Supplementary Table 1. Semen sampling schedules (YYMMDD of sampling). n = Total number of collected ejaculates.

Ram 1	Ram 2
200513	200513
200519	200527
200527	200602
200602	200609
200609	200616
210317	210317
210318	210318
210323	210323
210324	210324
210331	210331
210401	210401
210407	210407
210408	210408
210414	210414
210415	210415
210420	210420
210421	210421
210422	210422
210427	210427
210428	210428
210429	210429
210506	210506
210512	210512
210518	210518
210519	210519
210520	210520
210601	210601
210602	210602
210603	210603
210609	210609
210610	210610
210617	210617
210618	210618
n = 33	n = 33

Supplementary Table 2. Information about primers for qRT-PCR. Bt = *Bos taurus*.

Target gene, ID	Primer name (cat. number)	Localisation on chromosome (Reference ARS-UCD1.2 Primary Assembly)	Amplicon size (bp)
ACTB, ID:280979	Bt03279174_g1 (4331182)	Chr.25: 38799230 – 38802643 (NC_037352.1)	141
PANX1, ID: 528169	Bt04307406_m1 (4351372)	Chr.29: 611569 - 670980 (NC_037356.1)	67
PANX2, ID: 539070	Bt03274007_m1 (4351372)	Chr.5: 119546481 – 119554076 (NC_037332.1)	66
PANX3, ID: 538977	Bt03816868_m1 (4351372)	Chr.29: 28089645 – 28097175 (NC_037356.1)	60

All chemicals, primers and equipment for qRT-PCR analysis were purchased from ThermoFisherScientific, USA, if not stated otherwise. Total RNA was isolated using TRI REAGENT (MRC/Molecular Research Centre, USA) according to the manufacturer's protocol with slight modification (overnight precipitation at -20°C with isopropanol; initial spermatozoa concentration 750^6/mL, in average). The RNA concentration and purity were determined by the NanoDrop-1000 Spectrophotometer. The A260/A280 ratio ranged from 1.7 to 2.0. One hundred nanogrammes of total extracted RNA was reverse-transcribed with the High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor. Quantitative real-time PCR exploited TaqMan Universal Master Mix II, with UNG and specific TaqMan assay bovine primers that already include specific probes with FAM-MGB dye. The cross-specificity of the used bovine primers to ovine PANX1 and PANX2 genes was confirmed (relative expression = 0.099 ± 0.011 S.D. and 0.184 ± 0.015 S.D., correspondingly) using the samples of ovine brain tissue (gyrus), isolated on a farm, from a sheep died due to natural reason, as a positive control. qRT-PCR was performed on a 7900HT Fast Real-Time qRT-PCR system, in 8 µl of total volume reactions and with the following cycling program: 95°C 15s and 60°C 1'; 40 cycles. Three to nine technical replicates (wells) were analyzed for each sample. For the qRT-PCR data analysis was used $2^{\Delta\Delta}$(Ctr-Cts) (r = reference gene; s = sample) method. PANX1 and PANX2 were normalized on the expression levels of the reference gene ACTB (we tested also GAPDH and PPIA but the Ct levels were not as stable as for ACTB). Interestingly, in the present study we were not able to detect mRNA level of PANX3 by qRT-PCR (UNDETERMINED), possibly due to its different tissue expression.

Primers were ordered by ThermoFisherScientific, for more details follow the link below:

ACTB

https://www.thermofisher.com/taqman-gene-expression/product/Bt03279174_g1#assay-details-section

PANX1

https://www.thermofisher.com/taqman/gene-expression/assay/query?keyword=Bt04307406_m1%20&productType=ge&productSubtype=ge

PANX2

https://www.thermofisher.com/taqman/gene-expression/assay/query?keyword=Bt03274007_m1%20primer&productType=ge&productSubtype=ge

PANX3

https://www.thermofisher.com/taqman/gene-expression/assay/query?keyword=Bt03816868_m1%20primer&productType=ge&productSubtype=ge

Detailed protocol of qRT-PCR method

*1. Reverse transcription reactio*n was performed by High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (cat.no: 4374966, ThermoFisherScientific):

Chemicals	1x reaction
10x buffer	1.5 μL
25xdNTP (100 nM)	0.4 μL
RNAse inhibitors	0.1 μL
RT random primers (10x)	0.5 μL
Multiscribe reverse transcriptase	1.0 μL
100 ng RNA and H_20	11.5 μL
Total volume of 1 reaction	15 μL

Program in thermal cycler: 30 min 16°C, 30 min 42°C, 5 min 85°C, at the end of the cycle keep at 4°C

After added 35 μL of ddH_2O to get the final volume 50 ul of each cDNA sample. Stored at -20°C

2. qPCR MM reaction:

Chemicals	1x reaction
TaqMan MM	2.0 μL
cDNA	0.5 μL
H_2O	1.5 μL
Total volume of 1 reaction	4.0 μL

Chemicals	1x reaction
TaqMan MM	2.0 μL
Primers (20 μM)	0.4 μL
H_2O	1.6 μL
Total volume of 1 reaction	4.0 μL

The final volume for one qRT-PCR reaction was 10 uL. Samples were runned on Fast Real Time PCR machine 7900HT (ThermoFisherScientific) by program: 95°C 15s and 60°C 1’; 40 cycles