Housekeeping genes for RT-qPCR during ovine parthenogenetic embryos

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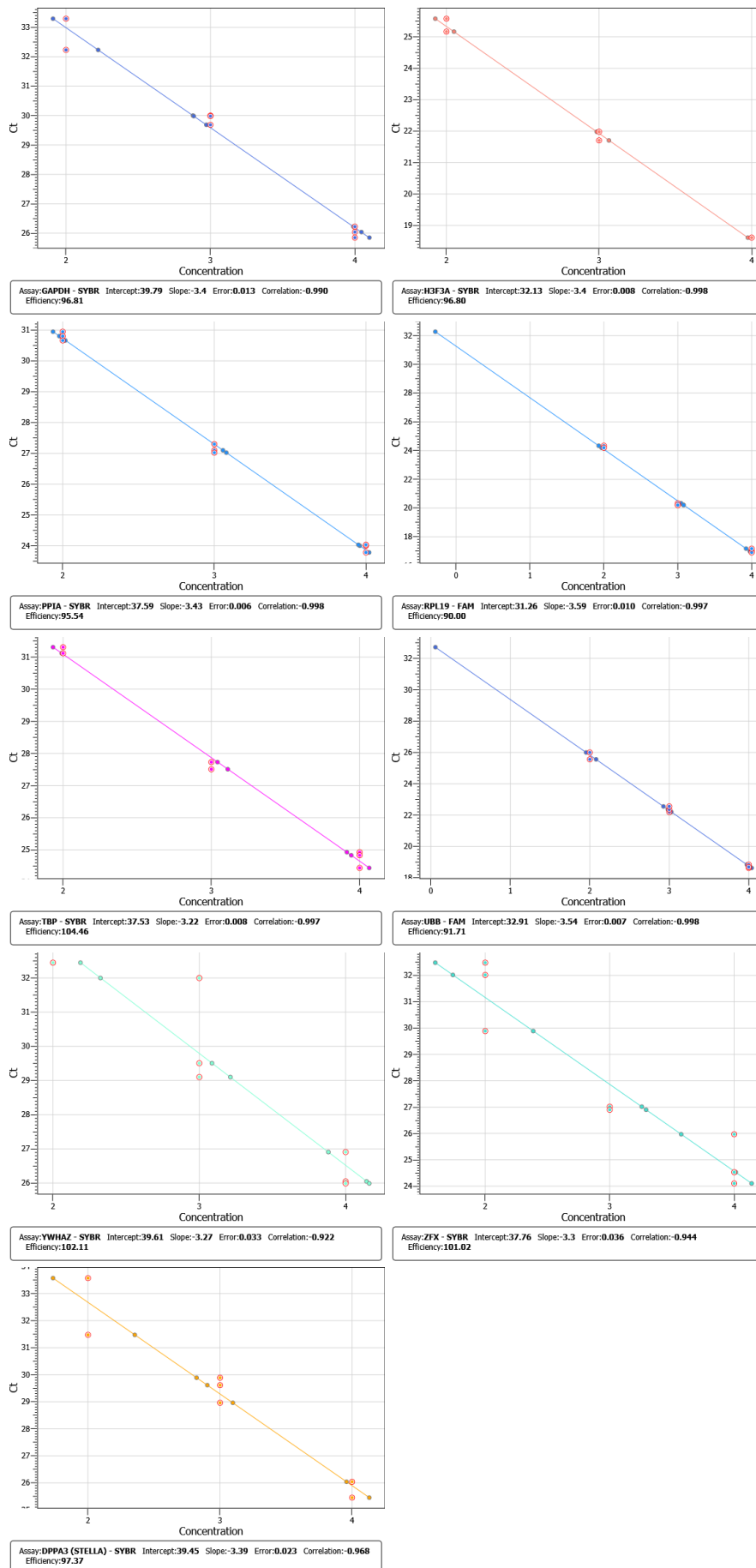
Supporting information

Supplementary Table 1. Developmental stage of biological material, number of oocytes/ embryos per sample, RNA quantification by Qubit assay, and RNA observance by Nanodrop 2000 C.

Developmental Stage	Samples (n)	RNA quantification (ng µL^{-1})	Absorbance (260-280)
Mature egg	50	6.5	1.74
	50	6.0	1.53
	50	4.4	1.35
Cleavage-stage	20	4.5	1.44
	20	3.6	1.69
	20	10.9	1.46
Morulae	10	3.4	1.50
	10	2.4	1.49
	10	2.8	1.93

Supplementary Figure 1. Melting curves for candidate reference genes (*GAPDH, H3F3A, PPIA, RPL19, TBP, UBB, and YWHAZ*) and target genes (*DPPA3* and *ZFX*).
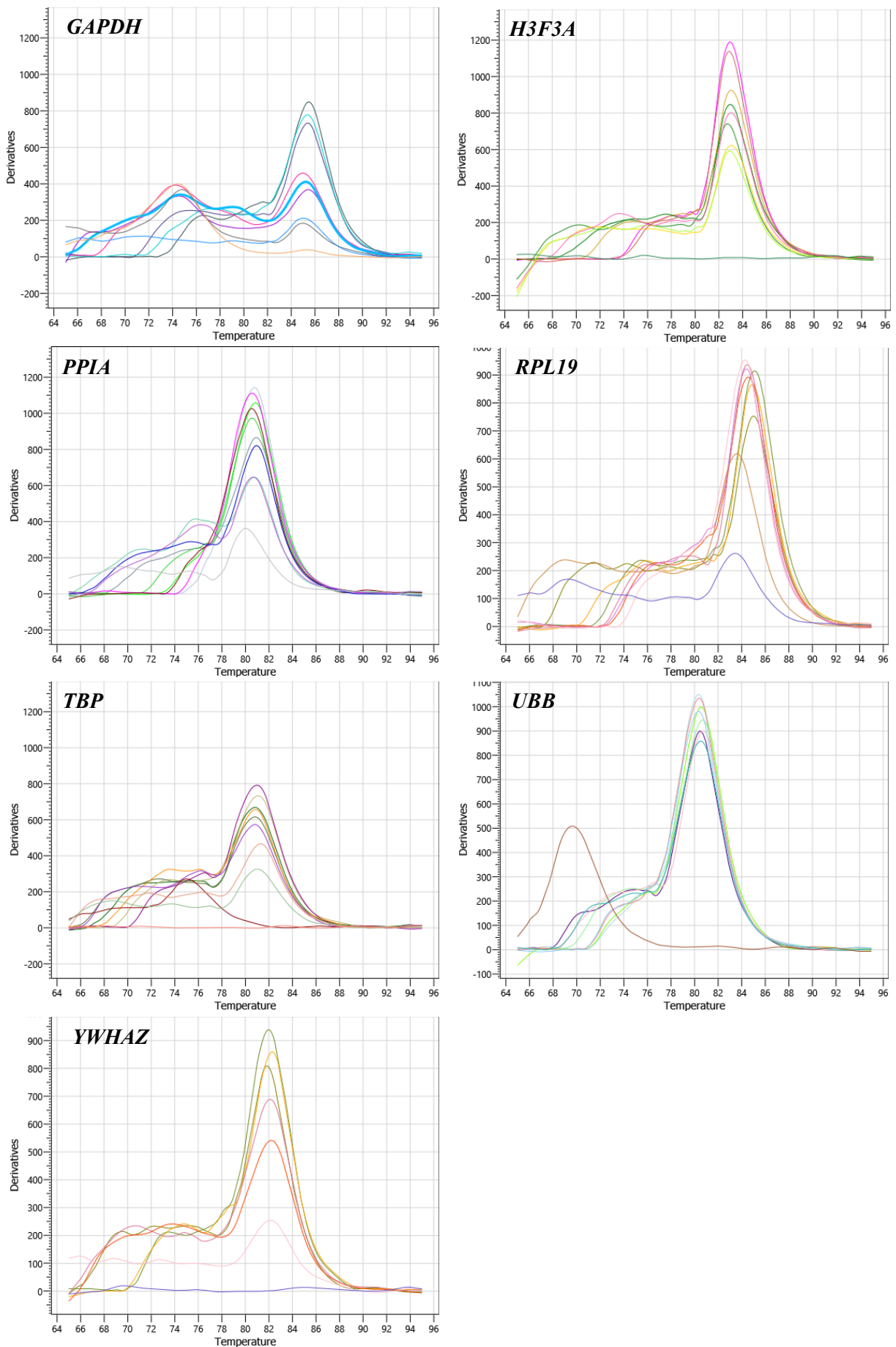
Supplementary Figure 2. Standard curve for candidate reference genes (*GAPDH, H3F3A, PPIA, RPL19, TBP, UBB,* and *YWHAZ*) and target genes (*DPPA3* and *ZFX*).

Assay:**GAPDH - SYBR**   Intercept:**39.79**   Slope:**-3.4**   Error:**0.013**   Correlation:**-0.990**
Efficiency:**96.81**

Assay:**H3F3A - SYBR**   Intercept:**32.13**   Slope:**-3.4**   Error:**0.008**   Correlation:**-0.998**
Efficiency:**96.80**

Assay:**PPIA - SYBR**   Intercept:**37.59**   Slope:**-3.43**   Error:**0.006**   Correlation:**-0.998**
Efficiency:**95.54**

Assay:**RPL19 - FAM**   Intercept:**31.26**   Slope:**-3.59**   Error:**0.010**   Correlation:**-0.997**
Efficiency:**90.00**

Assay:**TBP - SYBR**   Intercept:**37.53**   Slope:**-3.22**   Error:**0.008**   Correlation:**-0.997**
Efficiency:**104.46**

Assay:**UBB - FAM**   Intercept:**32.91**   Slope:**-3.54**   Error:**0.007**   Correlation:**-0.998**
Efficiency:**91.71**

Assay:**YWHAZ - SYBR**   Intercept:**39.61**   Slope:**-3.27**   Error:**0.033**   Correlation:**-0.922**
Efficiency:**102.11**

Assay:**ZFX - SYBR**   Intercept:**37.76**   Slope:**-3.3**   Error:**0.036**   Correlation:**-0.944**
Efficiency:**101.02**

Assay:**DPPA3 (STELLA) - SYBR**   Intercept:**39.45**   Slope:**-3.39**   Error:**0.023**   Correlation:**-0.968**
Efficiency:**97.37**

Supplementary Figure 3. Alignment of ovine (*Ovis aries*) and bovine (*Bos taurus*) *DPPA3* mRNA orthologs and annotation of regulatory sequences. Red: open reading frame (ORF). Green: polyadenilation sites (PA; longer PA site predicted by RegRNA 2.0 software and the shorter PAs described by Thélie *et al.*, 2007). Blue: CPE-like sequences described by Thélie *et al.*, 2007. Orange: Musashi Binding element predicted by RegRNA 2.0 software. Black: ATTTA motif described by Thélie *et al.*, 2007.

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CLUSTAL multiple sequence alignment by MUSCLE (3.8)


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NM_001111108.2      --GCGGGGATGGCTACTCTTCATC-----------------------------CCCT


XM_012175459.3      ------------------------------------------------------------
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NM_001111108.2      ACA----------AAAGCAGT----------------------------------------


XM_012175459.3      ------------------------------------------------------------
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NM_001111108.2      ------------------------------------------------------TATTGA-


XM_012175459.3      -----------------------------CCCTCT-------------------------
NM_001111109.2      CCCCTAATAGGACTACGCCCATTCACCTTATCCTTTTACTCATTAGTCATTTACATTAAA
NM_001111108.2      -CCCAAATG------------TCAACTTATGCTTT-------------------------
                                                       ** *


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XM_012175459.3      ------------------------------CTTTCTTAGCTC ATAGT CTTCTAGG
NM_001111109.2      ACCACGGCGTGTCAGCCTGGGTTTTTTTCCCCCCCTTTCTTAGTTC ATAGT CTTCCAGG
NM_001111108.2      ------------------------------CTGCTTTTGTTC ATAGT CTTCCAGG
                                       **    ** * ** ***** **** ***


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NM_001111109.2      CTTTTTTGGAGCAGTTTGAGCCTACAGCATCACCTTCCACTGGCACCCAAAGATCTAGGA
NM_001111108.2      CTTTTTTGGAGCAGTTTGAGCCTACAGCATCACCTTCCACTGGCACCCAAAGATCTAGGA
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