

# Characterization of Normal Skin Thickness For Various Body Regions, Ages, and Genders of Yucatan Miniature Swine

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## INTRODUCTION

Dermatology is an extremely important field, as the skin of humans and animals plays a life-sparing, protective role. The skin is the largest organ of the body and is metabolically active. It is constantly shedding and regenerating. Skin from various regions of the body may vary morphologically, including in physical thickness. Dermatological studies in miniature swine provide a unique opportunity for risk assessment because of demonstrated similarities to human cutaneous anatomy, biochemistry, and physiology. The Yucatan is a popular miniature swine often used in biomedical research today. Yucatan skin is slate grey-black in color, slightly or moderately pigmented, and contains a sparse haircoat (sometimes called the Mexican hairless miniature swine). The physical thickness of miniature swine skin may impact drug absorption during *in vivo* or *ex vivo* studies, or affect wound healing or phototoxicity studies. Therefore, a good understanding of the relative dimensions of each major skin application or collection site is indicated. This study of Yucatan skin was undertaken to better document this area to give researchers more information about the skin thickness of miniature swine. Few citations on porcine or miniature swine skin thickness exist. Eggleston et al. (2000) reported mean  $\pm$  SD thickness of the Yucatan miniature swine flank, and dorsal neck epidermis were  $68 \pm 34$  and  $68 \pm 25$   $\mu$ m, respectively. Thickness of the Yucatan miniature swine skin were closely comparable to the thickness of human epidermis from the face ( $68 \pm 26$   $\mu$ m), neck ( $65 \pm 24$   $\mu$ m) and arms ( $68 \pm 21$   $\mu$ m). The domestic Yorkshire epidermis from seven sites was not as thick (Eggleston et al., 2000). Monteiro-Riviere et al. (1990) reported back skin components of the domestic Yorkshire swine, including a  $12.28 \pm 0.72$   $\mu$ m stratum corneum,  $51.89 \pm 1.49$   $\mu$ m cellular epidermis, and  $3.94 \pm 0.13$  epidermal cell layers. Grabau et al. (1995) reported on a laboratory histological survey of research animal skin thickness, including a mixed breed domestic farm pig. This study will add additional standard Yucatan miniature swine data to the research database.

## MATERIALS AND METHODS

Eight-millimeter punch biopsies of Yucatan miniature swine skin from freshly sacrificed animals were collected, placed into cassettes, and fixed in 10% neutral buffered formalin (Protocol formalin, Fisher Diagnostic). Body surface sites evaluated for 18 animals included: neck, back, flank, and abdomen for all animals except two five-month-old intact males, which also had rump skin collected. Samples were core down to the hypodermis (fat layer) by gentle turning of the disposable punch biopsy device, eight millimeters in diameter (Miltex, York, PA), then elevating and snipping the underlying fat layer. The subcutaneous fat layer (hypodermis) was not measured. Both genders were represented. Ages varied depending upon availability, but in general ranged from 5.0 to 36.5 months (males) or 3.5 weeks to 10.3 months (females). From the 18 heads examined, sample data were pooled into age categories or groups. Skin samples were also collected from ear, axillary, and inguinal areas, but will not be reported here.

The abdomen sample was taken approximately one to two inches anterior to the umbilicus on the midline. The back or dorsum sample was anterior to the pelvis in the lumbar area off to one side of the spinal column by about one to two inches. The neck sample was taken from the lateral side of the neck about one to two inches anterior to the scapula. The flank sample was collected from the caudal lateral flank three inches anterior to the rear leg. The rump skin was taken caudal to the ilium, anterior to the ishium, and off to one side of the spinal axis.

Following adequate time for fixation, the hairs on the surface of the eight-millimeter-in-diameter skin punch biopsy specimen were counted under an Olympus SZH10 Zoom Stereo Microscope. The skin biopsy was trimmed, processed, embedded in paraffin, sectioned at 4  $\mu$ m, and stained with hematoxylin and eosin (H&E).

Two microphotographs (one 20X and the other 40X) were taken from each skin biopsy specimen by using an Olympus DP70 digital camera mounted on an Olympus BX41 microscope. Five thickness measurements of the stratum corneum (s.c.), cellular epidermis, and dermis were randomly taken. All measurements were digitally calculated using an image-analyzing system (Olympus MicroSuite™ Basic Edition) and were represented as micrometers ( $\mu$ m, microns).

The thickness of the dermis was measured on the surface of the 20X microphotographs. The epidermis and stratum corneum were measured on the 40X microphotographs.

The number of cell layers was estimated for the cellular epidermis (excluding the s.c.). Full-thickness epidermis and full-thickness skin calculations were performed by adding the stratum corneum and cellular epidermis values, and the full-thickness epidermis and dermis values, respectively.

The five readings for each skin category (stratum corneum, epidermis less s.c. [cellular epidermis], dermis) were averaged ( $\pm$ SD) for each gender and age group. Number of cell layers, hair counts, and full-thickness calculations was also recorded and averaged ( $\pm$ SD). Gender, age, and body region specific means (in  $\mu$ m),  $\pm$  SDs, and observed ranges were reported. Relative ratios of cellular epidermis and dermis to full-thickness measurements were performed. Data were compared to published measurements of adult human and domestic Yorkshire skin, illustrating similarities or differences.

## RESULTS

Table 1 presents mean ( $\pm$ SD) Yucatan skin component thickness measurements by biopsy site, gender, and age category. Table 2 presents full-thickness Yucatan epidermis and full-thickness skin calculations and skin component relative ratios (percentages) of these two total thickness estimates (epidermal, total skin). Minimum and maximum observed (range) values for all parameters measured are presented in Table 3. Gender, age, and body-region-specific means ( $\mu$ m),  $\pm$  SDs, and observed ranges are presented for five inter-follicular skin components (dermis thickness, stratum corneum thickness, cellular epidermis thickness, number epidermal cell layers, and hair count).

Table 1. Yucatan Miniature Swine Skin Component Thickness and Counts

Site	Gender/N	Group	Age	Fn	Dermis ( $\mu$ m)	Cellular Epidermis ( $\mu$ m)	Stratum Corneum ( $\mu$ m)	# of Epidermal Cell Layers*	Hair Count**
Neck	Males (1 cas) N=3	A	5-6.5 mo	Mean	4363.66	65.1	26.38	4.33	7
				SD	699.7	15.66	5.56	0.82	-
Neck	Males (cas) N=3	B	22.2-24 mo	Mean	3965.17	62.15	28.98	4.4	2
				SD	734.85	16.95	5.61	0.63	1
Neck	Male (cas) N=1	C	36.5 mo	Mean	5596.73	47.48	22.11	4	1
				SD	836.28	4.82	2.78	0.71	-
Neck	Male (cas) N=1	D	10.3 mo	Mean	3757.31	67.76	66.39	4.8	5
				SD	183.93	9.18	18.7	0.84	-
Neck	Females N=4	E	3.5 wk	Mean	1334.06	54.15	12.97	5.15	19
				SD	181.62	12.27	4.52	0.93	6
Neck	Females N=3	F	4-5.4 mo	Mean	3450.17	75.46	20.07	5.4	5
				SD	843.58	13.95	5.81	0.63	2
Neck	Females N=2	G	8.9-10.3 mo	Mean	2256.25	54.22	9.77	5	7
				SD	1717.83	41.49	7.55	0.82	0
Back	Males (1 cas) N=3	A	5-6.5 mo	Mean	4468.01	77.89	24.86	5.2	3
				SD	169.18	11.16	2.12	0.84	-
Back	Males (cas) N=3	B	22.2-24 mo	Mean	4343.19	63.84	35.34	4.2	1
				SD	634.19	15.44	11.63	0.56	1
Back	Male (cas) N=1	C	36.5 mo	Mean	4809.62	67.8	28.71	3.8	2
				SD	250.21	7.42	8.08	0.45	-
Back	Male (cas) N=1	D	10.3 mo	Mean	3418.45	77.83	23.53	3.8	7
				SD	216.39	9.47	4.02	0.55	-
Back	Females N=4	E	3.5 wk	Mean	1164.2	77.77	13.72	5.45	21
				SD	134.49	25.41	3.58	0.94	14
Back	Females N=3	F	4-5.4 mo	Mean	3503.94	87.37	23.03	4.6	3
				SD	1145.7	20.39	9.03	0.83	3
Back	Females N=3	G	8.9-10.3 mo	Mean	3580.52	92.15	37.78	5.4	4
				SD	423.3	24.96	6.12	0.99	2
Flank	Males (1 cas) N=3	A	5-6.5 mo	Mean	3872.94	59.59	47.3	4.4	3
				SD	415.12	15.96	9.61	0.89	-
Flank	Males (cas) N=3	B	22.2-24 mo	Mean	3990.93	53.51	45.12	3.87	2
				SD	391.6	8.24	5.81	0.35	0
Flank	Male (cas) N=1	C	36.5 mo	Mean	5008.9	57.2	32.96	4.8	2
				SD	402.01	6.51	6.22	0.84	-
Flank	Male (cas) N=1	D	10.3 mo	Mean	3614.24	48.41	13.95	3.8	3
				SD	85.61	4.37	2.02	0.55	-
Flank	Females N=3	E	3.5 wk	Mean	909.22	34.72	11.89	4.33	10
				SD	230.23	22.53	7.82	0.49	4
Flank	Females N=3	F	4-5.4 mo	Mean	2698.85	63.47	19.02	4.73	4
				SD	356.83	13.01	5.3	1.1	2
Flank	Females N=3	G	8.9-10.3 mo	Mean	3355.12	72.6	25.4	4.8	3
				SD	249.52	30.35	10.08	1.21	1
Abdomen	Male (1 cas) N=3	A	5-6.5 mo	Mean	3072.99	61.8	65.3	6.2	3
				SD	593.53	5.57	11.8	1.1	-
Abdomen	Male (cas) N=3	B	22.2-24 mo	Mean	2283.02	53.37	35.55	4.2	0
				SD	216.61	8.46	7.68	0.41	1
Abdomen	Male (cas) N=1	C	36.5 mo	Mean	2647.14	69.99	33.92	5	1
				SD	262.4	5.12	1.35	0.71	-
Abdomen	Male (cas) N=1, except dermis block missing	D	10.3 mo	Mean	Not read	38.8	21.75	3.4	0
				SD	N/A	3.51	1.9	0.55	-
Abdomen	Females N=4	E	3.5 wk	Mean	1020.37	66	19.32	5.15	2
				SD	369.67	17.67	8.33	0.81	2
Abdomen	Females N=3	F	4-5.4 mo	Mean	2343.19	66.75	15.36	4.47	1
				SD	722.45	11.03	3.56	0.64	1
Abdomen	Females N=3	G	8.9-10.3 mo	Mean	2015.31	61.6	31.76	4.67	1
				SD	2702.54	86.32	23.11	5.3	8.5
Rump	Males N=2	H	5 mo	Mean	287.05	19.22	6.43	0.82	4.9
				SD	-	-	-	-	-

\*Cell layers of epidermis; \*\*hairs evident on surface of 8mm (50.24 sq mm or 0.5024 sq cm) biopsy punch. Male (cas) = castrated; Five measurements were taken per skin site for each animal and averaged. Age group averages included the following: A, B, F & G = 3; E = 4; rump skin include N of 2 intact males. Animal #1289 (female, 10.3 months) was missing neck block and #2052 missing flank samples; animal #1291 (male castrate, 10.3 months) abdomen reading was not performed due to lower tissue disruption. Animal #1289 was OVXed; Animals #12913 & #1289 were acute Type 1 alloxan-induced diabetics



Figure 1. 40X image of Yucatan skin (H&E) illustrating five random measurements of cellular epidermal thickness, with overlying stratum corneum, also a component of the epidermis, and underlying pink, eosinophilic dermis containing abundant collagen and vascular supply.

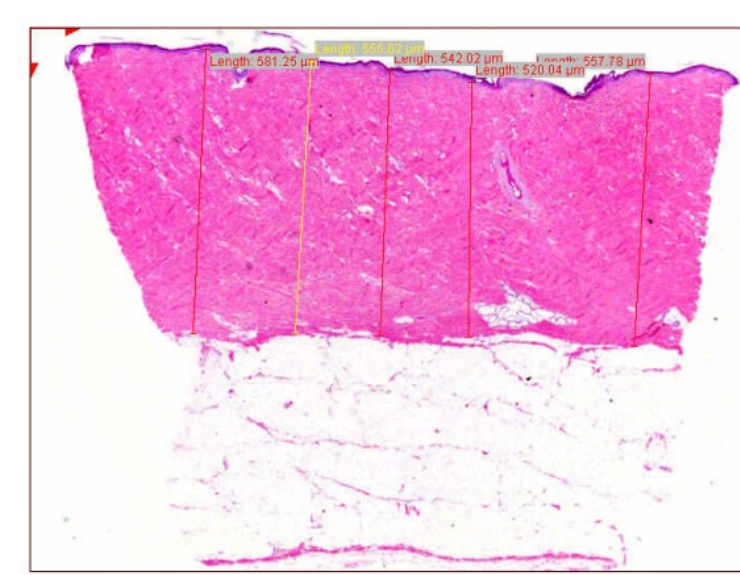


Figure 2. 20X image of Yucatan skin (H&E) illustrating five random measurements of dermis, with overlying epidermis and stratum corneum, and underlying hypodermal fat.

Table 2. Yucatan Epidermis and Full-Thickness Skin—Thickness and Component Relative Ratios

Site	Gender	Group	Age	Full-Thickness Epidermis* ( $\mu$ m)	Full-Thickness skin** ( $\mu$ m)	Ratio Full-Thickness Epidermis to Dermis	Ratio Dermis to Full-Thickness Skin
Neck	Male (1 cas) N=3	A	6.5 mo	91.47	4455.13	0.021	0.979
Neck	Male (cas) N=3	B	22.2-24 mo	91.14	3956.31	0.024	0.977
Neck	Male (cas) N=1	C	36.5 mo	69.59	5666.32	0.012	0.988
Neck	Male (cas) N=1	D	10.3 mo	134.15	3891.46	0.036	0.966
Neck	Females N=4	E	3.5 wk	67.12	1401.18	0.05	0.952
Neck	Females N=3	F	4-5.4 mo	95.53	3545.7	0.028	0.973
Neck	Females N=2	G	8.9-10.3 mo	63.99	2320.25	0.028	0.972
Back	Male (1 cas) N=3	A	6.5 mo	109.72	4274.39	0.026	0.974
Back	Male (cas) N=3	B	22.2-24 mo	99.18	4442.37	0.023	0.978
Back	Male (cas) N=1	C	36.5 mo	94.51	4904.13	0.02	0.981
Back	Male (cas) N=1	D	10.3 mo	101.36	3519.81	0.03	0.971
Back	Females N=4	E	3.5 wk	91.49	1255.7	0.079	0.927
Back	Females N=3	F	4-5.4 mo	110.4	3614.34	0.032	0.969
Back	Females N=3	G	8.9-10.3 mo	129.93	3710.45	0.036	0.965
Flank	Male (1 cas) N=3	A	6.5 mo	102.72	3982.27	0.027	0.974
Flank	Male (cas) N=3	B	22.2-24 mo	98.63	4089.56	0.025	0.976
Flank	Male (cas) N=1	C	36.5 mo	90.15	5099.06	0.018	0.982
Flank	Male (cas) N=1	D	10.3 mo	62.36	3676.6	0.017	0.983
Flank	Females N=3	E	3.5 wk	46.6	955.83	0.051	0.951
Flank	Females N=3	F	4-5.4 mo	82.48	2781.33	0.031	0.97
Flank	Females N=3	G	8.9-10.3 mo	98	3453.12	0.029	0.972
Abdomen	Male (1 cas) N=3	A	6.5 mo	108.31	3391.61	0.033	0.968
Abdomen	Male (cas) N=3	B	22.2-24 mo	98.92	2371.94	0.039	0.963
Abdomen	Male (cas) N=1	C	36.5 mo	103.91	2751.05	0.039	0.962
Abdomen	Male (cas) N=1, except dermis not read	D	10.3 mo	60.55	N/A	N/A	N/A
Abdomen	Females N=4	E	3.5 wk	85.32	1105.7	0.084	0.923
Abdomen	Females N=3	F	4-5.4 mo	82.11	2425.31	0.035	0.966
Abdomen	Females N=2	G	8.9-10.3 mo	93.36	2108.67	0.046	0.956
Rump	Males N=2	H	5 mo	109.43	2811.97	0.04	0.961

\*Stratum corneum plus cellular epidermis = Epidermis thickness \*\*Epidermis plus dermis = Skin thickness

Table 3. Minimum and Maximum Observed Values (Ranges) by Parameter

Site	Gender	Group	Age	Fn	Dermis ( $\mu$ m)	Cellular Epidermis ( $\mu$ m)	Stratum Corneum ( $\mu$ m)	# of Epidermal Cell Layers	Trichogram/ # of Hairs
Neck	Male (1 cas) N=3	A	6.5 mo	Min	3298.86	50.26	17.96	3	7
				Max	5644.62	97.86	37.95	6	16
Neck	Male (cas) N=3	B	22.2-24 mo	Min	2701.39	40.31	20.14	4	1
				Max	5334.3	89.8	38.69	6	2
Neck	Male (cas) N=1	C	36.5 mo	Min	4648.91	42.97	18.54	3	1
				Max	6741.67	55.09	26.26	5	-
Neck	Male (cas) N=1	D	10.3 mo	Min	3583.02	54.84	45.37	4	5
				Max	3950.42	76.6	36.13	6	-
Neck	Females N=4	E	3.5 wk	Min	1004.53	36.88	6.96	4	