

Table 1 Incidence of reproductive tract abnormalities and SIX1 expression in mice exposed neonatally to GEN or DES¹

Location	Pathology	Age (months) ²								
		6			12			18		
		CON	GEN	DES	CON	GEN	DES	CON	GEN	DES
Vagina	Adenosis	0/33 (0%)	0/30 (0%)	2/31 (6%)	0/29 (0%)	0/30 (0%)	2/26 (8%)	0/30 (0%)	1/30 (3%)	2/30 (7%)
Uterus	Cystic Change	2/33 (6%)	12/30* (40%)	4/31 (13%)	7/29 (24%)	23/30* (77%)	10/26 (38%)	15/30 (50%)	27/30* (90%)	23/30 (77%)
	Adenomyosis	0/33 (0%)	6/30* (20%)	13/31* (42%)	3/29 (10%)	10/30 (33%)	13/26* (50%)	4/30 (13%)	13/30* (43%)	18/30* (60%)
	Basal Cell Metaplasia	3/33 (9%)	19/30* (63%)	30/31* (97%)	2/29 (7%)	26/30* (87%)	25/26* (96%)	0/30 (0%)	30/30* (100%)	29/30* (97%)
	Squamous Metaplasia ³	1/33 (3%)	11/30* (37%)	7/31* (23%)	0/29 (0%)	16/30* (53%)	12/26* (46%)	0/30 (0%)	24/30* (80%)	14/30* (47%)
	Atypical Hyperplasia	0/33 (0%)	3/30 (10%)	14/31* (45%)	0/29 (0%)	9/30* (30%)	14/26* (54%)	0/30 (0%)	15/30* (50%)	18/30* (60%)
	Carcinoma	0/33 (0%)	2/30 (7%)	5/31 (16%)	0/29 (0%)	7/30* (23%)	9/26* (35%)	0/30 (0%)	10/30* (33%)	12/30* (40%)
	Sarcoma	0/33 (0%)	0/30 (0%)	1/31 (3%)	0/29 (0%)	1/30 (3%)	0/26 (0%)	0/30 (0%)	2/30 (7%)	0/30 (0%)
SIX1 IHC										
Vagina	Epithelium ⁴	33/33 (100%)	30/30 (100%)	31/31 (100%)	29/29 (100%)	30/30 (100%)	26/26 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
	Average SIX1 severity index ⁵	2.2	1.8	1.5	1.6	1.1	1.2	1.9	1.7	1.7
Uterus	Nonneoplastic Glands ⁶	3/33 (9%)	21/30* (70%)	30/31* (97%)	2/29 (7%)	26/30* (87%)	25/26* (96%)	0/30 (0%)	30/30* (100%)	30/30* (100%)
	Average SIX1 severity index ⁵	0.1	0.9	1.5	0.1	1.5	2.0	0.0	2.0	2.6
	Neoplastic Glands ⁷	na	2/2 (100%)	5/5 (100%)	na	7/7 (100%)	9/9 (100%)	na	10/10 (100%)	12/12 (100%)
	Average SIX1 severity index ⁵	na	2.5	2.8	na	2.0	2.7	na	2.1	2.4

* Statistically significant using one-tailed Fisher's Exact Test $p < 0.05$ compared to corresponding age-matched CON mice. $n = 26-33$ mice per treatment and age group as indicated in the table.

¹ Mice were given injections of GEN (50 mg/kg/day) or DES (1 mg/kg/day) on days 1-5 of neonatal life.

² Mice were necropsied at indicated ages.

³ Stratified squamous cells in place of glandular epithelium; distinct from a single layer of basal cells underlying luminal glandular cells.

⁴ Predominantly basal and suprabasal cells.

⁵ Average qualitative severity score from 0 to 4+ for SIX1 across the group.

⁶ Number of animals with positive staining out of all animals within that group.

⁷ Number of animals with positive staining in neoplastic lesions out of animals with carcinoma.

Table 2 Incidence of endometrial SIX1 expression in patients with different histopathological diagnoses.

Patient Category	Pathologic Diagnosis	# Patients	# SIX1-Positive	# SIX1-Negative	% SIX1-Positive
Normal	Normal	28	0	28	0%
Nonneoplastic	Normal cancer adjacent tissue	13	0	13	0%
	Hyperplasia	21	0	21	0%
	Inflammation	5	0	5	0%
Preneoplastic	Atypical hyperplasia	3	1	2	33%
Neoplastic	Endometrioid adenocarcinoma	171	43	128	25%
	Unspecified adenocarcinoma	87	28	59	32%
	Serous carcinoma	11	4	7	36%
	Clear cell carcinoma	4	0	4	0%
	Mucinous carcinoma	1	1	0	100%
	Adenocarcinoma metastasis	5	0	5	0%
	Adenosquamous carcinoma	7	1	6	14%
	Squamous carcinoma	8	3	5	38%
	Undifferentiated carcinoma	2	0	2	0%
	Stromal sarcoma	1	0	1	0%
Chorionic carcinoma	2	0	2	0%	

Supplemental Table 1. Uterine mRNA expression of Six1 (displayed in Figure 2B)

<i>6 months</i>			
	n	mean	SE
CON	33	55.0	8.5
GEN	31	383.1	66.7
DES	32	717.8	81.1

<i>12 months</i>			
	n	mean	SE
CON	29	166.8	23.6
GEN	29	1310.8	119.0
DES	27	1895.1	212.4

<i>18 months</i>			
	n	mean	SE
CON	31	459.3	54.8
GEN	29	4439.5	475.8
DES	30	4496.4	541.1

Supplemental Table 2. Percentage of SIX1-positive cases (displayed in Figures 3B-3D)

	SIX1+	SIX1-
Normal	0	28
Neoplastic	80	299

	SIX1+	SIX1-
Grade 1	24	60
Grade 2	27	83
Grade 3	28	55

	SIX1+	SIX1-
Early Stage	59	189
Late Stage	19	15