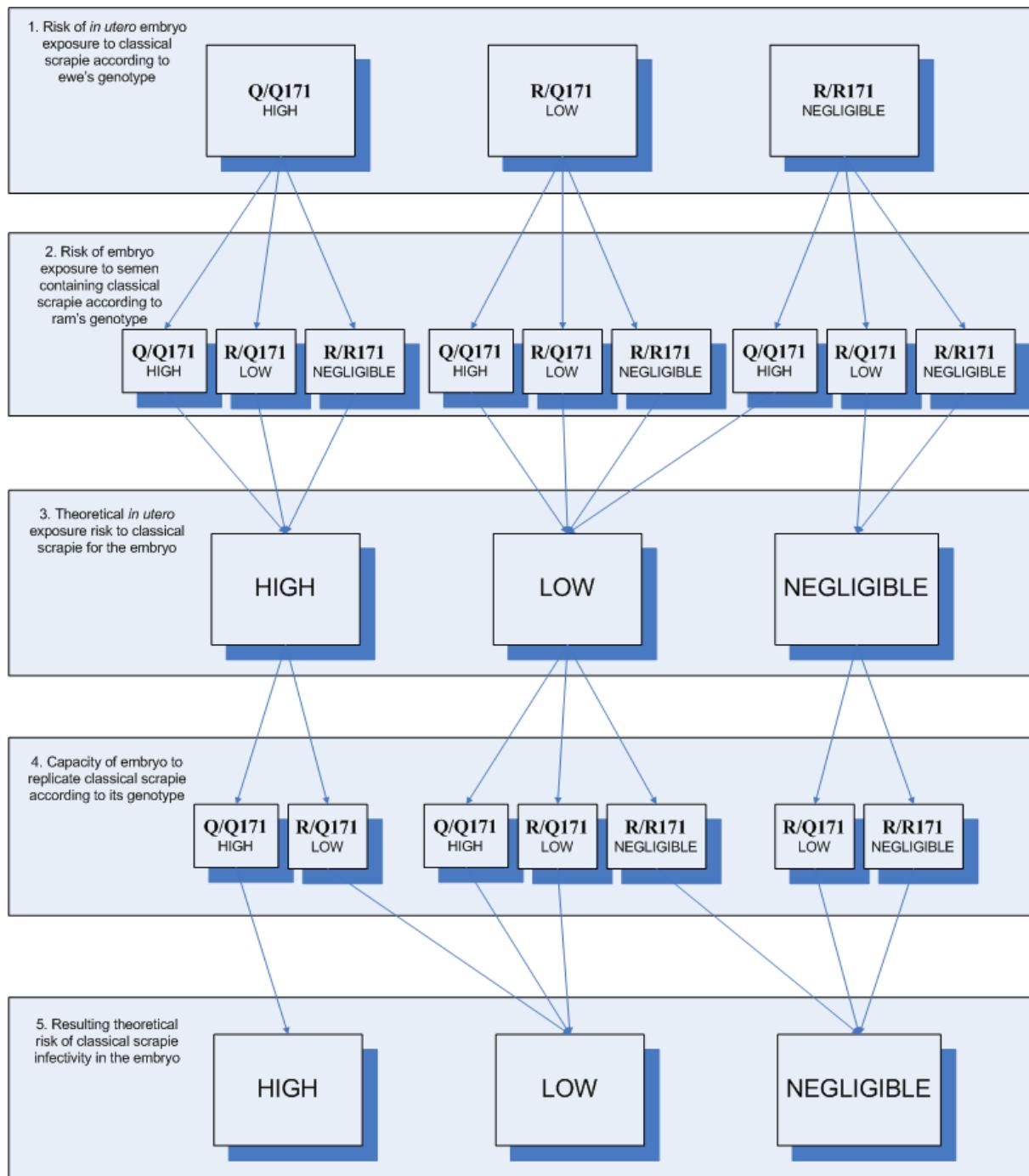


Figure 1: Theoretical risk for an embryo to carry infectivity or to be infected with classical scrapie infectivity according to the combination of its parents and its own genotype