Follicle depth, straight length, and curved length in plain and wrinkly sheep

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27 Aug 2018

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2 Materials and Methods

2.1 Sheep studied

This is a small study of 36 sheep from two Flocks, 18 from each Flock. Wthin each Flock there were 9 sheep chosen to represent the "loose" Skintype, and 9 sheep chosen to represent the "wrinkly" Skintype.

On each sheep 25 follicles chosen at random were measured on a vertical skin section taken fron a skin biopsy sample obtained by the technique of Maddocks and Jackson (1988) [8]. The measurements were of follicle depth, t, and h, as defined below.

2.2 Measurements

The following measurements and scores were available

SkinType visual scores for sheep skin type. Two grades "loose" and "wrinkly".

Follicle depth perpendicular distance from skin surface to bottom of follicle bulb

Follicle straight length(t) distance from skin surface to bottom of follicle bulb, measured at the angle of the follicle to the surface.

Follicle sagitta(h) If the straight follicle length is considered to be the chord of a circle, the sagitta is the height of the arc above the centre of the chord.

From these measurements it was possible to calculate

Radius of curvature of follicle given by $R = h/2 + t^2/(8h)$

Angle subtended by the arc given by $\theta = 2asin(2/(2R))$

Follicle curved length given by $S = R\theta$

The measurement of fibre curvature used by the wool industry is the reciprocal of radius of curvature and is in degrees per mm. The reciprocal of our R would be in radians per mm.

2.3 Statistical analysis

Data were imported into the R statistical program [12] and analysed using the *aov()* function for analysis of variance.

Table 1: Means for follicle measurements separately for each Flock and each Skintype

Flock	Skin.type	Folldepth	Straightlen	Curvlen	Radcurv
1	loose	91.95	91.37	91.95	348.5
2	loose	92.68	92.32	92.68	449.5
1	wrinkly	111.10	106.80	111.10	146.2
2	wrinkly	101.74	96.23	101.74	107.5

3 Results

3.1 Means

Means for each Skintype within each Flock are given in Table 1

All four traits (Follicle depth, straight follicle lenfgth, curved follicle length, and radius of curvature) would appear to differ substantially between loose and wrinkly Skintypes, but the two Flocks would seem to be similar.

We do not present standard deviations or standard errors here. The experimental design has multiple error levels. There are two random effects, Sheep, and Follicles within Sheep. Each of these contributes to the standard error of a mean. We need to estimate variance components for Sheep and Follicles to do the standard errors properly.

However we can test the significance of the Skintype difference and the Flock difference with an appropriate analysis of variance. We show these analyses of variance in Table 3.1   The first thing to note is that there are multiple error levels - corresponding to variation between sheep and variation between follicles within a sheep. The appropriate error level for testing Flock and Skintype differences is Error:Sheep - the line labelled Residuals. We can see that the analyses have used this error level for the F tests. These show that the Skintype difference was significant for all four traits, althought the probability level for Follicle straight length was only .027 (ie between 1 percent and five percent significance). None of the four traits showed a significant Flock difference.

This result is , of course, what we expected to find. Based on our understanding of the histology of wrinkle formation, we expect that wrinkled sheep will have a layer of hard collagen in the dermis which interferes with the downward growth of follicles from the dermis (ie reduces their length) and which also causes the downgrowing follicles to curve sideways in an attempt to complete their length growth (ie increases Radius of curvature). So these results support our hypothesis regarding the role of collagen in wrinkle formation and its effect on developing follicles.

3.2 Variance components

With two Error levels (Sheep and Follicles within sheep) we need to estimate variance components for each level, in order to calculate the standard error of

a mean. In Table 3.1 the mean square for Residual1 is the Follicles withn sheep variance component(σ_F^2). To get the Sheep variance component σ_S^2 we calculate

$$\sigma_S^2 = (MS(sheep) - MS(follicleswithinsheep))/N_F$$

where N_F is the number of follicles per sheep.

We can then calculate the variance of a mean as

$$Var(mean) = \sigma_S^2/N_S + \sigma_F^2/(N_S N_F)$$

where N_S is number of sheep in the mean and N_F is number of follicles per sheep. This gives the variance of a mean. To get a standard error we take its square root.

Table 3 gives the variance component estimates calculated as above for each of the four traits, plus a standard error calculated for a mean of 9 sheep with 25 follicles per sheep.

These are the standard errors which apply to the means given in Table 1 The significance tests in Table 3.1 indicate a higher level of significance because they are for the Skintype difference averaged across both Flocks - ie they correspond to means of 18 Sheep rather than 9. We have not presented the means for Skintype differences averaged across both Flocks, so we present these and their standard errors in Table 4

We see that these means are indeed significantly different, as indicated in the analyses of variance in Table 3.1.

3.3   Correlations

Figure 1 shows a scatterplot of curved length of follicles against follicle depth.

It suggests that follicle depth is a rather poor indicator of the curved length of a follicle. The points seem to cluster at or below the 1 to 1 line, indicating that curved length is either equal to or longer than follicle depth, depending on how much the follicle is curved. In this situation a correlation is not a good descriptor.

Figure 2 shows a similar scatterplot of curved length against straight length of follicles.

We see a similar picture to Figure 1 but with much closer agreement. The points again cluster at or below the 1 to 1 line. Curved length is either equal to straight length or slightly longer. Straight length is a better indicator of curved length than is follicle depth, but it misses out if the follicles are curved.

Figure 3 shows a scatterplot of curved length against radius of curvature.

Here we see something radically diffferent. Why the stepped spacing of points, especially those from the loose Skintype with high radius of curvature. Well, the issue is that measuring the follicle sagitta (h) gets to be rather imprecise when the follicle is not curved. Values of 1, 2, 3 for the sagitta produce the grouping effect when converted to radius of curvature.

What Figure 3 shows is that wrinkly sheep have curved follicles that may also be long, while loose sheep have straighter follicles that on average are shorter.
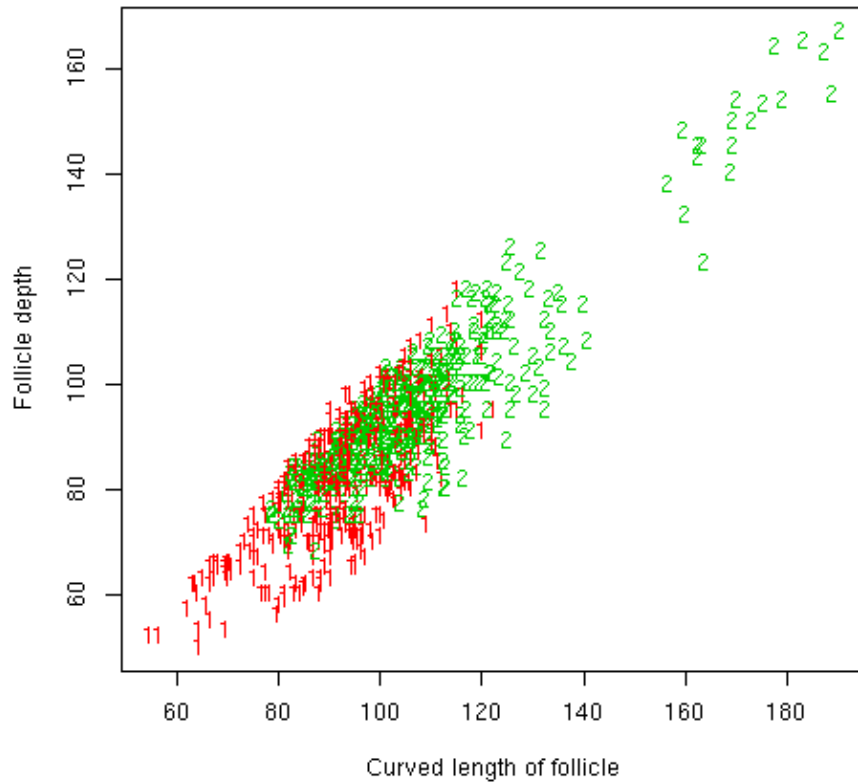
Figure 1: Plot of follicle depth against curved length of follicles. The loose and wrinkly skintypes are shown as points labelled 1 (red) and 2(green) respectively.

This is not quite what we expected. Those very long follicles in the wrinkly group bear further investigation.

The relationship between length and curvature is complicated. The points occupy the bottom left corner of the plot. Clearly there are other factors involved.
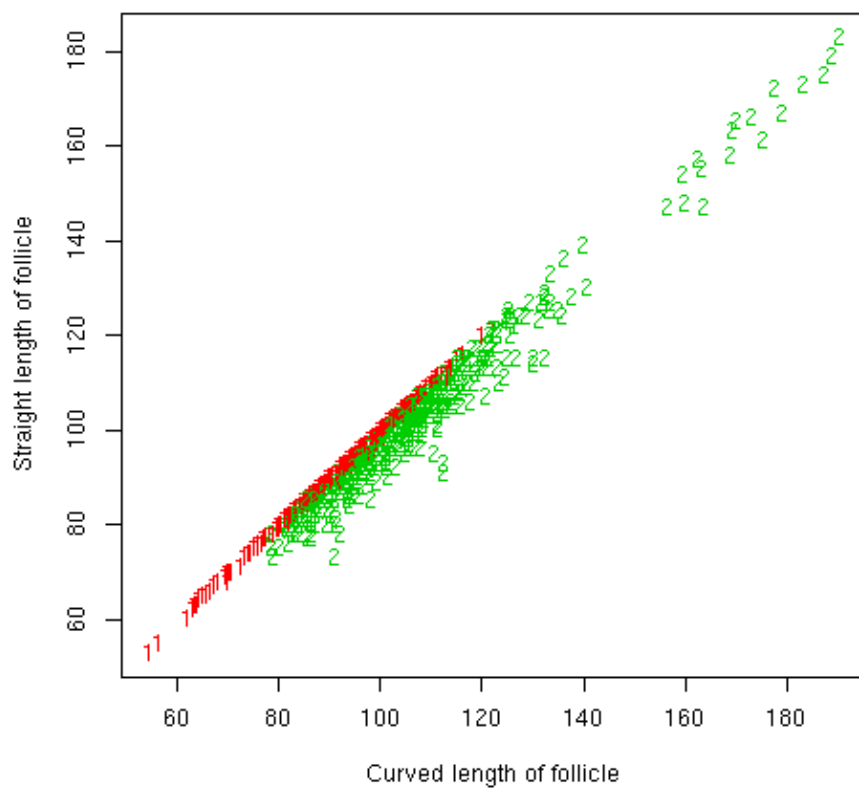
Figure 2: Plot of straight length against curved length of follicles. The loose and wrinkly skintypes are shown as points labelled 1 (red) and 2(green) respectively.

Table 2: Analyses of variance of Follicle depth, Follicle straight length, Follicle curved length, and Follicle radius of curvature

		Follicle depth			
Effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Error: Sheep					
Flock	1	1.08	1.08	0.00	0.9861
Skin.type	1	35822.56	35822.56	10.16	0.0031
Residuals	33	116320.51	3524.86		
Error: Within					
Residuals1	860	59641.23	69.35		
		Follicle straight length			
Effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Error: Sheep					
Flock	1	5218.12	5218.12	1.32	0.2591
Skin.type	1	21042.58	21042.58	5.32	0.0275
Residuals	33	130612.06	3957.94		
Error: Within					
Residuals1	860	66226.12	77.01		
		Follicle curved length			
Effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Error: Sheep					
Flock	1	4251.50	4251.50	1.03	0.3180
Skin.type	1	44711.74	44711.74	10.81	0.0024
Residuals	33	136454.62	4134.99		
Error: Within					
Residuals1	860	75424.61	87.70		
		Follicle radius of curvature			
Effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Error: Sheep					
Flock	1	239208.35	239208.35	1.31	0.2603
Skin.type	1	16554724.49	16554724.49	90.79	0.0000
Residuals	33	6017368.02	182344.49		
Error: Within					
Residuals1	860	20509483.53	23848.24		

Table 3: Sheep and follicle within sheep variance components for four traits. Standard error of a mean of 9 sheep with 25 follicles per sheep

	Folldepth	Straightlen	Curvlen	Radcurv
Sheep component	138.22	155.24	161.89	6339
Follicles component	69.35	77.01	87.70	23848
Standard error of mean	3.95	4.19	4.28	28.46

Table 4: Means and standard errors for follicle measurements separately for each Skintype

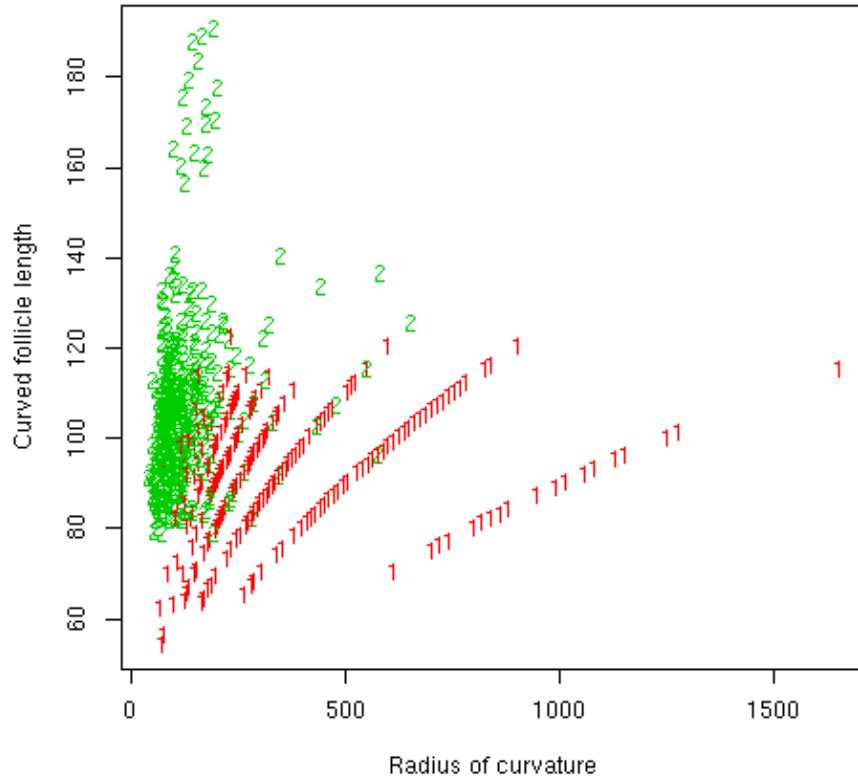| Skin.type	Folldepth	Straightlen	Curvlen	Radcurv
Mean loose	82.5	91.86	92.32	399.0
Mean wrinkly	95.14	101.6	106.5	127.1
Standard error	2.79	2.96	3.03	20.12

Figure 3: Plot of radius of curvature against curved length of follicles. The loose and wrinkly skintypes are shown as points labelled 1 (red) and 2(green) respectively.

4 Discussion

We wanted to show that wrinkled sheep have curved follicles, and we wanted to use something other than a subjective score for follicle curvature. We have achieved that - the radius of curvature differs significantly between loose and wrinkly sheep.

There are also differences in follicle length, regardless of how it is measured. The wrinkled sheep have longer follicles. This begs an explanation. If the presence of collagen in wrinkled sheep interferes with follicle development causing them to grow curved, that is one thing. But how would presence of collagen cause follicles to grow longer? Perhaps the folding of the epidermal and dermal layers which occurs as wrinkles form 'stretches' the follicles. Perhaps the collagen contracts and pulls on the follicles. Perhaps it is not a simple physical effect at all. Perhaps the collagen moves the papilla cells toward which the follicle downgrowth develops, so that the intital infolding of the epidermis and the papilla cells get out of line, causing the follicle to both curve sideways and grow longer in order to reach the papilla cells. We do not know.

So what needs to be done from here? Well we need to sort out whether the length of follicles is at all important in relation to fibre growth. There has been one experiment selecting for follicle depth (Jackson (2017) [4]) and it was a failure in relation to improving wool production. It changed follicle depth and fibre diameter and variance of fibre diameter. The experiment was started when there was a feeling that 'deep' follicles had a better blood supply and a more vigorous wool growth. That sort of thinking has since disappeared. 'Deep' follicles are just long follicles, not anything special. Having an elongted shaft is not going to help a follicle grow more wool.

There also needs to be some consideration as to whether follicle curvature is the same thing as intrinsic fibre curvature, and whether the current commercial curvature measurement technology (Laserscan or OFDA) measures intrinsic fibre curvature. It comes down to whether follicle curvature is the only factor determining intrinsic fibre curvature, or whether there are other factors perhaps related to the fibre cross sectional distribution of cortical cell types. Understanding the effect of collagen on follicle curvature is a half-way house. We need to know the effect of collagen on intrinsic fibre curvature, not the 'set' we observe in staple crimp, not some 'set' we measure under Laserscan od OFDA standardised measuring conditions, but the real intrincic curvature which is wahat a fibre can always be relaxed to and which is determined by different growth rates of the ortho and para cortical cells in a fibre with a bilaterally segmented cortex.

That being said, this study has progressed our understanding of why sheep with wrinkle and hard collagen differ from plain bodied sheep in wool growth. They differ because the presence of a layer of hard collagen in the dermis affects the size and shape of wool follicles. In our discussion we have explored possible mechanisms.

5 Conclusion

Of all the aspects discussed above, one stands out. Here is what we think is actually happening to follicle development in wrinkly sheep.

Follicles develop in the foetus between 60 and 150 days. Papilla cells migrate to or agglomerate at sites. Sites are thought to be determined by the collagen network. Above each site, at the skin surface, a downgrowth of epidermal tissue occurs, directed toward the site. The papilla cells at the site act as an attractor. Now, if the site moves sideways after the downgrowth commences, the partially completed downgrowth will curve toward the new site location, resulting in a curved follicle. The downgrowth will always be toward the attractor. The curved follicle will also need to be longer in order for the bulb to reach the papilla cells at the new site. What would cause the site to move? Well it would seem that development of hard collagen causes the sites to move. This would have to happen at the critical time while the follicle downgrowths were part complete.

Only P and So follicles have sites. So only P and So follicles would be affected by the 'moving site effect'. So look at Figure 3. For the wrinkly sheep (which are the green points) there are the P and So follicles with their curved length ¿ 140 and tiny radius of curvature, at the top left corner of the scatterplot. All the other green points are SD follicles; they do not have defined sites; they curve because the papilla cells aggregate around other follicles and they have to curve to belong to a common follicle opening. They are not long because the papilla cells aggregate close to other follicles.

So what happens in loose or non-wrinkly sheep? Well, the sites dont move, so the P and So follicles are not forced to curve to follow the papilla cells. So in Figure 3 there are the P and So follicles at the red points on the lower right corner of the scatterplot; they are quite straight (high radius) and normal length. The rest of the red points are loose sheep Sd follicles, and they are similar to the wrinkly sheep Sd follicles, because they form in the same way, and the collagen hardening does not interfere with Sd's.

So there we have it. Our 'moving site hypothesis' explains follicle size and shape, and how hard collagen affects follicle development. It does not explain why a curved follicle grows a curved fibre. That, however is quite simple - in order to form inside a curved follicle a fibre must have either more cortical cells or longer cortical cells on the side of the fibre cross section which corresponds to the outer side of the curve. This is simple material science, the outer side of a curved object has to be longer than the inner side. Once a fibre forms in that way, it retains the intrinsic curvature permanently. That is what we observe. Fibre curvature is a complicated phenomenon because, apart from this intrinsic curvature, fibres can be 'set' into other other degrees of curvature, for example in a staple where the crimp observed is not just intrinsic curvature but a complicated phenomenon involving stretching or unfolding of the intrinsic curvature combined with various setting conditions. Staple crimp is semi-permanent. Fibre intrinsic curvature is permanent (ie is always recoverable) and is the same thing as follicle curvature.

So why all this concern about crimp and curvature? Well, wrinkly sheep (and low density sheep with hard collagen that are not wrinkly, such as Downs Wool Breeds) all have characteristically highly crimped fleeces. This is one of the outstanding features of wrinkled sheep, so we must explain crimp and curvature, or we have not grasped the wrinkle issue.

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