

Summary of treated dams and offspring used in each experiment*

Control Dam ID	Neonatal		Adolescent		Adult (6 months)		
	PN3 (Freeze/Fix)	PN10 (Freeze/Fix)	Vaginal Opening [^]	First Estrous [^]	Estrous cycle tracking	Implantations + Progesterone	Estradiol
S2255	-	-	6F	6F	1F	1F	2F
S2256	-	-	6F	6F	1F	1F	2F
S2257	-	-	7F	7F	1F	1F	2F
S2264	-	-	6F	6F	1F	1F	2F
S2269	-	-	6F	6F	1F	1F	2F
S2273	-	-	7F	7F	1F	1F	2F
S2274	-	-	7F	7F	1F	1F	2F
S2276	-	-	6F	6F	1F	1F	2F
S2280	2F/2F	2F/1F	-	-	-	-	-
S2278	2F/1F	1F/1F	-	-	-	-	-
S2281	2F/2F	2F/2F	-	-	-	-	-
S2453	2F/2F	1F/2F	-	-	-	-	-
S2546	2F/1F	2F/1F	-	-	-	-	-
S2279	0F	2F/1F	-	-	-	-	-

No offspring at this time point due to aging error

EtOH Dam ID	Neonatal		Adolescent		Adult (6 months)		
	PN3 (Freeze/Fix)	PN10 (Freeze/Fix)	Vaginal Opening [^]	First Estrous [^]	Estrous cycle tracking	Implantations + Progesterone	Estradiol
E2258	-	-	7F	6F	1F	1F	2F
E2259	-	-	7F	7F	1F [#]	1F	2F
E2263	-	-	5F	5F	1F	1F	2F
E2265	-	-	6F	6F	1F	1F	2F
E2268	-	-	7F	7F	1F	1F	2F
E2270	-	-	7F	7F	1F	1F	2F
E2271	NB: Offspring from E2271 were not used as litter size was too small (n=7). Excluded from experiment.						
E2272	-	-	7F	7F	1F	1F	2F
E2275	-	-	7F	6F	1F	1F	2F
E2277	-	-	7F	7F	1F	1F	2F
E2266	1F/2F	2F/2F	-	-	-	-	-
E2261	2F/2F	2F/1F	-	-	-	-	-
E2544	1F/2F	2F/2F	-	-	-	-	-
E2545	2F/1F	2F/1F	-	-	-	-	-
E2282	2F/2F	2F/1F	-	-	-	-	-
E2547	2F/1F	2F/1F	-	-	-	-	-

[#]Not used in analysis as estrous was tracked for less than 21 days

Offspring used for adult experiments

Maternal data for litters providing adolescent and adult offspring are reported in Nguyen et al 2019 J Physiol 597(23):5619-5637.

Additional male and female offspring from these litters were used in a separate study examining metabolic outcomes (Nguyen et al 2019 J Physiol 597(23):5619-5637.)

Offspring used for neonatal experiments

[^] Average per litter was used for analysis

F = Female; PN = postnatal age; S = saline; E = EtOH (ethanol)

**Figure 2A: Oocytes in nests
(per PN3 ovary)**

<i>Control</i>	<i>EtOH</i>
3391	3670
1795	1955
5943	4188
479	0
638	160
399	4188
359	439
0	957
	3430

Figure 2B: Primordial follicles (per ovary)

<i>PN3</i>		<i>PN10</i>	
<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>
4348	4587	7818	7300
2513	5584	8536	4388
10291	7659	7380	4547
8098	5704	4308	5624
8576	8776	6582	6901
5505	8975	6941	5146
10092	6661	5664	7898
10730	5225	7100	4747
	5186		

Figure 2C: Growing follicles (per PN3 ovary)

<i>Transitional</i>		<i>Primary</i>	
<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>
40	239	40	40
319	239	80	0
239	160	80	120
359	239	0	80
638	359	0	0
399	359	40	0
160	718	80	279
199	479	0	80
	519		80

Figure 2D: Growing follicles (per PN10 ovary)

<i>Transitional</i>		<i>Primary</i>		<i>Secondary</i>		<i>Antral</i>	
<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>
917	1157	1476	957	792	684	180	216
957	558	838	917	828	792	72	360
1596	798	1237	678	900	1116	144	36
558	638	1356	957	720	1044	0	0
1556	917	1356	1197	648	756	180	288
638	1556	678	1316	1008	504	108	144
678	798	1635	2314	864	1008	36	0
798	439	917	439	828	468	0	144

Figure 3A: Age at vaginal opening (days)

Control	EtOH
38.2	41.0
38.9	37.6
36.4	37.2
37.8	37.0
39.2	34.3
37.3	38.7
35.7	37.3
38.8	39.9
	34.9

Figure 3B: Cumulative % open

Age (days)	Control			EtOH		
	Open	Not open	% Open	Open	Not open	%
30-32	2	49	3.9	2	58	3.3
33-35	10	41	19.6	19	41	31.7
36-38	33	18	64.7	38	22	63.3
39-41	46	5	90.2	56	4	93.3
42-44	50	1	98.0	59	1	98.3
45-47	51	0	100.0	60	0	100.0

Figure 3C: Age at first estrous (days)

Control	EtOH
43.5	46.7
44.9	47.0
46.0	45.2
48.6	43.2
49.8	40.0
43.6	47.0
41.1	42.4
43.2	47.0
	42.9

Figure 3D: Cumulative % with estrous cycles

Age (days)	Control			EtOH		
	Cycling	Not cycling	% Cycling	Cycling	Not cycling	% Cycling
36-40	5	46	9.8	13	46	22.0
41-45	35	16	68.6	40	19	67.8
46-50	46	5	90.2	51	8	86.4
51-55	48	3	94.1	57	2	96.6
56-61	51	0	100.0	59	0	100.0

Raw data from each litter

Animal ID	vag open	1st estrous	Animal ID	vag open	1st estrous		
S2255.10	35	43	E2258.10	39	41		
S2255.12	39	43	E2258.12	41	56		
S2255.2	41	44	E2258.14	46	49		
S2255.4	39	43	E2258.16	43	43		
S2255.6	35	44	E2258.18	38	41		
S2255.8	40	44	E2258.20	40	55		
		38.16667	43.5	E2258.8	40	42	
S2256.10	38	42				41	46.71429
S2256.14	40	46	E2259.10	38	55		
S2256.18	40	44	E2259.12	39	46		
S2256.2	38	43	E2259.14	37	45		
S2256.4	34	53	E2259.16	38	41		
S2256.6	43	43	E2259.18	38	42		
S2256.8	39	43	E2259.20	35	51		
		38.85714	44.85714	E2259.8	38	49	
S2257.10	37	48				37.57143	47
S2257.12	34	46	E2263.10	39	49		
S2257.14	38	48	E2263.2	37	45		
S2257.2	36	53	E2263.4	39	44		
S2257.4	37	42	E2263.6	37	45		
S2257.6	37	43	E2263.8	34	43		
S2257.8	36	42				37.2	45.2
S2264.10	34	59	E2265.10	34	44		
S2264.2	38	44	E2265.12	38	43		
S2264.4	43	48	E2265.2	40	42		
S2264.6	37	45	E2265.4	35	39		
S2264.8	37	47	E2265.6	40	51		
			E2265.8	35	40		
S2269.10	45	59				37	43.16667
S2269.12	38	49	E2268.10	36	39		
S2269.14	43	49	E2268.12	32	39		
S2269.4	36	49	E2268.14	35	42		
S2269.6	34	48	E2268.2	32	38		
S2269.8	39	45	E2268.4	35	42		
		39.16667	49.83333	E2268.6	36	41	
S2273.10	39	42	E2268.8	34	39		
S2273.12	38	42				34.28571	40
S2273.14	37	42	E2270.12	39	48		
S2273.2	34	38	E2270.14	42	47		
S2273.4	36	42	E2270.16	35	43		
S2273.6	39	42	E2270.18	37	47		
S2273.8	38	57	E2270.20	38	48		
		37.28571	43.57143	E2270.22	40	45	
S2274.10	36	45	E2270.8	40	51		
S2274.12	38	45				38.71429	47
S2274.14	32	40	E2272.10	35	40		
S2274.2	38	40	E2272.12	38	42		
S2274.4	39	40	E2272.14	41	42		
S2274.6	32	37	E2272.2	37	40		
S2274.8	35	41	E2272.4	38	42		
		35.71429	41.14286	E2272.6	35	50	
S2276.10	39	43	E2272.8	37	41		
S2276.12	38	41				37.28571	42.42857
S2276.2	43	46	E2275.10	39	44		
S2276.4	37	41	E2275.12	40	44		
S2276.6	39	43	E2275.14	42	missing		
S2276.8	37	45	E2275.2	41	61		
			E2275.4	38	41		
		38.83333	43.16667	E2275.6	40	52	
				E2275.8	39	40	
						39.85714	47
				E2277.10	35	39	
				E2277.12	35	43	
				E2277.14	35	40	
				E2277.2	36	48	
				E2277.4	34	50	
				E2277.6	34	40	
				E2277.8	35	40	
						34.85714	42.85714

Figure 4A: Example estrous cycle tracking (animal E2275.10)

Day of monitoring	Impedance (kW)
1	11.5
2	2.7
3	3.2
4	2.1
5	7.2
6	3.1
7	2.1
8	2
9	7.1
10	3.2
11	3.6
12	2.4
13	6.5
14	3.3
15	2.5
16	3
17	8.8
18	3.2
19	3.7
20	2.5
21	10.9

Estrous cycle tracking data (data used for 4B and 4C)

Offspring ID	Cycle length	Average
S2255.6	3, 9, 4, 4	5
S2269.14	4, 4, 4, 4, 4	4
S2264.4	2, 4, 4, 3, 3, 3	3.17
S2273.14	16, 4	10
S2256.10	8, 4, 6, 2	5
S2274.4	2, 4, 4, 4, 4	3.6
S2276.12	4, 4, 4, 4, 4	4
S2257.8	2, 3, 4, 5, 4	3.6
E2263.4	4, 4, 4, 4, 4	4
E2270.22	4, 4, 4, 4, 4	4
E2268.6	4, 4, 4, 4, 2	3.6
E2265.12	4, 4, 4, 4, 4	4
E2275.10	4, 4, 4, 4, 4	4
E2277.14	13, 3	8
E2258.20	4, 4, 4, 4	4
E2272.2	8, 8, 4	6.67

Figure 4B: Number of estrous cycles

Control	EtOH
4	5
5	5
6	5
2	5
4	5
5	2
5	4
5	3

Figure 4C: Estrous cycle length (days)

Control	EtOH
5	4
4	4
3.17	3.60
10	4
5	4
3.60	8
4	4
3.60	6.67

Figure 4D: Estradiol (pmol/L)

Control	EtOH
219.17	189.81
256.25	62.39
218.04	101.70
168.42	219.06
149.06	223.43
234.60	215.27
228.67	220.76
205.32	132.89
227.44	150.80
223.27	191.94
212.17	170.75
199.91	173.67
315.43	88.13
214.53	206.62
202.83	284.69
247.79	317.11
	170.61
	257.53

Figure 4E: Implantation sites

Control	EtOH
17	19
13	18
13	17
17	11
9	15
15	15
17	12
17	18
	17

Figure 4F: Progesterone (nmol/L)

Control	EtOH
75.05	110.35
59.47	85.22
69.64	93.81
160.59	95.40
83.32	70.60
87.45	103.67
85.54	79.50
86.50	99.85
	94.76

Table 2: Maternal parameters

Maternal body weight (g)

At mating		At E13.5		Total weight gain pre-gavage (E0.5-E12.5)		Total weight gain post-gavage (E15.5 to birth)	
Control	EtOH	Control	EtOH	Control	EtOH	Control	EtOH
239	261	307	313	38	62	78	90
261	270	324	305	67	59	99	84
268	235	305	306	49	39	103	111
262	242	286	340	75	52	86	95
239	283	306	316	50	48	64	80
248	245	323	322	43	61	62	110

Maternal water consumption (mL/day)

Pre-gavage (E12.5 only)		During gavage (E13.5 and E14.5)		Post-gavage (E15.5-birth)	
Control	EtOH	Control	EtOH	Control	EtOH
21.3	26.5	28.8	28.6	33.9	31.1
31.6	32.7	23.5	28.2	35.1	30.1
34.2	28.9	32.8	30.0	39.4	37.9
39.4	27.6	44.2	32.8	49.7	38.6
27.2	35.5	29.0	31.1	34.1	39.5
36.8	28.3	34.0	30.5	39.6	36.5

Maternal chow consumption (g/day)

Pre-gavage (E12.5 only)		During gavage (E13.5 and E14.5)		Post-gavage (E15.5-birth)	
Control	EtOH	Control	EtOH	Control	EtOH
22.5	22.9	23.2	23.0	23.0	27.0
	25.6	21.3	21.0	26.3	25.2
22	24.7	20.6	23.7	21.3	26.2
26.8	23	28.0	25.6	30.1	25.3
27.2	28	29.7	21.9	27.5	26.0
24.8	31.2	22.0	20.4	23.8	25.7

Measurement error

Pregnancy variables

Progesterone (nmol/L)		Litter Numbers		Litter sex ratio (M:F)	
Control	EtOH	Control	EtOH	Control	EtOH
47.65	79.64	11	15	0.83	1.50
51.56	47.83	11	17	0.83	0.55
27.81	49.22	13	16	0.86	1.00
39.22	78.45	13	14	0.44	0.75
65.27	71.32	15	17	0.50	0.70
30.02	54.94	13	14	1.17	1.33
85.23	55.08				
39.75	31.96				
55.34	68.18				
95.03	163.23				
52.51	43.30				
53.10	40.98				
41.95	84.58				
	136.13				

NB: 1 sample not collected from each group

Blood Alcohol Concentration (BAC) (mg/dL)

E13.5 1h post-gavage		E13.5 5h post-gavage		E14.5 1h post-gavage		E14.5 5h post-gavage	
Control	EtOH	Control	EtOH	Control	EtOH	Control	EtOH
1.4	13.8	0.9	1.4	0.9	62.2	0.9	6.9
0.0	24.9	0.5	0.5	1.8	77.4	1.4	0.9
	17.5				24.9		
	51.6				20.3		
	41.5				3.7		
	53.5				6.5		

NB: Only 2 controls were used as a representative sample, as assumed to be below limit of detection (effectively 0).
Only 2 EtOH group measured at 5h, as previous experience indicated BAC would be at or below limit of detection.

Table 3: Offspring weights

Neonatal offspring weights (g)

PN3		PN10	
<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>
8.0	7.7	24.2	19.4
7.8	8.4	24.4	21.8
7.7	7.7	20.9	18.9
8.2	7.6	21.2	19.6
9.4	8.7	21.8	23.0
	9.4	23.5	22.5

Litter averages (2-4 per litter)

Adolescent weights at PN28 (g)

<i>Control</i>	<i>EtOH</i>
63.7	53.9
63.9	66.7
62.1	72.1
68.2	67.8
67.5	84.7
67.1	68.8
62.9	66.9
63.2	58.7
	69.1

Litter averages (5-7 per litter)

Adult weights at 6 months of age (g)

Estrous cycles/mating/progesterone

<i>Control</i>	<i>EtOH</i>
340	309
397	279
273	318
290	283
268	328
310	309
376	267
281	316
	300

1 female offspring per litter

Pro-estrous cull/estradiol

<i>Control</i>	<i>EtOH</i>
356	312
323	325
272	295
305	323
323	349
281	361
351	330
415	340
393	290
278	282
323	338
288	312
276	323
415	348
342	366
332	279
	330
	306

2 female offspring per litter

Table 4: PN3 gene expression (fold change relative to *Hprt* endogenous control and normalised to Control group)

Bax		Bak		Bcl2		Bcl2l1		Bcl2l11		Puma/Bb3	
<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>
1.339	0.924	0.768	1.094	0.725	1.025	0.749	1.292	0.595	1.089	1.295	1.827
0.629	0.643	0.627	0.636	0.522	0.440	0.653	0.483	0.407	0.435	0.473	0.643
0.937	0.864	1.055	0.711	1.241	1.038	0.989	0.783	1.139	0.789	0.802	0.833
0.965	1.108	1.018	1.036	0.568	0.942	0.970	0.992	0.879	1.002	1.292	1.703
0.999	0.779	1.333	0.947	1.231	0.748	1.451	0.721	1.135	0.854	1.444	1.309
0.776	1.515	0.851	1.219	0.821	0.973	0.874	1.296	0.744	1.333	0.444	0.909
1.702	0.647	1.171	0.543	1.240	0.356	1.132	0.502	1.155	0.301	0.747	0.685
0.977	1.173	1.071	0.860	1.583	0.699	1.123	0.650	1.395	0.515	1.181	1.506
0.838	0.770	1.034	0.760	0.860	0.972	1.047	0.775	1.440	0.875	1.689	1.905
0.838	1.214	1.072	1.322	1.211	1.090	1.013	1.157	1.112	1.052	0.632	

Inha		AMH		Stk11		Cxcl12		Cxcr4		Pten	
<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>
0.660	1.214	0.586	0.485	0.640	1.089	0.486	1.650	1.169	0.905	0.605	1.132
0.638	0.434	0.876	0.309	0.518	0.464	0.529	0.595	0.787	0.449	0.567	0.506
1.233	0.972	0.701	1.289	1.159	0.835	1.035	0.845	0.964	1.023	1.231	1.022
0.844	0.902	0.496	0.560	0.942	1.095	0.775	0.825	0.751	1.217	0.860	1.058
0.900	0.798	0.900	0.810	1.005	0.741	1.171	0.901	1.072	0.901	1.061	0.813
1.003	0.566	0.399	0.535	0.835	1.437	0.841	0.610	1.073	0.700	0.915	1.179
1.136	0.365	0.540	0.240	1.444	0.395	0.830	0.266	0.799	0.382	1.180	0.416
1.260	0.696	2.367	0.627	1.335	0.631	0.952	0.599	1.077	0.700	1.283	0.684
1.093	0.911	1.606	1.053	1.014	0.961	1.302	1.175	1.160	0.763	1.162	1.029
1.233	0.976	1.529	2.321	1.108	1.227	2.079	1.562	1.147	0.854	1.135	1.013

qPCR error

Table 5: PN10 gene expression (fold change relative to *Hprt* endogenous control and normalised to Control group)

Bax		Bak		Bcl2		Bcl2l1		Bcl2l11		Puma/Bb3	
<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>
0.973	0.886	0.932	0.973	1.466	0.728	0.984	1.040	1.386	0.911	0.886	0.819
0.358	1.239	0.738	1.164	0.460	1.114	0.587	1.266	0.625	1.163	0.826	1.217
1.182	1.151	0.922	1.360	0.889	1.365	0.864	1.530	0.939	1.773	0.880	1.489
1.279	0.395	1.209	1.252	1.082	0.534	1.394	0.991	1.003	1.524	1.238	1.186
1.093	0.825	0.854	0.669	1.072	1.014	0.960	0.862	0.788	0.691	1.228	0.873
1.132	0.183	0.913	0.485	0.877	0.375	1.029	0.407	1.055	0.553	1.078	0.378
0.924	1.116	0.862	1.229	0.909	1.030	0.882	1.223	0.658	1.342	0.920	0.885
0.962	1.021	0.930	1.486	0.936	1.268	0.909	1.609	0.862	1.580	0.728	1.209
0.878	0.769	0.579	0.568	1.290	0.788	0.674	0.608	0.704	0.543	0.682	1.081
1.219	0.968	2.060	1.029	1.019	0.983	1.718	1.102	1.980	1.079	1.532	0.668
	0.938		0.992		1.303		1.028		0.968		0.440
	0.923		1.049		1.313		1.199		1.638		1.128
Inha		AMH		Stk11		Cxcl12		Cxcr4		Pten	
<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>	<i>Control</i>	<i>EtOH</i>
0.961	0.899	0.820	0.572	1.407	0.832	1.280	0.508	1.186	0.589	1.201	0.886
0.532	1.409	0.217	0.976	0.612	1.072	0.716	0.894	0.594	1.103	0.745	0.940
1.068	1.706	0.629	1.175	0.943	1.482	1.338	2.097	1.072	1.212	1.168	1.862
1.074	1.222	1.331	0.545	1.044	1.155	0.715	1.686	0.928	0.874	0.974	1.414
1.021	0.665	0.919	0.506	0.821	0.706	0.561	0.796	0.901	1.942	0.864	0.769
1.270	0.475	0.852	0.304	1.020	0.490	0.893	0.747	0.592	0.663	0.936	0.771
0.749	1.633	0.767	0.512	0.743	1.463	0.737	1.068	0.841	1.136	0.727	1.141
0.720	1.688	0.932	0.753	0.793	1.570	0.988	2.261	0.795	0.931	0.797	1.841
0.574	0.520	0.658	0.792	0.801	0.616	0.843	1.058	1.771	1.038	0.855	0.750
2.032	1.219	2.875	1.165	1.817	0.991	1.928	0.645	1.319	1.140	1.732	0.878
	0.980		1.098		0.900		1.347		1.085		1.051
	1.114		1.075		1.267		1.946		0.737		1.234