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Supplementary Material

High oxygen tension increases global methylation in bovine 4-cell embryos and blastocysts but does not affect general retrotransposon expression

Wenwen Li[A], *Karen Goossens*[A], *Mario Van Poucke*[A], *Katrien Forier*[B,C], *Kevin Braeckmans*[B,C], *Ann Van Soom*[D] *and Luc J. Peelman*[A,E]

[A]Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke, Belgium.

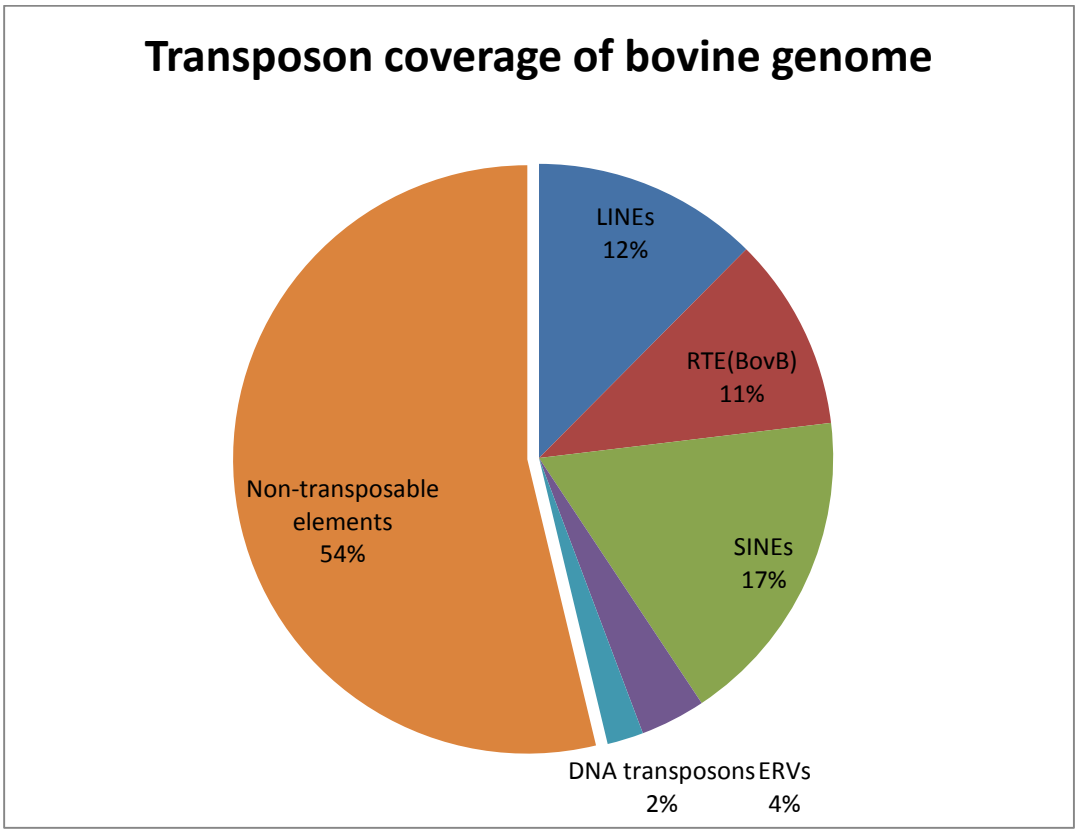
[B]Laboratory of General Biochemistry and Physical Pharmacy,Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium.

[C]Center for Nano- and Biophotonics, Harelbekestraat 72, 9000 Ghent, Belgium.

[D]Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium.

[E]Corresponding author. Email: luc.peelman@ugent.be

(a)


Transposon coverage of bovine genome
LINEs
12%
RTE(BovB)
11%
SINEs
17%
ERVs
4%
DNA transposons
2%
Non-transposable
elements
54%

(b)

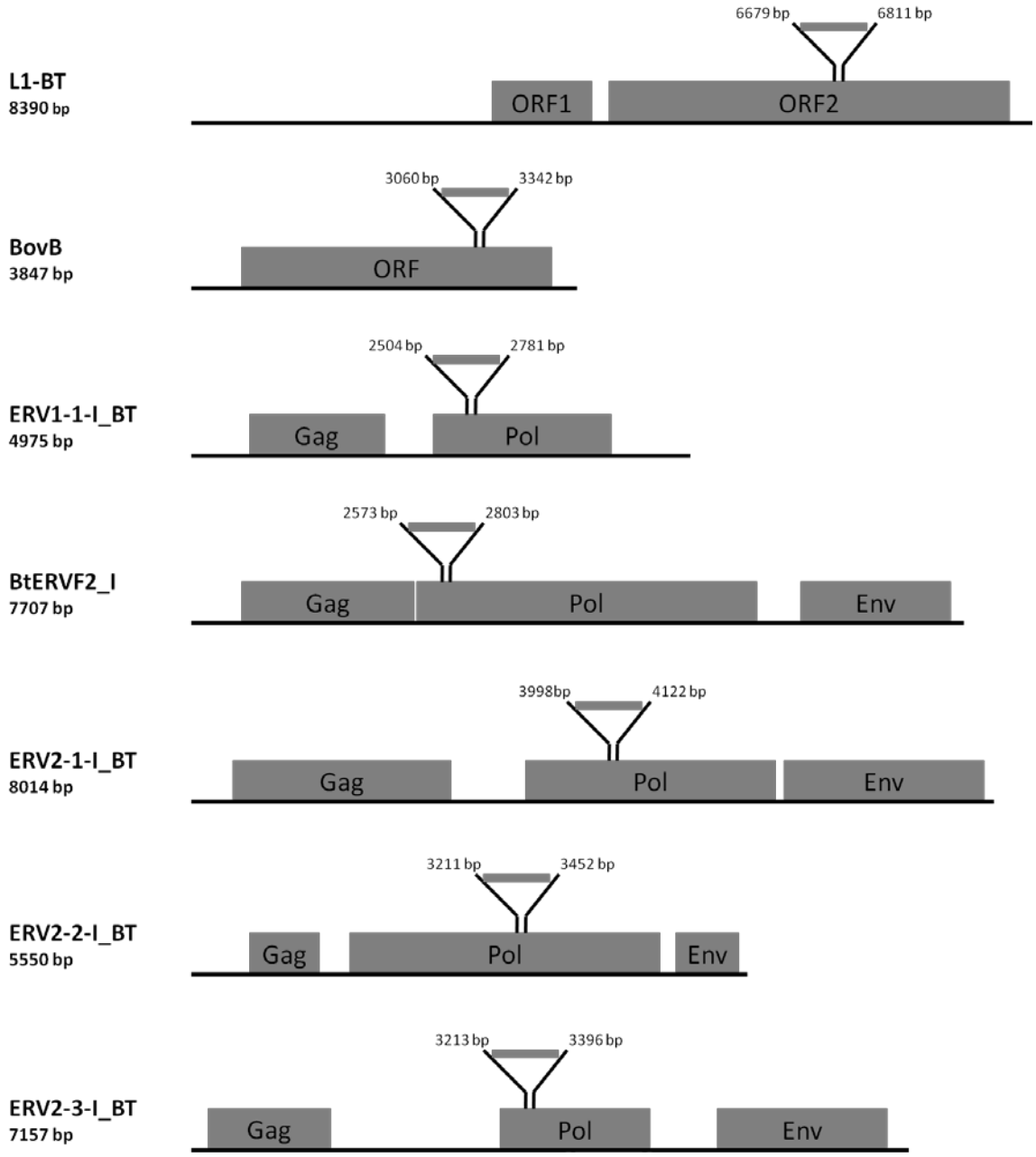


Fig. S1. (a) Percentage of bovine transposable elements. Retrotransposons include LINEs, RTE-BovB, SINEs and ERVs. Data of the pie chart are from (Adelson *et al*. 2009). (b) Location of PCR amplicons with retrotransposon ORFs.

(a)

(b)

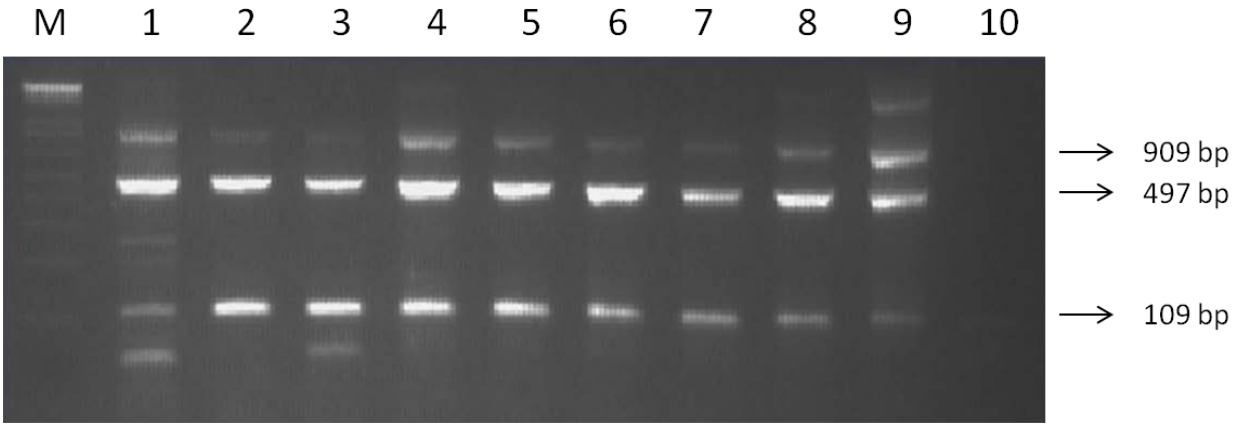


Fig. S2. RNA quality control primer assay. (a) YWHAZ primers design (b) embryo samples checked by the primer assay; samples are used when 909 bp can be amplified. M: DNA marker; 1-8: single preimplantation embryo cDNA; 9: bovine tissure cDNA; 10: negative control.

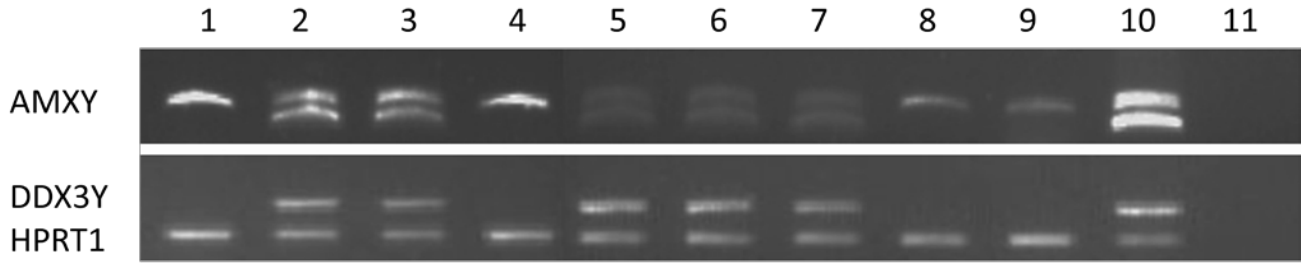
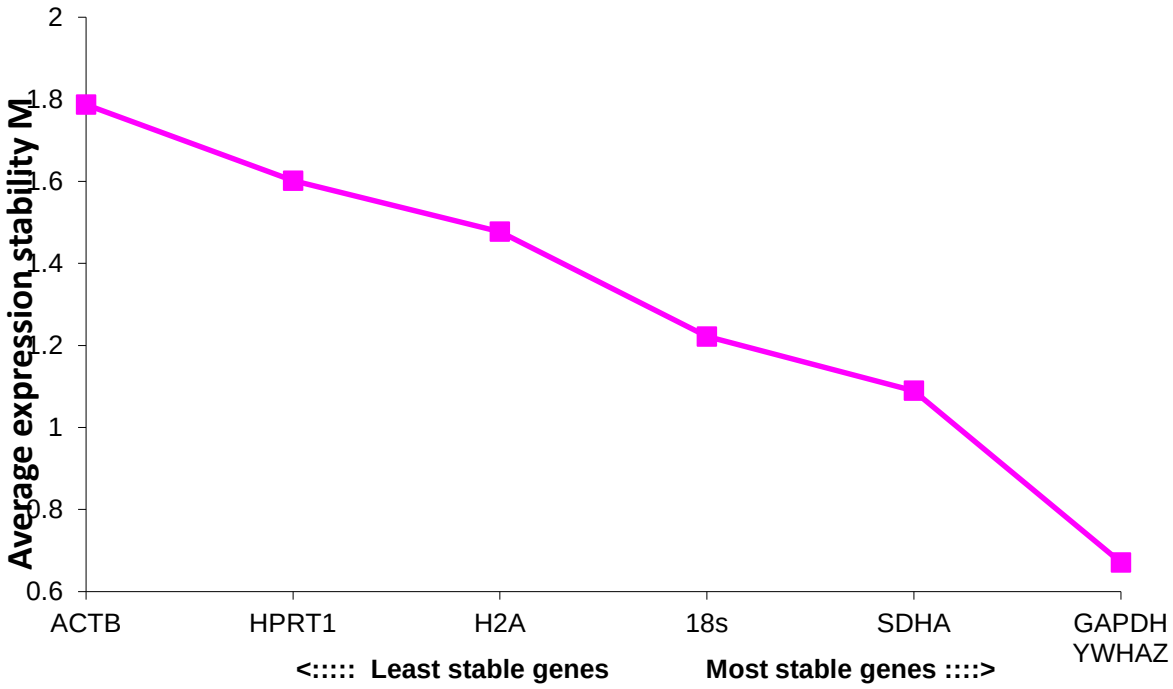


Fig. S3. Accordance of DNA-based and RNA-based embryo sexing results. Genomic AMXY was used as a gender marker (upper penal), and Y-specific gene DDX3Y expression was used for sexing while HPRT1 was added as an internal control. 1-4: 8-cell; 5-8 blastocyst; 9: female tissue DNA and RNA; 10: male tissue DNA and RNA; 11: negative control. 1, 4, 8 are female embryo and 2, 3, 5, 6, 7 are male embryos.

(a)


Average expression stability M
2
1.8
1.6
1.4
1.2
1
0.8
0.6
ACTB
HPRT1
H2A
18s
SDHA
GAPDH
YWHAZ
<::::: Least stable genes
Most stable genes ::::>

(b)

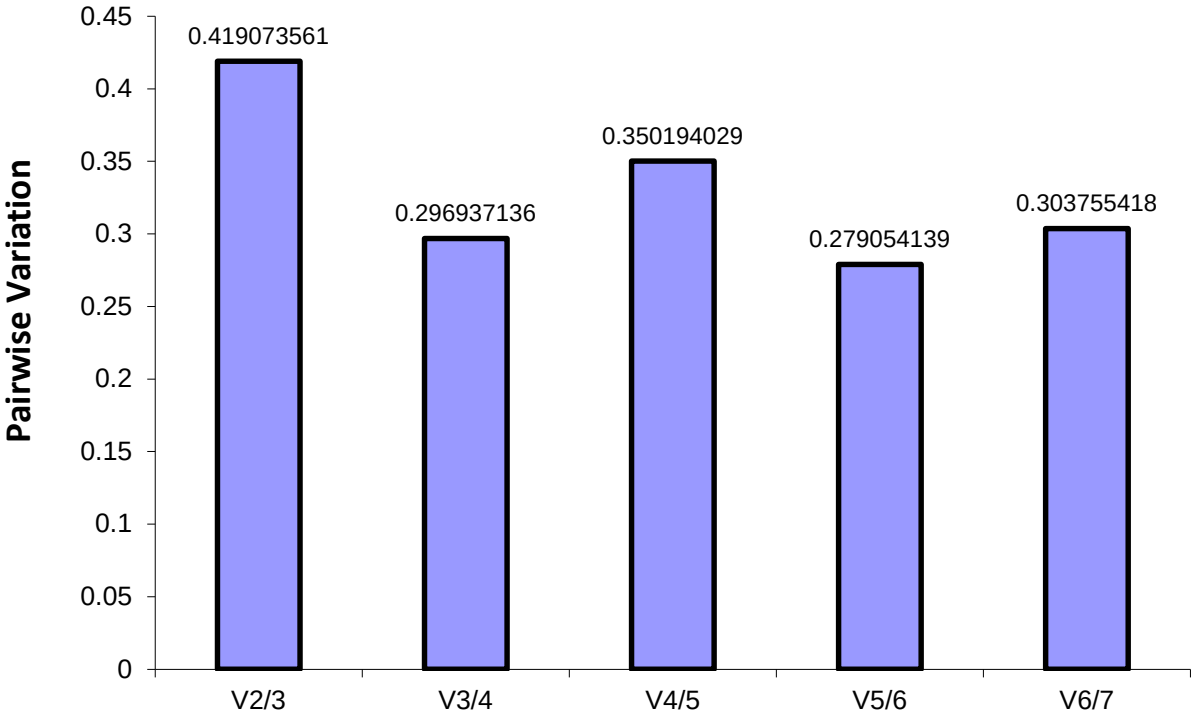


Fig. S4. Gene expression stability of candidate genes analyzed by geNorm programme. (a) Average expression stability values (M) of candidate genes spotted from the least stable (left) to most stable (right) from all 4-cell stage and blastocyst stage. (b) Pairwise variation analysis (V_n/V_{n+1}) between the normalization factors NF_n and NF_{n+1} to determine the optimal number of control genes for normalization of 4-cell stage and blastocyst stage.

Table S1. Sequences of retrotransposon families PCR amplicons

Retrotransposon families	Sequence
L1-BT (133 bp)	TGATTCCTCCAGTTCCATTCTTCTTTCTCAAGATCGCTTTGGCTATTCGAGGTTTTTTGTATTTCCATACAAATTGTGAAATTATTTGTTCTAGCTCTGTGAAAAATACTGTTGGTAGCTTGATAGGGATTGC
BovB (283 bp)	CCTCAGATATGCAGATGACACCACCCTTATGGTAGAAAGTAAAGAGGAACTAAAAAGCCTCTTGATGAAAGTGAAAGAGGAGAGTGAAAAAGCTGGCTTAAAGCTCAACATTCAGAAAATGAAGATCATGGCATCCGGTCCCATCACTTCATGGGAAATAGATGGGGAAACAGTGGAAACAGTGTCAGACTTTATTTTTCTGGGCTCCAAAATCACGACAGATGGTGACAGCAGCCATGAAATTAAAAGACGCTTACTCCTTGGAAGGAAAGTTATGACCAAC
ERV1-1-I_BT (277 bp)	TGTTAAGCTCAAAGACCCACACGTATTTCCGCATAAGAAGCAGTATCCACTGAAACCTGAAGTTAAGGAAGAGTTAAAACCCATCATCAAAAATTTAAAGGAGCAGGGACTATTAATTCCCTGTAACAGTCCTTGCAACACTCCTATTTTGGGTATAAAGAAATCGAATGGTAAATGAAGACTAGTTCAAGATTTATGAATAATAAATGAGGCTGTAGTTCCTTTACACCCCGTGGTGCCTAATCCTTATACTCTATTGTCTGAAATTCCTGAACGGAA
BtERVF2_I (231 bp)	ACCTTCCCTTGGATCTACCCACCTTANACCCCCAAGTCTGGGACACTGATCACCCATCCATAGCCAAACATCATCCCCCAGTCCACATTACCCTAAAAGACCCCTCGACTATAATCTCCCAACAGTACTCGCCACCCCGAAGACCCACAAGGGACTTAAGCCTATCATAGATCGTCTTCTTCAAGCCCCTATCCTAATTCCTAACCATTCACCACACAACACCCCTA
ERV2-1-I_BT (125 bp)	GGCTTAAAAATTGCCCCAGAGAAAATTCAAGTCAATCCGCCCATAACTTACTTAGGGCGGGTTATCAATTCAGAAACTGTGACTCATGCCCCATTAAAATTGAGAAAAGATCACCTTGTTACTTT
ERV2-2-I_BT (243 bp)	CCTGACCCTAATAGTCCTAGACAGCTAACAAAAGAAGTGGAAGAAGAATTAAAATTTGTTGAAAAATGCATTCAACAAGCTTTCACAACTCAGCTGGATCATACCCAACCGGTCTGTTTATATATATATATTCCCCACCAAACATTCACCTACTGCAATCATAGCTCAATACAGCCCGATAGAGTGGGTATATCTACAGACTAAACAGTTAAA-ATTTTCTTATCATACATTGAGAAAATAGGGCA
ERV2-3-I_BT (186 bp)	GCTTTTAGTGTCCCTTCTACAAATTATAAAGAACCTATGAAAAGATATCAGTGGCAAGTTTTACCTCAGGGAATGGCTAATAGTCCTACTCTCTGTCAGAAATTTGTTGCTCAAGCTTTAAAAACCACTAGGTCCTTGTACTCCCAAGTATATATTATTCATTATATGGATGATATTCTTTTGGCA

Table S2. Average expression stability M. Ranking of the reference genes in order of the average expression stability, decreasing from top to bottom

The reference genes chosen for normalization factor calculation are printed in bold

All stages (5%+20% F+M) [1]	4-cell (5%+20%)	Blastocyst (5%+20% F+M)	Male (5%+20%) [2]	Female (5%+20%)[2]	5%O2 (F+M) [3]	20% O2 (F+M) [3]	4cell+Blastocyst (5%+20%)[4]
GAPDH	GAPDH	YWHAZ	GAPDH	HPRT1	GAPDH	GAPDH	**GAPDH**
YWHAZ	YWHAZ	ACTB	YWHAZ	H2A	YWHAZ	YWHAZ	**YWHAZ**
18s RNA	ACTB	SDHA	18s RNA	YWHAZ	HPRT1	18s RNA	**SDHA**
SDHA	HPRT1	GAPDH	SDHA	GAPDH	H2A	SDHA	18s RNA
HPRT1	H2A	H2A	ACTB	18S RNA	18s RNA	ACTB	H2A
H2A	SDHA	18s RNA	HPRT1	SDHA	SDHA	HPRT1	HPRT1
ACTB	18s RNA	HPRT1	H2A	ACTB	ACTB	H2A	ACTB

[1]All embryo groups (from 2-cell to blastocyst) were considered.

[2]Male and female embryos were sexed 8-cell onwards.

[3]All embryo stages were considered.

[4]Unsexed 4-cell and sexed blastocyst were considered.