The effect of histological processing on sheep skin samples

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An experiment was undertaken to determine the amount of surface area shrinkage and its effect on follicle density estimations in sheep skin samples. Two methods were compared, namely the traditional formalin fixation, dehydration and wax-embedding technique as compared to the sectioning of fresh frozen skin samples with a cryostat. Surface area deminished by 20% and 21% after progessing with the two methods respectively. Significant differences were obtained in follicle densities (53,00/mm^2 and 44,06/mm^2 for the two techniques respectively).

Oppervlaktekrimping van skaapvelmonsters en die invloed daarvan op die bepaling van follikeldigthede is ondersoek. Twee histologiese metodes is toegepas en vergelyk, naamlik die tradisionele formalien fiksering, dehidrering en wasinbeddingsmetode teenoor die sny van vars velmonsters met 'n vriesmikrotoom. Die oppervlakte het na prosessering met 20% en 21% onderskeidelik afgeneem. Betekenisvolle verskille in follikeldigthede is waargeneem (53,00/mm^2 en 44,08/mm^2 vir die twee tegnieke onderskeidelik).

Keywords: Shrinkage, follicle density, sheep skin, histology

The aim of this study was to determine the shrinkage of skin samples from crossbred sheep using two histological processing techniques in order to estimate a possible correction factor for determination of follicle density in skin of live sheep.

Skin samples were taken from 47 crossbred rams according to the method described by Carter & Clarke (1957). Histological methods for the conventional formalin-fixed, wax-embedding method, outlined by Carter & Clarke (1957) and Gouws (pers. comm.) were followed. All sheep were sampled on the straight line between the hip (*Acetabulum*) and the shoulder bone (*Tuber scapulae*) and on the third rib cranial to the last thoracic vertebra. Two samples were taken on both right and left sides with a biopsy punch. Thus two samples were available from each sheep for application of the two histological methods. For freezing microtomy fresh skin samples were quickly frozen to –72°C such that the original state of the samples was retained. Two of the samples were sectioned at –20°C.

The experimental procedure is explained in Figure 1. Results of area measurements were obtained by measuring the circumference of each sample after each relevant procedure. The area was consequently calculated by formula. These measurements are presented in Table 1. The skin thickness of samples destined for the respective techniques did not differ significantly because duplicates for both methods originated from the same sheep.

Shrinkage of surface area of the wax-embedded samples (20%) did not differ significantly from that of the frozen sectioned samples (21%). Carter (1939) speculated that errors of measurements of skin tissue due to sampling and dehydration were insignificant, had it been carefully processed. The shrinkage percentages

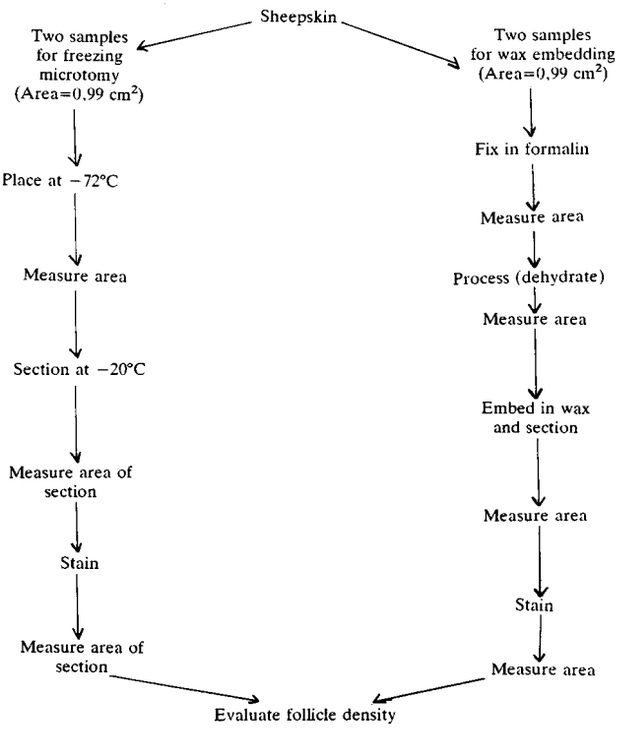

Figure 1 Method of determining shrinkage and follicle density during two histological techniques of processing sheep skin samples

Table 1 Differences of surface area during histological processing of sheep skin, follicle density and skin thickness

Procedure	Area (cm^2)					Shrinkage %	Follicle density (/mm^2)	Skin thickness (mm)
	Fresh sample	After fixation	After dehydration	After sectioning	After staining			
Wax embedding	0,99	1,12	1,0	0,69	0,79	20	53,00	2,50
Frozen sectioning	0,99	1,02[a]	–	0,76	0,78	21	44,06	2,00

[a] Measured on frozen sample (–72°C)

experienced in this experiment, however, were significant (Table 1). Carter & Dolling (1954) came to the conclusion, that the contraction of the biopsy specimen of cattle skin seems to be inversely related to the population density of hair follicles. Carter & Clarke (1957) recommended an adjustment for this shrinkage in order to reduce the follicle population estimates to those normally existing in the original living skin at the moment of biopsy. The factor of adjustment for sheep applied by these authors was a_1/a_2 where a_1 is the estimated area of the mounted and stained section and a_2 is the nominal area of the fresh skin specimen defined by the biopsy punch. The factors determined by above-mentioned authors ranged from 0,3 in sheep with low follicle population densities to 1,1 in sheep with high follicle population densities. The results in this study (0,79 and 0,78 for the wax embedding and frozen sectioning respectively) compare well with the expected values of Merino's (0,6 – 0,7) as defined by Carter & Clarke (1957).

The skin samples subjected to either technique maintained their surface area until sectioning was undertaken (Table 1). This observation was unexpected, as dehydration before wax embedding and sectioning, as well as freezing before sectioning should reduce the surface area of the samples. Staining did not affect the surface area of the frozen sectioned samples, whereas the area of the wax-embedded samples was increased highly significantly (Table 1). From these results the conclusion must be drawn that wax infiltration into the skin sample and the effect of wax embedding causes the sample to shrink to a larger extent as compared to the freezing method applied to the other half of the available samples. After dewaxing during the staining process, the sections regained surface area (0,79 cm^2), whereas the sections which underwent frozen sectioning did not increase significantly in surface area (0,78 cm^2) during the staining process.

Follicle density was significantly higher in samples processed by the wax-embedding technique (53,99/mm^2) as compared to the sectioning of frozen samples (44,06mm^2). The difference occurred although duplicate samples of the same sheep were processed and analysed in both techniques. The sectioning of fresh frozen specimens caused difficulties in evaluation as follicle populations became poorly distinguishable. The procedure of mounting the specimen absolutely vertical in order to obtain perfect horizontal sections for evaluation, is a necessity. The position of the specimen in the freezing microtome is difficult to adjust, which resulted in the mentioned distortions. The differences in follicle densities between the two techniques could thus be ascribed to these distortions.

The results of this experiment compare well with the correction factor proposed by Carter & Clarke (1957). Furthermore the conclusion can be made that the wax-embedding technique would produce more accurate results in the determination of follicle density than the freezing microtome technique.

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