

Supporting Information

Fiddymment et al. 10.1073/pnas.1512264112

The challenge for destructive sampling methods is finding sufficient appropriate samples. Recently, a collection of more than 100 sacrificial historic parchment samples (1480–1965) has been made available for analysis (45). However, many scholars wish to explore specific documents; Toniolo et al. (11) admit that the remaining leaves of the “Marco Polo Bible” (which could not be subjected to destructive analyses) may have been from sheep, as indeed were all three of the family’s archival documents that we have analyzed (Table S1).

Uterine Skins?

William Horman (46), writing in 1519, uses the term “abortyue” to refer to the rendered hide of a fetal animal. This material is called “abortive,” he claims, “bycause the beest was scante perfecte” in the uterus (46). Latin phrases, such as *charta virginea* and *pergamena virginea*, appear regularly in medieval and early modern sources alongside the formulation *charta non nata* (literally “unborn sheet”), strongly implying a material made from the skin of an aborted or miscarried fetus.

Researchers in the field of manuscript studies have long disputed the derivation of this ultrafine writing material. Some older scholarship suggested that uterine vellum probably derived from the hides of smaller, more thin-skinned mammals; thus, Pollard (1) spoke of what he believed were “probably rabbit-skin duodecimos,” whereas Thompson (2) suggested that “some of what now masquerades as ‘uterine vellum’ may actually be rabbit or squirrel parchment.” Most paleographers continue to view the notion of medieval uterine vellum as a myth or superstition, on the grounds that its production on the scale implied by extant manuscripts would have entailed an untenably high number of aborted fetuses. As Christopher de Hamel (3) stated definitively, “It is very difficult to believe that thousands of cows miscarried for generations, or were deprived of their foetuses in such numbers to supply the booktrade economically. . . . If the term uterine parchment must be used at all, it should perhaps refer to a quality of skin and not to its origin.” Other proposed solutions to the derivation of this material have involved specific production processes. Clarkson (4), for example, argues that uterine vellum may have entailed the splitting of skins. The importance of “proper analysis of parchment species” and, more specifically, the origin of uterine vellum has also been highlighted as a priority research question by Mark Clarke (5), who states “The question of ‘uterine vellum’ versus the use of small animals would be one historic debate it would be well to resolve once and for all, and would provide useful localisation data.”

If uterine vellum was sourced from finely prepared skins of domestic animals, we might suspect a predominance of calfskin, as implied by the etymology of the word *vellum*, although medieval terminology is notoriously inconsistent in this regard. Indeed, it has been suggested that the term “vellum” itself may well have been used to refer to the quality of the writing surface as much as to the calf it came from. If the production of uterine vellum represented instead a specialized craft skill and available skins could be worked to produce ultrafine parchment sheets, then the selection of animals would be similar to the selection of animals evident in other coarser membranes from the same geographic region.

SI Results

We explored the use of liquid chromatography (LC)-tandem MS (MS/MS) to see if differential biomarkers for uterine skin could be identified. Four samples were taken from a modern uterine

calfskin and compared with four nonuterine calfskins (two modern samples and two historic samples dated to 1776). Isobaric tags for relative and absolute quantification (iTRAQ) labeling was used on both sets of samples to determine quantitative differences between uterine and nonuterine parchment (Fig. S1). Uterine samples showed elevated expression of four proteins: dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 2, hemoglobin fetal subunit beta, heat shock protein 90- α , and collagen α -1(III) chain. Unfortunately, none of the peptide markers observed in eZooMS mapped to any of these proteins, so it is not possible to report the presence of these proteins in our Bible samples. The elevated expression levels of fetal hemoglobin and collagen III are consistent with previous studies (47, 48). Brandt et al. (49) identified calfskin leather from Neolithic capes from Denmark based upon the presence of fetal calf hemoglobin in one sample. However, fetal hemoglobin clears after 8 to 10 wk in cattle (48, 50) and 6 to 8 wk in sheep and goats (48, 51), preventing distinction between fetal and calf hides.

SI Materials and Methods

Four samples of uterine calfskin, each measuring 0.5 cm $\times$ 0.25 cm, were prepared, as well as four control “nonuterine” calfskins, also measuring 0.5 cm $\times$ 0.25 cm. Samples were incubated in 0.5 M triethylammoniumbicarbonate (TEAB) (pH 8.5) at 65 °C for 1 h to solubilize the collagen. The supernatant was quantified using a Qubit (Thermo Fisher Scientific). One hundred fifty micrograms of protein from each sample was digested overnight with trypsin at 37 °C at a ratio of 1:75 (trypsin to protein). The tryptic digest was transferred over C18 resin to desalt and concentrate peptides by washing with 0.1% TFA. Peptides were eluted in a final volume of 50 μ L of 50% acetonitrile (ACN)/0.1% TFA (vol/vol). Digested samples were labeled with the iTRAQ reagents following the protocol provided by the vendor (Applied Biosystems). Briefly, one vial of iTRAQ labeling reagent was used for every 100 μ g of protein. Ethanol was used to solubilize the iTRAQ reagent and then added to the peptide sample, ensuring a final organic concentration of at least 60% (vol/vol). The labeling reaction was performed by 2 h of incubation at room temperature. Samples were cleaned up before LC-MS/MS analysis using a cation-exchange cartridge system (SCIEX) and desalted using a C18 cartridge.

Dried peptides were resuspended in 2% (vol/vol) ACN and 0.1% TFA and analyzed by nano-LC-MS/MS as described previously (52). In brief, samples were separated using nano-ultra-performance liquid chromatography (Easy spray C18 column with a 75 μ m $\times$ 500 mm, 2.1- μ m particle size; Thermo Fisher Scientific) coupled to a Q Exactive tandem mass spectrometer (Thermo Scientific). MS data were acquired with a resolution of 70,000 at an m/z of 200 and by selecting the top 15 precursor ions. The ion target in MS1 mode was 3×10^6 , and it was 5×10^5 in MS2 mode. Ions with an m/z between 380 and 1,800 were accumulated for up to 100 ms in MS1 and for up to 128 ms in MS2. We used a stepped normalized collision energy at 31%, 34%, and 37%; a precursor isolation window of 1.6 m/z with an offset of 0.3 m/z ; a fixed first mass of 100 m/z ; and a resolution of 35,000 (profile) for MS2 spectrum acquisition. The samples were loaded in 0.1% TFA in 1% CH₃CN and eluted with a gradient of 3–35% (vol/vol/vol) CH₃CN in 5% DMSO and 0.1% formic acid for 60 min at a flow rate of 250 nL·min⁻¹.

Identification/quantitation of iTRAQ signals was processed with PEAKS 7 (Bioinformatics Solutions, Inc.). Raw files were

Table S1. Sample details for three of Marco Polo's family archival documents

Sample no.	Library	Date	Type of document	Location of production	Species
VVK 26	Biblioteca Marciana	August 5, 1280	Testament of Marco Polo "il vecchio" (Marco Polo's uncle)	Italy	Sheep
VVK 29	Biblioteca Marciana	August 31, 1300	Testament of Matteo Polo (Marco Polo's step-brother)	Italy	Sheep
VVK 31	Biblioteca Marciana	January 9, 1324	Testament of Marco Polo "il viaggiatore"	Italy	Sheep

Table S2. Sample details for 72 13th century pocket Bibles

Sample no.	Shelf-mark	Library	Date	Size, mm	No. of leaves	Thickness, mm	No. of pages sampled	Location of production	Species
CCC 31–32	CCCC MS 246	Corpus Christi College Cambridge	1200–1300	156 × 102	410	0.05–0.08	2	England	Calf
CL 001	Gg.6.15	Cambridge University Library	1225–1250	184 × 130	504	0.06–0.09	1	France	Calf
CL 002	Gg.6.45	Cambridge University Library	1225–1250	136 × 92	532	0.07–0.1	1	France	Calf
CL 003–005	Add.1848	Cambridge University Library	1225–1250	156 × 109	407	0.07–0.2	3	France	Goat
CL 006	Add.6159	Cambridge University Library	1225–1250	137 × 88	594	0.04–0.06	1	France	Calf
CL 057–059	Pembroke 268	Cambridge University Library	1200–1300	198 × 128	480	0.06–0.2	3	France	Calf
CL 064–067	Add.8438	Cambridge University Library	1200–1300	185 × 130	424	0.07–0.13	4	France	Calf
CL 082–086	Hh.1.3	Cambridge University Library	1250–1275	198 × 195	367	0.06–0.15	5	England	Calf
CL 087–091	li.6.22	Cambridge University Library	1250–1300	142 × 102	299	0.06–0.12	5	Italy	Goat
CL 092–094	li.6.12	Cambridge University Library	1200–1300	165 × 104	469	0.06–0.14	3	France	Calf
CL 100–101	Dd.5.52	Cambridge University Library	1200–1300	210 × 140	333	0.1–0.18	2	England	Calf and goat
CL 102–104	Gg.1.27	Cambridge University Library	1225–1250	224 × 153	406	0.07–0.22	3	England	Calf and goat
CL 105–106	Dd.10.29	Cambridge University Library	1250–1275	235 × 160	439	0.08–0.15	2	England	Calf
CL 107–108	Ee.1.16	Cambridge University Library	1200–1300	208 × 150	349	0.08–0.13	2	Northern France or England	Calf
CL 109–110	li.4.10	Cambridge University Library	1225–1250	242 × 170	346	0.1–0.2	2	England	Calf
CL 111–112	Ee.1.9	Cambridge University Library	1200–1300	210 × 150	285	0.08–0.18	4	England	Calf
CL 118–119									
CL 113–114	Mm.1.2	Cambridge University Library	1200–1300	245 × 163	610	0.06–0.1	2	Northern France or England	Goat
CL 120–121	Gg.1.3	Cambridge University Library	1225–1275	211 × 140	375	0.08–0.11	2	England	Calf
CL 122–123	Ff.6.19	Cambridge University Library	1200–1300	146 × 97	567	0.04–0.09	2	—	Calf
CL 124–125	Ff.6.20	Cambridge University Library	1200–1300	146 × 98	453	0.03–0.06	2	—	Goat
CL 126–127	Dd.15.35	Cambridge University Library	1200–1300	148 × 94	476	0.06–0.1	2	—	Goat
CL 128–129	Dd.12.47	Cambridge University Library	1200–1300	150 × 106	502	0.04–0.08	2	Northern France or England	Calf
CL 130–131	Ee.6.26	Cambridge University Library	1220–1230	198 × 138	459	0.07–0.28	20	England	Calf and sheep
CL 179–196									
CL 132–136	Ff.6.45	Cambridge University Library	1225–1275	144 × 103	478	0.05–0.09	5	England	Goat
DCL 05–06	A.IV.37	Durham Cathedral Library	1200–1300	235 × 147	376	—	2	England?	Calf
DCL 07–10	A.IV.30	Durham Cathedral Library	1225–1275	159 × 106	576	—	4	France?	Calf
DUL 01–03	DUL MS Cosin V.V.17	Durham University Library	1200–1300	127 × 92	322	0.08–0.08	3	Southern France or Northern Italy	Goat
DUL 04–06	DUL MS Cosin V.V.18	Durham University Library	1200–1300	132 × 87	420	0.05–0.05	3	France	Calf
FLP 01–03	Lewis E 029	Free Library of Philadelphia	1230–1240	182 × 113	160	—	3	England	Calf
FLP 04–06	Lewis E 028	Free Library of Philadelphia	1270–1270	159 × 105	659	—	3	France	Calf
FLP 07–09	Lewis E 030	Free Library of Philadelphia	1225–1250	142 × 98	539	—	3	England	Goat
FLP 10–12	Lewis E 033	Free Library of Philadelphia	1250–1250	150 × 104	341	—	3	France	Calf
FLP 13–15	Lewis E 036	Free Library of Philadelphia	1260–1280	166 × 123	370	—	3	Italy	Goat
FLP 16–18	Lewis E 039	Free Library of Philadelphia	1250–1250	186 × 126	403	—	3	Italy	Goat
JRL 158–159	MSM0163	Morgan Library	1229–1229	220 × 150	454	0.09–0.15	2	France	Goat
KRPB	Ms. 56	University of St. Louis	1200–1300	150 × 100	236	0.11–0.18	1	France	Calf
MAGGS 02	14739	Maggs Bros. Ltd.	1200–1300	—	—	—	1	—	Goat
MAGGS 03	14754	Maggs Bros. Ltd.	1200–1300	—	—	—	1	—	Goat
MAGGS 05	12307	Maggs Bros. Ltd.	1200–1300	—	—	—	1	—	Goat
NRO W 14	PARIS BIBLE	Private collection	1225–1275	158 × 112	395	0.12–0.12	1	France	Calf
OSU 01–18	OSU.MS.MR. Frag.74	Ohio State University	1220–1220	220 × 145	440	0.1–0.2	18	France	Calf
RLB 01–03	Ms 2053	Royal Library of Belgium	1200–1300	223 × 157	443	—	3	France (north)	Goat
RLB 04–06	Ms 4911	Royal Library of Belgium	1200–1300	130 × 93	180	0.093–0.149	3	France (north), Low Countries, or England	Calf
RLB 07–09	Ms 5627	Royal Library of Belgium	1200–1300	140 × 94	588	0.061–0.078	3	France (north)	Calf
RLB 10–12	Ms 8428	Royal Library of Belgium	1200–1300	208 × 159	412	0.082–0.174	3	England	Calf
RLB 13–15	Ms 8882	Royal Library of Belgium	1200–1300	140 × 96	486	0.067–0.088	3	France (north)	Calf
RLB 16–18	Ms 10517	Royal Library of Belgium	1200–1300	150 × 95	664	—	3	France (north)	Calf
RLB 19–21	Ms 10518	Royal Library of Belgium	1200–1300	140 × 90	642	—	3	France (north)	Calf
RLB 22–24	Ms 10520	Royal Library of Belgium	1200–1300	140 × 85	790	—	3	France (north)	Calf

Table S2. Cont.

Sample no.	Shelf-mark	Library	Date	Size, mm	No. of leaves	Thickness, mm	No. of pages sampled	Location of production	Species
RLB 25–27	Ms 10521	Royal Library of Belgium	1200–1300	154 × 97	592	—	3	France (north)	Calf
RLB 28–30	Ms 10522	Royal Library of Belgium	1200–1300	178 × 122	481	0.074–0.126	3	France or Italy	Calf and goat
RLB 31–33	Ms 10523	Royal Library of Belgium	1200–1300	178 × 116	359	0.101–0.172	3	France (north)	Calf
RLB 34–36	Ms 10524	Royal Library of Belgium	1200–1300	169 × 117	418	0.087–0.147	3	France (north), Low Countries, or England	Calf
RLB 37–39	Ms 10545	Royal Library of Belgium	1200–1300	221 × 149	462	0.031–0.105	3	France (north)	Calf
RLB 40–42	Ms 10753	Royal Library of Belgium	1200–1300	211 × 147	550	0.06–0.083	3	France (north)	Calf
RLB 43–45	Ms 10860	Royal Library of Belgium	1200–1300	180 × 124	604	0.056–0.104	3	France (north) or Low Countries or England	Calf and goat
RLB 46–48	Ms 14680	Royal Library of Belgium	1200–1300	143 × 95	545	0.06–0.083	3	France (north)	Calf
RLB 49–51	Ms 21700	Royal Library of Belgium	1200–1300	125 × 91	583	0.05–0.084	3	France (north)	Calf
RLB 52–54	Ms 2663–64	Royal Library of Belgium	1200–1300	193 × 133	456	0.087–0.124	3	France (north)	Calf
RLB 55–57	Ms IV 290	Royal Library of Belgium	1200–1300	244 × 175	478	0.135–0.211	3	Spain?	Goat
UPenn 02	Ms. Codex 236	Schoenberg Institute, University of Pennsylvania	1235–1240	218 × 148	465	—	1	France	Calf
UPenn 03	Ms. Codex 1065	Schoenberg Institute, University of Pennsylvania	1240–1250	178 × 117	356	—	4	England?	Goat
DNA UPenn 04–06									
UMLB 07–08	Bible Vulgata RARES 091 B47C	University of Melbourne	1250–1300	130 × 90	595	—	2	France or England	Calf
UVA 15	M.MS.N	Albert and Shirley Small Special Collections Library, University of Virginia	1200–1300	158 × 95	603	0.07–0.07	1	France	Calf
VV 17–18	Holkham Hall MS 10	Holkham Hall	1240–1260	226 × 152	—	—	2	France	Calf
VV 19–20	Holkham Hall MS 11	Holkham Hall	1200–1300	178 × 134	—	—	2	France	Calf
VV 21–22	Holkham Hall MS 12	Holkham Hall	1200–1300	185 × 123	—	—	2	France	Goat
VV 23–25	Holkham Hall MS 13	Holkham Hall	1230–1250	225 × 144	—	—	3	France	Goat
VV 26–27	Holkham Hall MS 1	Holkham Hall	1300–1400	163 × 108	—	—	2	England	Calf
YML 04–06	XVI.N.6	York Minster Library	1225–1275	203 × 143	333	0.1–0.14	3	England	Calf
YML 13–15	XVI.N.5	York Minster Library	1200–1250	200 × 140	420	0.07–0.11	3	France	Calf
YML 16–18	XVI.N.10	York Minster Library	1225–1275	151 × 99	665	0.06–0.1	3	France?	Calf

Table S3. Sample details for seven 13th century nonpocket pandect Bibles

Sample no.	Shelf-mark	Library	Date	Size, mm	No. of leaves	Thickness, mm	No. of pages sampled	Location of production	Species
CL 07–08	Add.7801	Cambridge University Library	1225–1275	243 × 164	515	0.08–0.12	2	France	Calf
DCL 01–02	C.III.22	Durham Cathedral Library	1200–1300	342 × 233	438	—	2	England	Sheep
DCL 03–04	A.II.3	Durham Cathedral Library	1200–1300	415 × 265	415	—	2	Northern France	Calf
RLB 58–60	Ms 9111–9114	Royal Library of Belgium	1286–1286	421 × 314	972	0.125–0.28	3	Germany	Calf
RLB 61–63	Ms II 2523	Royal Library of Belgium	1200–1300	495 × 358	588	0.186–0.261	3	Low Countries	Calf
U Penn 04	Ms. Codex 724	Schoenberg Institute, University of Pennsylvania	1275–1299	375 × 246	330	—	1	France	Calf
YML 01–03	XVI.D.13	York Minster Library	1200–1250	277 × 195	326	0.09–0.22	3	England	Sheep

Table S4. Sample details of English archival documents held at the Borthwick Archive, York

Sample no.	Shelf-mark	Date	Country	Type of document	Species
BA 61	YM/D/CAMP 1	1322–1322	England	Legal deed	Sheep
BA 26	MOR 2	1326–1326	England	Legal deed	Sheep
BA 81	YM/D/PO	1330–1330	England	Legal deed	Sheep
BA 41	YM/D/ASK 1	1332–1332	England	Legal deed	Sheep
BA 141	YM/D/HAT 1	1376–1376	England	Legal deed	Sheep
BA 06	YM/D/BAL a. 1	1377–1377	England	Legal deed	Sheep
BA 27	MOR 3	1380–1380	England	Legal deed	Sheep
BA 01	YM/D/BAL(M) 1	1382–1382	England	Legal deed	Sheep
BA 28	MOR 4	1392–1392	England	Legal deed	Sheep
BA 21	BED 1	1393–1393	England	Legal deed	Sheep
BA 42	YM/D/ASK 2	1404–1404	England	Legal deed	Sheep
BA 29	MOR 6	1415–1415	England	Legal deed	Sheep
BA 62	YM/D/CAMP 2	1427–1427	England	Legal deed	Sheep
BA 30	MOR 7	1443–1443	England	Legal deed	Sheep
BA 82	YM/D/PO	1447–1447	England	Legal deed	Sheep
BA 31	MOR 8	1448–1448	England	Legal deed	Sheep
BA 32	MOR 10	1455–1455	England	Legal deed	Sheep
BA 63	YM/D/CAMP 3	1456–1457	England	Legal deed	Sheep
BA 136	YM/D/YOR 1	1466–1466	England	Legal deed	Sheep
BA 33	MOR 11	1481–1481	England	Legal deed	Sheep
BA 142	YM/D/HAT 2	1484–1484	England	Legal deed	Sheep
BA 43	YM/D/ASK 4	1492–1492	England	Legal deed	Sheep
BA 44	YM/D/ASK 7	1494–1495	England	Legal deed	Sheep
BA 83	YM/D/PO	1503–1503	England	Legal deed	Sheep
BA 64	YM/D/CAMP 4	1507–1507	England	Legal deed	Sheep
BA 34	MOR 12a	1511–1511	England	Legal deed	Sheep
BA 09	YM/D/BAL a. 5	1513–1513	England	Legal deed	Sheep
BA 161	YM/D/OUS 1	1518–1518	England	Legal deed	Sheep
BA 137	YM/D/YOR 2	1520–1520	England	Legal deed	Sheep
BA 10	YM/D/BAL a. 6	1525–1525	England	Legal deed	Sheep
BA 22	BED 2	1529–1530	England	Legal deed	Sheep
BA 35	MOR 13	1534–1534	England	Legal deed	Sheep
BA 139	YM/D/YOR 6	1537–1537	England	Legal deed	Sheep
BA 65	YM/D/CAMP 5 (b)	1540–1540	England	Legal deed	Sheep
BA 101	YM/D/LANC 1	1542–1542	England	Legal deed	Sheep
BA 102	YM/D/LANC 2	1546–1546	England	Legal deed	Sheep
BA 66	YM/D/CAMP 10	1547–1547	England	Legal deed	Sheep
BA 144	YM/D/HAT 5	1549–1550	England	Legal deed	Sheep
BA 121	YM/D/KIRK 1	1552–1552	England	Legal deed	Sheep
BA 67	YM/D/CAMP 13	1554–1554	England	Legal deed	Sheep
BA 46	YM/D/ASK 11	1556–1556	England	Legal deed	Sheep
BA 84	YM/D/PO	1557–1557	England	Legal deed	Sheep
BA 85	YM/D/PO	1558–1558	England	Legal deed	Sheep
BA 103	YM/D/LANC 3	1561–1561	England	Legal deed	Sheep
BA 68	YM/D/CAMP 16	1563–1564	England	Legal deed	Sheep
BA 02	YM/D/BAL(M) 4	1565–1565	England	Legal deed	Sheep
BA 11	YM/D/BAL a. 9	1567–1568	England	Legal deed	Sheep
BA 47	YM/D/ASK 13	1571–1571	England	Legal deed	Sheep
BA 70	YM/D/CAMP 21	1573–1573	England	Legal deed	Sheep
BA 107	YM/D/LANC 9	1574–1575	England	Legal deed	Sheep
BA 03	YM/D/BAL(M) 10	1576–1576	England	Legal deed	Sheep
BA 124	YM/D/KIRK 4	1577–1577	England	Legal deed	Sheep
BA 109	YM/D/LANC 14	1579–1580	England	Legal deed	Sheep
BA 127	YM/D/KIRK 8	1580–1581	England	Legal deed	Sheep
BA 48	YM/D/ASK 16	1581–1581	England	Legal deed	Sheep
BA 87	YM/D/PO	1582–1582	England	Legal deed	Sheep
BA 169	YM/D/OUS 9	1586–1587	England	Legal deed	Sheep
BA 170	YM/D/OUS 10	1589–1589	England	Legal deed	Sheep
BA 14	YM/D/BAL a. 18	1591–1591	England	Legal deed	Sheep
BA 130	YM/D/KIRK 12	1592–1593	England	Legal deed	Sheep
BA 153	YM/D/HEN 5	1595–1596	England	Legal deed	Sheep
BA 154	YM/D/HEN 6	1596–1597	England	Legal deed	Sheep
BA 115	YM/D/LANC 24	1598–1598	England	Legal deed	Sheep
BA 52	YM/D/ASK 21	1599–1599	England	Legal deed	Sheep

