

Supplementary Information

Comparison of rat fetal sex determination using placental gDNA and mRNA via qRT-PCR

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Supplemental Table 1: Random assignment of treatment to each rat.

Rat Number	Batch Number	Treatment	Rat Number	Batch Number	Treatment
1	1	TCE	13	3	TCE + AOAA
2	1	NAC	14 ^a	3	AOAA
3	1	Control	15	3	NAC
4	1	AOAA	16	3	TCE
5	1	TCE + NAC	17	3	TCE + NAC
6	1	TCE + AOAA	18	3	Control
7	2	NAC	19	4	TCE + NAC
8	2	TCE + AOAA	20	4	AOAA
9	2	TCE	21	4	TCE + AOAA
10	2	TCE + NAC	22	4	Control
11	2	Control	23	4	NAC
12	2	AOAA	24	4	TCE

^aRat 14 was not pregnant.

Supplemental Table 2: Primer sequences used in the current study.

Sry (works for both genomic DNA and cDNA input)	
Forward	5'-AAGCGCCCCATGAATGCAT-3'
Reverse	5'-CGATGAGGCTGATATTTATA-3'
Source: [18] (Schulte et al. 2012)	
B2m (for cDNA input) – spans exon-exon junction	
Forward	5'-TCGTGCTTGCCATTCAGAAAAC-3'
Reverse	5'-GCAGTTGAGGAAGTTGGGCT-3'
Source: NCBI nucleotide database	
B2m (for genomic DNA input) – does not span exon-exon junction	
Forward	5'-CCCACCCTCATGGCTACTTC-3'
Reverse	5'-TGGGTGATGAAAACCGCACA-3'
Source: NCBI nucleotide database	

Supplemental Table 3: Raw data of *Sry/B2m* values for gDNA and mRNA analyses of rat placenta.^a

Sample	gDNA <i>Sry</i> (<i>Sry/B2m</i>) Values (Mean \pm SEM)	gDNA Sex Classification	mRNA <i>Sry</i> (<i>Sry/B2m</i>) Values (Mean \pm SEM)	mRNA Sex Classification
Batch 1				
Rat 1, P1	1.8553 \pm 0.36465	Male	1.75816 \pm 0.15779	Male
Rat 1, P2	1.35091 \pm 0.07932	Male	1.25365 \pm 0.08332	Male
Rat 1, P3	0.0076 \pm 0.00101	Female	N/A ^b	Female
Rat 1, P4	0.00057 \pm 0.00025	Female	N/A	Female
Rat 2, P1	0.00076 \pm 0.00005	Female	N/A	Female
Rat 2, P2	0.00048 \pm 0.00011	Female	N/A	Female
Rat 2, P3	1.26595 \pm 0.11618	Male	1.4044 \pm 0.02926	Male
Rat 2, P4	0.28803 \pm 0.01949	Male	0.6447 \pm 0.05727	Male
Rat 3, P1	0.00014 \pm 0.00007	Female	N/A	Female
Rat 3, P2	0.00017 \pm 0.00002	Female	N/A	Female
Rat 3, P3	0.00003 \pm 0.00001	Female	N/A	Female
Rat 3, P4	1.09624 \pm 0.01201	Male	1.34494 \pm 0.37041	Male
Rat 4, P1	0.00239 \pm 0.0003	Female	N/A	Female

Rat 4, P2	0.98607 ± 0.07636	Male	2.40693 ± 0.1271	Male
Rat 5, P1	0.00046 ± 0.00005	Female	N/A	Female
Rat 5, P2	1.43599 ± 0.236	Male	2.77016 ± 0.50183	Male
Rat 5, P3	1.5735 ± 0.09954	Male	1.88023 ± 0.83241	Male
Rat 5, P4	0.0064 ± 0.00054	Female	N/A	Female
Rat 6, P1	0.00105 ± 0.00029	Female	N/A	Female
Rat 6, P2	0.00055 ± 0.00006	Female	N/A	Female
Rat 6, P3	0.81686 ± 0.22515	Male	0.69736 ± 0.17313	Male
Rat 6, P4	0.78434 ± 0.176	Male	0.60509 ± 0.15653	Male
Batch 2				
Rat 7, P1	0.45842 ± 0.06073	Male	2.66218 ± 0.41164	Male
Rat 7, P2	0.7068 ± 0.05002	Male	0.37329 ± 0.06506	Male
Rat 7, P3	1.25384 ± 0.35354	Male	0.15523 ± 0.01555	Male
Rat 7, P4	0.00115 ± 0.00013	Female	N/A	Female
Rat 8, P1	1.86859 ± 0.84474	Male	0.0868 ± 0.03842	Male
Rat 8, P2	0.00014 ± 0.00011	Female	N/A	Female
Rat 9, P1	0.00612 ± 0.00047	Female	N/A	Female

Rat 9, P2	0.00132 ± 0.00036	Female	N/A	Female
Rat 9, P3	1.68139 ± 0.2327	Male	0.07279 ± 0.04347	Male
Rat 9, P4	2.60087 ± 0.55096	Male	0.29862 ± 0.01583	Male
Rat 10, P1	1.23832 ± 0.47824	Male	0.53257 ± 0.17415	Male
Rat 10, P2	0.00015 ± 0.00004	Female	N/A	Female
Rat 10, P3	0.00014 ± 0.00017	Female	N/A	Female
Rat 10, P4	1.24547 ± 0.19657	Male	2.44634 ± 0.18713	Male
Rat 11, P1	1.45054 ± 0.19347	Male	1.28074 ± 0.09505	Male
Rat 11, P2	0.00022 ± 0.00006	Female	N/A	Female
Rat 11, P3	1.29619 ± 0.16158	Male	4.4707 ± 0.64153	Male
Rat 11, P4	0.00193 ± 0.00051	Female	N/A	Female
Rat 12, P1	1.12085 ± 0.22752	Male	0.13099 ± 0.01466	Male
Rat 12, P2	0.00094 ± 0.00027	Female	N/A	Female
Rat 12, P3	1.16822 ± 0.24648	Male	0.14971 ± 0.07	Male
Rat 12, P4	0.00038 ± 0.00007	Female	N/A	Female
Batch 3				
Rat 13, P1	0.74347 ± 0.17855	Male	0.4197 ± 0.01839	Male

Rat 13, P2	0.00074 ± 0.00009	Female	N/A	Female
Rat 13, P3	1.23645 ± 0.07571	Male	0.71871 ± 0.0339	Male
Rat 13, P4	1.17863 ± 0.45061	Male	0.31068 ± 0.00479	Male
Rat 15, P1	0.00059 ± 0.00014	Female	N/A	Female
Rat 15, P2	0.80526 ± 0.01546	Male	0.51277 ± 0.0129	Male
Rat 15, P3	0.01917 ± 0.00092	Female	N/A	Female
Rat 15, P4	1.53205 ± 0.39272	Male	1.44913 ± 0.00404	Male
Rat 16, P1	1.501 ± 0.22721	Male	0.38445 ± 0.21339	Male
Rat 16, P2	1.87178 ± 0.22419	Male	1 ± 0.20161	Male
Rat 16, P3	1.42063 ± 0.28622	Male	2.31211 ± 0.14533	Male
Rat 16, P4	0.00258 ± 0.00038	Female	N/A	Female
Rat 17, P1	0.66446 ± 0.40248	Male	2.4979 ± 0.26512	Male
Rat 17, P2	0.00063 ± 0.00032	Female	N/A	Female
Rat 17, P3 (excluded) ^c	0.95096 ± 0.04092	Male	N/A	Female
Rat 17, P4	1.48153 ± 0.14384	Male	0.36141 ± 0.09401	Male
Rat 17, P5	0.00181 ± 0.00058	Female	N/A	Female
Rat 18, P1	0.00006 ± 0.00007	Female	N/A	Female

Rat 18, P2	0.00108 ± 0.00035	Female	N/A	Female
Rat 18, P3	0.00068 ± 0.00021	Female	N/A	Female
Rat 18, P4	0.00016 ± 0.00002	Female	N/A	Female
Rat 18, P5	1.0585 ± 0.13765	Male	1.7926 ± 0.03128	Male
Rat 18, P6	0.00035 ± 0.00013	Female	N/A	Female
Batch 4				
Rat 19, P1	N/A	Female	N/A	Female
Rat 19, P2	1.52719 ± 0.52702	Male	4.84862 ± 4.29553	Male
Rat 19, P3	N/A	Female	N/A	Female
Rat 19, P4	0.10017 ± 0.05816	Male	20.19149 ± 3.61191	Male
Rat 20, P1	0.1697 ± 0.05717	Male	1.72119 ± 0.15518	Male
Rat 20, P2	N/A	Female	N/A	Female
Rat 20, P3	1.49923 ± 0.93382	Male	2.20147 ± 0.5672	Male
Rat 20, P4	6.36962 ± 4.45239	Male	1.30547 ± 0.0608	Male
Rat 21, P1	1.0000 ± 0.08748	Male	0.27991 ± 0.22172	Male
Rat 21, P2	N/A	Female	N/A	Female
Rat 21, P3	0.00128 ± 0.00038	Female	N/A	Female

Rat 21, P4	0.00016 ± 0.00011	Female	N/A	Female
Rat 22, P1	0.00347 ± 0.00077	Female	N/A	Female
Rat 22, P2	2.0135 ± 0.28085	Male	0.53195 ± 0.14442	Male
Rat 22, P3	1.60734 ± 0.09553	Male	1.77879 ± 1.01151	Male
Rat 22, P4	0.00042 ± 0.00014	Female	N/A	Female
Rat 23, P1	1.86871 ± 0.95455	Male	0.96302 ± 0.05467	Male
Rat 23, P2	1.27642 ± 0.22452	Male	0.43457 ± 0.02941	Male
Rat 23, P3	0.00036 ± 0.00012	Female	N/A	Female
Rat 23, P4	1.39197 ± 0.03806	Male	1.13474 ± 0.20542	Male
Rat 24, P1	1.40109 ± 0.21905	Male	3.36553 ± 0.21195	Male
Rat 24, P2	1.37048 ± 0.14204	Male	0.99113 ± 0.07919	Male
Rat 24, P3	0.00111 ± 0.00012	Female	N/A	Female
Rat 24, P4	0.00419 ± 0.00094	Female	N/A	Female

^a*Sry/B2m* values used to arrive at sex classification and for analyses in this manuscript. P refers to placenta.

^bN/A refers to a zero *Sry/B2m* value derived from a corresponding *Sry* Cq value of ≥ 40 .

^cRat17, P3 had a discordant gDNA and mRNA classification and was excluded from statistical analysis requiring definitive sex classification.

Supplemental Table 4: Raw data of *Sry* and *B2m* Cq values.^a

Sample	gDNA <i>Sry</i> Cq (Mean ± SEM)	gDNA <i>B2m</i> Cq (Mean ± SEM)	gDNA Sex Classification	mRNA <i>Sry</i> Cq (Mean ± SEM)	mRNA <i>B2m</i> Cq (Mean ± SEM)	mRNA Sex Classification
Batch 1						
Rat 1, P1	21.92 ± 0.01887	23.6 ± 0.28293	Male	33.78 ± 0.12649	19.51 ± 0.02767	Male
Rat 1, P2	23.06 ± 0.00135	24.27 ± 0.0847	Male	34.23 ± 0.0954	19.47 ± 0.00957	Male
Rat 1, P3	30.27 ± 0.18842	24.02 ± 0.03329	Female	N/A ^b	19.74 ± 0.0257	Female
Rat 1, P4	32.98 ± 0.63834	22.99 ± 0.04696	Female	N/A	19.56 ± 0.00892	Female
Rat 2, P1	33.43 ± 0.08035	23.97 ± 0.04997	Female	N/A	19.48 ± 0.01798	Female
Rat 2, P2	34.83 ± 0.31499	24.72 ± 0.10211	Female	N/A	20.38 ± 0.06633	Female
Rat 2, P3	22.57 ± 0.0661	23.81 ± 0.11472	Male	33.66 ± 0.01071	19.06 ± 0.02809	Male

Rat 2, P4	25.3 ± 0.08379	24.4 ± 0.05014	Male	34.24 ± 0.12135	18.52 ± 0.04124	Male
Rat 3, P1	35.74 ± 0.74351	23.77 ± 0.05175	Female	N/A	20.31 ± 0.0569	Female
Rat 3, P2	36.08 ± 0.04991	24.32 ± 0.18513	Female	N/A	20.76 ± 0.03181	Female
Rat 3, P3	37.07 ± 0.63976	22.71 ± 0.14194	Female	N/A	20.49 ± 0.0173	Female
Rat 3, P4	23.27 ± 0.00931	24.3 ± 0.01277	Male	34.92 ± 0.39561	20.27 ± 0.0369	Male
Rat 4, P1	31.22 ± 0.01509	23.42 ± 0.18041	Female	N/A	20.15 ± 0.02269	Female
Rat 4, P2	24.03 ± 0.11036	24.91 ± 0.01738	Male	33.99 ± 0.01114	20.17 ± 0.07536	Male
Rat 5, P1	35.19 ± 0.1325	25 ± 0.08685	Female	N/A	21.37 ± 0.08777	Female
Rat 5, P2	22.77 ± 0.17165	24.19 ± 0.16356	Male	33.77 ± 0.12438	20.88 ± 0.22986	Male
Rat 5, P3	22.68 ± 0.06007	24.24 ± 0.06871	Male	33.75 ± 0.63584	20.3 ± 0.06037	Male

Rat 5, P4	31.01 ± 0.02326	24.62 ± 0.11878	Female	N/A	21.43 ± 0.05797	Female
Rat 6, P1	34.68 ± 0.39402	27.16 ± 0.01983	Female	N/A	19.55 ± 0.02019	Female
Rat 6, P2	33.59 ± 0.15446	25.13 ± 0.01664	Female	N/A	19.74 ± 0.00735	Female
Rat 6, P3	23.14 ± 0.3901	25.23 ± 0.07706	Male	34.71 ± 0.00982	19.83 ± 0.35804	Male
Rat 6, P4	23.9 ± 0.30404	25.93 ± 0.1112	Male	34.47 ± 0.37298	19.39 ± 0.01314	Male
Batch 2						
Rat 7, P1	24.22 ± 0.18849	25.48 ± 0.03159	Male	34.32 ± 0.21414	21.38 ± 0.06251	Male
Rat 7, P2	23.02 ± 0.07548	24.9 ± 0.06875	Male	35.84 ± 0.24862	20.06 ± 0.03774	Male
Rat 7, P3	24.47 ± 0.40676	25.04 ± 0.0051	Male	36.45 ± 0.14011	19.41 ± 0.03539	Male
Rat 7, P4	34.88 ± 0.08945	25.36 ± 0.1354	Female	N/A	19.75 ± 0.02065	Female

Rat 8, P1	24.46 ± 0.65194	26.48 ± 0.01864	Male	36.51 ± 0.63456	20 ± 0.07163	Male
Rat 8, P2	37.82 ± 1.05704	26.15 ± 0.41231	Female	N/A	19.89 ± 0.0161	Female
Rat 9, P1	31.36 ± 0.07365	24.79 ± 0.08381	Female	N/A	19.4 ± 0.00419	Female
Rat 9, P2	33.38 ± 0.31878	24.6 ± 0.22625	Female	N/A	20.55 ± 0.0485	Female
Rat 9, P3	22.76 ± 0.06811	24.29 ± 0.18769	Male	35.99 ± 0.86155	19.22 ± 0.00655	Male
Rat 9, P4	22.14 ± 0.07757	24.3 ± 0.29561	Male	35.01 ± 0.0168	20.28 ± 0.0746	Male
Rat 10, P1	25.17 ± 0.1835	26.6 ± 0.52608	Male	32.59 ± 0.47139	18.7 ± 0.01882	Male
Rat 10, P2	37.83 ± 0.2869	26.28 ± 0.21514	Female	N/A	18.29 ± 0.04336	Female
Rat 10, P3	37.26 ± 1.7428	25.58 ± 0.47701	Female	N/A	18.47 ± 0.077	Female
Rat 10, P4	24.68 ± 0.21145	25.24 ± 0.08446	Male	31.28 ± 0.03651	19.58 ± 0.10414	Male

Rat 11, P1	24.26 ± 0.13405	25.58 ± 0.13805	Male	31.7 ± 0.06396	19.87 ± 0.08586	Male
Rat 11, P2	35.36 ± 0.38169	23.97 ± 0.13075	Female	N/A	18.59 ± 0.06195	Female
Rat 11, P3	22.6 ± 0.17978	23.76 ± 0.0049	Male	30.32 ± 0.20671	20.29 ± 0.01133	Male
Rat 11, P4	31.95 ± 0.35994	23.72 ± 0.12762	Female	N/A	18.13 ± 0.0769	Female
Rat 12, P1	25.65 ± 0.24345	26.06 ± 0.16277	Male	34.5 ± 0.15951	19.19 ± 0.02465	Male
Rat 12, P2	35.9 ± 0.3981	26.96 ± 0.10345	Female	N/A	19.17 ± 0.04136	Female
Rat 12, P3	24.71 ± 0.26939	26.05 ± 0.14172	Male	34.94 ± 0.67423	20.01 ± 0.02102	Male
Rat 12, P4	36.98 ± 0.15652	26.75 ± 0.21302	Female	N/A	19.35 ± 0.02473	Female
Batch 3						
Rat 13, P1	26.62 ± 0.34621	26.44 ± 0.01362	Male	32.24 ± 0.06091	18.61 ± 0.01693	Male

Rat 13, P2	34.87 ± 0.15871	24.72 ± 0.07211	Female	N/A	18.84 ± 0.06757	Female
Rat 13, P3	25.1 ± 0.08653	25.65 ± 0.0178	Male	32.18 ± 0.01025	19.33 ± 0.06726	Male
Rat 13, P4	25.53 ± 0.52475	26.01 ± 0.1699	Male	32.66 ± 0.02222	18.6 ± 0.0005	Male
Rat 15, P1	35.76 ± 0.32357	25.26 ± 0.08427	Female	N/A	20.42 ± 0.02258	Female
Rat 15, P2	26.55 ± 0.02747	26.48 ± 0.00362	Male	32.36 ± 0.01716	19.03 ± 0.03197	Male
Rat 15, P3	30.42 ± 0.01688	25.2 ± 0.06745	Female	N/A	19.69 ± 0.04793	Female
Rat 15, P4	24.39 ± 0.36955	25.49 ± 0.01395	Male	30.69 ± 0.00152	18.86 ± 0.00372	Male
Rat 16, P1	22.07 ± 0.2031	23.44 ± 0.08025	Male	34.65 ± 0.80046	18.68 ± 0.02191	Male
Rat 16, P2	22.53 ± 0.16597	24.21 ± 0.04808	Male	32.46 ± 0.28472	17.87 ± 0.05945	Male
Rat 16, P3	22.41 ± 0.03137	23.7 ± 0.28897	Male	32.59 ± 0.05556	19.2 ± 0.07167	Male

Rat 16, P4	31.71 ± 0.18042	23.9 ± 0.11089	Female	N/A	18.97 ± 0.14989	Female
Rat 17, P1	26.22 ± 0.87349	26.11 ± 0.02569	Male	32.64 ± 0.05629	19.37 ± 0.1424	Male
Rat 17, P2	36.31 ± 0.71925	25.92 ± 0.09101	Female	N/A	18.84 ± 0.07659	Female
Rat 17, P3 (excluded) ^c	24.46 ± 0.06055	24.87 ± 0.01366	Male	N/A	19.97 ± 0.04591	Female
Rat 17, P4	24.44 ± 0.1342	25.49 ± 0.04011	Male	35.03 ± 0.32222	18.97 ± 0.19236	Male
Rat 17, P5	34.3 ± 0.45648	26.19 ± 0.07166	Female	N/A	19.02 ± 0.14877	Female
Rat 18, P1	36.92 ± 1.69648	23.59 ± 0.05661	Female	N/A	19.49 ± 0.01468	Female
Rat 18, P2	33.77 ± 0.27205	24.69 ± 0.3864	Female	N/A	20.98 ± 0.04915	Female
Rat 18, P3	33.37 ± 0.37724	23.63 ± 0.22007	Female	N/A	20.05 ± 0.02208	Female
Rat 18, P4	35.72 ± 0	23.98 ± 0.1541	Female	N/A	20.47 ± 0.0872	Female

Rat 18, P5	23.14 ± 0.10239	24.13 ± 0.15721	Male	35.28 ± 0.02243	20.58 ± 0.01143	Male
Rat 18, P6	36.7 ± 0.53377	26.2 ± 0.06348	Female	N/A	23.37 ± 0.30469	Female
Batch 4						
Rat 19, P1	N/A	28.34 ± 0.85129	Female	N/A	22.92 ± 0.02949	Female
Rat 19, P2	27.18 ± 0.04694	28.81 ± 0.49564	Male	36.42 ± 1.27771	23.15 ± 0.0325	Male
Rat 19, P3	N/A	26.95 ± 0.18782	Female	N/A	23.92 ± 0.01275	Female
Rat 19, P4	31.08 ± 0.8005	28.73 ± 0.24659	Male	34.63 ± 0.25719	23.42 ± 0.02136	Male
Rat 20, P1	30.52 ± 0.25971	28.93 ± 0.41086	Male	32.21 ± 0.04317	19.31 ± 0.12269	Male
Rat 20, P2	N/A	28.11 ± 0.11903	Female	N/A	18.8 ± 0.03566	Female
Rat 20, P3	30.22 ± 0.82431	27.54 ± 0.35775	Male	32.19 ± 0.37153	19.66 ± 0.01158	Male

Rat 20, P4	26.75 ± 0.48793	30.44 ± 0.88255	Male	32.33 ± 0.06622	19.04 ± 0.0114	Male
Rat 21, P1	26.46 ± 0.05184	27.47 ± 0.11506	Male	37.17 ± 1.13892	20.24 ± 0.09365	Male
Rat 21, P2	N/A	26.45 ± 0.05097	Female	N/A	20.35 ± 0.00788	Female
Rat 21, P3	35.53 ± 0.0031	26.89 ± 0.43114	Female	N/A	24.19 ± 0.09587	Female
Rat 21, P4	38.05 ± 0.98704	26.39 ± 0.06206	Female	N/A	20.75 ± 0.02139	Female
Rat 22, P1	31.38 ± 0.31462	24.11 ± 0.05129	Female	N/A	20.49 ± 0.20358	Female
Rat 22, P2	23.27 ± 0.17419	25.18 ± 0.10076	Male	36.82 ± 0.39166	22.23 ± 0.00418	Male
Rat 22, P3	22.51 ± 0.06806	24.1 ± 0.05216	Male	35.24 ± 0.81905	22.39 ± 0.04687	Male
Rat 22, P4	34.63 ± 0.48753	24.31 ± 0.00557	Female	N/A	21.23 ± 0.19734	Female
Rat 23, P1	25.53 ± 0.01099	27.4 ± 0.73686	Male	33.08 ± 0.02998	18.26 ± 0.07621	Male

Rat 23, P2	26.26 ± 0.12801	27.58 ± 0.21912	Male	35.49 ± 0.07076	19.53 ± 0.06726	Male
Rat 23, P3	38 ± 0.39133	27.51 ± 0.26325	Female	N/A	18.65 ± 0.1243	Female
Rat 23, P4	24.65 ± 0.02447	26.09 ± 0.03094	Male	33.52 ± 0.25137	18.95 ± 0.07088	Male
Rat 24, P1	22.56 ± 0.18862	23.95 ± 0.12368	Male	33.02 ± 0.04866	20.01 ± 0.07673	Male
Rat 24, P2	23.18 ± 0.14793	24.54 ± 0.02181	Male	34.25 ± 0.1149	19.48 ± 0.00915	Male
Rat 24, P3	32.87 ± 0.14018	23.95 ± 0.07308	Female	N/A	19.37 ± 0.06038	Female
Rat 24, P4	31.55 ± 0.32449	24.55 ± 0.01048	Female	N/A	19.31 ± 0.01025	Female

^a*Sry* and *B2m* Cq raw values used in analyses of the current study. P refers to placenta.

^bN/A refers to any Cq value ≥ 40 .

^cRat 17, P3 had a discordant gDNA and mRNA classification and was subsequently excluded from statistical analysis requiring definitive sex classification.