

Tissue Collection Methods for Antler Research

C. Li and J.M. Suttie

AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand

Abstract

The rapid growth of deer antlers makes them potentially excellent models for studying tissue regeneration. In order to facilitate this, we have developed and refined antler tissue sampling methods through years of antler research. In the study, antler tissues were divided into three main groups: antler stem tissue, antler blastema and antler growth centre. For sampling stem tissue, entire initial antlerogenic periosteum (around 22 mm in diameter) could be readily peeled off from the underlying bone using a pair of rat-toothed forceps after delineating the boundary. Apical and peripheral periosteum/perichondrium of pedicle and antler could only be peeled off intact when they were cut into 4 quadrants and 0.5 cm-wide strips respectively. Antler blastema included blastema per se, and potentiated and dormant periosteal. Blastema per se was sampled after it was divided into 4 quadrants using a disposable microtome blade. Potentiated and dormant periosteal were collected following the same method used for sampling peripheral periosteum of pedicle and antler. The antler growth centre was divided with a scalpel into 5 layers according to distinctive morphological markers. The apical skin layer could be further separated into dermis and epidermis using enzyme digestion for the study of tissue interaction. We believe that the application of modern techniques coupled with the tissue collection methods reported here will greatly facilitate the establishment of these valuable models.

Keywords: Antler, pedicle, deer, blastema, antlerogenic periosteum, growth centre, tissue sampling, growth centre.

Introduction

The annual renewal of deer antlers offers a unique opportunity for studying mammalian epimorphic regeneration. The extraordinary rate of growth of antlers provides us with a rare

system where rapid cell proliferation is elegantly regulated without becoming cancerous. Thus the self-differentiation ability of antlerogenic periosteum can serve as a valuable model for stem cell research. Although the potential for using antlers in biomedical research has been appreciated for decades, the establishment of suitable models has been slow. This is partly because of the lack of a complete set of comprehensive methods for sampling different tissue types.

During our years of studying antler biology at Invermay, we have developed and refined tissue sampling methods using biopsy techniques and animal heads recovered after slaughter. The tissue samples collected using these methods have been successfully used for many techniques – transplantation, histology, immunohistochemistry, reverse transcription polymerase chain reaction, Northern blot analysis, *in situ* hybridization, 2-dimensional electrophoresis, and tissue and cell culture and co-culture *in vitro* and *in vivo*. In this paper, we describe these methods of tissue collection. General precautions should be taken when collecting tissue samples for different purposes, such as aseptic conditions for tissue or cell culture, RNase free when handling tissue samples for Northern blotting and *in situ* hybridization, and completion of tissue collection within the required time frame for gene expression studies. Importantly, if tissue samples are collected via biopsy, the procedure must comply with regulations pertaining to animal welfare. All the tissue collections described in this paper via biopsy were approved by Invermay Animal Ethics Committee.

Tissue collection

1. Antler stem tissue

The type of tissue that meriting the description ‘antler stem tissue’ is subject to debate. The periosteum overlying the two frontal crests (Fig. 1A) of a prepubertal deer has been

Received: 1 July, 2003

Accepted: 21 October, 2003

Address correspondence to: C. Li, AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand. E-mail: chunyi.li@agresearch.co.nz

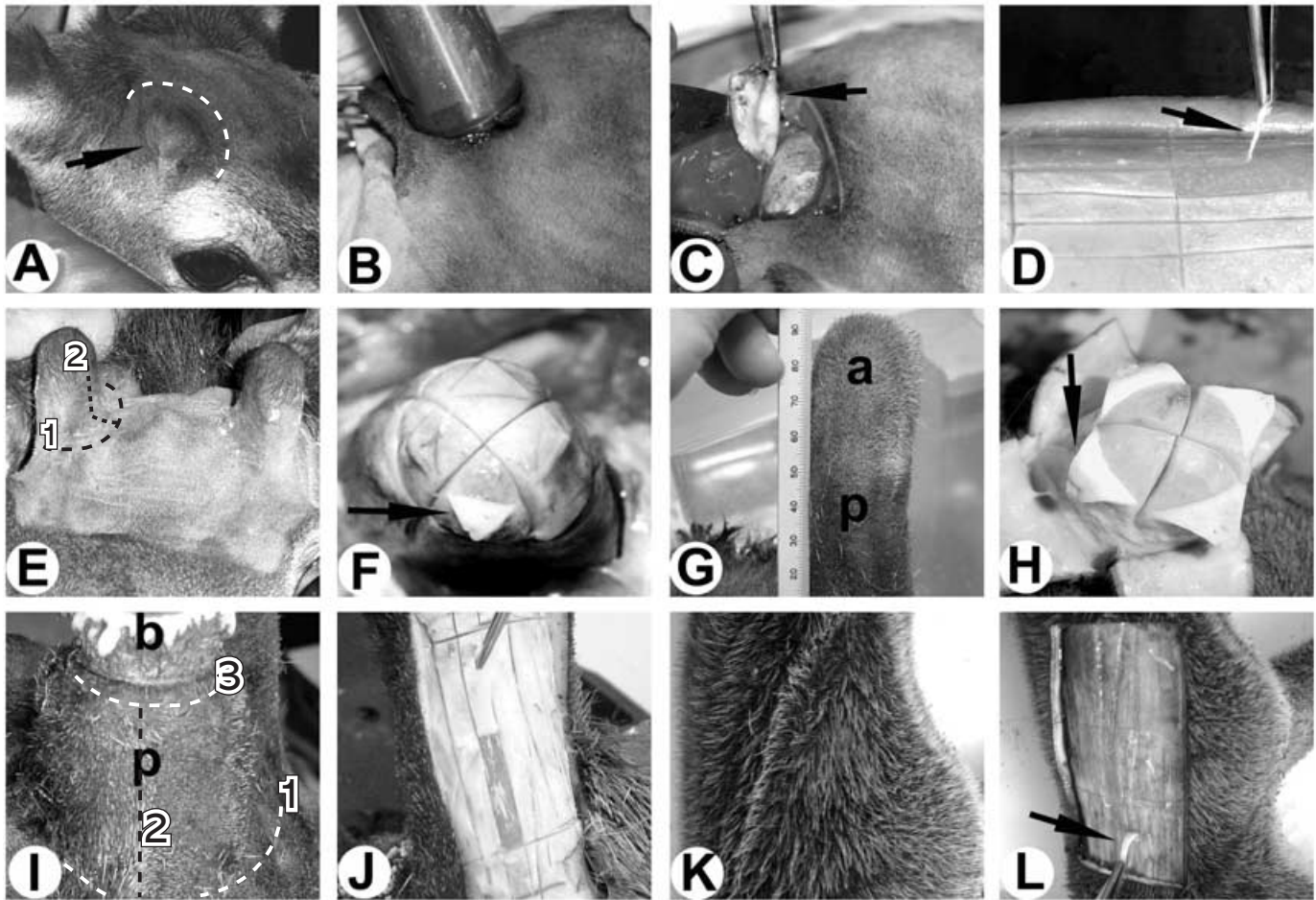


Fig. 1. Antler stem tissue sampling.

- A. A frontal crest (arrow) from a 4-month-old red deer stag calf. The broken line marks the incision made to expose initial antlerogenic periosteum.
- B. Territory of initial antlerogenic periosteum being marked using a 22 mm diameter punch.
- C. Initial antlerogenic periosteum (arrow) was detached using a pair of rat-toothed forceps.
- D. A piece of facial periosteum strip was peeled away using a pair of rat-toothed forceps.
- E. Mid-growth-stage pedicles from a 7-month-old red deer stag. Broken lines mark the skin incisions to expose apical and peripheral periosteum/perichondrium. 1, the first incision; 2, the second incision.
- F. The partly-detached apical perichondrium of a pedicle (arrow) divided into four quadrants.
- G. An early-growing-stage antler from an 8-month-old red deer stag. a, antler; p, pedicle.
- H. The partly-detached apical perichondrium of an antler (arrow) divided into four quadrants.
- I. A pedicle (p) with a hard button (b) on top from a 2-year-old red deer stag calf in September (spring in southern hemisphere). The broken lines mark the skin incisions to expose the peripheral periosteum of the pedicle. The first, second and third incisions are marked 1, 2, and 3.
- J. Peripheral periosteal strips from the pedicle were detached using a pair of rat-tooth forceps.
- K. A section from the mid main beam of a 60-day growing antler from a 3-year-old red deer stag.
- L. Antler peripheral periosteal strips were detached using a pair of rat-tooth forceps.

demonstrated to possess full antlerogenic potential (Goss & Powel, 1985). Removal of this periosteum from a presumptive antler growth region abolishes future pedicle and antler formation. Transplantation of this periosteum elsewhere on the deer body (Li & Suttie, 2001), or even to a nude mouse (Li et al., 2001) induces ectopic pedicles and antlers to grow. Therefore, this periosteum is called initial antlerogenic periosteum (IAP), and is the true antler stem tissue.

In addition to IAP, both the apical periosteum/perichondria and the peripheral periosteum from pedicles or antlers are also considered as antler stem or progenitor tissues. These tissues have undoubtedly further differentiated from the IAP, and may have partly or completely lost the ability to induce ectopic antler formation. However, each has specific properties that only belong to stem or progenitor tissues (see below).

For histology, immunohistochemistry or *in situ* hybridization, a scalpel should be used to remove the IAP, apical stem tissue or peripheral periosteum/perichondrium together with a thin layer of underlying tissue. For RNA extraction, cell culture or transplantation, these stem tissues should be separated from surrounding tissues and sampled separately. The methods described in the following sections mainly relate to the separate sampling of each type of stem tissue.

1. Initial antlerogenic periosteum (IAP)

a) Definition The time when IAP acquires full antlerogenic potential prior to pedicle initiation (7–9 month-old in red deer) is not known. However, this process may not be accurately determined solely by knowing the deer age, as pedicle initiation also relies on body weight (Suttie & Kay, 1982). We have successfully used IAP from 4-month-old stag calves with average body weight for our various studies including transplantation. Our results indicate that IAP from a well nourished 4-month-old red deer has acquired the potential to induce ectopic antler formation. Thus, IAP can be sampled for the aforementioned studies at any time from 4 months of age up to the time of pedicle initiation.

The actual boundary between deer frontal periosteum with and without antlerogenic potential has never been clearly delineated in the history of antler research. However, it is known that antlerogenic potential is not evenly distributed within the IAP and gradually decreases away from the centre of the presumptive antler growth region (Goss & Powel, 1985). Based on our studies, the highest antlerogenic potential in the IAP is located in the posterior half of each frontal crest. We have successfully carried out a variety of studies using samples of IAP up to 22 mm in diameter. This indicates that IAP can be sampled anywhere within this 22 mm in diameter region in red deer.

b) Sampling methods Before sampling, the hairs around the area of the presumptive antler growth region should be shaved, and the shaved area should be thoroughly cleaned and sterilized. A crescent-shaped incision is made through the scalp skin 2 cm medial to the frontal crest (Fig. 1A) ensuring that laterally-located, major blood vessels and nerves are avoided (Li et al., 1993). Frontal crests can be readily palpated and observed – which aids the placement of the incision. The skin can then be separated from the frontal bone by blunt dissection and reflected laterally to expose the IAP. Subcutaneous loose connective tissue can be trimmed away if necessary. The exposed IAP can be peeled away from the underlying bone with a pair of rat-toothed forceps following a scalpel incision or a punch (Fig. 1B) used on the periosteum. Compared to somatic periosteum, IAP is denser and more than three times as thick (Li & Suttie, 1994). Therefore, IAP can be readily peeled off using a pair of forceps (Fig. 1C).

Antlerogenic periosteum varies in size among deer species, as does the maturation time of IAP for antler induc-

tion. In order to sample true mature IAP from species other than red deer, the investigators should collect the periosteum from a region as near to the posterior half of a frontal crest as possible, and as close to the time of pedicle initiation time as practical.

As for all biomedical research, appropriate control tissue for the study of IAP or any other type of antlerogenic periosteum is indispensable. In our previous related studies, we used facial periosteum for this purpose. After IAP collection, a 4–6 cm long skin incision is made parallel to the midline of the nose on one side of the deer face. This incision is then continued medially from both ends, meeting in the midline at the nose. A flap of skin is separated by blunt dissection and reflected medially to expose one side of the nasal bone. Facial periosteum can only be peeled away from the underlying bone after it is cut into small strips (0.5×3 cm), as it is substantially thinner and more fragile than IAP. Each strip is then detached using a pair of forceps (Fig. 1D).

2. Pedicle apical or antler apical periosteum/perichondrium (PAP or AAP)

a) Definition Pedicle apical and antler apical periosteum/perichondrium (PAP and AAP) are actively proliferating tissues. Based on the results of our antler cell lineage tracing using a gene marker (Li & Suttie, 2001), almost every cell type in pedicle or antler tissue is differentiated from this tissue. The results of our BrdU labelling experiment (Li et al., 2002) showed that this tissue consists of 2 layers: outer and inner reserve mesenchyme. Outer reserve mesenchyme contains the cells that are mitotically quiescent, whereas inner reserve mesenchyme is essentially made up of proliferating cells. We hypothesized that outer reserve mesenchyme supplies cells continuously to the rapidly proliferating cell pool in the inner reserve mesenchyme. In turn, these proliferating cells further differentiate into a variety of cell types found in pedicles and growing antlers.

To study features of PAP (Fig. 1E) or AAP (Fig. 1G) or to make a comparison between them, it is critical to define precisely the stage at which PAP or AAP is sampled. In a previous study, we reported that pedicle elongation in red deer proceeds through three ossification stages (Li & Suttie, 1994). These are intramembranous (pedicle height, <1.0 cm), transitional (pedicle height, 1.0–3.0 cm) and endochondral (pedicle height over 3.0 cm) ossification. Antler elongation is achieved solely through endochondral ossification. PAP or AAP can be sampled using the following techniques according to the purpose.

b) Sampling methods Two incisions (coronal and sagittal) are made through the central point of the apex of a sterilized pedicle or an antler using a scalpel. These are extended proximally to about 2 cm in length and made deep enough to cut through the apical skin and leave the marks on the underlying

ing tissue. The four quadrants of apical skin are then reflected laterally to expose the PAP or AAP. Two further incisions are made on the PAP or AAP following the marks left from the initial skin incisions. These incisions must be deep enough to cut through the PAP or AAP. Then, the four quadrants of the PAP or AAP divided by these incisions on a pedicle (Fig. 1F) or an antler (Fig. 1H) can be easily peeled away from the tip where the 2 incisions meet, with a pair of rat-toothed forceps.

In our previous studies, we have sampled apical periosteum/perichondrium from 1 cm incipient pedicles to 10 cm long growing antlers (Li & Suttie, 1998; Li et al., 1999). Histological studies showed that PAP or AAP sampled following our procedure did not include underlying tissue (data not shown).

3. Pedicle peripheral or antler peripheral periosteum (PPP or APP)

a) Definition The peripheral periosteum of pedicles and growing antlers is a mitotically-quiescent tissue. However, once a pedicle or antler stump is created naturally or artificially with the enveloping skin, this type of periosteum has the full (pedicle) or partial (antler) potential to regenerate antlers. Therefore, these periosteum may also be called antler stem or progenitor tissues.

b) Sampling methods Collection of peripheral pedicle periosteum (PPP) from pedicles prior to transformation to antlers is undertaken by making a crescent-shaped incision through the scalp skin 2 cm medial to the base of a pedicle. A second incision is made on the medial surface of the pedicle. This cut starts 2 cm distal to the pedicle tip and terminates when it meets the first at the base of the pedicle (Fig. 1E). To expose pedicle bone, the enveloping skin can be separated from the bony core via the two incisions and reflected laterally using both hands. By so doing, major blood vessels and nerves located laterally are avoided. For pedicles carrying antlers or hard buttons (Fig. 1I), the first incision is made as above, but the second is located proximally just below the pedicle-antler junction. It terminates when it meets the first incision at the pedicle base. The third skin incision on the medial surface of the pedicle is made just below and parallel to the pedicle-antler junction at the level of the starting point of the first incision (Fig. 1I). PPP is exposed after the enveloping skin is reflected away. Again the major blood vessels and nerves supplying the pedicle and antler should be avoided. The exposed PPP from different developmental stage pedicles is then divided into strips around 0.5 cm in width along the longitudinal axis of the pedicle (Fig. 1J). The periosteal strips now can be readily peeled off with a pair of rat-toothed forceps.

APP is normally collected from the main beam of several antlers (Fig. 1K). Following velvet skin removal, the exposed APP is divided into strips (Fig. 1L) similar in size to those of PPP using a scalpel. We have found that it is harder to peel

APP away as strips as neatly as PPP, because the former is much thinner and more fragile than the latter.

2. Antler blastema

The rapidly regenerating tissue mass on a pedicle stump, which forms after previous hard antler casting, constitutes the biggest initial regenerating tissue mass in the animal kingdom (Figs. 2A,B). Antler blastema is crucial for investigating antler patterning and the mechanism underlying antler regeneration – the only example of mammalian epimorphic regeneration. This was recognised when Goss (1961) discovered the uneven distribution of antler growth potential in antler blastema and pedicles by deletion experiments. The results showed that removing the posterior half of an antler blastema truncates the subsequent antler from the level above the brow line, whereas deletion of the anterior half only affects brow line formation. Ablation of the medial half of an antler blastema causes no morphogenetic abnormality, whereas loss of the lateral half completely prevents antler growth. Thus, antler growth and patterning information are undoubtedly features of the early antler regenerating tissue.

1. Blastema per se

a) Definition The period within which regenerating antler tissue mass can be called antler blastema has not been clearly defined. For the purpose of the antler regeneration study, we have sampled regenerating tissue from the casting date of previous hard antlers to the stage before antler bifurcation can be readily visualized on top of the regenerating antler bud. The reason we include the hard antler casting date is because antler regeneration has already started sometime before the day of hard-antler casting.

b) Sampling methods For histology and gene expression studies, an antler blastema (Fig. 2C) is removed first using the New Zealand standard velvet harvesting procedures. Antler blastema is a soft and rubber-like tissue (Figs. 2C and 2D), that can be sampled easily. The isolated blastemas are divided into 4 quadrants using a scalpel or preferably a disposable microtome blade as shown in Figure 2D. These quadrants are anterolateral (la), posterolateral (lp), anteromedial (ma) and posteromedial (mp).

2. Blastema perichondrium and pedicle stump periosteum

The sampling procedure described above is simple and works well for evaluating the histological make-up and gene expression in different parts of an antler blastema. However, it is crucial to be able to dissect the tissue components of a blastema in order to address the fundamental question of what type of tissue or cells from a pedicle stump initiates and predominately participates in antler blastema formation.

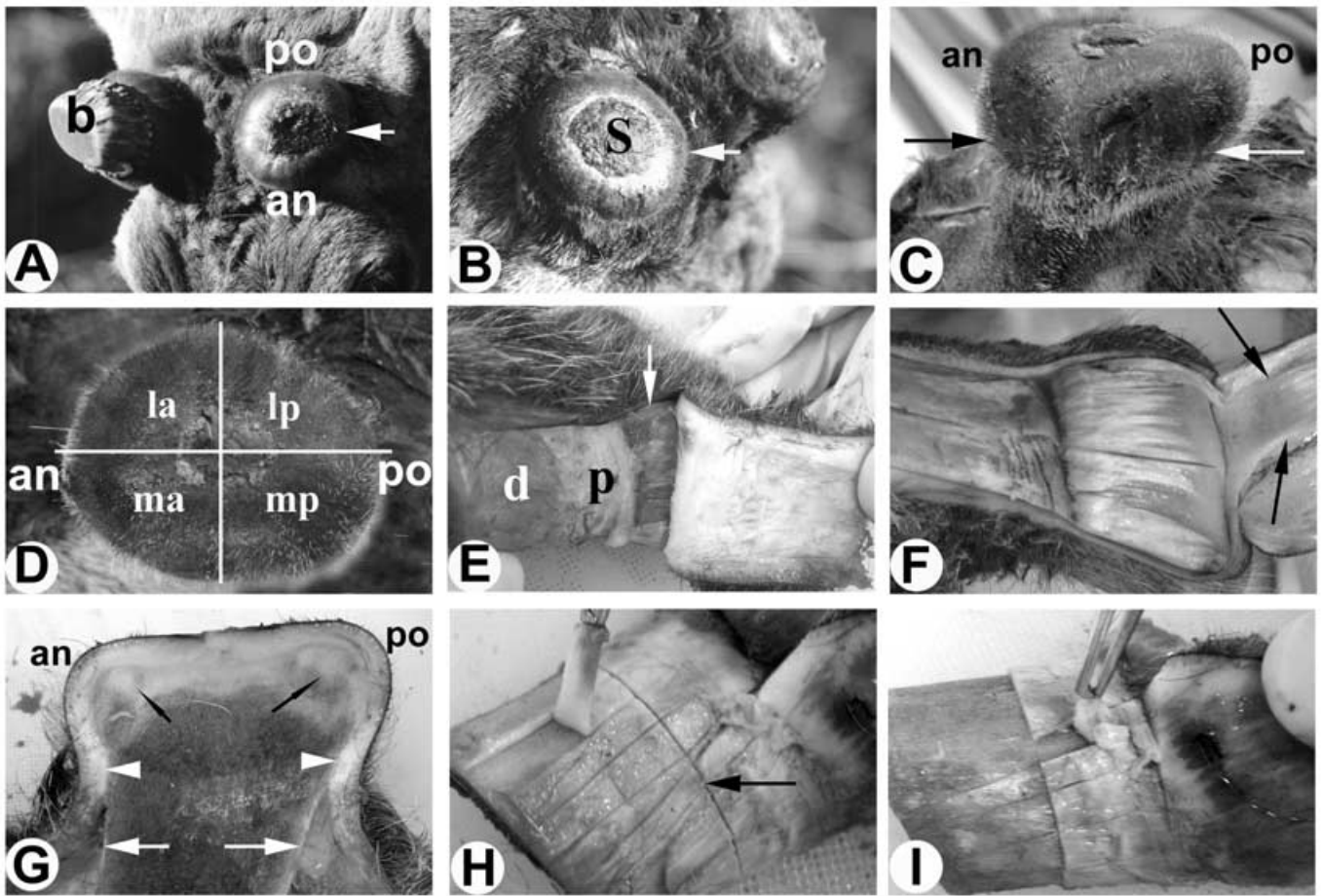


Fig. 2. Antler blastema tissue sampling from 3-year-old red deer stags.

- A. Superior view of red deer pedicles. The pedicle on the right of the photo is a stump created after previous hard antler casting. That on the left of the photo still had the hard antler button (b) attached. It is the remnant of the hard antler remaining following the surgical removal of the velvet antler during the previous season. Note that at the casting time of the hard antler or button, the healing process over a pedicle stump has already started (arrow). an, anterior; po, posterior.
- B. Top view of a pedicle stump (arrow) from a 3-year-old stag. A scab (S) was formed in the central region of the casting surface on the pedicle stump.
- C. Lateral view of a well-formed antler blastema. The boundary between the blastema and the pedicle is readily discernable (arrows). However, at this stage, anterior (an) and posterior (po) cannot be distinguished morphologically.
- D. Top view of a well-formed antler blastema. To sample different types of tissue for histology, immunohistochemistry and *in situ* hybridisation, a blastema can be divided into four quadrants by two incisions. One incision is made coronally and the other sagittally. These four quadrants are anterolateral (la), posterolateral (lp), anteromedial (ma) and posteromedial (mp). an, anterior; po, posterior.
- E. A whole pedicle with the fully-formed antler blastema. Note that after lifting up the pedicle skin, dormant (d) and potentiated (p) pedicle periosteum can be readily discerned by colour. The arrow points to the antler blastema.
- F. Distal part of a pedicle and fully-formed antler blastema. Note that blastema perichondrium (arrows) was detached together with the peeled-off skin from the blastema.
- G. A sagittally-cut pedicle and an antler blastema. From the cutting surface, the pedicle shaft can be divided into two clearly-different parts. The proximal part has the skin loosely attached to the bone (white arrows); whereas the distal part has the skin tightly attached (white arrowheads). The periosteum to which skin is loosely attached is termed *dormant progenitor tissue*, and that to which skin is tightly attached is called *potentiated progenitor tissue* (see text). In addition, the anterior (an) and posterior (po) growth centres can be readily identified on the cutting surface (black arrows).
- H. A piece of dormant pedicle periosteal strip being detached using a pair of rat-toothed forceps. An incision (arrow) marks the boundary between dormant and potentiated pedicle periosteum.
- I. A piece of potentiated periosteal strip was detached using a pair of rat-toothed forceps.

A pedicle stump mainly consists of bone, peripheral periosteum (PPP) and enveloping skin. There is controversy as to whether skin or periosteum is more important for the histogenesis of antler blastema. PPP would seem to be the leading candidate as the initiator of antler regeneration, as it is the derivative of IAP – the tissue that gives rise to pedicles and first antlers (see above). To address this fundamental issue, we developed a procedure to sample separately antler blastema skin and the PPP derivatives, antler blastema perichondrium, and pedicle stump periosteum.

a) Definition In the defined period, the interior component of an antler blastema is covered by a layer of perichondrium. This perichondrium is tightly associated with the enveloping skin and more fragile than its antecedent, pedicle periosteum. Although all PPPs have the potential to regenerate an entire antler, there is a difference in the degree of association between skin and PPP along the long axis of a pedicle shaft. The skin proximal to the pedicle base (approximately 2/3 of the total pedicle length) is loosely attached to the PPP, whereas the skin distal to the antler blastema (about 1/3 of the total pedicle length) is tightly bound to the pedicle bone. Because both antler generation and regeneration rely on the close association of the progenitor tissue, pedicle periosteum/perichondrium, and the covering skin (Goss, 1990; Li & Suttie, 2000; Li et al., 2001), the region closely associated with the skin may have moved one step further toward antler regeneration than the area loosely contacted with the skin. Therefore, we have called the PPP to which the skin is loosely attached *dormant PPP* (d); and that to which the skin is tightly attached *potentiated PPP* (Fig. 2E). Blastema perichondrium (the derivative of the potentiated PPP) has essentially fused with the covering skin and is actively forming cartilage. It is thus called *activated progenitor tissue* (Fig. 2F). We think that the level (dormant, potentiated or blastema level) at which the pedicle stump is created may affect the timing of antler regeneration.

The dormant, potentiated and activated progenitor tissues can be readily identified from the sagittally-cut surface of an early-antler-regenerating pedicle (Fig. 2G). A comparative study of dormant, potentiated and activated periosteum/perichondrium may hold the key to revealing the underlying mechanism by which the progenitor tissue is geared up step by step for antler regeneration.

b) Sampling methods The first incision is made through the skin at the junction between the pedicle and the frontal bone. The major blood vessels and nerves that are located on the lateral side of a pedicle should be avoided if the tissue is collected by biopsy. The cut should be deep enough to pass through the skin. A second incision starting from the apical scab (Figs. 2A–D) of the blastema is made along the long axis of the pedicle toward the proximal end and terminates when it meets the first incision at the pedicle base. All subsequent incisions are parallel to the second and made at intervals of 1.5 cm. The divided strips of skin can now be lifted

up from the proximal end using a pair of rat-toothed forceps. In the dormant PPP region (approximately 2/3 of the total pedicle length), the skin can be separated using a scalpel with little effort. However, the skin in the potentiated PPP region (the remaining 1/3 of the total pedicle length) is hard to detach. Both mechanical force and scalpel cuts have to be used to separate this part of the skin from the pedicle bone. Once a skin strip is lifted from the pedicle bone to the junction between the pedicle and the antler blastema (Fig. 2E), it can readily be peeled away from the antler blastema without any need for cutting. In most cases, a layer of whitish tissue about 1–2 mm thick (Fig. 2F) detaches from the blastema together with the skin strip. Histological studies have shown that this tissue is the blastema perichondrium (data not shown). The partial detachment of the perichondrium together with the peeled skin reflects the intimate association of these tissues. Subsequently, the blastema perichondrium can be separated from the attached skin using a scalpel. Both tissue types can be collected for regeneration study.

To sample the dormant and potentiated periosteum, following the same procedure for sampling blastema perichondrium, an incision is made through the periosteum when the pedicle skin is lifted up from the proximal end to the point where tight association with underlying periosteum is reached to delineate the dormant and potentiated PPP (Fig. 2H). Subsequent periosteal incisions are made parallel to each other along the long axis of the pedicle at 3–5 mm intervals to divide the periosteum into strips. Each periosteal strip (dormant [Fig. 2H] or potentiated [Fig. 2I]), can be readily detached with a pair of rat-toothed forceps.

3. Antler growth centre

The astonishing growth rate of deer antlers offers an opportunity for discovering new potent growth factors and unique regulatory systems. The antler growth centre is located in the antler tip and consists of different tissue types. To address the issues of rapid antler growth at the molecular level, we recognize a need to locate and separately sample different types of tissue from a piece of unstained antler tip tissue. In a recent paper (Li et al., 2002), we reported a generally applicable and standardized sampling technique to meet this demand.

However, the figures published in that paper only permit these tissue types to be distinguished at a micro-scopic level. The figures provided in this paper allow these types to be visualized at a macroscopic level with confidence.

a) Definition The antler growth centre consists of 3 layers of tissue. From distal to proximal these are: reserve mesenchyme, precartilag and cartilage (Banks & Newbrey, 1982). However, we have also found that the apical antler skin may be involved in fast antler formation through the interaction with underlying reserve mesenchyme (Li & Suttie, 2000; Li et al., 2001; Li & Suttie, 2001). Thus, apical antler skin should also be collected if tissue interactions are to be studied.

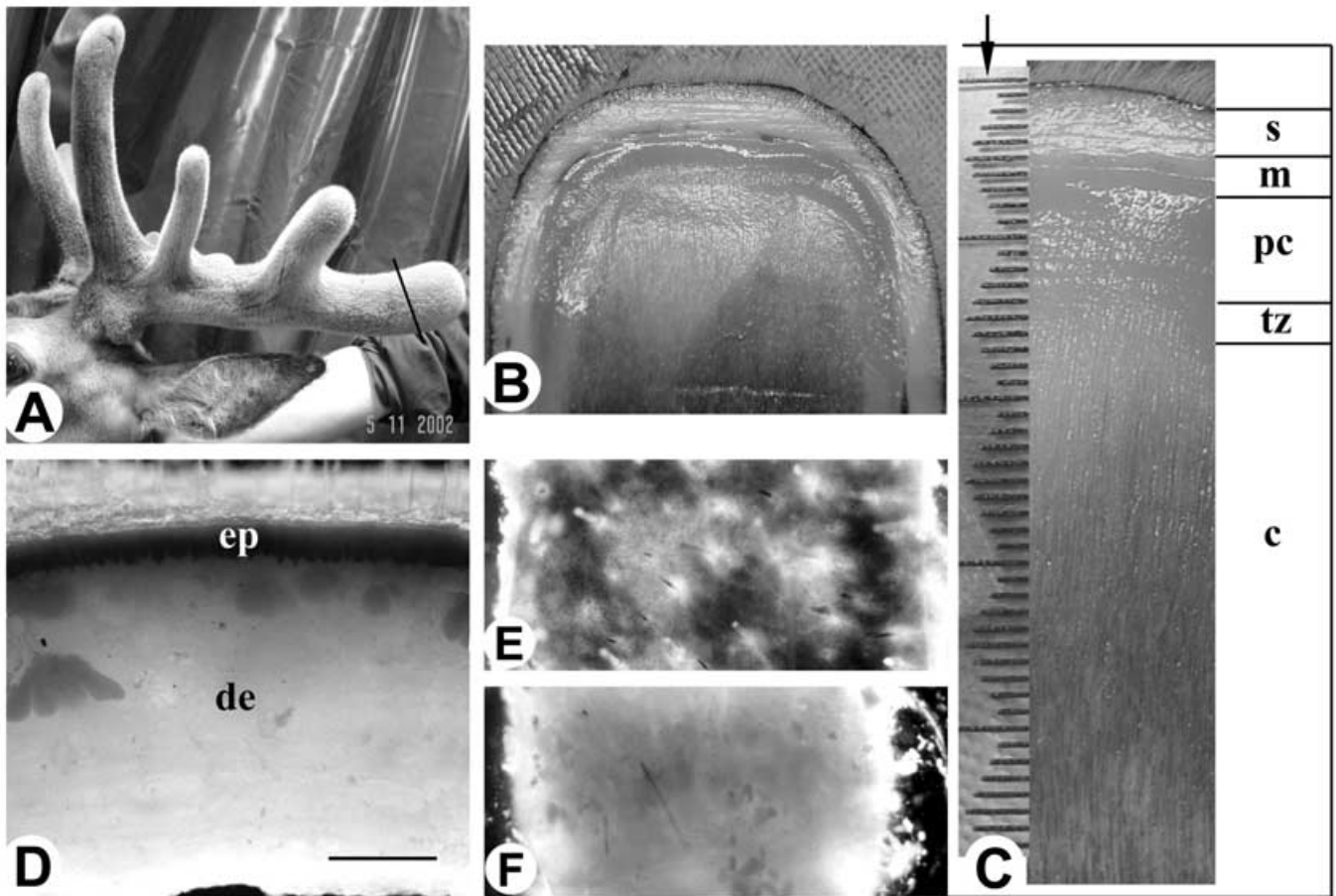


Fig. 3. Antler tip tissue sampling.

- A. A 60-day-growing antler from a 3-year-old red deer stag ready to be harvested. The line indicates the amount of tissue that will be removed from the tip.
- B. An antler tip that has been cut sagittally.
- C. The central portion of a sagittally-cut antler tip. Layers of skin (s), mesenchyme (m), precartilage (pc), transition zone (tz) and cartilage (c) can be identified by distinct morphological markers (see text). The arrow points to a ruler with 1 mm increments.
- D. A cross section of apical antler skin. ep, epidermis; de, dermis. Bar = 1.3 cm.
- E. The separated epidermal layer of tissue.
- F. The separated dermal tissue.

The epidermal and dermal layers of apical antler skin can be further separated in order to investigate mesenchymal-epithelial interactions *in vitro* or to isolate RNA.

b) Sampling methods The procedure for sampling 3 tissue layers in an antler growth centre from a piece of unstained antler tip tissue has been described in detail previously (Li et al., 2002). Briefly, antlers are collected at the half way point of their growth stage (around 60 days in red deer) using the New Zealand standard antler harvesting procedures. The distal 5 cm tip from the main beam (Fig. 3A) is then cut off and sagittally sectioned in the median plane (Fig. 3B). The tip is then cut into 5-mm-thick slices along the same plane. The slices are further cut into strips 1–2 cm across. The identification and sampling of these tissue layers is then carried out on these strips (Fig. 3C).

After sampling, the apical antler skin is diced into small tissue blocks (Fig. 3D) around 1 mm × 4 mm. These are then digested overnight at 4°C in Ca²⁺ and Mg²⁺ free phosphate buffered saline containing 0.2% trypsin. Epidermis (Fig. 3E) and dermis (Fig. 3F) can then be separated using two pairs of fine forceps under a dissecting microscope.

Conclusion

Deer antlers are a fascinating model for investigating mammal epimorphic regeneration, systems in which normal growth occurs at an astonishing rate, and self-differentiation capable stem tissue. While molecular biology techniques will have to be used to study these models, results can only be meaningful if correct tissue sampling methods are used. We

believe that the adoption of the collection methods reported here will greatly facilitate the establishment of deer antlers as a valuable biomedical research model.

In: Brown RD, ed., *Antler Development in Cervidae*. Kingsville, Texas, Caesar Kleberg Wildl Res Inst, pp. 61–71.

Acknowledgements

The authors wish to thank our following colleagues who have either directly or indirectly participated in tissue collection processes during 12 years of antler research at Invermay. They are Dr Colin Mackintosh, Mrs Shirley Martin, Mr Ian Corson, Mrs Marion Labes, Dr Dawn Clark, Dr Eric Lord, Mr Jason Gray, Ms Wenying Wang and Ms Yu-Jen Chen.

References

- Banks WJ, Newbrey JW (1982): Light microscopic studies of the ossification process in developing antlers. In: Brown RD, ed., *Antler Development in Cervidae*. Kingsville, Texas, Caesar Kleberg Wildl Res Inst, pp. 231–260.
- Goss RJ (1961): Experimental investigations of morphogenesis in the growing antler. *J Embryol Exp Morph* 9: 342–354.
- Goss RJ (1990): Of antlers and embryos. In: Bubenik G, Bubenik A, eds., *Horns, Pronghorns, and Antlers*. New York, Springer-Verlag, pp. 299–312.
- Goss RJ, Powel RS (1985): Induction of deer antlers by transplanted periosteum. I. Graft size and shape. *J Exp Zool* 235: 359–373.
- Li C, Clark DE, Lord EA, Stanton JL, Suttie JM (2002): Sampling technique to discriminate the different tissue layers of growing antler tips for gene discovery. *Anat Rec* 268: 125–130.
- Li C, Harris AJ, Suttie JM (2001) Tissue interactions and antlerogenesis: new findings revealed by a xenograft approach. *J Exp Zool* 290: 18–30.
- Li C, Littlejohn RP, Suttie JM (1999): Effects of insulin-like growth factor 1 and testosterone on the proliferation of antlerogenic cells *in vitro*. *J Exp Zool* 284: 82–90.
- Li C, Sheard PW, Corson ID, Suttie JM (1993): Pedicle and antler development following sectioning of the sensory nerves to the antlerogenic region of red deer (*Cervus elaphus*). *J Exp Zool* 267: 188–197.
- Li C, Suttie JM (1994): Light microscopic studies of pedicle and early first antler development in red deer (*Cervus elaphus*). *Anat Rec* 23: 198–215.
- Li C, Suttie JM (1998): Electron microscopic studies of antlerogenic cells from five developmental stages during pedicle and early antler formation in red deer (*Cervus elaphus*). *Anat Rec* 252: 587–599.
- Li C, Suttie JM (2000): Histological studies of pedicle skin formation and its transformation to antler velvet in red deer (*Cervus elaphus*). *Anat Rec* 260: 62–71.
- Li C, Suttie JM (2001): Deer antlerogenic periosteum: a piece of postnatally retained embryonic tissue? *Anat. Embryol (Berl)* 204: 375–388.
- Suttie JM, Kay RNB (1982): The influence of nutrition and photoperiod on the growth of antlers of young red deer.