Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 69 (2008) 408–415
Theriogenology
www.theriojournal.com

Lack of risk of transmission of caprine arthritis-encephalitis virus (CAEV) after an appropriate embryo transfer procedure

M.Z. Ali Al Ahmad [a], Y. Cheblounе [b,c], B.A. Bouzar [c], G. Baril [d], F. Bouvier [e], G. Chatagnon [a], B. Leboeuf [f], M. Pepin [g], J.M. Guibert [h], P. Russo [h], E. Manfredi [i], J. Martin [j], F. Fieni [a,*]

[a] *Department of Research into the Health Risks and Biotechnology of Reproduction ENVN/DGER, National Veterinary School, Nantes Cedex 03, France*
[b] *MMD Labs, Kansas University of Medical Center, USA*
[c] *INRA, Department of Animal Health, France*
[d] *INRA, UMR85 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France*
[e] *INRA Unité expérimentale caprine, Bourges, France*
[f] *INRA de Rouillé, France*
[g] *AFSSA-LERPAZ, Maisons-Alfort, France*
[h] *AFSSA Sophia Antipolis, France*
[i] *UR631-INRA de Toulouse, France*
[j] *Station caprine de Moissac, France*

Received 8 August 2007; received in revised form 6 October 2007; accepted 10 October 2007

Abstract

The aim of this study was to demonstrate that embryo transfer can be used to produce CAEV-free kids from CAEV-infected biological mothers when appropriate procedure is implemented.

Twenty-eight goats that had tested positive for CAEV using PCR on vaginal secretions were used as embryo donors. Embryos with intact-ZP were selected and washed 10 times; they were then frozen and used for transfer into CAEV-free recipient goats. Nineteen of the 49 recipient goats gave birth, producing a total of 23 kids. Three blood samples were taken from each recipient goat, 10 days before, during, and 10 days after parturition; these were tested for CAEV antibodies using ELISA and for CAEV proviral DNA using PCR. The mothers were then euthanized. Tissue samples were taken from the lungs, udder, and retromammary and prescapular lymph nodes.

The kids were separated from their mothers at birth. Seven of them died. At 4 months of age, 16 kids were subjected to drug-induced immunosuppression. Blood samples were taken every month from birth to 4 months of age; samples were then taken on days 15, 21, and 28 after the start of the immunosuppressive treatment. The kids were then euthanized and tissue samples taken from the carpal synovial membrane, lung tissue, prescapular lymph nodes, inguinal and retromammary lymph nodes, and uterus.

All samples from the 19 recipient goats and 23 kids were found to be negative for CAEV antibodies and/or CAEV proviral DNA.

Under acute conditions for infection this study clearly demonstrates that embryo transfer can be safely used to produce CAEV-free neonates from infected CAEV donors.

Keywords: Caprine arthritis-encephalitis virus; Embryo transfer; IETS; Polymerase chain reaction

* Corresponding author. Tel.: +33 2 40 68 77 10; fax: +33 2 40 68 77 48.
E-mail address: fieni@vet-nantes.fr (F. Fieni).

0093-691X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2007.10.009

1. Introduction

Caprine arthritis-encephalitis virus (CAEV) is a lentivirus that causes chronic arthritis, interstitial pneumonia, and indurative mastitis with decreased milk production in adult goats [1]. Less commonly, CAEV causes leukoencephalomyelitis in young goats [2]. The high prevalence of CAEV infection is a major concern in many parts of the world, particularly in industrialized countries where 80–95% of the breeding stock may be infected [3,4].

The major route of CAEV transmission is via the ingestion of virus-laden colostrum or milk from infected does [5]. Less efficient routes of transmission, such as the vertical route, need to be investigated to improve control and eradication methods [6]. Embryo manipulation has become a routine technique for fundamental research such as gene transfer or cloning and for commercial exchanges. The risk of viral infection of the goat embryo during embryo transfer has been demonstrated *in vivo* through the demonstration of CAEV proviral DNA in the embryo harvesting fluid [7], as well as in the cells of the cumulus oophorus surrounding the oocytes in the ovarian follicle [8]. *In vitro*, the epithelial cells of the oviduct [9], the cells of the granulosa [10], and the embryonic blastocytes [11] were shown to be sensitive to infection with CAEV and capable of producing virus. However, washing *in vitro*-infected embryos with an intact zona pellucida (intact-ZP) 10 times over [12], or washing oocytes with intact-ZP, in which the *in vivo* CAEV-infected cumulus oophorus cells have been physically and enzymatically removed, 10 times over [8], resulted in the production of virus-free female gametes or embryos that can be used for *in vitro* fertilisation or embryo transfer.

In goats, few studies have been undertaken to examine the consequences of embryo transfer (ET) on the vertical transmission of CAEV. In 1987, Wolf et al. [13] did not report any cases of seroconversion in 4-month-old kids issued from embryo transfer, originating from seropositive donor goats that had been inseminated by seropositive bucks. Later, a Brazilian study confirmed the absence of seroconversion at 6 months of age in kids born after embryo transfer and issued from seropositive goats with clinical disease, Cavalcante et al. [14]. These are the only two studies to have reported serological findings in kids obtained by embryo transfer. However, it has been demonstrated that delayed seroconversion may be observed following natural or experimental infection, up to 8 months of age [15]. Molecular biology now enables earlier and more sensitive diagnosis, as well as the detection of animals with latent infection and who are seronegative despite the presence of proviral DNA in the cells of their genital apparatus [16].

The aim of this experiment was to determine whether embryo transfer can be used to produce CAEV-free kids from CAEV-infected biological mothers.

2. Materials and methods

2.1. Production and freezing of embryos

Thirty goats that had repeatedly tested seropositive for CAEV using ELISA, and that had been confirmed as positive using PCR on leucocytes and on vaginal secretions, were divided into groups of 10 animals and used as embryo donors. Each group of 10 females underwent two embryo-collection procedures at an interval of around 2 months. Oestrus was synchronized in the donor goats by the insertion on day 0 of a vaginal sponge impregnated with 45 mg of Fluorogestone acetate FGA (Chronogest, Intervet, Angers, France). On day 9, each goat received 50 μg of Cloprostenol (IM) (Estrumate®-Schering-Plough Veterinary, Levallois Perret, France). The sponges were removed on day 11. Superovulation was induced by the IM-injection of 160 μg of porcine FSH (Merial, University of Liege, Belgium) given twice daily for 3 days (Days 9–11) in decreasing amounts (40, 40, 20, 20, 20 and 20 μg). Porcine LH was added to the FSH preparation on the last two injections (66 μg per injection). The total dose of FSH used per goat was equivalent to 16 Armour units [17].

The donor goats were artificially inseminated under endoscopic control with 200 million previously frozen spermatozoa collected from certified uninfected bucks, 48–52 h after the withdrawal of the vaginal sponge.

The embryos were collected surgically 7 days after the onset of oestrus [18]. The lumen of each uterine horn was infused with 40 ml of modified phosphate-buffered saline (PBS) (PBS–BSA 4% IMV Technologies, France) containing 4% BSA with Kanamycin sulphate 50 mg/l. This washing liquid was collected into a sterile flask and a mucosal swab was taken from each uterine horn. After collection, a smear of uterine cells was made for each horn using a cytobrush. The cytobrushes were stored in 15 ml tubes containing 10 ml of PBS until they arrived at the laboratory. At the laboratory, the tubes containing the cytobrushes were centrifuged to detach the cells. The cells were then recovered by centrifugation (1900 × *g* for 15 min) and washed twice in PBS.

The embryos were identified using a binocular microscope and assessed for quality (magnification 70×

to 80×). All of the embryos and oocytes collected were washed in batches in 10 successive baths of a phosphate buffer (PBS–BSA 4% IMV Technologies, France) in accordance with the IETS guidelines [19]: each wash corresponding to a dilution of 1/100 of the previous medium, with a new sterile pipette used for each bath.

Embryos that were at a correct stage of development (compacted morula or blastocyst), and which had a perfectly intact *zona pellucida*, were selected for freezing. Selected embryos were immersed for 5 min in three successive baths of PBS/BSA 4%, with increasing concentrations of ethylene glycol (0.5, 1.0, 1.5 M) [20]. After the final bath, the embryos were aspirated with 20–30 μl of PBS–ethylene glycol into the central compartment of a 0.25 ml straw (IMV Technologies, France). The two extremities of the straw were filled with a solution of PBS/BSA 4% and ethylene glycol 1.5 M. The straws were placed in a programmable freezer (Agrogen, EFI, Freiburg, Switzerland), and then cooled at a rate of 4 °C/min from 25 to −7 °C. Crystallization was induced 5 min later and after 10 min at this temperature, cooling was continued to −30 °C (0.3 °C/min). After 15 min at this temperature, the straws were plunged into liquid nitrogen and stored.

The cells in suspension in the harvesting medium and on the uterine swabs were harvested in pellets following centrifugation (1900 × *g* for 15 min). The supernatant was discarded and the cell pellets washed twice with 1 ml of sterile PBS, before being frozen at −80 °C for subsequent CAEV proviral DNA testing using PCR. The 10 embryo washing solutions were also concentrated by ultracentrifugation at 100,000 × *g* for 0.5 h. Each pellet was re-suspended in 140 μl of PBS and stored at −80 °C for subsequent examination, following extraction, for viral DNA using RT-PCR.

2.2. Embryo transfer

The recipient goats were selected from a herd of 53 goats comprising 3–6-year-old females, born into CAEV-free certified herds and raised for the last 2 years away from any new introduction or risk of contamination. The absence of infection in these females was checked using four independent ELISA serology tests per year from birth. Prior to selection as a recipient, two blood samples and vaginal swabs were taken from each goat, at an interval of 1 month. PCR testing on these samples revealed that 8/53 of these goats harboured CAEV proviral DNA. These animals were removed from the recipient herd. A further blood test was taken from the 45 remaining goats to detect the presence of CAEV following monocyte-derived macrophage cultivation, a technique that has been proven to promote the replication of any latent provirus [21,22]. Mononuclear cells (around 10^8 cells) were cultured in Teflon bottles containing a macrophage differentiation medium (MDM) composed of RPMI 1640 (Invitrogen, France) supplemented with 10 mM of HEPES, pH 7.3, 50 μg/ml of gentamycin, 5×10^{-5} M β-mercaptoethanol, 2 mM of glutamine, and 20% heat-inactivated newborn lamb serum. These were cultured at 37 °C with 5% CO_2 for 2 weeks. The matured macrophages were transferred from the Teflon bottles to a culture flask for 2 weeks. Portions of cells were then examined using PCR to detect proviral DNA, and the remaining portions were used for the *in situ* detection of the characteristic cytopathic effects (CPE) of CAEV. Two of the 45 goats tested positive on PCR and demonstrated CPE; these were excluded. The 43 goats that tested negative for CAEV on all detection methods were selected as recipients; some of them were used twice over for embryo transfer.

The recipient goats underwent oestrus synchronization/induction to ensure that they were compatible with the stage of development of the embryos. This involved the insertion of vaginal sponges impregnated with 45 mg of fluorogestone acetate for 11 days (Chronogest®, Intervet, Angers, France), combined with the intramuscular injection, 48 h prior to the withdrawal of the sponges, of 50 μg of a prostaglandin F2α analogue (Cloprostenol) (Estrumate®) and 600 IU of eCG (Intervet, Angers, France).

The transfer procedures were performed on day 7 after the onset of oestrus. The straws containing the embryos were thawed by immersion in a water-bath at +37 °C for 20 s. The cryoprotective agent was removed by passing the straws through two baths of PBS/BSA + 0.25 M of sucrose, then through two baths of PBS/BSA (5 min/bath). The embryos were assessed using morphological criteria under a binocular microscope; the transferable embryos were surgically implanted (two or three embryos) into the lumen of the uterine horns [18]. One hundred and thirty-nine embryos were transferred during three transfer procedures at 1-month interval.

2.3. Gestation and parturition

Pregnancy was diagnosed in the recipient female goats using serum progesterone assays 21–22 days after the onset of oestrus, and was confirmed ultrasonographically at between 45 and 60 days of gestation. The kids were born over a 3-month period, as a function of the dates of the embryo transfer procedures.

Three blood samples were taken from the mothers into heparinized tubes, 10 days before, during, and 10 days after parturition; a placental swab was also taken, using a cytobrush, at the time of parturition. Ten days after parturition, the mothers were euthanized. Tissue samples were taken from the lungs, udder, and retromammary and prescapular lymph nodes. These samples were stored at −80 °C for subsequent PCR testing for CAEV proviral DNA.

2.4. Neonates

The kids ($n = 23$) were separated from their mothers at birth and isolated to prevent all risk of contamination. They were fed with milk replacer. At 4 months of age, the kids ($n = 16$) were subjected to drug-induced immunosuppression combined with antibiotic treatment for the prevention of opportunistic pathogens [23–25]: 5 mg of dexamethasone (VOREN® injectable solution, Boehringer Ingelheim, France) were injected intramuscularly every 2 days for 10 days, combined with the subcutaneous injection of 75 mg of amoxicillin (CLAMOXYL LA© Pfizer Animal Health, France) every 2 days for 3 weeks. Serological testing for CAEV antibodies using ELISA and PCR testing of the leucocytes for CAEV proviral DNA was performed from blood samples taken every month, from birth to 4 months of age, then three times at days 15, 21, and 28 after the start of the immunosuppressive treatment. The kids were then euthanized and tissue samples taken from known preferential sites for CAEV infection: carpal synovial membrane, lung, prescapular lymph nodes, inguinal and retromammary lymph nodes, and uterus (in females). These samples were stored at −80 °C for subsequent PCR testing for CAEV proviral DNA.

2.5. Nested-PCR and RT-PCR

The extraction of cellular DNA from blood samples, thawed vaginal smears, and various tissue samples, was performed using the "QIAamp tissue kit®" (Qiagen, Courtaboeuf, France). After thawing, the ten embryo washing solutions from each goat were concentrated by ultracentrifugation at 100,000 × g for 0.5 h. The supernatant was discarded; RNA extraction was performed on the cells of the pellet using the "QIAamp® Viral RNA" kit (Qiagen, Courtaboeuf, France).

The nested-PCR technique was used to detect the presence of CAEV proviral DNA in the blood and tissue samples from the mothers and kids, as well as in the collection fluid. RT-PCR was used to detect viral RNA in the 10 embryo washing fluids.

Proviral CAEV DNA was examined using nested-PCR [26]. CAEV gag sequences were amplified using primers GEX5 (5′-GAA GTG TTG CTG CGA GAG GTG TTG-3′) and GEX3 (5′-TGG CTG ATC CAT GTT AGC TTG TGC-3′), corresponding to bases 393–416 and the complement of bases 1268–1291 of CAEV-CO [27]. Samples of 10 μl of isolated DNA (containing 0.5–1 μg) were used as templates for PCR amplification (94 °C for 1 min, 46 °C for 1.5 min, and 60 °C for 2.5 min). Amplification was preceded by an initial denaturation at 94 °C for 5 min and terminated with a final extension at 60 °C for 15 min. Five microliters of the PCR products of this reaction were used as a template for a second round of amplification using the internal primers GIN5 (5′-GAT AGA GAC ATG GCG AGG CAA GT-3′) and GIN3 (5′-GAG GCC ATG CTG CAT TGC TAC TGT-3′), located at positions 524–546 and 1013–1036 in CAEV-CO. DNA integrity was checked by amplifying the β-actin housekeeping gene using primers based on the human sequence [28]. Amplified products were visualized by ethidium bromide staining after electrophoresis in 1.5% agarose gel. This technique has been shown to be capable of detecting fewer than 10 infected cells in samples containing 10^6 or 10^7 cells [29].

RT-PCR amplification of viral RNA was used to detect the CAEV genome in embryo washing fluids. RNA was purified using a "QIAamp RNA" kit (Qiagen, Courtaboeuf, France) in accordance with the manufacturer's instructions. For each sample, 5 μl (containing 10–100 ng) of the total RNA extracted was used as a template for RT with 15 μl of mix solution containing: 1 μl of a dNTP mixture (25 mM each of: dATP, dGTP, dCTP, dTTP), 1 μl of Random primers for RT (Biolabs, S1230S, Ozyne, France), 4 μl of 5× RT buffer (Kit M-MLV Reverse-Transcriptase, Promega, 3681, Charbonniéres Les Bains, France), 2 μl of RT (Kit M-MLV Reverse Transcriptase, Promega, 3681) and 7 μl RNase-free water. The mixture was incubated at 37 °C for 30 min. The reaction was then stopped following incubation at 95 °C for 5 min and the samples were stored at −80 °C for subsequent PCR analysis. The latter consisted of nested-PCR with two amplifications of the gag gene [30].

3. Results

Samples analyzed for CAEV proviral DNA using PCR were considered as being positive when a 512 bp band, corresponding to the positive control, was seen on

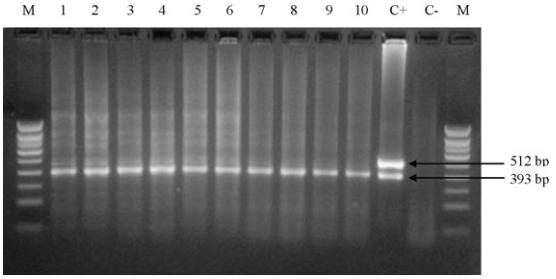

Fig. 1. Example of nested-PCR amplification of proviral DNA. DNA from tissues taken from kids was used to perform nested-PCR using specific sets of oligonucleotide primers to amplify both the 512 bp CAEV gag and 393 bp actin fragments. Following nested-PCR reactions, each PCR product was separated on 1.5% agarose gel and the bands visualized by staining with ethidium bromide. M: smart ladder used as a molecular weight standard. Lanes 1–10: tissue samples (lanes 1–5: lung, negative; lanes 6–10: retromammary lymph nodes, negative). C+: positive tissue control (infected retromammary lymph node). C−: negative control (distilled water).

agarose gel electrophoresis under UV light, between the 600 and 400 bp molecular weight bands. The 393 bp band on the other hand, generated from the amplification of the endogenous actin gene, was present in all tissues with correct DNA extraction. An example of the results is displayed in Fig. 1, showing the analysis of DNA isolated from sample tissues taken from the kids.

3.1. Embryo collection

The CAEV provirus genome was identified in DNA isolated from cells harvested from the embryo collection fluid and the uterine smear samples of 28/30 donor goats (93.3%). Samples were negative in two animals that had only been harvested once and had then been excluded from the protocol.

Table 1
Results of PCR viral diagnostic tests performed on female recipients 10 days before, during, and 10 days after parturition

Tissue samples	10 days before parturition	At parturition	10 days after parturition
Blood	Negative	Negative	Negative
Vaginal smear	Negative	Negative	Negative
Udder			Negative
Lung			Negative
Prescapular lymph nodes			Negative
Retromammary lymph nodes			Negative

From the six harvesting procedures 158 oocytes and 334 embryos were recovered, equivalent to 6.7 ± 4.6 embryos per goat. Following examination under the binocular microscope, 191 embryos were selected, washed, frozen, and used for transfer into the recipient goats. RT-PCR analysis using RNA harvested from ultracentrifuged products from the embryo washing fluids, demonstrated that although CAEV-RNA was detected in the first three baths, the seven following baths were free from CAEV.

3.2. Embryo transfer

Three embryo transfer procedures were performed, at 3-month intervals, on the 43 CAEV-negative recipient goats. A total of 49 embryo transfers were performed ($n = 32$, 12, 5 for each of the three transfer procedure, respectively). At day 22 of gestation, 36/49 goats had a progesterone level that was compatible with gestation. Uterine ultrasonography confirmed that 20/49 goats were pregnant between 45 and 60 days of gestation. One goat died 10 days before term from

Table 2
Results of monthly PCR testing for viral detection in neonates during the first 4 months of life, and then 15, 21, and 28 days after the start of immunosuppressive treatment

Tissue sample	Time after birth (days)				Time after start of immunosuppressive treatment (day 0 = 4 months after birth) (days)		
	30	60	90	120	15	21	28
Blood	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Synovial membrane							Negative
Lung							Negative
Superficial cervical lymph nodes							Negative
Pelvic lymph node							Negative
Retromammary lymph node							Negative
Uterus[a]							Negative

[a] In female.

enterotoxaemia and three died during parturition. The 19 goats that gave birth produced a total of 23 kids. Seven of them died (three within the first 5 days after birth, three others at week 4, and the last one at week 7 after birth). Sixteen kids underwent the entire screening protocol for CAEV infection.

All of the samples taken in accordance with the protocol (blood samples, placental samples, tissue samples) from the 19 recipient goats that gave birth at term, and the recipient goat that died from enterotoxaemia 10 days before term, were negative for CAEV proviral DNA using PCR (Table 1). All of the samples taken in accordance with the protocol (blood and tissue samples) from the seven kids that died before 4 months of age, and the sixteen 5-month-old kids euthanized 4 weeks after the beginning of the immunosuppressive treatment, were found to be negative for CAEV proviral DNA (Table 2).

4. Discussion

To determine the risk of CAEV transmission through embryo transfer and in the context of an *in vivo*/*in vivo* approach, we transferred embryos from CAEV seropositive goats, whose blood and uterine tissues were infected with CAEV, into CAEV-free recipient females, in accordance with the guidelines of the International Embryo Transfer Society (IETS) [19]. These guidelines are as follows: embryos with an intact zona pellucida, washed 10 times over in culture medium, with a new pipette for each wash, and with each wash representing a dilution of 1/100.

This study, performed under field conditions, clearly demonstrates that embryo transfer can be used to produce CAEV-free kids from CAEV-infected biological mothers. Indeed, none of the 16 kids collected from infected mothers at the embryonic stage, transferred to CAEV-free recipient goats, and subjected to immunosuppressive treatment at 4 months of age, were found to be positive for CAEV with any of the diagnostic methods used in all of the analyzed target tissues of the virus. Similarly, none of the 20 recipient goats seroconverted, and none of the sampled tissues tested positive for CAEV proviral DNA.

The results of this study, using powerful molecular biology techniques, bring a unique experimental demonstration that confirms and supports the two previous studies of Wolf et al. [13] and Cavalcante et al. [14], who did not detect any seroconversion at 4 and 6 months, respectively in kids issued from embryo transfer from seropositive donors and/or with clinical disease. In these two studies, CAEV-infected donor goats were selected on the basis of serological tests. Therefore, the donors used in these studies were not tested for viraemia or latent infection of the genital tract target cells.

Our study, performed on a significant number of donors, has the originality of examining females with confirmed uterine infection, and which therefore present with a maximal risk of viral transmission. The second particularity of this study is the panel of techniques used to detect viral infection in the recipient goats and kids. In Wolf et al.'s study [13], viral infection was studied by testing the recipients and kids (up to 4 months of age) for seroconversion and performing viral cultures on colostrum, placenta, and tissue samples from still-born kids and those that died as neonates. In their study, Cavalcante et al. [14] looked for viral infection by testing the kids for seroconversion up to 6 months of age. However, the lack of detection of seroconversion in the kids could be explained by delayed seroconversion as reported previously [15,31,32], and false negatives may be observed in kids over 6 months of age [15]. Thus, animals that are seronegative on ELISA might still be healthy carriers of the virus [33]. Indeed, this was observed during the selection of donors, where eight seronegative females were eliminated after repeatedly testing positive on blood and vaginal smears using PCR, and a further two following monocyte-derived macrophage cultivation and detection of virus-induced CPE. The cell culture of differentiated macrophages demonstrated the presence of infectious viral particles, when it was not possible to detect the markers of infection such as proviral DNA. It is well known that the latter may remain quiescent during the latent period of the infection, notably in neonates that have been contaminated in the early embryonic stages, where gene hypermethylation may disable proviral expression. Thus, in the monocyte/macrophage cell lineage, which is the main target of the virus *in vivo*, CAEV remains latent in the form of proviral DNA in the monocytes with no infectious particles being produced; the differentiation of these cells into macrophages is a prerequisite for viral replication [34]. The full replication cycle of CAEV continues in the differentiated macrophages, within specific target tissues [35]. Various factors affect the expression of viruses responsible for latent infections: stress, immunodepression, or a significant physiological modification (gestation) can cause viral reactivation, its expression, and the onset of lesions over subsequent weeks [36].

In the present study, physical viral detection was based on the demonstration of CAEV proviral DNA

using PCR. The sensitivity of this technique is well proven [26,37], and it enables the detection of one infected goat synovial membrane (GSM) cell, one caprine oviduct epithelial cell (COEC), and a minimum of 10 granulosa cells *in vitro* [22]. We also used iatrogenic dexamethasone-induced immunosuppression to induce viral reactivation in the kids [25,38].

Under acute conditions for infection and using highly sensitive diagnostic techniques, combined with a protocol for the exacerbation of CAEV virulence, this study clearly demonstrated, under field conditions, that embryo transfer performed in accordance with the IETS protocol and using good quality embryos, can be safely used to produce CAEV-free neonates from infected CAEV donors. This technique of artificial reproduction can therefore play a useful role in a prophylactic health programme against CAEV in the goat.

Acknowledgments

The authors would like to thank Myriam Larrat, Sandrine Destrumelle, Cécile Roux, Sylvie Anezo and Hervé Guilloteau from the Department of Research into the health risks and biotechnology of reproduction, National Veterinary School of Nantes, France, for their technical assistance. This research was supported by Institut de l'Elevage, France. The authors would also like to thank Mr. J.P. Sigwal who managed the funding program.

References

[1] Cheevers WP, Knowles DP, McGuire TC, Cunningham DR, Adams DS, Gorham JR. Chronic disease in goats orally infected with two isolates of the caprine arthritis-encephalitis lentivirus. Lab Invest 1988;58:510–7.

[2] Cork LC, Hadlow WJ, Crawford TB, Gorham JR, Piper RC. Infectious leukoencephalomyelitis of young goats. J Infect Dis 1974;129:134–41.

[3] Rowe JD, East NE. Risk factors for transmission and methods for control of caprine arthritis-encephalitis virus infection. Vet Clin North Am Food Anim Pract 1997;13:35–53.

[4] Peretz G, Bugnard F, Calavas D. Study of a prevention programme for caprine arthritis-encephalitis. Vet Res 1994;25:322–6.

[5] East NE, Rowe JD, Dahlberg JE, Theilen GH, Pedersen NC. Modes of transmission of caprine arthritis-encephalitis virus infection. Small Ruminant Res 1993;10:251–62.

[6] Peterhans E, Greenland T, Badiola J, Harkiss G, Bertoni G, Amorena B, Eliaszewick M, Juste RA, Krabnig R, Lafont JP, Lenihan P, Petursson G, Pritchard G, Thorley J, Vitu C, Mornex JF, Pépin M. Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes. Vet Res 2004;35:257–74.

[7] Fieni F, Rowe J, Van Hoosear K, Burucoa C, Oppenheim S, Anderson G, Murray J, Bondurant R. Presence of caprine arthritis-encephalitis virus (CAEV) infected cells in flushing media following oviductal-stage embryo collection. Theriogenology 2002;57:931–40.

[8] Ali Al Ahmad MZ, Fieni F, Martignat L, Chatagnon G, Baril G, Bouvier F, Chebloune Y. Proviral DNA of caprine arthritis encephalitis virus (CAEV) is detected in cumulus oophorus cells but not in oocytes from naturally infected goats. Theriogenology 2005;64:1656–66.

[9] Lamara A, Fieni F, Mselli-Lakal L, Tainturier D, Chebloune Y. Epithelial cells from goat oviduct are highly permissive for productive infection with caprine arthritis-encephalitis virus (CAEV). Virus Res 2002;87:69–77.

[10] Lamara A, Fieni F, Chebloune Y, Tainturier D. Efficient replication of caprine arthritis-encephalitis virus in goat granulosa cells. Virus Res 2001;79:165–72.

[11] Ali Al Ahmad MZ, Fieni F, Guiguen F, Larrat M, Pellerin JL, Roux C, Chebloune Y. Cultured goat embryos and cells are susceptible to infection with caprine encephalitis virus. Virology 2006;353:307–15.

[12] Lamara A, Fieni F, Mselli-Lakal L, Chatagnon G, Bruyas JF, Tainturier D, Battut I, Formazero C, Chebloune Y. Early embryonic cells from in vivo produced goat embryos transmit the caprine arthritis-encephalitis virus (CAEV). Theriogenology 2002;58:1153–63.

[13] Wolf DF, Nusbaum KE, Lauerman LH, Mysinger PW, Ridell MG, Putnam MR, Shumway LS, Powe TA. Embryo transfer from goats seropositive for caprine arthritis-encephalitis virus. Theriogenology 1987;28:307–16.

[14] Cavalcante TV, Salles HO, Freitas VJF. Ovulatory response and embryo production after superovulatory treatment in goats seropositive for caprine arthritis-encephalitis virus (CAEV): preliminary results. In: Proceedings of the fourteenth meeting of European embryo transfer association; 1998.p. 136 (abstract).

[15] Rimstad E, East NE, Torten M, Hinggins J, DeRock E, Pederson NC. Delayed seroconversion following naturally acquired caprine arthritis-encephalitis virus infection in goats. Am J Vet Res 1993;54:1859–62.

[16] Fieni F, Rowe J, Van Hoosear K, Burucoa C, Oppenheim S, Anderson G, Murray J, Bondurant R. Presence of caprine arthritis-encephalitis virus (CAEV) proviral DNA in genital tract tissues of superovulated dairy goat does. Theriogenology 2003;59:1515–23.

[17] Baril G, Vallet JC. Time of ovulations in dairy goats induced to superovulate with porcine follicle stimulating hormone during and out of the breeding season. Theriogenology 1989;34:303–11.

[18] Tervit HR, Goold PG, McKenzie RD, Clarkson DJ. Techniques and success of embryo transfer in Angora goats. N Z Vet J 1983;31:67–70.

[19] Stringfellow DA. In: Stringfellow DA, Seidel SM, editors. Recommendation for the sanitary handling of in vivo-derived embryos. Manual of the International Embryo Transfer Society (IETS); 1998. p. 79–84.

[20] Le Gal F, Baril G, Vallet JC, Leboeuf B. *In vivo* and *in vitro* survival of goat embryos after freezing with ethylene glycol or glycerol. Theriogenology 1993;40:771–7.

[21] Gorrell MD, Brandon MR, Sheffer D, Adams RJ, Narayan O. Ovine lentivirus is macrophagetropic and does not replicate productively in T lymphocytes. J Virol 1992;66:2679–88.

[22] Chebloune Y, Sheffer D, Karr BM, Stephens E, Narayan O. Restrictive type of replication of ovine/caprine lentiviruses in ovine fibroblast cell cultures. Virology 1996;222:21–30.

[23] Guiguen F, Lerondelle C, Favier C. Responses of kids to monocytes infected *in vitro* by the caprine arthritis-encephalitis virus. Ann Rec Vet 1990;21:179–85.
[24] Buonavoglia C, Tempesta M, Cavalli A, Voigt V, Buonavoglia D, Conserva A, Corrente M. Reactivation of caprine herpesvirus-1 in latently infected goats. Comp Immunol Microbiol Infect Dis 1996;19:275–81.
[25] Morin T, Guiguen F, Bouzar BA, Villet S, Greenland T, Grezel D, Gounel F, Gallay K, Garnier C, Durand J, Alogninouwa T, Mselli-Lakhal L, Mornex JF, Chebloune Y. Clearance of a productive lentivirus infection in calves experimentally inoculated with caprine arthritis-encephalitis virus. J Virol 2003;77:6430–7.
[26] Guiguen F, Mselli-Lakhal L, Durand J, Du J, Favier C, Fomazero C, Grezel D, Balleydier S, Hausmann E, Chebloune Y. Experimental infection of mouflon-domestic sheep hybrids with caprine aethritis-encephalitis virus. Am J Vet Res 2000;61: 456–61.
[27] Saltarelli MJ, Querat G, Konings DA, Vigne R, Clements JE. Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. Virology 1990;179:347–64.
[28] Joag SV, Stephens EB, Adams RJ, Foresman L, Narayan O. Pathogenesis of SIV mac infection in Chinese and Indian rhesus macaques: effects of splenectomy on virus burden. Virology 1994;200:436–46.
[29] Chebloune Y, Sheffer D, Karr BM, Stephens E, Narayan O. Restrictive type of repication of ovine/caprine lentiviruses in ovine fibroblast cell cultures. Virology 1996;222:21–30.
[30] Leroux C, Lerondelle C, Chastang J, Mornex JF. RT-PCR detection of lentiviruses in milk or mammary secretions of sheep or goats from infected flocks. Vet Res 1997;28: 115–21.
[31] Adams DS, Kleyjer-Anderson P, Carlson JL, McGuire TC, Gorham JR. Transmission and control of caprine arthritis-encephalitis virus. Am J Vet Res 1983;44:1670–5.
[32] Knowles DP. Laboratory diagnostic tests for retrovirus infections of small ruminants. Vet Clin North Am Food Anim Pract 1997;13: 1–10.
[33] Vitu C, Russo P, Vignoni M, Delor V. L'Arthrite Encéphalite en enzootique caprine en France: Recherches épidémiologiques et expérimentales. Comp Immun Microbiol Infect Dis 1988;11:27–34.
[34] Narayan O, Cork LC. Caprine arthritis-encephalitis virus. In: Levy JD, editor. The retroviridae. New York: Plenum Press; 1995 p. 441–51.
[35] Clements JE, Zink MC. Molecular biology and pathogenesis of animal lentivirus infection. Clin Microbiol Rev 1996;9:100–17.
[36] Narayan O, Sheffer D, Clements JE, Tennekoon G. Restricted replication of lentiviruses: visna viruses induce a unique interferon during interaction between lymphocytes and infected macrophages. J Exp Med 1985;162:1954–69.
[37] Barlough J, East N, Rowe JD, Van Hoosear K, Deock E, Bigornia L, Rimstad E. Double-nested polymerase chain reaction for detection of caprine arthritis-encephalitis virus proviral DNA in blood, milk and tissues of infected goats. J Virol Methods 1994;50:101–14.
[38] Charlety P, Fleury C, Guiguen F. Immunodépression et virus de l'arthrite et de l'encéphalite de la chèvre (CAEV). Sci Vet Med Comp 1987;89:59–65.