East African Medical Journal Vol. 80 No. 5 May 2003
HUMAN ENDOGENOUS RETROVIRUS-R (ERV 3) *ENV*-LIKE ANTIGENS EXPRESSED IN BABOON TESTES AND EPIDIDYMIDES
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HUMAN ENDOGENOUS RETROVIRUS-R (ERV 3) *ENV*-LIKE ANTIGENS EXPRESSED IN BABOON TESTES AND EPIDIDYMIDES

J.M. MWENDA, M.W. SICHANGI, D. ODUOR-OKELLO, E.O. WANGO and D.K. LANGAT

ABSTRACT

Background: **A substantial component of the vertebrate genome comprise of retrovirus-related sequences named as endogenous retroviruses (ERVs). The role of these ERV-related sequences in the biological processes of the host species is still unknown. However, they have been associated with tumourigenesis, autoimmune diseases and placental morphogenesis in primates.**

Objective: **To determine the expression of ERVs in male baboon reproductive tissues.**

Design: **The testes and other reproductive tissues from sexually immature and mature male olive baboons (*Papio anubis*) were investigated for the expression of endogenous retrovirus-related particles. Immunohistochemical staining was performed using antibodies raised against human immunodeficiency virus (HIV)-1/2, simian immunodeficiency virus (SIV) and human ERVs. Biochemical properties were determined by western blot, and reverse transcriptase (RTase) activity in epididymal spermatozoa, ejaculate spermatozoa and seminal fluid was evaluated.**

Setting: **Institute of Primate Research, Nairobi, Kenya.**

Results: **ERV3 *env*-like antigens were detected on spermatogenic cells in mature baboon testes and on epididymal spermatozoa. Similarly, antigens cross-reactive with antibodies to HIV structural and envelope glycoproteins were expressed in mature and juvenile baboon testes. In addition, reverse transcriptase activity was detected in ejaculate spermatozoa, seminal fluid and epididymal spermatozoa.**

Conclusion: **These results indicate that retroviral-related genes were expressed in normal male baboon testes and spermatozoa, similar to humans. The functions of these ERVs in vertebrates remains unclear.**

INTRODUCTION

Endogenous retroviruses (ERVs) are viral elements carried vertically in the germ line and comprise a significant proportion of most vertebrate genomes(1). Most of these viral sequences are defective due to the presence of multiple termination codons, preventing translation to functional proteins(2-4). However, there are some retroviral sequences that produce viral particles, which in most cases are non-infectious(4). In human genome, approximately 1% consists of retroviral sequences(4) but their origin remains unknown. These particles have structural homologies to exogenous retroviruses and are highly expressed in reproductive tissues, particularly the placenta(5-7) and to a lesser extent in the testes(5,8). Recent evidence suggests that ERVs could be involved in normal cellular processes like differentiation as well as in tumour formation (9,10) and immunomodulation(11). Attention has focused on the potential roles of ERVs in reproductive physiology(3,8,12), development and germ cell tumours(4). Studies have demonstrated expression of intracisternal type A-like particles in mice epididymis(13) and ERV3 *env* transcripts in human testis(5). The role of these retroviral particles in normal male reproductive tissues requires further investigations.

Previous studies have shown that the pattern of spermatogenesis in olive baboons (*Papio anubis*) is intermediate between that in rats and man(14). Therefore, the baboon can be used as a model to study the roles of these ERVs in human disease and reproductive physiology. Since ERVs have formed part of vertebrate genome for millions of years(15), it's likely that some of them have acquired some important biological functions in the cells in which they exist. In this study, we examined the pattern of expression of ERVs in reproductive tissues of male baboons, with a view of

developing the baboon as a model for studies to elucidate the role of ERVs in human disease and physiology.

MATERIALS AND METHODS

Sample collection: Male olive baboons (*Papio anubis*) were obtained from the wild and housed at the Institute of Primate Research, Nairobi, Kenya. All animals were screened for antibodies against SIV and STLV-1 as part of the institute routine screening procedure, and the virus-antibody free animals were used in this study. A total of six male olive baboons (4 mature and 2 juveniles) were used.

The adult animals were sedated by administration of ketamine (10mg/kg body weight) and xylazine (0.5mg/kg) intramuscularly. Semen was obtained by rectal probe stimulation using the spaceage ejaculator (Standard electronics Inc. Littleton, CO). Electroejaculation was performed once every week for three weeks. The semen was collected in sterile pre-warmed 15ml centrifuge tubes and allowed to liquefy at room temperature for 10-15 minutes. The semen was then centrifuged at 500g for 10 minutes to separate seminal plasma from the sperm pellet. The seminal fluid was aliquoted then frozen at –20°C, and spermatozoa were frozen at –70°C until the time of analysis. To obtain tissues, the animals were euthanised using 20% pentobarbitone (Euthatal®, Rhone Poulenc, Germany), and testicular and epididymal tissues surgically removed. The tissues were cut into small pieces, washed in PBS (pH 7.4) and either fixed in 10% formaldehyde or embedded in TissueTek® OCT compound (Miles Diagnostics Inc., Elkhart, IN), snap-frozen in liquid nitrogen and stored at –70°C until the time of sectioning for histology. The epididymides spermatozoa were squeezed out using forceps into 15ml tubes containing PBS and stored at –70°C until the time of analysis.

Immunohistochemistry: The frozen OCT-embedded tissues were sectioned and stained as previously described(6,7). Briefly, 6μm sections were cut using a cryostat (Frigocut 2800N, Cambridge Instruments, UK) and placed on gelatin-coated slides. The sections were air-dried at room temperature for one hour and subsequently fixed in cold acetone (at 4°C) for 10 minutes. Sections were stained using the streptoavidin-biotin peroxidase method (Histo-SP kit, Zymed Laboratories, South San Francisco, CA) as described by the manufacturer. Briefly, slides were placed in a moist chamber and non-specific binding sites on the sections were blocked using 10% non-immune goat serum for 10 minutes. The mouse and rabbit primary antibodies were diluted as shown in Table 1, using 3% fat-free skimmed milk in PBS. One hundred microlitres was added to the sections and incubated for 60 minutes at room temperature in the moist chamber. The sections were washed for 2 minutes with PBS, then 100μl of biotinylated goat anti-mouse or anti-rabbit IgG antibody was added to the sections and incubated for 10 minutes, washed as above and followed by incubation for another 10 minutes with streptoavidin-peroxidase-conjugate (all reagents from Zymed). The sections stained with human primary antibodies were incubated with goat anti-human horse-radish-peroxidase (HRP)-conjugated secondary antibodies (Sigma Immunochemical Co., St. Louis, MO) for 20 minutes. The sections were then washed with PBS, and reactivity in all the sections was detected using 3% hydrogen peroxide as enzyme substrate and 3-amino-9-ethyl carbazole as chromogen. The sections were counterstained with haematoxylin and mounted with a coverslip using glyceryl vinyl alcohol (GVA) mountant, dried overnight at room temperature and examined using a light microscope. A monoclonal antibody (W6/32) raised against human HLA class I monomorphic heavy chain determinant was used as a positive control. This antibody reacts with major histocompatibility complex class I antigens that are ubiquitously expressed in most nucleated cells. Negative controls included isotype controls for monoclonal antibodies (Dako Corp., Carpinteria, VA; Table 1), non-immune sera for polyclonal antibodies and PBS instead of primary antibody as reagent control.

Reverse Transcriptase assay: Reverse transcriptase (RTase) activity in the spermatozoa from the ejaculum and epididymides, and in seminal plasma was tested using commercial kits optimised for semi-quantitative screening for retroviruses (Cavidi HS-kit Mn^{2+} RT and Cavidi HS-kit Mg^{2+} RT kits, Cavidi Tech AB, Uppsala Sweden). Supernatants and cell lysates from Molt-4 cell line infected with HIV (clones MN and KS, gift from Mr. Siongok, Kenya Medical Research Institute, Nairobi) were used as positive controls. The procedure was performed according to the manufacturer's instructions.

Briefly, a reaction mixture containing 20mM HEPES pH 7.6, 4mM $MgCl_2$ (or 6 mM $MnCl_2$), 4mM spermine, 1% (v/v) Triton X-100, 0.4 mM EGTA, 1mg/ml BSA, 0.1 g/l dextran sulphate, 1mM GTP, 40 ng/ml oligo $(dT)_{22}$, and 32 μM BrdUTP was prepared, and 140-200μl added to each well of two poly (A) plates from each kit, and incubated at 33°C for 60 minutes. Ten microlitres of undiluted samples (sperm pellet lysates or seminal fluid) were added to corresponding wells of both poly (A) plates, and the standards and controls added in a similar manner. The plates were incubated at 33°C on an orbital shaker set on gentle agitation. One of the two plates was incubated for three hours and the other one for overnight. The RTase reaction in the plates was stopped after the specified times by washing with wash buffer containing Triton X-100.

The presence of double-stranded DNA in the wells (positive reactivity) was detected by incubating the plates with 100μl of RTase product tracer (BrdU binding antibody, conjugated to alkaline phosphatase enzyme and containing 1% Triton X-100) for 90 minutes at 33°C on an orbital shaker. The excess tracer was removed by washing the plates with wash fluid as above, then 100μl of alkaline phosphatase substrate (*p*-nitrophenyl phosphate) was added, and incubated at room temperature under dark cover. The optical density of both plates was read after 30 minutes of incubation at 405 nm, repeated after 2 hours, and on the following day after 24 hours of incubation using a DYNEX microtitre plate ELISA reader (Dynatech, Germany). The measured absorbance at 405 nm of each standard dilution was plotted against its concentration of standard present, and the RTase activity from the unknown samples was estimated from the standard curve.

Table 1

Antibodies used in immunohistochemistry

Antibody	Isotype	Host	Dilution	Source
Monoclonal antibodies				
Anti-SIV mac p27(55-2F12)	IgG_{2b}	Mouse	1:200	NIH
Anti-HIV-1 RT	IgG	Mouse	1:200	NIH
Anti-HIV-1 gp120 (ID6)	IgG	Mouse	1:200	NIH
Anti-HIV-2 CP	IgG	Mouse	1:100	NIH
Anti-HIV-1 gp41(md-1)	IgG	Human	1:200	NIH
Anti-HERV-K *env*	IgG	Mouse	1:10	Gift from W. Vogetseder (Univ. of Innsbruck, Austria)
Anti-HIV-1 gp120 (Chessie)	IgG_1	Mouse	1:20	NIH
Anti-HIV-1 gp41	IgG_3	Mouse	1:200	NIH
Anti-SIV mac 251 *gag* (kk59)	IgG_1	Mouse	1:200	NIH
Anti-SIV mac 251 *gag* (kk64)	IgG_1	Mouse	1:200	NIH
Anti-HIV-1 p24	IgG	Mouse	1:400	NIH
Anti-HIV-1 p24/25 *gag*	IgG_{2b}	Mouse	1:200	NIH
Polyclonal antibodies				
Anti-HIV-2ST gp120		Rabbit	1:200	NIH
Anti-ERV3 *env*		Rabbit	1:200	Gift from Prof. P. Venables (Kennedy Inst. Rheumatol., UK).
Anti-HIV-1 p24/25 SF2		Rabbit	1:200	NIH
Controls				
W6/32 (positive control)	IgG_{2a}	Mouse	1:10	Sera Lab, UK
Negative control	IgG_{2a}	Mouse	1:1000	Dako
Negative control	IgG_{2b}	Mouse	1:1000	Dako
Negative control	IgG_1	Mouse	1:1000	Dako

KEY

CP = Core protein
gp = Glycoprotein
HIV = Human-immunodeficiency virus
RT = Reverse transcriptase
SIV = Simian-immunodeficiency virus
ERV = Endogenous retrovirus
HERV = Human endogenous retrovirus
NIH = National Institute of Health, USA.

RESULTS

Immunohistochemistry

Testes: The expression and distribution of endogenous retroviral proteins in baboon reproductive tissues was assessed by immunohistochemistry using antibodies to human exogenous and endogenous retroviruses. A polyclonal antibody against human endogenous retrovirus-R (ERV3) envelope protein reacted with spermatogenic cells (spermatogonia/primary spermatocytes) within the peripheral compartment of seminiferous tubule epithelium from all mature animals (Figure 1, Table 2). However, this antiserum did not react with spermatozoa within the same tubules. This staining demonstrated the expression of ERV3 envelope protein by germ cells during early phases of spermatogenesis in baboons. Anti-HIV-1 p24/25 *gag*, (a monoclonal antibody against a human immunodeficiency virus type-1 core protein) showed weak non-specific staining on all mature testes tested in this study (Table 2). Similarly, anti-HIV-2 ST gp120, a polyclonal antibody against a human HIV-2 envelope protein, non-specifically bound to all testicular cells, both within the seminiferous tubules and interstitium (Table 2). The rest of the antibodies tested did not show any reactivity with testicular sections.

Table 2

Immunostaining on mature baboon testes

Antibody	Peritubular cells	Spermatogonia spermatocytes	Spermatozoa	Sertoli cells	Leydig cells
Anti-ERV3 env	-	+	+ (acrosome)	-	-
W6/32	++	-	-	-	-
Anti-HIV-1 gp 120 (ID6)	-	-	-	-	-
Ant-HIV-1 RT	-	-	-	-	-
Anti-HIV-2 CP	-	-	-	-	-
Anti-HIV-2 ST gp120	+/-	+/-	-	-	-
Anti-ERV3 env	-	+	-	-	-
Anti-HIV-1 p24/25 SF2	-	-	-	-	-
Anti-SIV mac p27 (55-2F_{12})	-	-	-	-	-
Anti-HIV-1 gp41(md-1)	-	-	-	-	-
Anti-HIV-1p24/25 gag	+/-	+/-	-	-	-
Anti-SIV mac 251 p17 gag	-	-	-	-	-
Anti-HERV-K env	-	-	-	-	-

Key

++ = Strong staining
\+ = Low intensity staining
+/– = Indeterminate generalised staining
– = No staining

Figure 1

Anti-ERV3 env (a polyclonal antibody against a human endogenous retrovirus-R envelope) staining on mature baboon testis. Early phases of spermatogenic cells were stained (arrow). mag x400). Sp = spermatogonia, PS = Primary spermatocyte

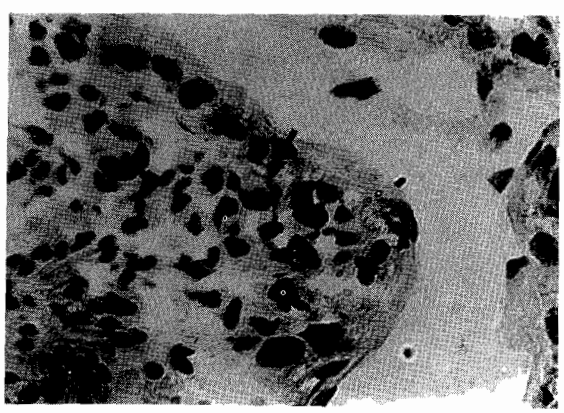


Figure 2

Anti-ERV3 env (a polyclonal antibody against a human endogenous retrovirus-R envelope) staining spermatozoa within mature baboon caput epididymis. Strong staining of spermatozoa heads was observed (arrow), mag x400.

Figure 3

W6/32 (a monoclonal antibody against HLA-A, B and C) staining on mature baboon testis. The smooth muscle cells within the interstitium were strongly stained (shown by arrow). mag x200, St = seminiferous tubule epithelium, It = Interstitial tissue

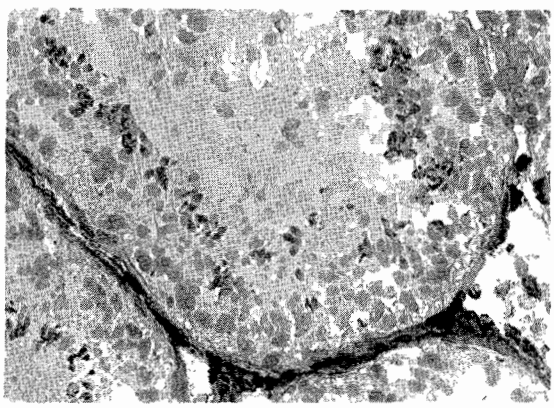
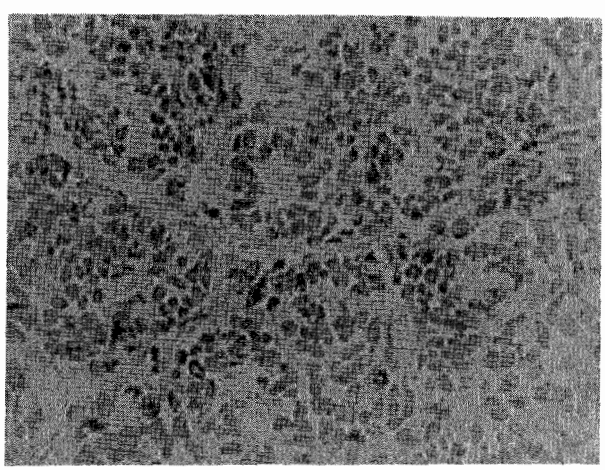


Figure 4

A tissue section of baboon testis stained with the negative control antibodies to demonstrate the specificity of the test antibodies



Anti-HIV-2 ST gp120 showed some weak staining on juvenile baboon testis. The pattern of staining was similar to that observed in the adult baboon testis (Figure 1). Similarly, anti-HIV-1 p25/24 *gag* showed some weak non-specific staining on juvenile baboon testes. Weak non-specific staining of spermatogonia and interstitial tissue was also observed. Anti-ERV3 *env* antibody, which strongly stained adult baboon testes, did not stain any cells in juvenile testes.

A monoclonal antibody (W6/32) that binds human monomorphic class I MHC molecules (HLA-A, B and C) was used as a reagent control. This antibody reacted strongly with the smooth muscle cells within the interstitium (Figure 3). The sections stained with the isotype control antibodies or non-immune serum did not show any reactivity in all the tissues tested (Figure 4).

Epididymis: In the epididymis, anti-ERV3 *env* reacted weakly with spermatozoa within the epididymal lumen. The head of the spermatozoa, particularly the acrosome region strongly bound this antibody (Figure 2). This reaction was consistent in all the four mature animals. This antibody also weakly stained the epithelial cells of the cauda epididymal duct. The remaining antibodies tested were negative. W6/32 reacted with cells in the basement membrane, smooth muscle cell fibres surrounding the epididymal duct and those within the connective tissue stroma. This antibody also stained the three muscle layers of the tunica muscularis in the vas deferens.

Reverse Transcriptase (RTase) activity: To further characterise the ERV particles expressed in testicular tissues, we tested seminal fluids extracted from the testis and epidydimis for presence of reverse transcriptase activity. We used two different commercial kits, one optimised for RTase which uses magnesium ions as a co-factor and the other one optimised for RTase which utilises manganese as co-factor. We used cell culture extracts from HIV-infected cells as a positive control and uninfected cell culture extracts as a negative control.

RTase activity was detected in the ejaculate and epididymal spermatozoa, including the seminal plasma of ejaculum (Table 3).

Table 3

RTase activity using magnesium and manganese dependent kits

Sample type	RTase concentration	
	Mg^{2+}(pg/ml)	Mn^{2+}(μU/ml)
HIV-1 SUP (MN clone)	1371	79195
HIV-1 SUP (KS clone)	1356	29100
Pan 1669 seminal plasma	861	3287
Pan 1669 ejaculate sperm	231	3110
Pan 1669 epididymal sperm	–	2917

The culture extracts from the two HIV-1 clones (HIV-1 MN and HIV-1 KS) used as positive controls had the highest RTase activity among the samples tested. The values were 1,371 and 1,356 pg/ml (Mg^{2+} dependent kit) and 79,195 and 29,100 μU/ml (Mn^{2+} dependent kit) for HIV-1 MN and HIV-1 KS clones respectively (Table 3).

DISCUSSION

The human genome contains numerous endogenous retroviral sequences with partial relatedness to animal retroviruses(4). However, most of sequenced ERVs are defective and cannot be expressed, and their function in the vertebrate genome still remains a mystery(2,3). One of the unique features of these particles is their expression is specific tissues in vertebrates(16,17). ERV particles have been detected in reproductive tissues, especially in the placenta, in a number of species, including human(16,18) and baboon(6,7). In male reproductive tissues, ERV particles have been detected in the testis and epididymis in human(5) and mice(13). Their role in reproductive processes is still unknown.

In this study, ERV 3 *env*-like antigens were demonstrated on the early phase spermatogenic cells. The strong association of ERV3 *env* expression with spermatogenic cells suggests that there may be a physiologic role for ERV3 *env* in spermatogenesis. Several studies have shown a possible role for ERV3 in placental development. Lin *et al,*(19) detected a decreased cellular proliferation, increased differentiation-related changes in cellular morphology and a significant increase in intercellular fusion in BeWo cells transfected with ERV3 *env*. In a related study, the same research group(12) showed, using a similar placental trophoblast model (BeWo) that ERV3 *env* decreases cyclic B (a positive cell cycle regulator) and increases P21 (a negative cell cycle regulator) expression, which in turn inhibits cell growth. We postulate that ERV3 *env* –like proteins detected in the testes may inhibit cellular division (proliferation) among the spermatocytes and subsequent clones undergoing spermatogenesis resulting in syncytia formation(20). In addition, it is possible that ERV3 *env* may enhance cellular differentiation in early phases of spermatogenesis, but there is no evidence for this yet.

Studies done using syncytiotrophoblasts have indicated that a protein termed syncytin is responsible for cell fusion among cytotrophoblasts. This protein was also found expressed at lower levels in the human testis, but not in any other tissues(21). The protein has also been shown to share a 34% identity with BaEV envelope protein (21). DNA nucleotide analysis revealed sequences on human chromosome 7 that contained an intact open reading frame (ORF) that possessed 100% identity to syncytin cDNA. This observation concurs with the fact that ERV3 gene sequences are located on human chromosome 7 and share homology to BaEV (15,22). These previous studies strengthen the possibilities that ERV3 *env*-like proteins demonstrated in this study may have a role in syncytia formation among spermatogenic cells and/or spermatogenic cellular differentiation. It's also possible that the ERV3 *env*-like proteins described in this paper are actually BaEV gene products but only cross-reactive with anti-ERV3 *env* antibodies.

The expression of ERV3 *env* antigen on the epididymal spermatozoa demonstrated in this study was also striking. We postulate that this could play a role in sperm-zona binding during fertilization. This is based on the virus envelope fusogenic properties, as earlier demonstrated(8). In addition, possibilities for existence of ERV 3 *env* receptors on the oocyte cannot be ruled out. Viral envelope proteins have properties that enable the virus to bind a specific receptor on a target cell, and so mediate viral entry. Such properties could mediate spermatozoa-oocyte fusion, entry of the sperm nucleus into the oocyte and eventual fertilization.

We also demonstrated the presence of reverse transcriptase activity in baboon seminal plasma, spermatozoa from ejaculum and epididymides. Earlier studies have also demonstrated reverse transcriptase activity within the reproductive tract of mice(23) suggesting activation of ERV sequences in mouse and baboon epididymidis. But whether RTase activity is ubiquitous in the baboon epididymis or is accompanied by retroviral particle formation could not be established in the current studies. However, we can attribute the RTase activity to a possible existence of ERV particle expression. In addition, this study demonstrated ERV3 *env*-like proteins on spermatozoa within the epididymis, which can be associated with other viral genes such as the *gag,* and *pol*, although they were not demonstrated in this study. Reverse transcriptase enzyme is a product of pol gene expression, and hence it is possible that ERV-associated endogenous RTase exists in the epididymis and spermatozoa.

Retroviral particles have most often been detected in humans and other species in an environment associated with steroid hormones such as placenta, testis, adrenal and sebaceous glands(17). It is tempting to speculate that ERV expression is regulated through changes in the levels of steroid hormones. In fact, it has been shown that the long terminal repeats (LTR) of ERV3 contain two potential hormone-responsive elements. In addition, the LTRs of ERV3 contain one of these elements, and steroid hormones regulate ERV3 mRNA expression(2). Since the testis is an active site of androgen hormone synthesis and action, and ERV3 *env* has been demonstrated in the testis, the association between androgen activity and ERV expression cannot be ruled out. More so, the expression of ERV3 *env* as demonstrated in this study was associated with sexually mature baboons but not with juveniles. This leads to speculation for a steroid-hormone dependency in expression pattern, and restriction to sexually mature animals.

Another observation was that the cauda epididymis expressed ERV3 *env*-like particles within its epithelia. The cauda epididymis has vital role in concentrating and storing spermatozoa awaiting coitus and eventual ejaculation(20). The role of retroviral antigens has not been documented, but a possible role in genetic feedback mechanism from somatic to germ cells has been

speculated(13,17). The cauda epididymis could be a site for ERV3 *env* synthesis. But whether this is ubiquitous or has a role in enhancing fertilizing ability of spermatozoa or concentration of the same cannot be concluded from this data.

In summary, we have demonstrated the expression of retrovirus-like gene products in male baboon testis and epididymis. We postulate that the virus genome could be having a role in maintaining the syncytia that exists between spermatogenic cells originating from same stem spermatogonium. This in effect synchronizes the physiological and chemical changes among spermatogenic cells. For epididymal spermatozoa, the expression of ERV3 *env*-like molecules could be of relevance in maturation, storage and/ or fertilization. If ERV3 *env* antigen is proved to play a role in spermatogenesis and/or acquisition of fertilizing ability, then it might offer a novel target for fertility regulation. The converse also holds true; failure of expression of ERV may have adverse effects on fertility. Further studies are under way to address these important research questions.

ACKNOWLEDGEMENTS

To the National Institutes of Health (USA), Prof. Venables (Kennedy Institute of Rheumatology, UK) and W. Vogetseder (University of Innsbruck, Austria) for donating some of the antibodies used in this study. We also thank Prof. Simon Gronovitz (Research Director, Cavidi Tech AB, Sweden) for donating kits used in the RTase assays. This work was financially supported by a grant from WHO-Special Programme for Research, Development and Research Training in Human Reproduction to MWS through the Reproductive Biology Unit, University of Nairobi.

REFERENCES

1. Coffin, J. Endogenous viruses. In: Weiss, R., Teich, N., Vermus, H. and Coffin, J. (Ed): *RNA Tumour Viruses* (2nd Edition) New York: Cold Harbour Press, 1984; 1109-1203.
2. Larsson, E., Kato, N. and Cohen, M. Human endogenous proviruses. *Curr. Top. Microbiol. Immunol.* 1989; **148:**115-132.
3. Leib-Mosch, C., Brack-Werner, R., Werner, T. *et al.* Endogenous retroviral elements in human DNA. *Cancer Res.* (Suppl.) 1990; 50:5636s.
4. Casau, A.E., Vaughan, J.E., Lozano, G. and Levine, A.J. Germ cell expression of an isolated human endogenous retroviral long terminal repeats of the HERV-K/HTDV family in transgenic mice. *J. Virol.* 1999; **73:** 9976-9983.
5. Larsson, E., Anderson, A. and Nilsson, O. Expression of endogenous retrovirus (ERV3, HERV-R) in human reproductive and embryonic tissues-evidence for a function for envelope gene products. *Upps. J. Med. Sci.* 1994; **99:** 113-120.
6. Langat, D.K., Johnson, P.M., Rote, N.S., Wango, E.O., Owiti, G.O. and Mwenda, J.M. Immunohistochemical localization of retroviral-related antigens expressed in normal baboon placental villous tissue. *J. Med. Primatol.* 1998; **27:** 278-386.
7. Langat, D.K., Johnson, P.M., Rote, N.S. *et al.* Characterization of antigens expressed in normal baboon trophoblast and cross-reactive with HIV/SIV antibodies. *J. Reprod. Immunol.,* 1999; **42:** 41-58.
8. Mi, S., Lee, X., Li, X., Veldman, G.M., Finnerty, H. *et al.* Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature.* 2000; **403:** 785-789.
9. Morse, B., Rotherg, P., Spandorfer, J. and Astrin, S. Insertion mutagenesis of the *myc* locus by a LINE-1 sequence in a human breast carcinoma. *Nature,* 1988; **333:** 87-90.
10. Marrack, P., Kushnir, E. and Kappler, J. A maternally inherited superantigen encoded by a mammary tumour virus. *Nature,* 1991; **349:** 524-526.
11. Kato, N., Pfeifer-Ohlsson, S., Kato, M. *et al.* Tissue specific expression of human provirus ERV3 mRNA in human placenta: Two of the three ERV3 mRNAs contain human cellular sequences. *J. Virol.* 1987; **61:** 2182-2191.
12. Lin, L., Xu, B. and Rote, N.S. The cellular mechanism by which the human endogenous retrovirus ERV-3 *env* gene affects proliferation and differentiation in a human placental trophoblast model, BeWo. *Placenta,* 2000; **21:** 73-78.
13. Kiessling, A.A., Crowell, R. and Fox, C. Epididymis is the principal site of retroviral expression in the mouse. *Proc. Natl. Acad. Sci. (USA),* 1989; **86:** 5109-5114.
14. Chowdhury, A.K., Marshall, G. and Steinberger, E. Comparative aspects of spermatogenesis in man and baboon (*Papio anubis*). *Proceedings of the Fourth International Symposium on Comparative Biology of Reproduction: Reproduction and Evolution,* Australian academy of Sciences, Canberra, Australia, 1977; 143-150.
15. Van der Kuyl, A.C., Dekker, J.T. and Goudsmit, J. Full-length proviruses of baboon endogenous virus (BaEV) and dispersed BaEV reverse transcriptase retroelements in the genome of baboon species. *J. Virol.* 1995; **69:** 5917-5924.
16. Urnovitz, H.B. and Murphy, W.H. Human endogenous retroviruses: Nature, occurrence, and clinical implications in human disease. *Clin. Microbiol. Rev.* 1996; **9:** 72-99.
17. Taruscio, T. and Montavani, A. Human endogenous retroviral sequences: Possible roles in reproductive physiopathology. *Biol. Reprod.* 1998; **59:** 713-724.
18. Johnson, P.M., Lyden, T.W. and Mwenda, J.M. Endogenous retroviral expression in the human placenta. *Amer. J. Reprod. Immunol.* 1990; **23:** 115-120.
19. Lin, L., Xu, B. and Rote, N.S. Expression of endogenous retrovirus ERV 3 induces differentiation in BeWo, a choriocarcinoma model of human placental trophoblast. *Placenta* 1999; **20:** 109-118.
20. Sharpe, R.M. Regulation of spermatogenesis. In: Knobil, E. and Neill, J.D. (Ed), *The Physiology of Reproduction,* Vol. 1, 2nd Ed, Raven press Ltd, 1994; pp. 1363-1434.
21. Stoye, J.P. and Coffin, J.M. A provirus put to work. *Nature.* 2000; **403:**715-717.
22. Cohen, M., Powers, M., O'Connell, C. and Kato, N. The nucleotide sequence of the *env* gene from the human provirus ERV3, and isolation and characterization of an ERV3-specific cDNA. *Virology* 1985; **147:** 449-458.
23. Kiessling, A.A. Evidence that reverse transcriptase is a component of murine epididymal fluid. *Proc. Soc. Exp. Biol. Med.* 1984; **176:** 175-182.