

Development of the springbok skin — colour pattern, hair slope and horn rudiments in *Antidorcas marsupialis*

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In the foetal springbok skin, identifiable melanocytes are present before colour patterns are established. These pigment cells fail to appear in the pouch and belly areas, indicating that these zones are piebald and not albinotic. Areas of regional colour differences are sharply separated. Their distinctiveness is caused by a discontinuous change in brightness of the hairy coat. This is due to a stepwise shift in the average quanta of melanin in the hairs. The pigment itself does not alter its colour from one colour-zone to another, but the density of the pigment particles merely changes. It is suggested that the steps could correspond to a discontinuous difference in tissue dosage, resulting perhaps from differing activation among multiple melanizing genes. Anatomical peculiarities of the pouch area and horn rudiment are described. A proposal is made that hair slope is brought about by traction from mobile superficial fibroblasts. These cells travel preferentially in the direction of the greatest tissue strain. By dragging the papillary layer components along behind them, such as the hair buds and arrector pili muscles, the hair streams could arise. The tips of the hair shafts will then point away from each direction of greatest internal expansion, which the fibroblasts strive to cover.

By die fetale springbokvel word melanosiete identifiseerbaar voordat enige kleurpatrone neergelê word. Hierdie pigmentselle verskyn glad nie in die pronk- of maagareas nie. Dit dui op 'n blestoestand, en nie op albinisme nie. Gebiede van streekskleurverskille is skerp afgebaken. Hul kenmerke word deur plotselinge veranderinge in die helderheid van die pelshare veroorsaak. Die pigment self verander nie sy kleur van een kleurgebied na 'n ander nie. Net die pigmentdigtheid verskil. Daar word voorgestel dat hierdie stappe aan 'n trapsgewyse aktivering van melanserende gene toegeskryf mag word. Anatomiese eienskappe van die pronk- en die horingkiemgebiede word beskryf. 'n Voorstel word gemaak waardeur die helling van haarstrome aan die verskuiwing van oppervlakkige fibroblaste toegeskryf word. Hierdie selle beweeg in 'n rigting van maksimale stres, en sleep papillêre strukture agterna saam, soos die haarbotte en die arrector pili-spiere. Die punte van die haarskagte sal gevolglik van die gebiede van maksimum uitsetting wegwys, soos die fibroblaste moet beweeg om die oppervlak te dek.

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The skin of the adult springbok *Antidorcas marsupialis* is known for its elegant brown and white colour patterns, and also for its pouch or 'pronk' on the hinder part of the back. This distinctive pouch comprises a field of long white hair normally thatched over by brown hairs, but it will show itself as a blaze under conditions of display. These features, as well as other striking characteristics, are laid down in early foetal life.

No work has evidently been published on the development of the springbok skin. Nevertheless, Du Plooy (1973) investigated this subject, but his findings have lain obscurely in a departmental manuscript. Van Zyl & Skinner (1970) had already established standards for the weight, size and age of the springbok foetus. Gestation was found to last about 24 weeks. Using their data, Du Plooy calculated the foetal ages, ranging from 5 to 20 weeks and over, in a collection of 45 springbok foetuses. Part of this work was done in the writer's department, and consisted of a gross and microscopic study of pigment cells in the skin and skin appendages. Through the courtesy of J.D. Skinner, these findings are summarized here in an Appendix (q.v.).

Materials and Methods

In the present study, Du Plooy's slides were re-examined and extended by a further supply of springbok foetuses

(six specimens; 7–8 weeks to maturity). The adult springbok coat was studied on a tanned skin loaned from the Transvaal Museum. Hair samples from this skin were cleared in cinnamic aldehyde and examined microscopically under epi-illumination and by transmitted light. (With epi-illumination the light falls on the specimen from above through the microscope objective. It is comparable with visual perception of an object in incident light reflected from its surface.)

For colour analysis, a fibre-optic self-recording reflectance spectrophotometer (National Physical Research Laboratory, Pretoria) was used in the visible range of 400–700 nm. These reflectance curves were analysed by computer programme to transform them into data corresponding to the impression made on the normal human eye in sunlight. This is expressed by three values — (i) the dominant wavelength, (ii) the purity or dilution of the colour, (iii) the brightness or dimness, i.e. light energy flow to the eye.

A colour analysis was therefore made by reflectance spectrophotometry from five zones on the trunk of the springbok pelt. An average of six readings for each zone is given.

Scope of the present study

The aims of the present investigation were:

- to determine the type and source of colour differences in variously tinted zones of the springbok hairy coat;
- to examine the pouch area in the foetus for such peculiarities of structure as could be linked to its adult structure and function;
- to determine the origins and features of hair growth, colour and slope;
- to add a few observations on the horn primordium.

Results

For purposes of analysis the coat of the normal springbok can be divided into four main colour types (Kruger, Skinner & Robinson 1979), with usually quite sharp transitions between the adjoining areas. On the trunk are found:

- white (ventral, pouch, either side of the tail)
- light yellow-brown (legs, neck, flank)
- dark yellow-brown (back, and sides of pouch i.e. paravertebral)
- dark red-brown (stripe bordering white abdominal skin).

In the white hair of the pouch region the entire whiteness in the appearance arises by a general bright white reflection from the medulla. The medulla gives the impression of being encrusted everywhere with glistening white particles. It has a rippled surface and occupies roughly two-thirds to three-quarters of the hair diameter. No colour or other reflex comes from the narrow band of hair cortex lying on either side of the medulla as viewed by incident light. The thin outline of the cuticle is just detectable, but it is not coloured either. By transmitted light the medulla is opaque. The cortex contains no sharply distinctive melanosomes, although there are some vague brownish masses of a non-particulate type.

The tips of the hairs are tapered, and contain small spots of medulla, or none at all. The cortical cells are elongated and ribbon-like, and contain no pigment grains.

Pigment forms no integral part of these hairs. (Such hue as these hairs possess would apparently arise from non-particulate sources).

In the dark and lighter yellow-brown areas the main microscopic difference between these hair types lies in the differing proportions of the pigmented and unpigmented lengths of the outer facing of the hair, as well as the differences in pigment density. The lighter hair types also show many white hairs among the lighter pigmented hairs. The pigment particles form a pale and thin mantle in the cortex, insufficient to obscure the medulla when present. The tips of the hairs are dark, but below the tips the hair shafts are straw-coloured. The pigment continues for only a short distance, leaving the inner or basal portion white. Since some hairs contained almost no pigment, these lighter areas comprised a white- and straw-coloured hair mixture. In the darker areas the pigment is more prominent, and it spreads further down the hair shaft. With a sharper cut-off point below, the pattern reminds one of a bulrush. There is

also much more masking of the medulla by pigment.

Dark brown hair in the flank stripe is generally heavily pigmented, though variable in intensity, and the medulla is obscured. The pigment is concentrated on the outer facing and distal half of the shaft. In the depths of the shaft, most of the fully grown hairs are seen to be quite white where they emerge from the skin. The point at which the pigment ceases is variable, but in long hairs pigment is usually lacking in the proximal quarter to half of the hair.

Because of the overlap of hairs in the hairy coat, one finds that hair pigment is maximally deposited only on those regions of the hair shaft which are directly exposed towards the outside world, viz. the distal outer facing. Pigmentation of the hair is therefore clearly a property belonging to the externally visible part of the hair structure. There is evidently no expenditure of pigment on areas which would not be seen.

Colour analysis (Table 1)

Selected topics

White areas

Several features demonstrate that the white areas are piebald and not albinotic. In mammals generally, piebald areas are distributed along the dorsal (including frontal) midline, and on the belly, as well as in the 'stocking' area. This is roughly the case in the springbok. The microscopic absence of pigment-producing or of 'clear' cells in the pouch and belly areas of our material was manifest throughout. Furthermore there was in some specimens a banking-up of melanocytes against the border between the darkly pigmented and the piebald pouch areas. In albinism by contrast the melanocytes migrate, and remain identifiable, but are not fully functioning.

At the transition between the pigmented flank band

Table 1 Colour analysis of major patterned regions of the springbok trunk

Region	Wavelength (dominant) (nm)	Purity (%)	Brightness (%)
Flank (dark brown)	592	37	3,9
Paravertebral	585	37,3	20
Paler yellow	584	26,1	35,4
Belly and pouch	582	8,6	68,5

Explanatory note to Table

The dominant wavelength is the pure spectral colour which the human eye attributes to the complex surface reflection. In all regions the colour lies in the yellow to orange range. The percentage purity indicates the extent to which the dominant wavelength is diluted by white. The colour in dark brown hair is seen to be purer than in the paler shades. Brightness denotes the combined energy of the reflected light from the surface in the visible range. A pure white surface is taken as 100%. It measures the degree of darkening irrespective of the colour values.

and the belly, in the almost-mature foetus, no banking together of melanocytes was noted. The epidermal dendritic cells became sparse and had lost their dendrites while the hair roots, which normally show about six compact melanocytes, contained no trace of them.

None of these colour transitions as seen in the embryo could be related anatomically to any of the visible parts of the vascular system. At the pouch area in a 7–8 week foetus, we observed that the neural tube comes closer to the surface, making an arrowhead-like whitening, corresponding in shape to the borders of the pouch. The relationship was interesting, and could possibly be related to a suspension of directional fibroblast trends discussed below.

Pouch area

The white hair of this region differs in that it is implanted into the skin at right-angles to the surface. At all other sites, the hair lies at a slope. Where the hair slopes, a fixed pattern determines the three-dimensional relation of muscles and glands to the hair follicle. In the pouch however (Figure 1), the arrector pili muscles run in bundles, in a craniocaudal direction, lying between the hair follicles and not evidently attached to them. At the age of 14 weeks there are apocrine glands on either side, to the left and right, of the hair shafts. Some neutrophils and larger mesenchymal cells were also to be seen. On either side of the pouch the epidermis (Figure 2) is pigmented, leaving the dart-like prospective pouch area pale. Microscopically in the dark areas the slope of the hair roots points away from the midline. The hair tips will therefore ultimately point towards the midline. No structural obstacle to the invasion of melanocytes was visible, but none enter the area. The growth directions of all visible skin components (hair, glands, muscle) change abruptly in the pouch area.

The anatomical arrangement explains the way that the white hair would be thatched over by the surrounding brown, but on stiffening the longitudinal arrector muscle



Figure 2 Unpigmented pouch area, as seen from 11 weeks onward. Melanocyte-occupied territory is distinctly dark. The dart-shaped pouch area remains pale, broadening towards the tail.

mass, the white hair would become bunched together and stand out. The porcupine fans its quills by contracting a solid muscle plate in a similar way.

Horn rudiment

The horn primordium was examined microscopically in a single section, and a few simple observations recorded. It differs from the surrounding epidermis in the following respects:

the epidermis is thicker, with a periderm of twice the surrounding periderm thickness. The melanocytes (clear cells) are much more plentiful, causing basal but not suprabasal layer pigmentation. A somewhat mucoid and poorly cellular papillary layer, with a stratum of capillaries at its lower level, was noted. At its hairy edge, the horn primordium dips in, forming an outer ring. Some of these features are shown under high power view in Figure 3.

Hair slope

In the available developmental stages of the springbok, the hair slope is already evident when the hair bud starts to grow inward.

Since the slope is co-ordinated over wide areas, a controlled traction in a consistent direction is needed to establish the regular phenomenon of hair slope, and the hair streams which are thus created by a co-ordination of the slope. Studying the foetal sections for the source for such a pull, the only system qualified for the task would be the moving set of interlinked fibroblasts in the dermis. One has no difficulty in excluding the periderm, epidermis, hair papillae and ground substance from consideration, since none of these structures possesses the ability of conducting physical forces in a given direction over wide distances.

The following microscopic features could be seen in the springbok sections available. (It should be noted that these cellular features are only seen with careful focus adjustment under oil immersion). In Figure 4 the

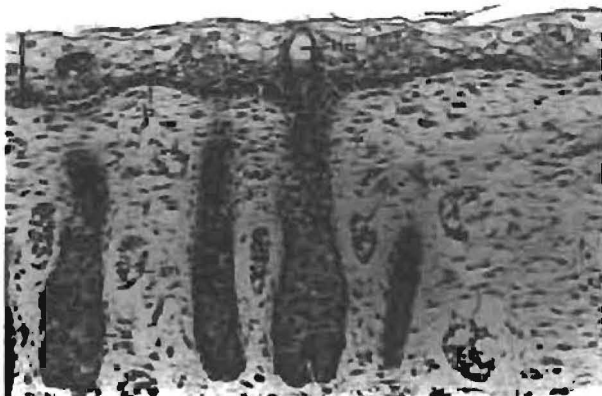


Figure 1 Transverse section through the pouch area at 14 weeks. Three vertical hair follicles are seen, surrounded in their lower halves by a mantle of sebaceous cells. The hair canal (hc) of the central shaft is visible. The epidermal basal layer (b) contains no clear cells. Muscle bundles (m) are interstitial.

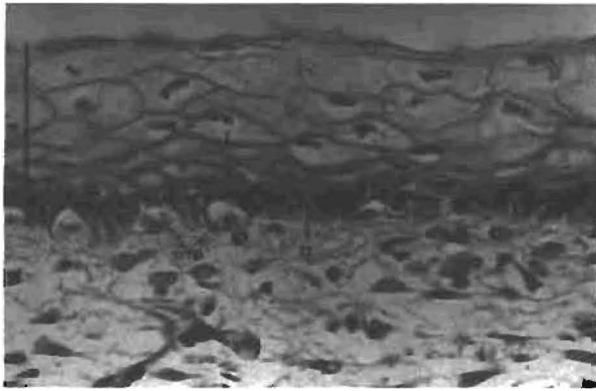


Figure 3 Horn rudiment at 13,3 weeks. A high-power view shows the special disposition of the basal layer, periderm and melanocytes in this region. Prominent melanocytes (me) occur in the basal (b) and epidermal (e) layer, and (c) indicates a cell wall in the periderm.

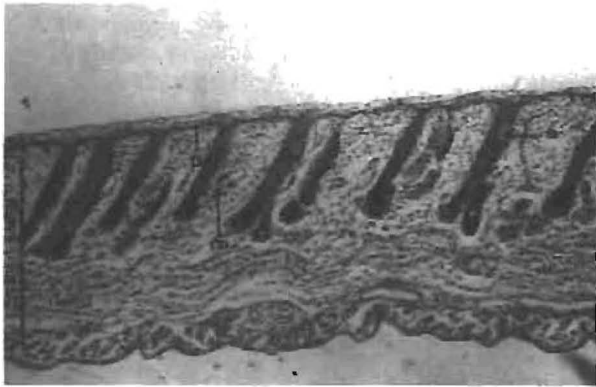


Figure 4 Pigmented side of the pouch area at 14 weeks showing hair slope. In the papillary layer (pl) of the dermis, the fibroblast streams run somewhat obliquely, parallel to the hair muscle (m). Sebaceous gland rudiment at (s).

fibroblasts and arrector pili muscles are arranged in lines between the acute and the obtuse angles of the hair slope.

- (i) Fibroblasts lying in the dermis between the epidermis and the superficial vascular plexus, were disposed with their long axes roughly horizontal in the early stages. Into this layer the hair follicles descend in sloping fashion.
- (ii) The fibroblasts were largely asymmetrical, when viewed at the angle of maximum hair slope.
- (iii) Their nuclei were placed asymmetrically in the cytoplasm, with a short unipolar cap of cytoplasm at one end and a trailing tangle of tendrils at the other. An array of cells was thus generated, with head-to-tail asymmetry. The cells were bipolar rather than stellate. Judging by the hair slope, the advancing edge would lie at the short cap of cytoplasm while the trailing portion would pull on the follicles dragging up the root and tilting the hair shafts to point in the opposite direction. The

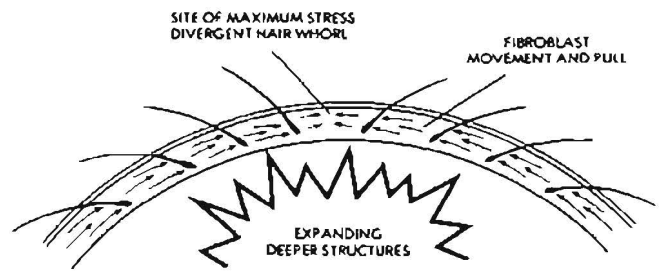


Figure 5 Diagram to illustrate a suggested correlation between internal growth, fibroblast migration and traction, and the production of hair slope with divergent hair whorls. The driving force in producing hair streams on the surface is the internal expansion of the embryo. This stretch leads to compensatory fibroblast spread with tissue contraction and consequent tilting of the hair.

arrector pili muscles would also tend to grow towards their insertion points in the same way.

This concept is illustrated in Figure 5 .

Discussion

In the springbok visual differences in the coat are attributable to melanin (differing in quantity and translucency but not in colour); to horny cells (an intrinsic pale yellow in white hairs visible only if the melanin is scanty or absent); and to the medulla (a diffusely scattering layer, which will contribute provided the melanin allows the light through sufficiently to reach it). Human colour vision would assess the colours of each zone as being 'different', but spectral analysis shows that the great changes are those of brightness, and not of actual hue. Brightness is directly altered by different quanta of melanin contained in the hair, with a corresponding masking or uncovering of the reflecting medulla. This does not imply that the power of camouflage is altered. If a predator is colour-blind, the contrasts in brightness will have the same disruptive effect.

Pigment deposits in the hair occupy a position which indicates a more important function in relation to light than to heat or temperature regulation.

The materials for colour production exist before the hair structures are fully developed. Later changes must determine the fashion through which colour differences arise. Hair pigmentation arises most probably by a 'demand' from the individual growing hair, timed and deposited in the hair according to its stage of growth. However, it is clear that the quanta of pigment supplied will depend on the regional placement of the hair.

Thus it appears that the demand for pigment must depend on the hair cycle but the supply depends on the hair placement for pattern production.

The colour pattern traced around the trunk of the springbok from the back to the belly changes in a series of zones, first from dark to light, and then from very dark to very light. While there is a limited colour variation in the individual hairs in any given zone, the mass effect is nevertheless sharply outlined and constant.

It expresses the stepwise fashion of hair melanization. The brightness of each of the three melanized zones lies at a distance of (roughly) 15% apart (see Table 1). In the visual sense the differences between them are quite abrupt.

Melanization has long been known to occur in the epidermis in steps or stages (*Stufen*, in German), giving rise in certain situations to several distinct grades of pigmentation. Since pigment is often controlled at more than one locus, these colour steps may possibly correspond to recruitment of differing genetic sources as the colour switches between one site and another. Patterns will thus appear on a notched scale rather than on a sliding scale, for which an epistatic system of genes may well be at work.

These considerations fail to take the fixed but regional nature of the pattern into account. A study in progress concerns the role of diffusible extracellular cues which may establish the melanocyte's functional level of pigment production.

Fibroblasts have the property of moving to cover an expanding growth beneath them, or to close a gap (wound) within their field. In either case they migrate towards the geometrical centre or the 'median chord' of a gap, or to the area of maximum stretch. It is likewise a property to pull up structures in the rear, best known from wound contraction. In foetal skin the growth direction of the superficial dermal connective tissue will be aimed towards the site where the stretch from the underlying expansion is greatest. The effect on the hair slope is to create a divergent whorl at the centre of expansion over a convex surface (Figure 5).

Our earlier attempts (Findlay & Harris 1977) to investigate the opposite theory, namely a growth centre in the middle of a divergent whorl in the human foetus, was a failure. The reason is now clear — growth takes place towards a divergent whorl and not away from it. Hair slope is therefore to be regarded as pointing away from the areas of greatest superficial stretch at the time of foetal expansion.

Some may question the possibility that migrating fibroblasts are capable of producing the necessary traction on surrounding structures. Besides wound contraction, fascial contractures (e.g. Dupuytren's contracture), and in foetal life the traction of the gubernaculum, causing migration of the mammalian testis, should not be overlooked as examples illustrating the possibilities of ordered contractility in fibroblasts.

Acknowledgments

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Appendix

Springbok skin development: Ages in weeks determined by calculation (formula of Huggett & Widdas 1951).

- 5 w Hair germs present.
- 6,5 w Follicle rudiments enlarge on head.
- 7,4 w Melanocytes visible; plentiful on eyelids. They are absent from the crest of the spine, the pouch area and belly. Per unit area, there are 15 melanocytes per follicle on the head.
- 8,1 w Stripe from upper lip to eye and to ear visible as a dark band of melanocytes. Horn rudiment visible but melanocytes few.
- 9 w Hair follicles visible at all potentially hairy sites. Melanocytes visible in grouped clusters in pigmented follicle regions but not in 'white' areas. Hooves becoming melanized. Epidermal surface shows ungrouped melanocytes.
- 9,9 w Intensification of pigment in all potentially pigmented structures. With increase of follicles, there is increase of hair clusters of melanocytes.
- 10,6 w Congregation and clustering of melanocytes along certain dark edges where there is diffuse pigmentation (eyelids, horn area except centrally, at edge of white belly skin). Increase in surface melanocytes. No pigment along crest of spine or pouch area.
- 11,2 w Increase in hair numbers.
- 12 w Hair emerges. Melanocytes, scattered and in clusters, lie in concentric circles round and on the horn rudiment. Clusters darker towards the horn edge.
- 12,4 w Melanocytes seen to stop at the edges of the belly and pouch areas.
- 13,3 w Pouch area contains unpigmented hairs in trios. Marginal hairs at submental pad are long and black.
- 14 w Unpigmented pouch hairs elongate.
- 15,6 w Increase in melanocyte clusters. Scattered melanocytes less obviously dendritic.
- 18 w Brown hair of the back overlies the white pouch hair.
- At later ages No distinctive additions.

In this material it was found that:

- (i) Pigment-producing melanocytes appear in the skin soon after the formation of the rudiments of hair, horn and hoof, to which they supply the melanin.

- (ii) Two sets of melanocytes are distinguishable — scattered epidermal cells and clustered groups in the hair rudiments.
- (iii) As the hairs multiply the clustered groups increase.
- (iv) Melanocytes are not evident in the pouch and belly areas.