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## ATTEMPTS TO ISOLATE BOVINE LEUKEMIA AND BOVINE SYNCYTIAL VIRUSES FROM BLOOD, UTERINE FLUSH FLUID, UNFERTILIZED OVA AND EMBRYOS FROM INFECTED DONOR CATTLE

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### Résumé

ISOLEMENT DU VIRUS DE LA LEUCÉMIE BOVINE ET DU VIRUS SYNCYTIAL BOVIN DES ÉCHANTILLONS DE LEUCOCYTES, DES LAVAGES UTÉRINS, DES ŒUFS NON FÉCONDÉS ET DES EMBRYONS OBTENUS DES VACHES INFECTÉES. — Le virus de la leucémie bovine (BLV) et le virus syncytial bovin (BSV) ont été isolés dans un troupeau de 25 vaches ayant des anticorps humoraux anti-BLV. L'isolement viral a été effectué par co-culture des cellules des échantillons avec des cellules de rate de foetus d'ovin et par les épreuves d'induction de syncytium et d'immonofluorescence. Le BLV et le BSV ont été respectivement décelés dans 11/15 (73,3 %) et 14/15 (93,3 %), des échantillons de leucocytes et 4/25 (16,0 %) et 21/25 (84,0 %) de ceux des lavages utérins, mais n'ont pas été isolés dans les 26 œufs et les 60 embryons obtenus de 20 des 25 vaches. L'isolement de BLV a pu être limité par la multiplication concurrentielle du BSV et par la croissance plus rapide des cellules de lavage utérin que celles de rate d'ovin. Quoique le BLV ne se multiplie pas dans les cultures pures de lavage utérin, il a été néanmoins isolé dans l'utérus et, par conséquent, pourrait être transmis au fœtus par la voie transplacentaire. Le BLV a été probablement isolé des leucocytes présents naturellement dans la lumière utérine ou d'un épanchement sanguin passé inaperçu lors des lavages utérins. Le BSV pourrait être très répandu chez les bovins et être la cause de troubles du système reproducteur si on tient compte du pourcentage élevé d'isolement du virus et de sa multiplication dans les cultures pures de cellules de lavage utérin.

Embryo transfer has evolved into a multimillion-dollar industry in North America. It is developing rapidly in Europe (Seidel, 1981), and interest is growing in its use for the international exchange of blood lines.

There have been a few studies on disease transmission or control by embryo transfer (Mitchell and Betteridge, 1977; Betteridge, 1978; Bowen, 1979; Waters, 1981), which

indicates that the probability of congenital transmission varies from one disease to another (reviewed by Eaglesome *et al.*, 1980).

The epizootiology of bovine leukemia virus (BLV) has been reviewed recently by Burny *et al.* (1980b), Ferrer (1980), Van Der Maaten and Miller (1979). Horizontal spread is the prevalent means of transmission (Ferrer, 1979; Van Der Maaten and Miller, 1979; Olson,

1979) but in one herd, 18 % of the calves had antibody evidence of BLV infection at birth (Piper *et al.*, 1979). These calves were probably infected *in utero* by the transplacental passage of BLV-infected lymphocytes, since : 1) the calves had BLV antibodies prior to receiving colostrum (Ferrer *et al.*, 1976 ; Piper *et al.*, 1979 ; Van Der Maaten *et al.*, 1980), 2) it appears that BLV is not transmitted by gametes (Burny *et al.*, 1978, 1980a ; Olson, 1979), 3) BLV has never been found in any cells other than lymphocytes of BLV-infected cattle (Kettmann *et al.*, 1978), and 4) normal lymphocytes are found in the uterus and have been shown to cross the uterine wall (Dubois *et al.*, 1980).

Like BLV, bovine syncytial virus (BSV) (Malmquist *et al.*, 1969 ; Dermott *et al.*, 1971) is a member of a family of Retroviridae (Matthews, 1979). Both viruses are commonly isolated from lymphocytes (Kenyon and Piper, 1977 ; Paul *et al.*, 1977 ; Malmquist *et al.*, 1969 ; Greig, 1979), and BSV has also been found in fetal and maternal tissues and in fetal blood (Scott *et al.*, 1973 ; Van Der Maaten *et al.*, 1973 ; Luther *et al.*, 1978).

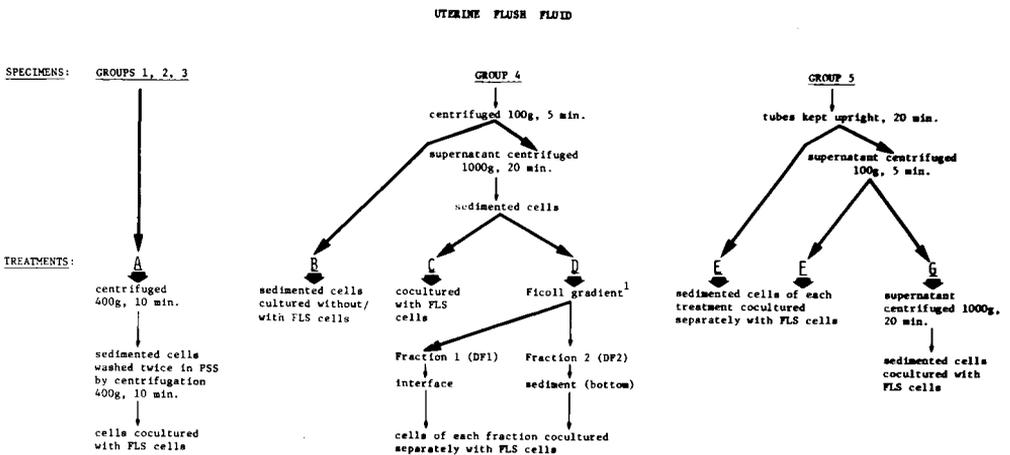
As a part of a larger study on the transfer of embryos from BLV-infected donor to BLV-free recipient cattle, we report studies of attempts to isolate BLV and BSV from blood cells, cells from the fluids (UFF) used to flush the uterus

when collecting the embryos and unfertilized ova and embryos collected from donor animals known to have BLV antibodies in their serum by agar gel immunodiffusion (AGID) test.

## Materials and Methods

Twenty-five donor cattle, BLV-positive by the AGID test (Samagh *et al.*, 1977), were divided into groups of five for convenience of embryo collection at slaughter. The BSV status in 19/25 of the cattle was evaluated before slaughter by the syncytium regression test for BSV antibodies in serum (Greig, 1979), or the co-cultivation of their leucocytes with fetal lamb spleen (FLS) cells followed by direct immunofluorescence (Malmquist *et al.*, 1969 ; Ruckerbauer *et al.*, 1967) to detect antigens *in vitro*. Sera of all cattle were also tested by serum-virus neutralization tests for antibodies to bovine virus diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) virus.

BLV and BSV isolations were attempted from the first three groups of animals from both the blood and UFF in Dubelcco's phosphate buffered saline (PBS) with 2 % fetal bovine serum, FBS heat-inactivated for 30 min at 56 °C, and 250 µg/ml neomycin sulphate, but only from the UFF of the last two groups. BLV and BSV isolations were also attempted



1. Van der Maaten and Miller, 1980.

(Fig. 1. — Recovery of cells from 50 ml of uterine flush fluid after removal of embryos.)

on some of the eggs and embryos collected from 20/25 of the animals.

BLV-indicator cells consisted of FLS cells grown in minimum essential medium (MEM) in Hanks' balanced salt solution supplemented with 20 % BLV-free fetal bovine serum and 100 µg/ml neomycin sulphate.

Buffy coat lymphocytes were obtained after lysis of erythrocytes by hypotonic shock (Stock and Ferrer, 1972), washed in Ca<sup>++</sup> and Mg<sup>++</sup>-free PBS, mixed with 1 to 2 × 10<sup>6</sup> FLS cells, seeded in 25 cm<sup>2</sup> plastic flask (Falcon, Oxnard, Calif., USA) and incubated at 37 °C. Cell cultures were split in a 1:2 ratio every week. Both BLV and BSV immunofluorescence tests were made at each passage 24 and 48 h after cell seeding (Ruckerbauer *et al.*, 1967). When a positive reaction was observed, one additional passage was made to confirm it. Co-cultures of leucocyte-FLS cells were treated for 24 h with 2.5 µg amphotericin B per ml of medium (Van Der Maaten and Malmquist, 1969) when trypanosomes were observed.

Cells contained in UFF were recovered and processed for virus isolation as shown in the flow chart (fig. 1). The procedures followed with groups 4 and 5 were intended to separate epithelial-like (uterine) cells from any leucocytes present in the uterus.

Embryos and unfertilized ova recovered from UFF were immediately transferred to PBS supplemented with 20 % heat-inactivated BLV-free FBS and 100 µ/ml penicillin. They were washed twice in PBS. Embryos from the same donor animal were pooled and deposited in a 2 cm<sup>2</sup> well of a tray (FB-16-24TC, Linbro Chemicals Co., New Haven, Conn., USA) seeded 24 h previously with FLS cells. Cultures were split in a 1:2 ratio every week and transferred successively from wells to petri dishes and 25 cm<sup>2</sup> plastic flasks. They were examined for both BLV and BSV antigens by immunofluorescence (Ruckerbauer *et al.*, 1967) 24 and 48 h after seeding the last two passages of embryo-FLS cell cultures.

Passages of all cultured material were carried out in duplicate except for the first embryo passage. Presence of BLV and BSV antigens was indicated by syncytium induction and detection of specific antigens by immunofluorescence. Supernatant fluids of three cultures were concentrated 50 x by ammonium sulphate (Samagh *et al.*, 1977) and

used as an antigen in the AGID test. Some cultures were examined by electronmicroscopy as previously described (Bouillant *et al.*, 1978).

When BLV was not identified at the fifth passage of an infected culture, up to 10 or more passages were carried out because it had previously been observed that BLV can be identified by immunofluorescence after 20 passages, although syncytia were seen in the early passages and subsequently (A.S. Greig, 1979, unpublished observations).

Table 1. — BSV<sup>1</sup>, BVD<sup>2</sup> and IBRV<sup>3</sup> status of 25 BLV-positive donor cattle 6 to 12 months before slaughter

Group	Cow No.	BSV <sup>4</sup>	BVD <sup>5</sup>	IBR <sup>5</sup>
1	241	+	+	+
	242	+	+	±
	251	+	+	+
	426	+	+	+
	464	+	+	+
2	60	+	+	—
	215	+	+	+
	233	+	+	+
	247	+	+	+
	484	+	+	+
3	64	ND	+	—
	238	+	+	+
	297	ND	+	—
	308	ND	+	—
	340	+	+	+
4	232	+	+	+
	247	+	+	+
	278	—	+	+
	493	—	+	+
	503	+	+	+
5	22	ND	+	+
	55	ND	+	—
	231	+	+	—
	257	+	+	+
	318	ND	—	+

1, 2, 3 : BSV bovine syncytial virus ; BVD bovine virus diarrhoea ; IBRV infectious bovine rhinotracheitis virus.

4 : BSV antibodies evaluated by syncytium regression tests and/or co-cultivation with FLS cells followed by immunofluorescence : + positive ; — negative ; ND not done.

5 : Antibodies evaluated by seroneutralization tests : + positive ; — negative ; ± suspicious.

## Results

The results of the serological tests for antibodies to BSV, IBR virus and BVD are given in table 1, and those of the virus isolation studies on leucocytes and UFF cells in groups 1, 2 and 3 and on UFF cells in groups 4 and 5 are given in tables 2 and 3 respectively.

Cell necrosis and growth of large epithelial-like cells and elongated cells, which were more refractile than FLS cells, were observed in the UFF sediment co-cultured with FLS cells. Cell cultures of the second group consisted entirely of epithelial-like and elongated cells at the fourth passage, and 4/5 were shown to be BSV-positive by immunofluorescence. In the third group of UFF cell cultures, 2/5 of the cultures (238, 308) consisted almost entirely of epithelial-like cells. BSV virions were observed by electronmicroscopy in the fifth passage of cultures 64, 238, and 340 from group 4. More than 90 % destruction of the cell sheet was observed in all cultures except number 64.

In cultures set up from group 4 animals, the only one to show BLV antigen by immunofluorescence was from BSV-free animal 278 at the thirteenth passage following UFF processing by method B as in figure 1.

The presence of BSV in cultures, indicated by syncytia (fig. 2 and 3) and immunofluorescence, was confirmed by observing BSV virions by electronmicroscopy in the sixth passage of UFF cells from animal 503 treated by method B and cultured without FLS cells (figs. 4 and 5), although they were not observed in similar material from animals 232 and 247.

To eliminate the possibility that UFF cells treated by method B and cultured without FLS cells could have become contaminated with FLS cells, a cytogenetic analysis was made of cultures from animal 247 and the cells found to have the normal 60, XX bovine karyotype. BLV antigens were not found in supernatant fluids harvested from passages 19 to 23 of DF1 and DF2 (fig. 1) cultures from animal 493 when

Table 2. — Detection of BLV and BSV in blood leucocytes and cells in uterine flush fluid (UFF) (groups 1, 2 and 3)

Group	Donor Animal	Leucocytes <sup>1</sup>			UFF cells		
		Syncytium	BLV <sup>2</sup>	BSV <sup>2</sup>	Syncytium	BLV <sup>2</sup>	BSV <sup>2</sup>
1	241	+ (1) <sup>3</sup>	+ (3) <sup>3</sup>	+ (3) <sup>3</sup>	+ (2) <sup>3</sup>	— (6) <sup>3</sup>	+ (3) <sup>3</sup>
	242	+ (1)	+ (5)	+ (3)	+ (2)	— (6)	+ (3)
	251	+ (1)	+ (5)	+ (3)	+ (2)	+ (3)	+ (3)
	426	+ (1)	+ (5)	+ (3)	+ (2)	+ (3)	+ (3)
	464	+ (1)	+ (4)	+ (3)	+ (2)	+ (3)	+ (3)
2	60	+ (1)	+ (4)	— (5)	+ (2)	— (4)	— (4) <sup>4</sup>
	215	+ (1)	+ (4)	+ (3)	— (4)	— (4)	+ (3) <sup>4</sup>
	237	+ (1)	+ (5)	+ (3)	+ (2)	— (4)	+ (3) <sup>4</sup>
	467	+ (1)	+ (3)	+ (3)	+ (2)	— (4)	+ (3) <sup>4</sup>
	484	+ (1)	— (4)	+ (3)	+ (2)	— (4)	+ (3) <sup>4</sup>
3 <sup>5</sup>	64	+ (2)	+ (12)	+ (2)	+ (2)	— (7)	+ (2)
	238	+ (7)	— (12)	— (12)	+ (2)	— (7)	+ (2) <sup>4</sup>
	297	+ (7)	— (12)	— (12)	+ (3)	— (7)	+ (2)
	308	— (12)	+ (12)	— (12)	+ (4)	— (7)	+ (5) <sup>4</sup>
	340	+ (2)	— (10)	+ (2)	+ (3)	— (7)	+ (2)

1: trypanosomes were found in all specimens except in those from animals 238, 297 and 308 of group 3.

2: BLV and BSV antigens were detected by immunofluorescence.

3: (in brackets) passage number of the 1st positive or last negative observation made.

4: extensive proliferation of uterine cells in cocultures with FLS cells.

5: Cocultures of leucocyte FLS-cells were re-fed with fresh FLS cells at the 3rd and 8th passages.

they were concentrated 50X and used as an antigen in the AGID test.

Likewise, they were not found in the supernatant fluids harvested from passages 13 to 17 of E and G (fig. 1) cultures from animal 55.

There was a great variation in packed-cell volume (PCV) of leucocytes from 5.0 to  $12.0 \times 10^6$  in 7 ml of blood as observed in counts in the five specimens of group 3 and of UFF cells from 5.2 to  $8.4 \times 10^6$  in 50 ml from two specimens of group 1 (data not shown).

The results of the unfertilized ova and the

embryo studies are given in table 4. Neither syncytia nor BLV and BSV immunofluorescence were observed in any co-cultured specimens. Nor were BLV antigens demonstrated in the AGID test when supernatant fluids from the culture of embryos from animal 278 were harvested from passages 16 to 21 and processed as described above.

### Discussion

The overall results show that BLV was found in leucocyte cultures from 11/15 (73.3 %) animals, in UFF cultures from 4/25

Table 3. — Detection of BLV and BSV in cells in uterine flush fluids (UFF) processed for culture as shown in Figure 1 (groups 4 and 5)

Group	Animal <sup>1</sup>	Donor Treatment <sup>2</sup>	Syncytium	BLV <sup>3</sup>	BSV <sup>3</sup>
4	232 (+)	B	+ (1) <sup>4</sup>	— (15) <sup>4</sup>	— (15) <sup>4,6</sup>
		C (FLS) <sup>5</sup>	+ (1)	— (7)	+ (2) <sup>6,7</sup>
	247 (+)	B	+ (1)	— (8)	+
		C (FLS)	+ (1)	— (5)	+ (2)
	278 (—)	B (FLS)	+ (4)	+ (13)	— (15)
		DF1 (FLS)	+ (8)	— (15)	— (15)
		DF2 (FLS)	+ (8)	— (15)	— (15)
	493 (—)	B	— (16)	— (16)	— (16) <sup>6</sup>
		DF1 (FLS)	+ (6)	— (17)	— (17) <sup>8</sup>
		DF2 (FLS)	+ (6)	— (17)	— (17) <sup>8</sup>
503 (+)	B	+ (3)	— (8)	+ (5) <sup>6,7</sup>	
	C (FLS)	+ (1)	— (5)	+ (2) <sup>7</sup>	
5	22 (ND)	EF	+ (2)	— (4)	+ (3) <sup>7</sup>
		G	+ (2)	— (4)	+ (3) <sup>7</sup>
	55 (ND)	E	+ (4)	— (12)	— (12) <sup>8</sup>
		F	+ (6)	— (12)	— (12)
		G	+ (4)	— (12)	— (12) <sup>8</sup>
	231 (+)	EF	+ (2)	— (4)	+ (3) <sup>7</sup>
		G	+ (2)	— (4)	+ (3) <sup>7</sup>
	257 (+)	EF	+ (3)	— (4)	+ (3) <sup>7</sup>
		G	+ (2)	— (4)	+ (3) <sup>7</sup>
	318 (ND)	E	+ (3)	— (4)	— (3) <sup>7</sup>
		F	— (2)	— (2)	— (2) <sup>9</sup>
		G	+ (3)	— (4)	— (3) <sup>7</sup>

1 : in brackets presence or absence of BSV antibodies in months preceding slaughter ; ND : not done.

2 : Treatment : see flow chart (Fig. 1).

3 : BLV and BSV antigens were detected by immunofluorescence.

4 : in brackets passage number of the first or last negative observation.

5 : (FLS) specimens of groups 4 which were cocultured with FLS cells.

6 : Extensive proliferation of uterine cells in cultures.

7 : Complete necrosis : destruction of the cell sheet.

8 : Replenished with fresh FLS cells at the 16th passage (specimen 493 DF1 and DF2) and at the 10th passage (specimen 55 E and G).

9 : Discarded (fungi).

(16.0 %) animals, but not in 26 unfertilized ova and 60 embryos from 20 donors after coculture for nine or more passages with FLS cells. BSV was detected in more animals than was BLV in both leucocytes and UFF cell cultures, 14/15 (93.3 %) and 21/25 (84.0 %), respectively, but like BLV it was not found in any of the ova or embryo cultures.

Syncytia were generally observed in the first five passages of leucocytes and UFF cells, except in those from animals 278 and 493 which were not infected by BSV (table 3), and most were probably induced by BSV based on the immunofluorescence studies. However, detection of BLV antigens was possibly hampered by two simultaneous competitions: firstly, competition for virus replication could have occurred between these two viruses to the disadvantage of BLV because BSV appeared to replicate faster than BLV as indicated by immunofluorescence. Secondly there was cell competition between the FLS and UFF uterine cells with the latter overgrowing the former, and immunofluorescent and electronmicrosc-

copy studies have shown that UFF are permissive to BSV (247 and 503, table 3) but apparently not to BLV. It could be that BLV would be isolated from UFF more frequently if the Ficoll treatment was applied directly to the original UFF, thereby separating out most of the uterine cells and leaving the leucocytes.

The likelihood that BLV was isolated from lymphocytes contained in UFF rather than uterine cells is supported by three arguments. Firstly, BLV sequences have been found only in leucocyte-DNA of BLV-positive cattle (Kettman *et al.*, 1978). Secondly, normal lymphocytes are found in the uterus and have been shown to cross the uterine wall (Dubois *et al.*, 1980) and, in the case of UFF, there is always the possibility that trauma to the tunica mucosa caused by the insertion of the uterine catheter produced small hemorrhages and the entry of infected lymphocytes. Thirdly, BLV antigens and virions were not detected by immunofluorescence and electronmicroscopy in uterine cell cultures. However, recovery of BLV from UFF undoubtedly depends upon the

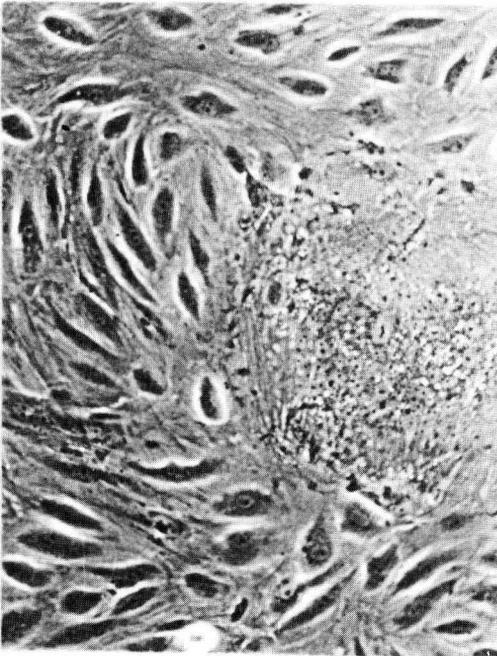


Fig. 2. — Uterine cells of the 2nd passage with fusiform morphology. Note a syncytium with more than 20 nuclei from BSV-positive but BLV-negative culture as determined by immunofluorescence. X 527.

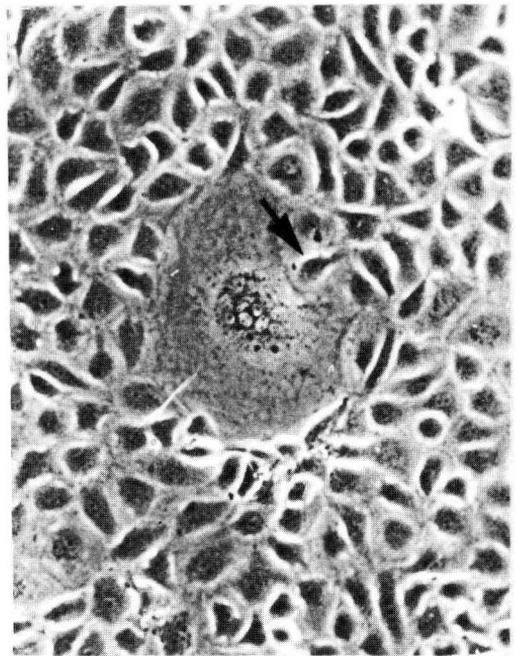


Fig. 3. — Uterine cells of the 6th passage with epithelial morphology. Note a syncytium with more than 10 nuclei from the same culture specimen of Fig. 1 and migration of a cell entering into the syncytium (arrow). X 527.

number of harvested lymphocytes, the capability of lymphocytes for expressing infectious virions, and the sensitivity of the techniques used during collection of UFF.

The occurrence of syncytia in the eighth and sixth passages of UFF, DF1 and DF2 treated cells from BSV-negative animals 278 and 493 respectively (table 3), suggests the presence of BLV, inasmuch as the animals were BLV-positive. Failure to demonstrate BLV antigens by immunofluorescence in the seventh to tenth following passages could have been due to there being too little antigen present to be detected by this test. The lack of detection of BLV antigens by immunofluorescence was probably related to inadequate amounts of antigens in B-treated cells of BLV-positive but BSV negative UFF cultures 278 (table 3) which had syncytia for nine passages. Also syncytium-negative culture 308 (table 2) was found BLV-positive by immunofluorescence at the twelfth passage. Syncytium-inducing viruses other than BSV can be also isolated from leucocytes (Van Der Maaten and Boothe, 1972 ; Van Der Maaten *et al.*, 1972 ; Coulter

and Storz, 1979). This possibility cannot be completely eliminated since we made no special attempts to identify them.

Ferrer *et al.* (1976), Piper *et al.* (1979) and Van Der Maaten *et al.* (1980) have shown that some calves born to BLV-infected dams are BLV infected. In this study, BLV was isolated from the UFF of 16 % of BLV-infected cattle, but it should not be assumed that one figure has a bearing on the other in view of the BLV-BSV and FLS-uterine cell competitions and the trauma factor referred to above. However the UFF isolations do bring additional evidence to the concept of transplacental transmission of BLV, as discussed by other groups (Ferrer, 1979 ; Olson, 1979 ; Van Der Maaten and Miller, 1979).

To our knowledge, data have not been presented on the possible role of mechanical trauma or infectious agents in causing unrecognized breaks without endangering the development of the fetus. However, it is of interest that BSV, which has been found in the uterus (Luther *et al.*, 1978), possesses a fusion factor which allows it to induce syncytia. Is it possi-

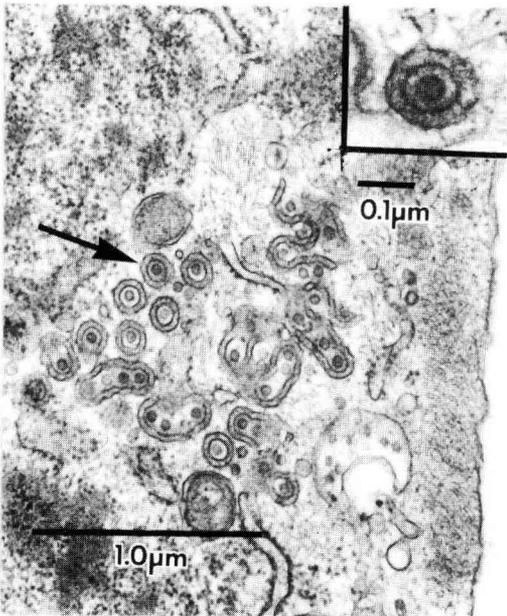


Fig. 4. — Bovine syncytial virions emerging from the endoplasmic reticulum: double ring structure with central core (arrow). Insert: virion at the plasma membrane.

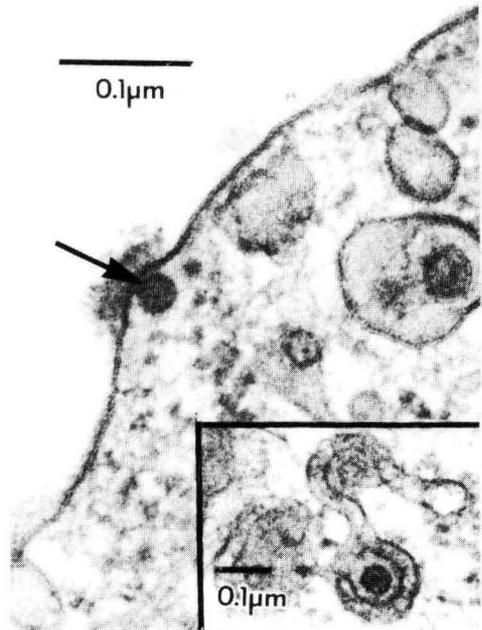


Fig. 5. — Bovine syncytial virion budding at the plasma membrane (arrow). Insert: virion in continuity with endoplasmic reticulum.

ble that syncytial cells located on the boundary of fetal and maternal circulations could detach and create a break which, in turn, would allow passage of lymphocytes (BLV-positive) into the fetal circulation ? Alternatively, trypanosomes have frequently been observed in blood of BLV-positive cattle (Malmquist, 1965 ; Hare *et al.*, 1967) and they have also been found in fetal tissues (Lundholm *et al.*, 1959). Transplacental transmission has been suggested (Woo and Limebeer, 1971) and they have been observed in fetal fluids (Hare and Singh, unpublished observations), so they may be involved in the development of unrecognized breaks in the placenta which could facilitate the entry of maternal lymphocytes into the fetal circulation.

BLV and BSV appear to share some biological characteristics. Firstly, both were isolated from leucocytes and UFF sediments. Secondly, they were not detected in either unfertilized eggs or zona-pellucida intact embryos meaning that either cell-free virions or cells expressing BLV infectious virions on their membrane were not present or that

unfertilized eggs and early bovine embryos are not permissive to their attachment to the zona pellucida and/or their penetration through the zona followed by viral replication and release. It is known that the zona pellucida can act as a protective barrier against infectious agents, as discussed by Eaglesome *et al.* (1980), although data have not been provided to prove or refute this hypothesis of protection of the zona pellucida against BLV and BSV contaminations. However, there have been, to our knowledge, several observations which might support the view that BLV is not transmitted by embryos. To date, the BLV genome has been found only in lymphocytes or in tumors derived from them (Kettman *et al.*, 1978), and more than 80.0 % of calves born from BLV-infected cows were BLV-negative (Ferrer *et al.*, 1976 ; Piper *et al.*, 1979 ; Van Der Maaten *et al.*, 1980). Olson *et al.* (1981) have shown that seven embryos were transferred from BLV-positive cows, which are been artificially inseminated with semen from BLV-positive bulls, to BLV-negative recipients. The resultant calves were BLV antibody negative for more than

Table 4. — Status of unfertilized ova and embryos co-cultured with FLS cells from BLV-positive donor cows

Group	Cow No.	No. of passages	No. of ova	No. of embryos	Remarks
1	241, 264	20		7	1 morula, 3 blastocysts with attached debris, 4 degenerated.
	242	19		4	2 morulae, 1 blastocyst, 1 8-cells
	251	19		2	morulae
	426	19		2	morulae
2	60	14		3	1 morula, 1 8-cells, 1 degenerated
	215	14	1	3	2 morulae, 1 degenerated
	237	14		4	3 morulae, 1 degenerated
	484	14		4	morulae
3	64	12	1		
	238	12	15	8	degenerated
	297	12		7	degenerated
	308	12	2	1	morula
	340	12		2	morulae
4	232	9		3	2 morulae, 1 blastocyst with attached material
	247	9	1	1	4 6-cells
	278	20		3	morulae with attached material
	493	9	5	3	1 2-cells, 2 16-cells
	503	9	1	2	morulae
5	257	10		1	2 morula embedded in material
TOTAL	20		26	60	

a year. Similarly, there is strong evidence that the developing chick embryo can be completely surrounded by exogenous lymphoid leukaemia viruses and yet escape infection (Spencer *et al.*, 1980). However, it remains to be seen if more sensitive techniques for the detection of viral antigens or proviral DNA sequences (Gupta and Ferrer, 1980 ; Schmerr *et al.*, 1980 ; Burny *et al.*, 1980a) determine whether or not embryos play a role in BLV and BSV transmissions.

The infection of 84.0 % of UFF specimens with BSV is interesting in view of the hypothesis that this virus may cause disorders of the reproductive tract in cattle (Scott *et al.*, 1973 ; Luther *et al.*, 1978) and it suggests that BSV infection may be more prevalent in dairy cattle than was thought based on a previous study in England (Appleby, 1979).

In conclusion, BLV was not found in 26 unfertilized ova and 60 zona pellucida-intact embryos collected from 20 BLV-infected cattle. BLV antigens were detected in UFF from 4/25 of donor cattle in spite of a high rate of BSV infection. BLV was probably isolated from lymphocytes present in the lumen of the uterus, either through natural occurrence or

through trauma caused during flushing, rather than uterine epithelial cells.

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### Summary

Blood leucocytes, sediments of uterine flush fluid (UFF), eggs and embryos from 25 BLV-positive donor cows were tested for bovine leukemia (BLV) and bovine syncytial (BSV) viruses by cocultivation with fetal lamb spleen cells and by applying syncytium induction and immunofluorescence tests. BLV was diagnosed in 11/15 (73.3 %) leucocyte and 4/25 (16.0 %) UFF-sediment specimens as compared to BSV in 14/15 (93.3 %) and 21/25 (84.0 %) of the similar specimens and neither BLV or BSV were found in 26 eggs and 60 embryos collected from 20 of the 25 cows. Detection of BLV antigens by immunofluorescence was hampered by the competitive replication of both BLV and BSV and competitive growth of indicator cells and uterine cells. As BLV has not been observed in cells of UFF sediments, it was probably isolated from leucocytes present in the lumen of uterus or from blood seeping out from inapparent vessel damage during flushing. Isolation of BLV in UFF sediments gives additional evidence to the concept of a transplacental transmission by a not yet elucidated mechanism. The high rate of BSV recovery from cells of UFF sediments indicated that this virus is more wide-spread than previously shown and that it may play a role in causing disorders of the reproductive tract.

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