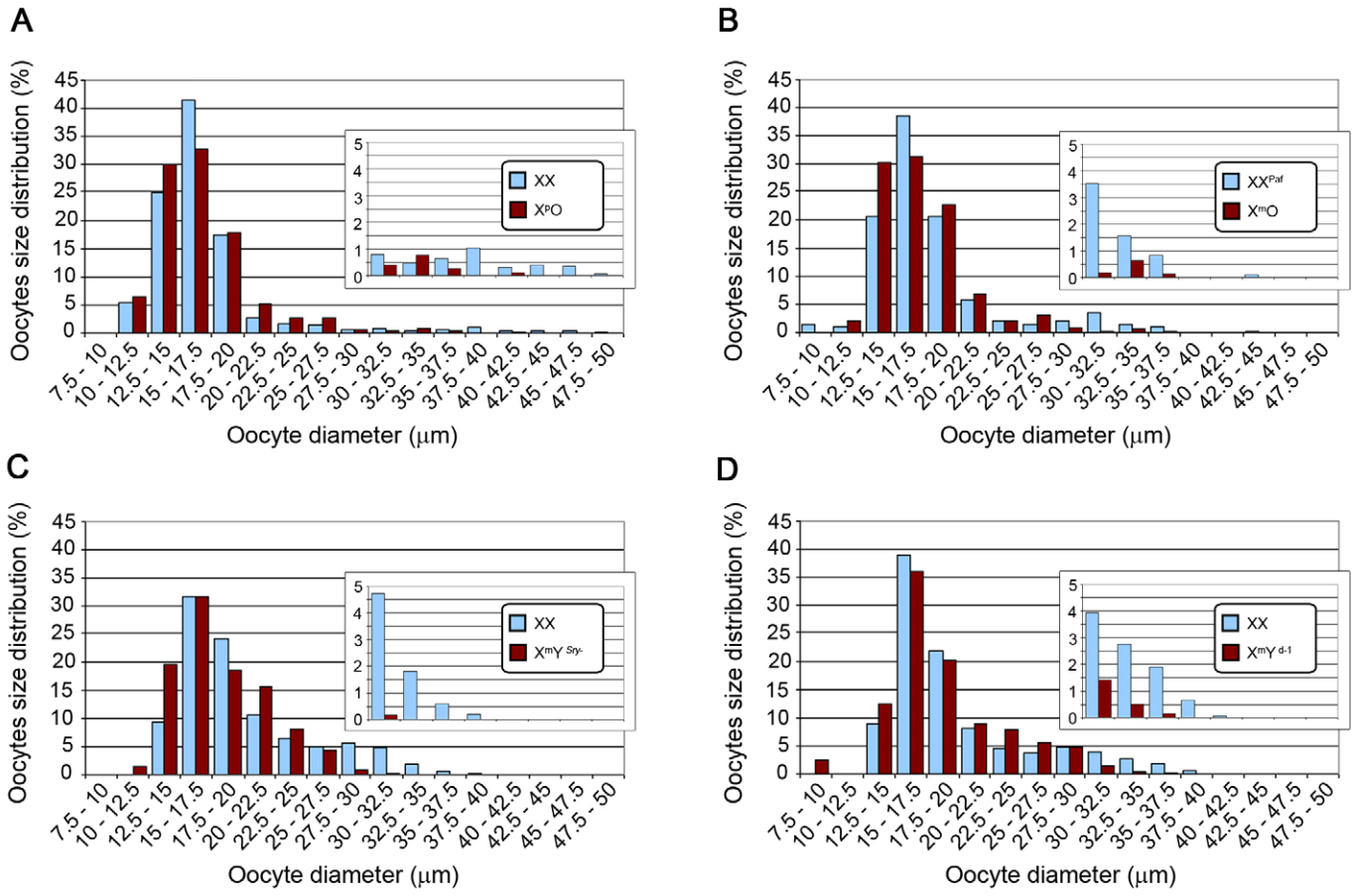

Fig. S1. Oocyte size distributions at 5 dpp comparing single X genotypes with XX from the respective crosses.

X^p, paternal X; X^m, maternal X; inset shows the distribution of 3050 ㎜ diameter oocytes with a magnified scale. Oocyte size data were collected during the 'distributed point count' procedure (see Materials and Methods). Throughout this procedure, whenever a 'hit' occurred on an oocyte that was judged to have been sectioned through the middle an outline of the oocyte was 'drawn' using a computer mouse and the number of pixels was obtained. Areas of oocytes were determined based on the number of pixels per square for a grid of known area 'drawn' at the same magnification. The cross-sectional areas were then used to generate a frequency distribution of oocyte diameters as previously described (Burgoyne and Baker, 1985).

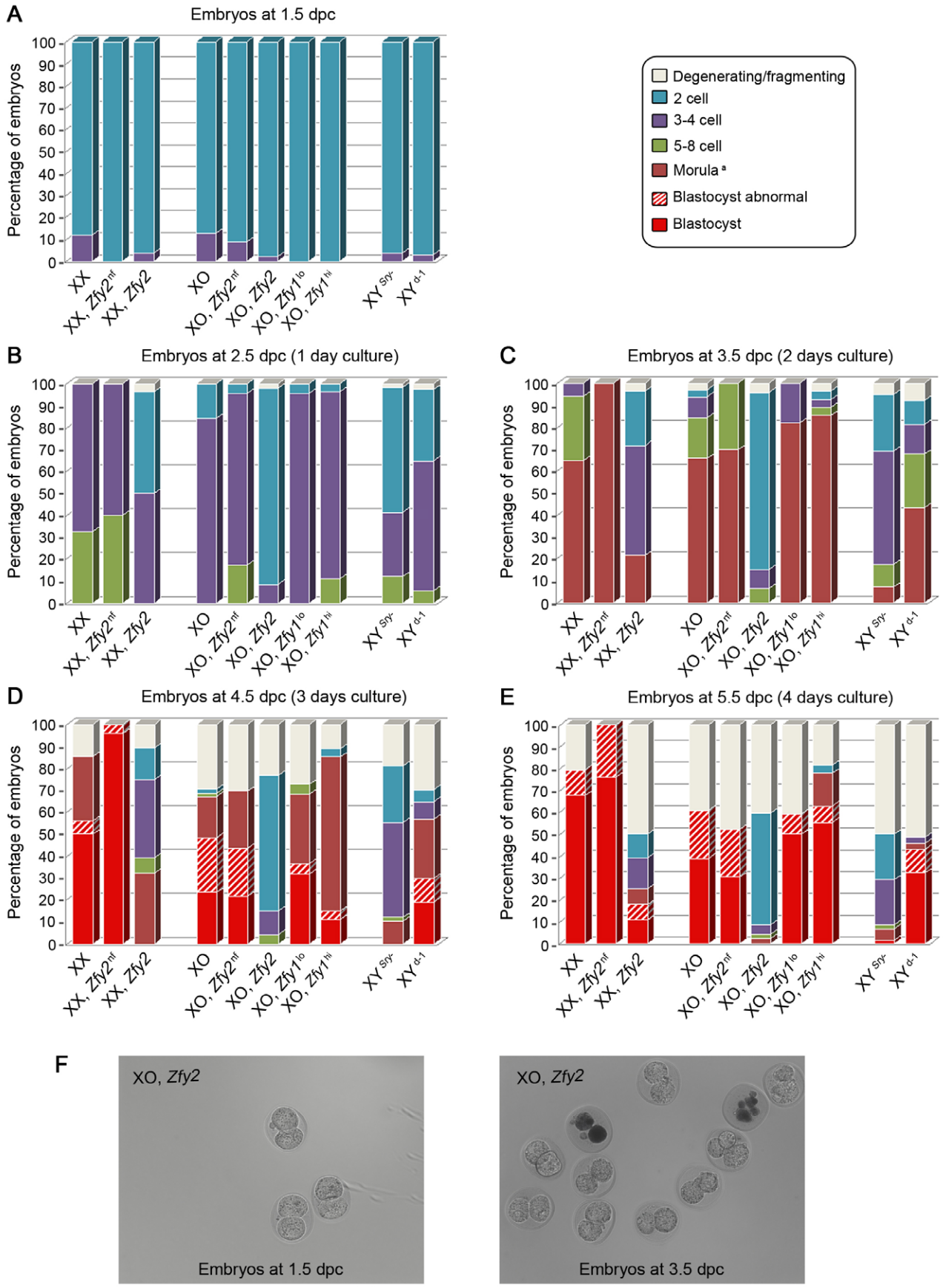

Fig. S2. **Embryo development in culture scored daily from 1.55.5 dpc.**

(**A-E**) Percentage of embryos by stage of development after 0, 1, 2, 3 and 4 days of culture. (**F**) Confirmation of visual viability assessments by dye exclusion tests. 2-cell embryos from XO,*Zfy2* at 1.5dpc exclude trypan blue, as do 2-cell embryos scored as viable after two days of culture (3.5dpc). However, embryos scored as dead or fragmenting are strongly stained with trypan blue.

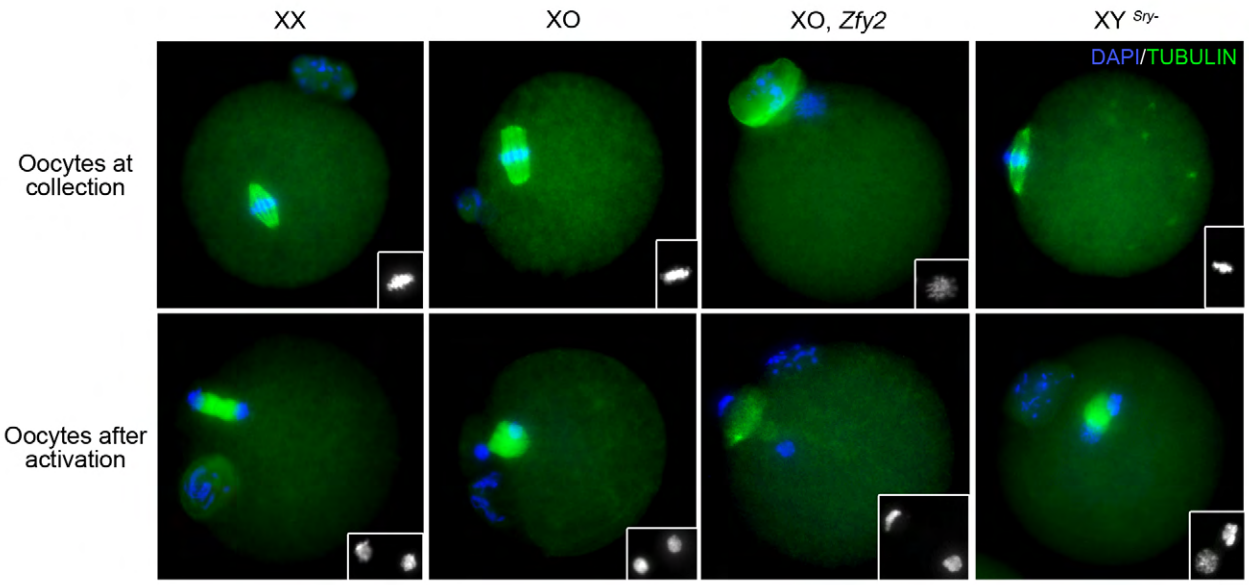

Fig. S3. Abnormal chromatin and spindle features at meiosis-2 in *Zfy2* carriers.

Chromatin is stained blue with DAPI (insets provide a black and white image), and tubulin (and thus the spindle) is stained green. (**A**) Examples of oocytes fixed and stained at collection. As expected, **XX** and **XO** have intact 1st polar bodies with characteristic multiple condensed small chromatin masses, and a well-organised MII spindle and metaphase plate in the oocyte. **XO,*Zfy2*** has a strongly tubulin positive 1st polar body suggesting it has attempted to divide, but has no organised spindle or metaphase plate (likely a consequence of polar body death). There is no clear spindle associated with the oocyte chromatin (all the XO,*Zfy2* oocytes were scored as having a faint or undetectable spindle - Table S2). **XY^{Sry-}** has no visible polar body but there is a well organised spindle and metaphase plate in the oocyte. Only 56% of XY^{Sry-} oocytes at collection had a clear well-organised spindle. (**B**) Examples of oocytes fixed and stained after activation. **XX, XO** and **XO,*Zfy2*** each have a 1st polar body and are at anapahase/telophase II, with the oocyte chromatin having separated into two masses with one mass moving into the forming 2nd polar body. 100% of XX oocytes, 50% of XO oocytes, and 76% of XO,*Zfy2* (and XY-$^{Sry-}$) oocytes reached this stage (Fig. 7F). **XY^{Sry-}** has a 1st polar body and the oocyte chromatin has separated into two masses. However, the lower of the two masses has precociously decondensed. Precocious decondensation is a prevalent feature in *Zfy2* carriers (Fig. 7F).

Table S1. Analysis of variance of oocyte data relevant to the single X genotype comparisons

A. Identification of a major maternal effect on oocyte pool size of their daughters

Analysis of Variance Table for oocyte pool

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level
A: Cross	2	3883733	1941866	0.42	0.671468
B(A): XX mother	9	4.196655E+07	4662950	4.95	0.006168*
S	12	1.131344E+07	942786.7		
Total (Adjusted)	23	5.716372E+07			
Total	24				

This ANOVA analysis shows that XX mothers from different crosses are equivalent but there are major differences between mothers irrespective of cross. Since each mother provided only one litter, the maternal effect may be a litter effect.

B. Identification of a reduced oocyte pool in XY^{Sry-} females at 5dpp

Analysis of Variance Table for oocyte pool

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level
A: Genotype	2	0.2276341	0.1138171	4.55	0.026177*
S	17	0.4254298	0.02502528		
Total (Adjusted)	19	0.653064			
Total	20				

Planned Comparison: XY^{Sry-} vs. XO (P=0.0078)

Group	Females	Mean	Comparison SE
XY^{Sry-}	8	0.4976	0.0902
XO	5	0.7695	

Planned Comparison: XY^{Sry-} vs. XY^{d-1} (P=0.1975, NS)

Group	Females	Mean	Comparison SE
XY^{Sry-}	8	0.4976	0.0819
XY^{d-1}	7	0.6074	

This ANOVA analysis was carried out using the oocyte counts of the single X genotypes expressed as a ratio to the mean value for their XX siblings, thus compensating for the maternal effect identified in **A**. The analysis shows a significant effect of genotype on the size of the oocyte pool at 5dpp, and the planned comparisons reveal that this is due to a significant reduction in the pool size of XY^{Sry-} females relative to XO females.

C. Single X genotype comparisons at 28dpp

Analysis of Variance Table for oocyte pool

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level
A: Genotype	2	0.05977543	0.02988772	0.98	0.391923 (NS)
S	21	0.6406197	0.0305057		
Total (Adjusted)	23	0.7003952			
Total	24				

Planned Comparison: XY^{Sry-} vs. XO (P=0.3979, NS)

Group	Females	Mean	Comparison SE
XY^{Sry-}	8	0.3877	0.0873
XO	8	0.4631	

Planned Comparison: XY^{Sry-} vs. XY^{d-1} (P=0.1803, NS)

Group	Females	Mean	Comparison SE
XY^{Sry-}	8	0.3877	0.0873
XY^{d-1}	8	0.5088	

Analysis of Variance Table for growing oocytes

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level
A: Genotype	2	0.02149437	0.01074719	0.24	0.785466 (NS)
S	21	0.9239169	0.04399604		
Total (Adjusted)	23	0.9454113			
Total	24				

Planned Comparison: XY^{Sry-} vs. XO (P=0.8014, NS)

Group	Females	Mean	Comparison SE
XY^{Sry-}	8	0.7247	0.1049
XO	8	0.6980	

Planned Comparison: XY^{Sry-} vs. XY^{d-1} (P=0.4971, NS)

Group	Females	Mean	Comparison SE
XY^{Sry-}	8	0.7247	0.1049
XY^{d-1}	8	0.6522	

There are no significant differences between the single X genotypes at 28dpp.

D. XY^{Sry-} vs. XY^{d-1} comparisons at 35-56dpp

Analysis of Variance Table for oocyte pool

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level
A: Genotype	1	0.03149262	0.03149262	3.29	0.099572
S	10	0.09559035	0.009559034		
Total (Adjusted)	11	0.127083			
Total	12				

Comparison: XY^{Sry-} vs. XY^{d-1} (P=0.0996, NS)

Group	Females	Mean	Comparison SE
XY^{Sry-}	6	0.1997218	0.0564
XY^{d-1}	6	0.3021793	

Analysis of Variance Table for growing oocytes

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level
A: Genotype	1	0.02241009	0.02241009	0.34	0.571028
S	10	0.6531672	0.06531672		
Total (Adjusted)	11	0.6755773			
Total	12				

Comparison: XY^{Sry-} vs. XY^{d-1} (P=0.5710, NS)

Group	Females	Mean	Comparison SE
XY^{Sry-}	6	0.5716591	0.1476
XY^{d-1}	6	0.6580885	

There are no significant differences between XY^{Sry-} and XY^{d-1} at 35-56dpp

Table S2. Zfy2-dependent abnormalities originating before the 2-cell stage.

Table S2A. Polar body counts from photographs such as those in Fig. 7A.

	Number of 2-cell embryos			Notes
Mouse	0 PB	1 PB	2-3 PB	
XO #1	0	4	7	The two blastomeres of the embryos were of normal shape and even in size.
XO #2	0	6	7	
XO # 3	0	5	5	
XO # 4	0	4	4	
Totals	0	19	23	
Percentages	**0%**	**45%**	**55%**	
XO, Zfy2 #1	8	6	1	Blastomeres and polar bodies often very aberrant in size and shape.
XO, *Zfy2* #2	8	3	3	
XO, *Zfy2* #3	4	4	1	
Totals	20	13	5	
Percentages	**53%**	**34%**	**13%**	
XY*Sry*- #1	2	5	1	Blastomeres often not of the same size and somewhat misshapen.
XY*Sry*- #2	11	2	1	
XY*Sry*- #3	4	4	0	
Totals	17	11	2	
Percentages	**57%**	**37%**	**6%**	

Table S2B. Polar body and blastomere nuclei data from DAPI-stained 1.5 dpc 2-cell embryos.

	Polar Body count			**Blastomere nuclei**		
Mouse	**0**	**1**	**2/3**	**Central**	**Eccentric**	**Multiple**
XO (1)	0	6	4	19	1	0
XO (2)	0	5	2	10	4	0
XO (3)	0	4	3	11	3	0
Totals	0	15	9	40	8	0
Percentages	**0.0%**	**62.5%**	**37.5%**	**83.3%**	**16.7%**	**0.0%**
XO,*Zfy2* (1)	9	3	1	15	10	1
XO,*Zfy2* (2)	9	3	1	9	15	1
Totals	18	6	2	24	25	2
Percentages	**69.2%**	**23.1%**	**7.7%**	**47.1%**	**49.0%**	**3.9%**
XY^{Sry-} (1)	3	6	0	9	7	2
XY^{Sry-} (2)	8	7	0	13	15	2
Totals	11	13	0	22	22	4
Percentages	**45.8%**	**54.2%**	**0.0%**	**45.8%**	**45.8%**	**8.3%**

Table S2C. Meiotic stage assessed when oocytes collected

Mouse	**Number of oocytes collected (females used)**	**Percentages of oocytes by stage***	
		MII	**Not MII / faint MII**
XX	17 (3)	100.0	0.0
XX,$Zfy2^{nf}$	14 (4)	71.4	28.6
XX,*Zfy2*	17 (4)	70.6	29.4
XO	11 (3)	54.5	45.5
XO,*Zfy2*	16 (4)	0.0	100.0
XY^{Sry-}	18 (4)	55.6	44.4

* The stage classification is based on the degree of spindle organisation: MII = complete, 'faint' MII is incomplete, and 'Not MII' is no spindle (also see Fig. S3).

Table S3. List of genes significantly regulated by *Zfy2* in GVoocytes.

- Tab1: 2655 genes found to be regulated by the functional *Zfy2* transgene in XX oocytes (batch 2) when compared to XX wild type.

- Tab2: 359 genes significantly regulated in XX,*Zfy2* and where the fold change was double that for any control genotype or batch effect.

- Tab3: Scatter plot comparison between Batch 1 XX,*Eif2s3y*,*Zfy2* and Batch 2 XX,*Zfy2* samples.

- Tab3: Clustering analysis using DAVID

- Tab4: Genes showing concordant regulation in both XX,*Zfy2* and XY^{dom} relative to XX control.

Download Table S3

Table S4. List of primers used for semi-quantitative and quantitative RT-PCR.

Gene	Primer ID	Primer sequence	Annealing Temp	Amplicon size	References
Primers for RT-PCR					
Zfy1	*Zfy1*-F	GCCAGTGCTCTCTTAAACCAA	60°c	386bp	(Vernet et al., 2011)
	Zfy1-R	TGAGTACACAAAGTCCCAGCA			
Uba1y	*Uba1y*-FP1	CGACAGCAACTTTCACATGG	60°c	226bp	(Royo et al., 2010)
	Uba1y-R	GAGCCAGAGGTGCAGAAAAG			
Smcy/Kdm5d	o96-*Smcy* 1F	GGATGAACTGCCTGCTATGCT	60°c	187bp	Current paper
	o97-*Smcy* 1R	CAATCGCTGAAGCAGCTCAC			
Eif2s3y	*Eif2s3y*-F1	CCAGGGACCAAAGGAAACTT	60°c	250bp	Current paper
	Eif2s3y-R1	TAGCCTGGCTTTCTTTCACC			
Uty	o50 *Uty*-F	ACCTGAAGATTATTGGGGTG	60°c	146bp	Current paper
	o51 *Uty*-R	CCAAATCACCAGGTCGTTGA			
Dby/Ddx3y	o46 *Ddx3y*-F	GTATGGCTTATGAACACCAC	60°c	111bp	Current paper
	o47 *Ddx3y*-R	CATTTGCAGAACCACTGCTC			
Usp9y	o42 *Usp9y* F	GGGAAGTTTCTGAACATGGG	60°c	145bp	Current paper
	o43 *Usp9y* R	GGTCCTTCATCCAAAGACAC			
Zfy2 Germ cell	*Zfy2*-F	GCCAGTGCTATGTTACACCAT	60°c	386bp	(Vernet et al., 2011)
	Zfy2-R	TCTGTATGCATTGTCCCAGCA			
Zfy2 Spermatid	o2696- *Zfy2* ex1a F	CTGTTGTGGTTCTCGTAGCAG	58°c	192bp	(Decarpentrie et al., 2012)
	o4018-Zfy2 ex5 R	CTATTCCATCAAATAACGAC			
H2al2y	*H2Al2y*-FP	CCGTGTAGATCGTTTCCTTG	60°c	206bp	Current paper
	H2Al2y-RP	AGAGTTGGTGGAGCTGTTCC			
Rbmy	*Rbmy*-F	GCGTCTTCCAGAAGAGATGAGT	60°c	113bp	(Ellis et al., 2005)
	Rbmy-R	GAGTGGTAATTGCCATAGTCACAG			
Dazl	*Dazl*-F	CCTCCAACCATGATGAATCC	60°c	234bp	(Vernet et al., 2011)
	Dazl-R	TGAACATTCATTGGGCAAAA			
β-actin	*β-actin*-F	GGCACCACACCTTCTACAATG	60°c	352bp	Current paper
	β-actin-R	GTGGTGGTGAAGCTGTAGCC			
Primers for qRT-PCR					
Zfy1 total	o4047-*Zfy1* ex8 F	AAGTCACAGATCAGAGCACT	58°c	278bp	(Decarpentrie et al., 2012)
	o4048-*Zfy1* ex10 R	CTCATCTTGGTTTAAGAGAGCA			
Zfy1 with exon6	o2836-*Zfy1* ex5 F	AAGAACTGAAGGCCATGGATG	60°c	132bp	(Decarpentrie et al., 2012)
	o2714-*Zfy1* ex6 R	TACTTCCACAACAATCTGGTCAC			
Zfy1 without exon6	o2832-*Zfy1* ex5/7 F	GATGGAATAGTGGATGAACC	60°c	205bp	(Decarpentrie et al., 2012)
	o2833-*Zfy1* ex8 R	CATTGCCATTTTTGGTCTCACTC			
Zfy2 total	o54-*Zfy2* ex7 F	GGATGAGCCTAGCAAAACAG	60°c	194bp	Current paper
	o55-*Zfy2* ex8 R	CATTGCCATTGTCGGTCTCAC			
Zfy2 total	o2985- *Zfy2* ex10 R	CTGGCAGTGGCATTCTGCAC	60°c	452bp	Current paper
	o2986- *Zfy2* ex7 F	GAGCCTAGCAAAACAGATCATG			

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