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Review

The risk of small ruminant lentivirus (SRLV) transmission with reproductive biotechnologies: State-of-the-art review

C. Cortez-Romero [a], J.L. Pellerin [b], M.Z. Ali-Al-Ahmad [c], Y. Chebloune [d], J. Gallegos-Sánchez [e], A. Lamara [b], M. Pépin [f], F. Fieni [b,*]

[a] Colegio of Postgraduados (Postgraduate College), Campus San Luis Potosí, Animal Science Program, Salinas of Hidalgo, San Luis Potosí, Mexico
[b] LUNAM University, Oniris, Nantes-Atlantic National College of Veterinary Medicine, Food Science and Engineering, Sanitary Security of Reproduction Biotechnology Unit, Nantes, France
[c] Department of Surgery and Obstetrics, Faculty of Veterinary Medecine - University of Al-Baath, Hama, Syrian Arab Republic
[d] LAPM, UMR 5163, CNRS, Grenoble, France
[e] Colegio of Postgraduados (Postgraduate College), Campus Montecillo, Animal Science Program, Mexico-Texcoco, Montecillo, Texcoco, Mexico
[f] VetAgroSup-Campus Vétérinaire de Lyon, Marcy-l'Étoile, France

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ABSTRACT

Reproductive biotechnologies are essential to improve the gene pool in small ruminants. Although embryo transfer (ET) and artificial insemination (AI) greatly reduce the risk of pathogen transmission, few studies have been performed to quantify this risk. The aim of this review is to contribute to the elements needed to evaluate the risk of lentivirus transmission in small ruminants (SRLV) during ET, from embryos produced *in vitro* or *in vivo*, and with the use of the semen destined for AI. The purpose is to consider the genetic possibilities of producing uninfected embryos from infected females and males or bearers of the SRLV genome. We have reviewed various studies that evaluate the risk of SRLV transmission through genital tissues, fluids, cells, and flushing media from female and male animals. We have only included studies that apply the recommendations of the International Embryo Transfer Society, to obtain SRLV-free offspring from infected female animals using ET, and the justification for using healthy male animals, free from lentivirus, as semen donors for AI. As such, ET and AI will be used as routine reproductive techniques, with the application of the recommendations of the International Embryo Transfer Society and World Organization for Animal Health.

1. Introduction

In recent years, the use of reproductive biotechnologies, such as embryo transfer (ET), has been essential for the creation and diffusion of genetic progress, and to rescue endangered species or those with reproductive problems. Similarly, artificial insemination (AI) has enabled the genetic improvement of small ruminants in production. This progress has been enhanced by sanitary guarantees and easier access to international commercial exchanges. Nevertheless, the development of these reproductive biotechnologies on an industrial scale is accompanied by concern over disease transmission via embryos and semen, which has therefore become the subject of numerous studies.

Pathogenic agents can be transmitted by crossing the zona pellucida (ZP) or via infected particles adhering to the surface of that same ZP [1,2]. Pathogens can also adhere to the surface of the spermatozoon or to nonspermatic cells in the seminal plasma [3,4]. Although some authors claim that certain pathogens can also be transported within the deoxyribonucleic acid (DNA) of those same spermatozoa (SPZ) [5,6]. Subsequently, the transfer of contaminated

* Corresponding author. Tel.: +33 2 40687711; fax: +33 2 40687748.
E-mail address: francis.fieni@oniris-nantes.fr (F. Fieni).

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embryos to uninfected recipients can result in the infection of the latter or the embryo [1,2] (Fig. 1).

At present, the protocols developed and established by the International Embryo Transfer Society (IETS) [7] and the World Organization for Animal Health (OIE) [8], have been accepted and applied for international exchanges of reproductive material. These protocols are designed to ensure the integrity of the ZP and the absence of any adherent epithelial cells. They involve washing the embryos with simple or associated media, rather than using trypsin and antibiotics. In the case of AI, the Human Fertilization and Embryology authority in the United Kingdom recommend washing and centrifugation through Percoll gradients, and Sephadex columns to separate the SPZ from other components of the semen [9], and trypsin washes [10], or washing procedures through density gradients with trypsin [11]. The efficacy of the IETS and OIE recommendations to reduce the risk of pathogen transmission via ET and AI requires verification; this involves studying each pathogenic agent in each species individually [12,13].

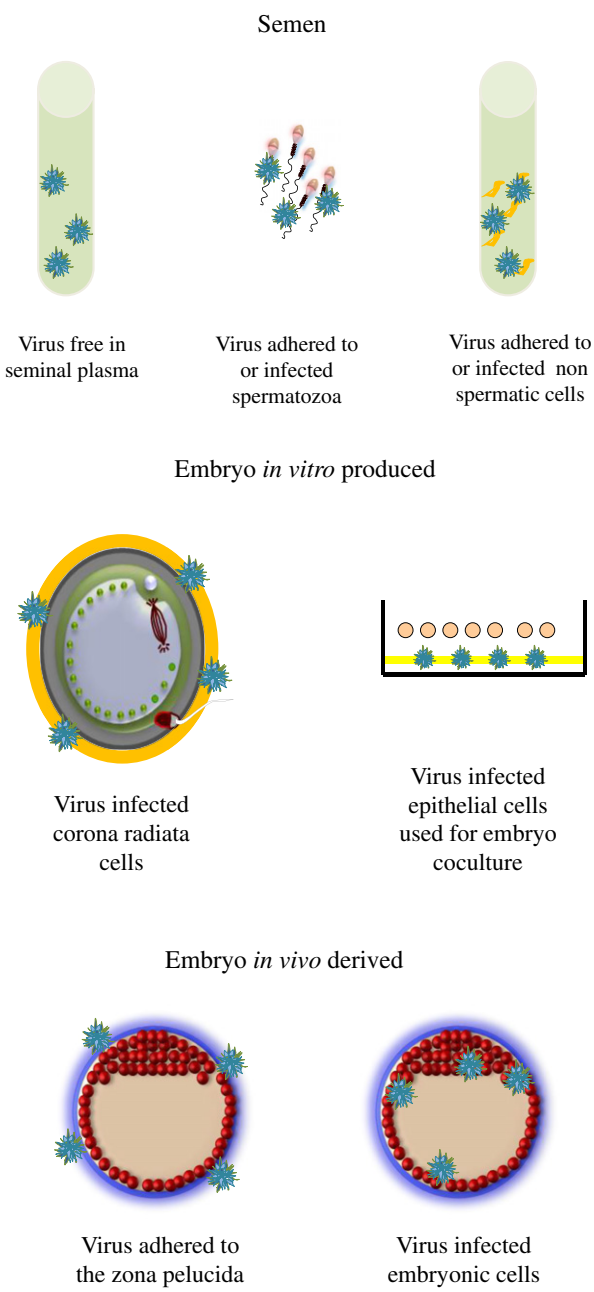

Fig. 1. Possible mechanisms of small ruminant lentivirus transmission via semen and embryos.

2. Small ruminant lentiviruses

The two main viral diseases in small ruminants, which are closely related, are Maedi-Visna virus (MVV) or pneumonia progressive virus in sheep and caprine arthritis-encephalitis virus (CAEV) in goats. They both belong to the group of diseases known as small ruminant lentiviruses (SRLV), and it is these that we have chosen to study in this review (Table 1).

Small ruminant lentiviruses are enveloped ribonucleic acid (RNA) viruses, belonging to the *Retroviridae* family and the *Lentivirus* genus [19,22,23] (Fig. 2). They cause persistent infections and irreversible, progressive, degenerative inflammatory disease, characterized by a long incubation period (1–3 years). This disease affects multiple organs including the lungs, synovium of joints and bursae, nervous system, and mammary glands [24,25]; only 30% to 35% of infected animals will develop clinical signs of the disease [26–29]. Small ruminant lentiviruses infect the monocyte-macrophage line as the main target cells *in vivo* [30–32] and the dendritic cells [33], with viral production inherent in the differentiation of monocytes to macrophages [30,31,34,35], and with the bone marrow serving as a reservoir of infected cells [36,37]. These diseases have a worldwide distribution (primarily Africa, America, Asia, and Europe), with a frequency ranging from 15% to 90% [38–40]. Importantly, recent studies show that these two lentiviruses can be transmitted between the two species, under both experimental [41,42] and natural conditions [43–46], which should be considered in programs designed to control and prevent these diseases in these two species. Small ruminant lentiviruses can be transmitted horizontally and/or vertically. Horizontal transmission occurs via direct contact between healthy and infected animals, via colostrum, aerosols, and natural mating. Vertical transmission, *in utero*, has also been demonstrated, but the exact mechanism of this transmission has yet to be elucidated (Table 2).

3. Health risk associated with ET and AI

Because of the risk of horizontal and/or vertical transmission of SRLV (Fig. 3), several countries are trying to obtain flocks that are free from lentivirus and other

Table 1
Lentiviruses in small ruminants (SRLV).

SRLV	Species	References
Maedi-Visna virus	Sheep	[14–16]
Ovine progressive pneumonia virus	Sheep	[17,18]
Caprine arthritis-encephalitis virus	Goat	[19–21]

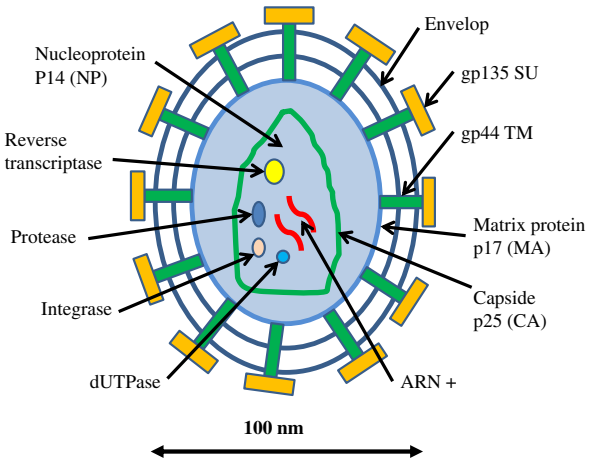

Fig. 2. Small ruminant lentivirus structure.

pathogenic agents, to facilitate the international exchange of certified disease-free animals, embryos, or semen. This review therefore examines the potential risks of SRLV transmission associated with the routine use of ET and AI in small ruminants.

3.1. Health risk in ET

The improvement in the quantity and quality of embryos obtained by assisted reproductive techniques is largely reliant on ET for the exchange of genetic material between different farms, regions, and countries [67]. The embryo surrounded by its ZP seems resistant to bacterial and viral infections and the risk of transmission by embryos is minimal or nonexistent [59]. The significant development of this technique, and the resulting dissemination of genetic material, raises the issue of the risk of introducing disease [60]. The apparition of certain infectious diseases in countries importing embryos of high genetic value has prompted the implementation of strict sanitary measures by those countries; these include certification from the exporting countries that the donors are free from specific pathogens. The current lack of understanding regarding the mechanism of transmission of pathogenic agents during ET, has only led to the enforcement of further restrictions by importing countries amid unfounded speculation [61].

To date, despite the sanitary (disease control), scientific (diffusion of ovine and caprine genetic progress), and economic (genetic value of small ruminants on an international scale and an increase in embryo export) concerns surrounding ET in small ruminants, few studies have been conducted to evaluate the potential risk of SRLV transmission to the embryo or recipient female. To promote ET in small ruminants as a safe method for genetic exchange for the creation of SRLV-free flocks [62], we need to assess the risk of lentivirus transmission, whether horizontal or vertical, understand the different methods for transferring embryos that are produced *in vivo* or *in vitro*, and verify the efficacy of the IETS safety regulations.

Table 2
Horizontal and vertical SRLV transmission.

Transmission	Contact	Modes	References
Horizontal	Direct	Consumption of colostrum and milk	[26,47–49]
		Inhalation of respiratory secretions	[48,50,51]
		Natural mating	[52–57]
	Indirect	Embryo transfer and AI	[13,58–62]
Vertical	Direct	*In utero* to the embryo or to the fetus	[26,48,63–66]

Abbreviation: SRLV, small ruminant lentivirus.

3.1.1. *In utero transmission (vertical transmission)*

Lentivirus can be transmitted via secretions from infected cells. Reports of cases of unexplained seroconversions in newborns separated from their mother, raise the suspicion of vertical transmission *in utero* or during delivery [26,48]. Maedi-Visna virus lesions were found at between 2 and 4 months of age in two of eleven rams delivered from infected ewes by hysterectomy [63], and uterine lesions were found in goats with clinical signs of CAEV [68], showing that the uterus can be the seat of viral multiplication. Brodie et al. [66] found that 11% of the offspring born to infected sheep in their study were infected *in utero*. Likewise, CAEV infected cells were detected in postpartum genital discharges in the goat [56] and in oviduct samples and wash media collected during embryo harvesting from infected goats [69]. Ovine lentivirus was isolated from three lambs born by cesarean section, which had been isolated for 8 to 9 months [64], and from a fetus of almost 100 days old born to an ewe that had been naturally infected by a ram [65]. Cases of seroconversion to CAEV were also observed in kids born to infected mothers, by cesarean section or by natural delivery, and which had not consumed any colostrum from their biological mother [48], and in kids fed with pasteurized milk or milk substitute inside a control program [49]. All of these results indicate the existence of *in utero* viral transmission from infected mothers to the embryo or fetus, with the participation of the tissues and cells of the female genital tract playing an important role.

3.1.1.1. Female genital tissue. Studies have reported caprine lentivirus infection in tissues and cells from the genital tract of superovulated goats [70,71]. Similar results *in vivo* were reported in goats by Fieni et al. [72], Ali-Al-Ahmad et al. [73], and in sheep by Cortez-Romero et al. [74,75], demonstrating SRLV-infected cells using nested-polymerase chain reaction in ovarian, oviduct, and uterine tissue from naturally infected females.

The presence of lentivirus-infected cells, whether of macrophagic or epithelial origin, explains the infection of lambs at birth, evaluated at 18% by Cross et al. [63] and 11% by Brodie et al. [66], and the *in utero* infection recorded by Cutlip et al. [65]. It also explains the positive CAEV viral sequence amplification results in caprine postpartum secretions [56].

According to Ali-Al-Ahmad [61] and Cortez-Romero [67] (unpublished results), although the cotyledonary epitheliochorial placentation of small ruminants normally prevents direct contact between fetal and maternal blood, cellular exchange does occur in the event of local inflammation with leukocytic infiltration [68,76]. The presence of

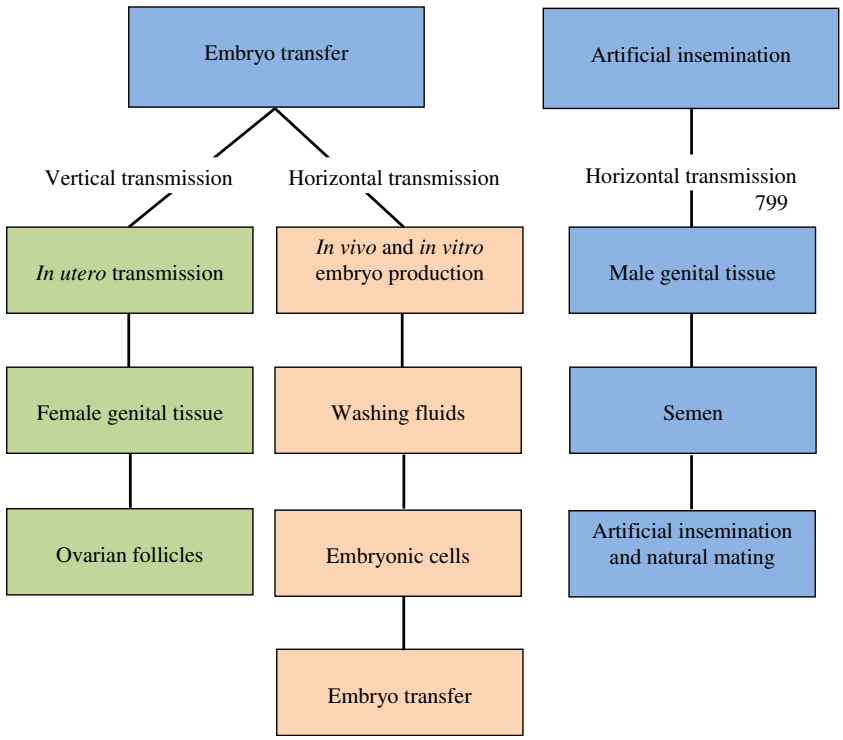

Fig. 3. Schematic diagram of small ruminant lentivirus transmission by embryo transfer and AI.

these leukocytes would favor the infection of the neonate at delivery, during placental maturation, and/or during distension of the cervix. This would also explain the seroconversions observed in neonates delivered by cesarean section [64] and in naturally born kids who are deprived of colostrum [48]. Early contamination of the oocyte, during its development in contact with contaminated ovarian cells is also discussed.

3.1.1.2. Ovarian follicles. There have been no studies to evaluate the risk of vertical SRLV transmission from mother to fetus during the oocytic phase. However, caprine lentivirus infects and replicates in granulosa cells *in vitro* [77]; CAEV and MVV-proviral DNA has been detected in granulosa cells recovered from naturally infected goats and sheep [78,79], respectively. There is therefore a risk of viral transmission during *in vitro* embryo production (IVP), because granulosa cells are commonly used for oocyte development and maturation [80,81]; if such cells are removed from the oocytes before maturation, their capacity for development is reduced [82].

Small ruminant lentiviruses therefore have the opportunity to be in direct contact with oocytes with an intact *cumulus oophorus* (CO) during the oogenesis or *in vivo* maturation phase after ovulation. The sanitary safety of reproductive biotechnologies is therefore conditioned by the quality of the oocyte-CO complex.

In recent studies, SRLV-proviral DNA was identified in CO cells from naturally infected sheep (2.8%) and goats (26.0%) [39,73]. These studies showed that, despite being surrounded by these infected cells and follicular fluid, the oocyte is lentivirus-free. Cumulus cells were then eliminated by enzymatic washing. These authors state that the most probable hypothesis for the resistance of the oocyte to lentivirus infection is the absence of oocyte membrane receptors, which are required for the internalization of lentiviruses, as reported by Mselli-Lakhal et al. [83]. Nevertheless, the structure of such receptors for cell internalization remains to be defined for such lentiviruses. It is evident that the presence of SRLV-proviral DNA in oviduct and uterine tissues, and in follicular and ovarian cells, presents a risk of lentivirus transmission to the embryo and/or fetus, although more studies are needed.

3.1.2. In vivo and IVP (horizontal transmission)

The risk of SRLV transmission is also present during IVP, because after *in vitro* fertilization (IVF), the early embryos are usually cultivated on oviduct epithelial cell layers [84,85], because their secretions are needed to ensure satisfactory embryonic development before implantation in the recipient [86,87]. In goats, it has been shown that oviduct epithelial cells are susceptible to CAEV infection *in vitro* [88]. Such cells are often harvested from sheep and/or goats in the abattoir, where the sanitary status is unknown.

In industrialized countries, between 60% and 80% of caprine flocks are bearers of caprine lentivirus [89]. It is also important to demonstrate whether the recovery and washing media obtained from the lumen of oviducts and uterus, are infected during embryo collection (*in vivo*).

3.1.2.1. Washing fluids. The presence of CAEV-infected cells in has been detected in oviduct washing media recovered

during embryo collection from superovulated goats [69]. More recent studies in goats found CAEV proviral DNA [61] and viral RNA [90] in washing media during embryo collection.

Caprine lentivirus has also been found in wash media from the genital tract, although to date, no such studies have been carried out in sheep. The presence of SRLV genome in the genital tract, the identification of active viral replication in the epithelial cells or macrophages of such tissues, and the detection of infected cells in wash media, presents a risk of early infection of the embryo and propagation of lentivirus infection via ET.

However, this risk seems limited because *in vitro* studies have shown that an intact ZP will protect the embryo from viral infection in goats [91]. In sheep and goats, it was confirmed that the IETS recommendations, which include using ZP-intact embryos and 10 wash cycles, enable the elimination of MVV and CAEV infection *in vitro* [92–94]. It is important to specify the protective properties of the ZP in *in vitro*-produced embryos, grown on granulosa cell or oviduct epithelial cell monolayers, which are usually taken from animals of unknown sanitary status. This risk now needs to be measured during the embryonic development phase.

3.1.2.2. Embryonic cells. In some studies, early embryonic cells taken from goat embryos produced *in vivo* (eight- to 16-cell stage) were found to transmit CAEV [91,95]. Lamara et al. [91] demonstrated that an intact ZP is a strong barrier that protects the caprine embryo from CAEV infection, but ZP-free embryos, when incubated with CAEV and washed extensively, could transmit the infection to the permissive indicator goat synovial membrane (GSM) cells. Ali-Al-Ahmad et al. [96] reported that caprine blastomeres are susceptible to CAEV infection and that those cells are viable: ZP-free embryos at the eight- to 16-cell stage produced at least $10^{3.25}$ median tissue culture infective doses ($TCID_{50}$) per mL over 24 hours in the acellular medium. These results clearly demonstrate that caprine early embryonic cells are susceptible to CAEV infection and that infection with this virus is productive.

However, Cortez-Romero [67] and Cortez-Romero et al. [93] reported that this phenomenon of viral replication in sheep embryonic cells from *in vitro*-produced embryos, in the absence of a ZP, is weak, meaning the susceptibility of ZP-free embryos to viral infection is variable. The adsorption of enveloped viruses is strictly regulated by the presence of functional receptors, expressed at the surface of the target cells. These authors thus all report using early embryos at the eight- to 16-cell stage, which are known to show active gene expression and protein synthesis necessary in sheep [97,98].

The risk of disrupting the ZP is minimal *in vivo* in early embryos, unlike during *ex vivo* and *in vitro* manipulation for ET, which therefore increases the risk of SRLV infection in early embryos. Data from Ali-Al-Ahmad et al. [96], Lamara et al. [91], and Cortez-Romero et al. [92,93], concur that the only barrier to prevent natural SRLV infection of female blastomeres is the presence of an intact ZP. The ZP of embryos is composed of three different glycoproteins, ZP1, ZP2, and ZP3, which create a mechanical barrier against viruses and bacteria [99].

3.1.2.3. Embryo transfer. There are some ET studies using embryos taken from SRLV-infected donors and transferred to SRLV-free recipients; Wolf et al. [100] took embryos from CAEV-positive donors with clinical disease (arthritis) mated to CAEV-positive males with early clinical signs, and implanted them into healthy, seronegative recipients. None of the recipients or resulting offspring (up to 4 months of age) seroconverted. Lentivirus was not detected in any of the samples taken from the recipients (colostrum, placenta) and live or dead neonates. A recent Brazilian study confirmed the absence of seroconversion, up to 6 months old, in kids obtained by ET from clinically infected CAEV-positive goats mated with CAEV-infected males [101]. Caprine lentivirus was not detected in the embryo collection media in either of the latter two studies. However, seroconversion can occur up to 8 months after infection [58], and these studies were conducted over only 4 and 6 months, thus the risk of infection via ET cannot be ruled out.

Woodall et al. [102] demonstrated that embryos and uterine wash media taken from MVV-infected sheep were virus-free. Recently, Vainas et al. [103] reported that embryos harvested from MVV-positive sheep according to IETS recommendations and transferred to seronegative recipients, produced MVV-free lambs. No subsequent seroconversion was detected in either the recipients or the lambs, which were all tested every 6 months for 3 years. In goats, Ali-Al-Ahmad et al. [90] reported that under acute infection conditions, ET can be safely used to produce CAEV-free neonates from infected CAEV donors. They used CAEV-positive goats as donors and kids were separated from their mothers at birth. All samples from the recipient goats and kids were negative for CAEV-antibodies and/or CAEV proviral DNA. Recently, a study reported that CAEV-free embryos can be produced by IVF using SPZ infected *in vitro* by CAEV [94].

These results emphasize the importance of the protective ZP during ET and thus confirm the validity of the IETS recommendations to use ZP-intact embryos and successive washings, with the additional restriction of the strict elimination of any non-ZP-intact embryos. Such precautions will minimize the risk of the emergence of endogenous SRLV genomes in animals produced by ET from lentivirus positive females. This is further supported by the absence of any substantiated report, anywhere in the world, of disease transmission to an uninfected recipient after the commercial transfer of *in vivo*-derived embryos [1,2], despite the high numbers of embryos that are transferred each year.

3.2. Health risk in AI

The reproduction of small ruminants by AI offers economic and genetic advantages to flocks specialized in milk or meat production. To become more widespread, the quantity and quality of the semen obtained must be improved, particularly in terms of storage. Semen represents a significant potential risk for spreading infectious diseases. This risk is linked to the numerous pathogenic microorganisms that can be present in the semen and to the multiplication factor because of the high numbers of

straws prepared from each individual ejaculate. This risk persists over time, because most microorganisms survive the freeze-thaw process [61]. The transmission of viral diseases via semen is a significant problem in both human and veterinary medicine.

SRLV can be transmitted horizontally from male to female and/or vertically from male to offspring.

3.2.1. Male genital tissue (horizontal transmission)

Caprine arthritis-encephalitis virus proviral DNA has been identified in preputial cells, seminal plasma, and in nonspermatic cells of the ejaculate of experimentally infected billy goats [55]. Recently, Peterson et al. [104] reported that tissue samples from testes, epididymis, ampullary, vesicular, prostate, and bulb-urethral glands were positive for SRLV proviral DNA (CAEV and MVV). Similarly, Ali-Al-Ahmad et al. [105] reported the presence of CAEV proviral DNA in various genital tissues (testis, epididymis [head, body, and tail], *vas deferens*, and vesicular glands) from goats. These results indicate that genital cells and tissue contribute to the horizontal transmission of SRLV.

3.2.2. Semen

Few studies have studied infection of the semen; results of earlier studies performed in a wide variety of tissues have demonstrated the infection of other cell types, such as epithelial cells, which are commonly found in the semen. Theoretically, SRLV can be found in the sexual organs and semen of infected rams and bucks in three different forms, namely incorporated proviral DNA (in macrophages), as virions (complete virus particles with their single-stranded (ss) RNA core and protein coat released by budding from the plasma membrane), and as free viruses (released by cell lysis) [104]. In the sheep, one of the first studies on the potential risk of lentivirus transmission by semen [52] revealed pathological lesions in the testes of MVV-infected rams. This lentivirus has also been found in the epididymis of rams that have been experimentally infected with *Brucella ovis* and its tropism by the epididymal epithelial cells would then be responsible for infection of the semen [54,57]. These results show that persistent infection or inflammation in the testes would induce the secretion of the lentivirus in the semen. These authors suggest that the presence of the virus in the semen might be because of excretion from the epididymal epithelial cells.

One study [55], tested the semen of experimentally infected male goats for CAEV-proviral DNA. The seminal fluid of those goats was found to be infected, but not the spermatic cells or SPZ. These results were confirmed 1 year later, using naturally infected male goats [106]. More recent studies [107–109], detected CAEV proviral DNA and viral RNA in the spermatic fractions of naturally infected males, nonspermatic cells, and seminal plasma [105]. Peterson et al. [104], using a group of naturally SRLV-infected individuals, reported the presence of proviral SRLV DNA in epididymal tissue and semen. The fact that epididymal semen and tissue samples from the testes, epididymis, ampullary, vesicular, prostate, and bulbourethral glands all tested positive for proviral DNA, indicates that various male sexual organs might directly contribute to the shedding of proviral SRLV DNA in ejaculated semen. They suggest that there is a seasonal shedding pattern, and a possible explanation for this phenomenon is an increase in sexual activity and stress coinciding with the optimal breeding period of these seasonal breeders. Increased stress is known to (re-) activate viral loads and subsequent viral excretion.

Nevertheless, no studies have shown positive results in the SPZ fraction. This *in vivo* resistance of SPZ to lentivirus infection could be because of epididymal proteins that protect the SPZ during their transit through the male genital tract. These proteins, by capping the SPZ, stabilize the plasma membrane and prevent a premature acrosomal reaction [110]. Ali-Al-Ahmad et al. [105] indicate that the most likely hypothesis to explain the resistance of SPZ to the infection is the absence of SPZ plasma membrane receptors, which are required for the internalization of the SRLV particle. The structure of these receptors remains to be elucidated for lentivirus, while in man it has been shown that SPZ do not express significant levels of surface receptors (CD4, CCR5, and CXCR4), indicating that they are unlikely to be the principal targets for HIV [3,111]. All these studies clearly indicate that the sexual organs might contribute to shedding proviral SRLV DNA in ejaculated semen and therefore the infectious nature of semen, found in both experimentally and naturally infected males, implying that SRLV transmission via semen is possible.

3.2.3. Artificial insemination and natural mating

Because homosexual activity is common when rams and bucks are housed together, studies should include both male–male and male–female contacts [104]. To confirm that sexual transmission is possible, we would need to inseminate noninfected females using positive ejaculates or natural mating with an infected male. One study bred MVV-infected rams to uninfected ewes via natural mating; the animals were housed separately before and after mating and none of the ewes seroconverted [112].

Similarly, when CAEV-infected bucks were bred to uninfected does directly or by AI, none of the does had seroconverted within 18 months of insemination [48]. It is likely that, in these studies, the semen did not contain enough viral particles to transmit the virus or that the animals did not remain in direct contact for long enough, despite the sexual contact. Rowe et al. [53] reported one seroconversion in a female goat bred to a CAEV-positive male goat.

A recent study comparing laparoscopic transabdominal intrauterine insemination using infected semen and natural mating with naturally infected male goats, demonstrated proviral DNA in uterine smears and embryo collection media [61] (unpublished data). This last author indicates that the high frequency of infection observed in the AI goats compared with natural mating, could be because of a high concentration of infectious particles and the absence of the natural defensive barriers normally provided by the cervix and vaginal secretions [113]. AI can be either transcervical or intrauterine, and laparoscopic transabdominal intrauterine insemination completely bypasses the host defenses; transcervical insemination (especially without complete penetration to the uterus) might leave more of these defenses intact.

In this context, it is clear that both natural mating and AI can be sources of lentivirus transmission, with the detection of proviral DNA in the nonspermatic cell fraction of the semen [61]. These cells primarily include monocyte macrophages, the main target cells of lentivirus replication *in vivo* [30–32], which can be present in the lumen of the spermatic tubes and the epididymis in sufficient concentrations to be detected, without altering tissue function or the fertility of the semen. Small ruminant lentivirus is thought to enter the semen from the circulation via infected macrophages [114,115]. According to Peterson et al. [104], proviral DNA is predominantly detected in the semen fraction that contains macrophages and cytoplasmic droplets. Finally, the risk of transmission through sexual contact appears to be low, despite studies demonstrating the presence of the virus in semen. However, too few studies have been performed to enable any definite conclusions to be drawn and further work is warranted [115].

4. Conclusions

Numerous studies have proven the presence of SRLV-proviral DNA in the genital tissues of naturally infected females. The possibility of vertical SRLV transmission from mother to embryo or fetus *in utero* and horizontally during the transfer of embryos produced *in vivo* or *in vitro*, explains the origin of a certain number of *in utero* and postnatal infections. The elimination of the CO cells surrounding the oocyte generates SRLV-free genetic material that can be used in an *in vitro* embryo production program.

Likewise, ET from infected donors to healthy females, in accordance with the IETS protocols (ZP integrity and 10 washes), confirms the validity of these IETS recommendations. These results also confirm the conclusions of Wrathall and Sutmöller [1], Stringfellow and Givens [2], and Blacklaws et al. [115], that ET appears to pose minimal risk, provided that the embryos retain their ZP and are washed to IETS standards. Although, we believe that more *in vivo* studies with a larger number of animals are needed to completely guarantee the safety of ET as a means of disseminating genetic material via the female line from infected to SRLV-free animals.

Nevertheless, it is well known that selection via the male line, using AI, is the most powerful route for genetic progress. Studies have shown that SRLV infect tissues from the male genital tract and have found lentivirus in the semen, which increases the possibility of horizontal transmission from male to female or vertical transmission from male to offspring, during natural mating or AI. Yet, despite infection of the seminal plasma and nonspermatic cells, the SPZ seem to resist lentivirus infection. Blacklaws et al. [115] indicated that the use of semen as a product for AI appears to represent a minor risk. We recommend further AI studies to determine the exact degree of risk, using washed semen from infected males to inseminate SRLV-free females. Meanwhile, the need for SRLV-free males, bred in special herds and regularly tested, is justified for use as sperm donors for AI in genetic selection programs.

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