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THE ONTOGENY OF NASAL HEAT EXCHANGE STRUCTURES IN ARCTIC ARTIODACTYLES

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Cover picture: one of the female reindeer with her calf born in late April 2014 at the research facility at the Arctic Biology building of the Department of Arctic and Marine Biology at the University of Tromsø.

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Abstract:

Reindeer (*Rangifer tarandus*) live in the Arctic and have evolved special adaptations to cope with hard environmental conditions. Nasal heat exchange (NHE), which is an efficient heat and water exchange mechanism to combat loss of energy, is well documented in adult reindeer (e.g. Blix and Johnsen (1983), Johnsen 1988). However, it is not known if this mechanism is fully developed from birth and if it has the same function in newborns as in adults. Dissections, CT-scans and histology preparations have been performed in this study in order to investigate if the structure responsible for the NHE is fully developed from birth. The study shows that the double scroll turbinate structure is present from birth. The mucosal surface area exposed to the air stream of the nasal cavity was calculated with a value of 772cm^2 in juvenile reindeer (male of 52.5kg). The discovery of a closed inner "bulb" (central lumen of the turbinate scroll is not open to air flux) has been important for the calculation of the surface area in relation to an efficient NHE. The most complex part of the nasal cavity, where the surface area is largest, is in the proximal slices, and it is probably the most important part of the nasal cavity for the nasal heat exchange. As a comparison, the relative mucosal surface area exposed to the air stream was much larger in muskoxen than in reindeer, although the turbinate is less complex in muskoxen than in reindeer. The ontogeny of turbinate geometry and histology was also analysed. In reindeer calf the size of vessels (arteries and veins) was smaller than in juvenile, but the density of vessels was higher. It can be speculated that a higher density is required to ensure the proper functioning of the nasal glands and because the calves have higher metabolic rates; or could be just a size fact, the juvenile is much higher so in 1cm there are less vessels than in the calf. Finally, it was concluded that the anatomy required for efficient NHE mechanism is developed from birth.

1. INTRODUCTION:

Life in the Arctic is a challenge for most organisms. The cold, dark and long winters are hard, not only due to low temperatures but also because of scarcity of food. Arctic animals have evolved special adaptations to fight against environmental conditions. Arctic artiodactyls like muskoxen (*Ovibos moschatus*) and reindeer (*Rangifer tarandus*) not only have to keep warm, they also have to be energy efficient to maintain a constant body temperature without losing too much energy. To do this they need to reduce heat dissipation and ensure supply of metabolites for heat production (Langman 1985).

Particularly in these environmental conditions the gas exchange of warm and humid air inside the lungs with cold and dry atmospheric air, may result in loss of large amounts of energy and water. To combat this loss of energy arctic artiodactyls have evolved efficient heat and water exchange mechanisms (Blix and Johnsen 1983).

In this study, aspects of these respiratory heat and water exchange mechanisms have been investigated. In particular, it was compared anatomical features of the nasal cavity in newborns and juveniles/adults in the only permanent resident species of Arctic ungulates: reindeer and muskoxen (Suttie and Webster 1998); with special consideration to reindeer.

1.1. Study animals:

1.1.1. Reindeer:

The reindeer (*Rangifer tarandus*) is a circumpolar species inhabiting the Arctic, subarctic tundra and boreal regions. It is present in several wild subspecies and domestic populations (fig1). Reindeer are ruminants and depend on highly digestible winter forage such as lichens (Skogland 1984). Lichens dominate winter diet while vascular plants including leaves of willow, forbs and sedges represent the summer diet (Klein 1992).

Adult reindeer have a total weight between 87-99kg, males are bigger than females. Also, males have bigger hoof size (mm^2) being 170-185 in males and 138-146 in females (Suttie and Webster 1998). Reindeer have long legs that give advantages in deep snow both for digging for food and locomotion (Suttie and Webster 1998). Reindeer are efficient in digging through snow to obtain forage (Klein et al. 1987).

Reindeer fur consists of thick and hollow permanent guard hairs and in winter they grow a low density of finer underwool (Klein 1992).

A specific characteristic of reindeer is that both sexes grow antlers. The antlers of old males are shed in December, those of young males in the early spring, and those of females in the summer. For female reindeer, this provides an advantage in intraspecific competition, particularly in winter (Henshaw 1969) when males lose their antlers. Due to this fact, females get higher dominance status than males (Espmark 1964; Suttie and Webster 1995) and use the antlers to protect their newborn calves (Henshaw 1969).

Most mating occurs in October and reindeer calves are usually born in May-June. After 45 days, calves are able to graze and forage but continue suckling until the following fall when they become independent from their mothers. The growth of reindeer calves is rapid during the first summer, when they can gain 400g per day, but it ceases almost completely during winter (Timisjärvi et al. 1979) when juvenile reindeer (6 months) are more or less physically fully mature.

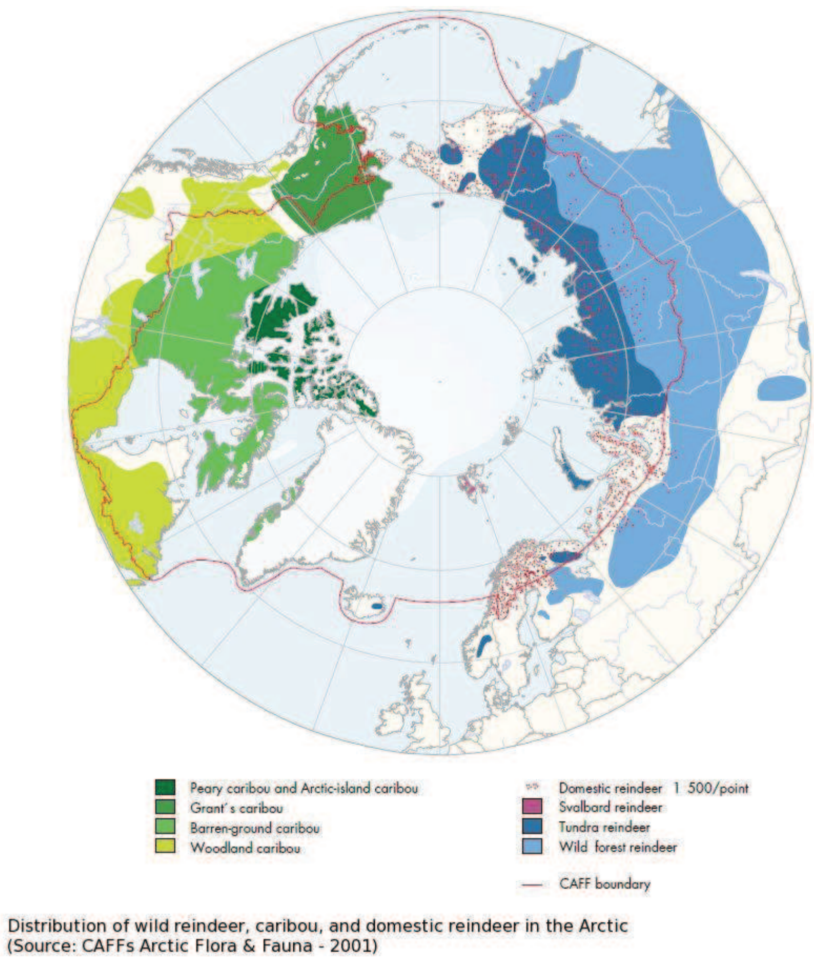

Fig 1: Distribution of wild reindeer, caribou and domestic reindeer in the Arctic. (CAFF 2001, with permission from the publisher)

1.1.2. Muskox:

The muskox (*Ovibos moschatus*) is a native species from Greenland and Canadian Northwest Territories, but has been introduced with differing success in Norway, Spitsbergen and Alaska (Blix and Steen 1979) as can be seen in figure 2.

Muskox, with their large rumen capacity and slow passage rates, can process a winter diet of low-quality and high-fiber content, usually dominated by graminoids (White et al. 1981, Klein 1992).

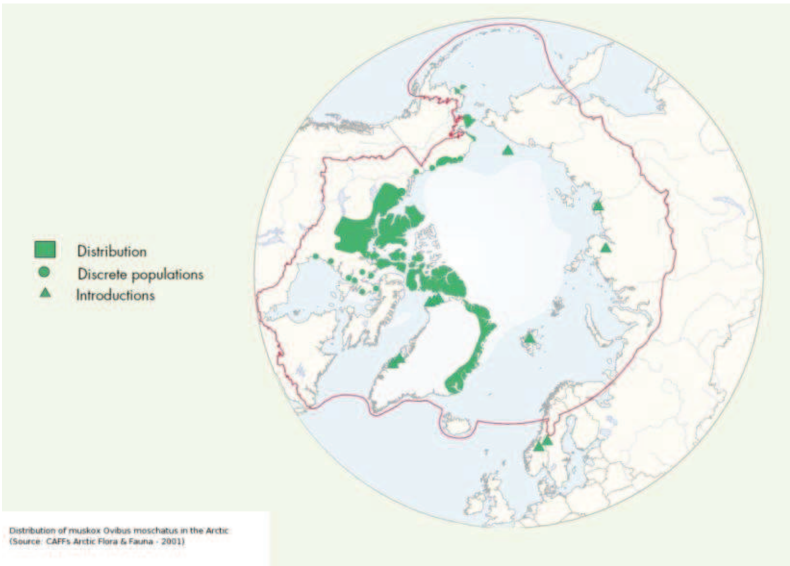

Fig 2: Distribution of muskox in the Arctic. (CAFF 2001, with the permission from the publisher)

Muskoxen have a large body size (total weight between 218-266 kg in adults) that gives a lower surface area/volume ratio resulting an advantage in energy efficiency (Suttie and Webster 1998). The weight in muskoxen is concentrated over the large forehooves and the large body size is used as a predator avoidance strategy (Klein 1992).

Muskoxen have a dense and thick underwool and is covered with long guard hairs. When muskox lie down, the long guard hairs form a blanket to the surface and the insulation efficiency is extremely high (Klein 1992).

Another characteristic of muskox is that both sexes have horns (Suttie and Webster 1998) which have function of social display and defence from predators (Klein 1992).

Newborn muskoxen calves are born in late April and they possess a large amount of brown adipose tissue (BAT) at birth (Blix et al. 1984).

Both adults and calves of both species are adapted to survive in extreme environmental conditions.

1.2. Thermoregulation in Arctic artiodactyls:

This section will mostly deal with reindeer because there is little information in the literature about thermoregulation in muskox.

1.2.1. Adult:

Body temperature

Arctic ungulates need to keep a high internal body temperature to survive. Normal deep-body temperature in reindeer is 38.9 ± 0.2ºC (Soppela et al. 1986).

Insulation

These animals are really well insulated by fur to protect themselves against cold. The insulation properties of fur depend on its thermal conductivity. The winter reindeer fur consists of thick guard hairs (with air-filled cavities separated by thin septa) and of thin and filled woollen hairs. The thickness of the fur varies during the year (Moote 1995). The summer fur is thinner and it contains less woollen hairs than the winter fur (Timisjärvi et al. 1984).

Also, resting reindeer typically choose a position in which the wind blows against the fur (Skjenneberg and Slagsvold 1968) to cover the head and minimize heat loss.

Due to these insulation properties, Nilssen et al. (1984) demonstrated that the lower critical temperature (T_{LC}) of reindeer is reduced from about 5ºC in the summer to about -30ºC during winter.

Heat production

Another way to reduce energy expenditure is to reduce the basal heat production itself. The metabolic rate of reindeer fed *ad libitum* shows a reduction from summer to winter due to a reduction in food intake (Nilssen et al. 1984).

As for reindeer, the resting metabolic rate of adult muskoxen is reduced from summer to winter associated with a reduction of food intake (Nilssen et al. 1994).

Peripheral heterothermia

Animals can decrease blood flow to the skin to reduce the heat loss. Reduction of blood flow reduces the skin temperature and is especially important in the extremities where the insulation is poorest and the area to volume ratio is largest

(Johnsen et al. 1985). Due to peripheral heterothermia (extremities of the animal have another temperature than the core of the animal) the blood in the extremities will be cooled and this could result in hypothermia. To avoid this possible hypothermia, reindeer have a vascular counter current heat exchange mechanism (Irving and Krog 1955, Olsson 2011).

The temperature of the venous return flow is lower than the arterial flow because of the energy transferred from the blood to the surroundings. Heat is transferred between the two vessels, so the arterial flow is cooled due to heat transfer to the venous flow. At the end the venous flow returns to the core of the animal at a higher temperature than if this heat transfer hadn't happened. The counter current heat exchange reduces the heat loss from the extremities and the whole animal (Mitchell and Myers 1968).

Nasal heat exchange

The respiratory gas exchange may result in loss of large amounts of energy and water, particularly in cold and dry environments. To combat this loss of energy, arctic ungulate species have evolved efficient heat and water exchange mechanisms. This mechanism is called the nasal temporal counter current heat exchange (nasal heat exchange (NHE) in short) and; was first described as a phenomenon in humans (Walker et al. 1961). Schmidt-Nielsen and colleagues discovered that this mechanism was important for body water conservation in desert mammals and birds (Jackson and Schmidt-Nielsen 1964; Schmidt-Nielsen et al. 1970), and it was later also shown by Blix and Johnsen (1983) that the NHE contributes to the maintenance of energy balance in reindeer, and that this process is, in fact, under physiological (thermoregulatory) control.

In brief, in a cold environment, NHE involves the following processes: cold and dry inhaled air is warmed and humidified through transfer of heat and evaporation of water from the nasal mucosa, which is cooled in these processes. During the exhalation the air passes over the cool mucosal surfaces and, heat and moisture is returned to the mucosa and the exhaled air leaves the animal at a temperature that may be much lower than deep body temperature (Walker et al. 1961; Schmidt-Nielsen et al. 1970; Blix and Johnsen 1983; Folkow and Blix 1987). Process showed in figure 3.

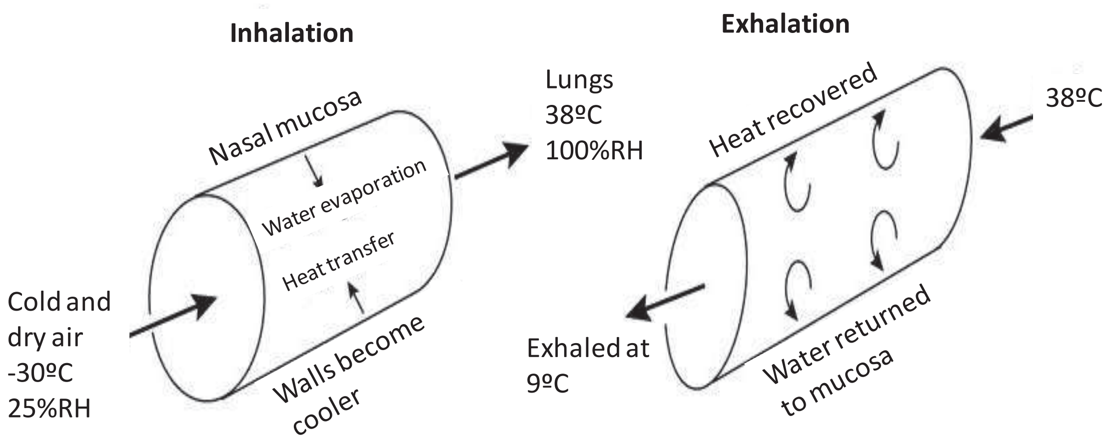

Fig 3: The principle of nasal countercurrent systems that recover heat and water from exhaled air. (Modified from Willmer 2005)

The efficiency of these processes, which are separated in time (thus the term nasal temporal counter current heat exchange), relies in part on the anatomical features of the nasal cavity (Jackson and Schmidt-Nielsen 1964, Collins et al. 1971, Mitchell and Myers 1968, Schroter and Watkins 1989).

Many species have evolved complex and convoluted nasal turbinate structures that serve to increase the surface area of the nasal mucosa, the interface for exchange of heat and water between the animal and the respired air (Negus 1958).

Like other artiodactyls, adult reindeer have turbinates with a double scroll design that according to Blix et al. (1983) exposes a large mucosal surface area of about $0.12m^2$ to the air stream, and which allows recovery of up to 70 % of the heat and 80 % of the water that is added on inspiration of cold, dry air in the resting state (Blix and Johnsen 1983). Few quantifications of the mucosal surface area have been done along the years.

The respiratory chamber of nasal cavity in reindeer is filled with a complex system of scrolled structures (fig4) which are covered by a richly vascularized mucosal layer (Johnsen 1988). The nasal mucosa is irrigated by an arterial and venous rete that communicates by capillaries and arteriovenous anastomoses. The effluent from the venous rete can be drained both from its anterior end (through dorsal nasal vein) and from its posterior end (through the sphenopalatine group of veins). In an extreme heat conservation state, the blood runs in opposite directions in these retia, maintaining the temperature gradient along the nasal mucosa due to countercurrent heat exchange (Johnsen et al. 1985).

It was not possible to find information about nasal heat exchange or nasal turbinates in adult muskoxen.

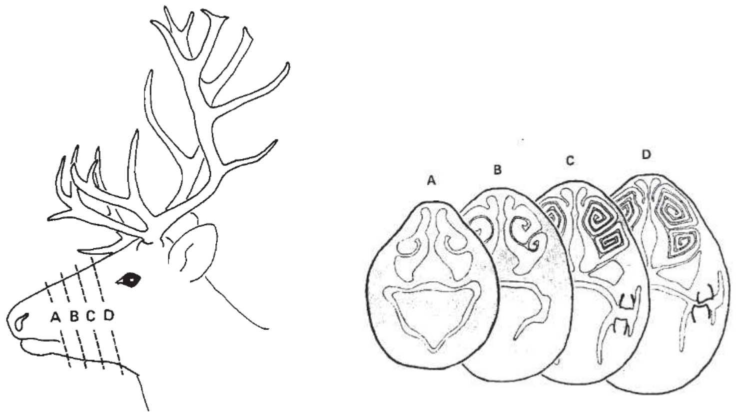

Fig 4: Cross sections of the reindeer nose obtained at four different levels (A, B, C and D). Sections were made at approximately 1cm intervals and illustrate the elaborate organization of the maxilloturbinates which project into each nasal cavity from the lateral wall (Johnsen 1988, with permission from the author).

1.2.2. Newborn/calf:

Body temperature

Newborn reindeer are born in late April, when pastures are still snow covered. (Markussen et al. 1985). Survival of calves in this environment demands to keep a high body temperature (40.2ºC) (Soppela et al. 1986). Calves have higher body temperature than adults due to higher metabolic rate (Soppela et al. 1986).

Newborn muskoxen calves are born in late April and can tolerate an ambient temperature (T_a) of -35ºC due to high metabolic heat production (Blix et al. 1984).

Insulation

Reindeer calves have a birth weight of about 5kg and are insulated by a fur of air-filled hairs that offers prime insulation while it´s dry. There is no layer of subcutaneous fat, but brown adipose tissue (BAT) is interspersed among the skeletal muscles (Blix and Steen 1979).

Muskoxen calves are also insulated with a large amount of brown adipose tissue (BAT) at birth (Blix et al. 1984).

Heat production

Heat production by metabolism of BAT, or non-shivering thermogenesis (NST), is assumed to have an important role in cold resistance (Hissa et al. 1981). This metabolism of BAT is stimulated by the cold-induced noradrenaline release (Soppela et al. 1986) and contributes as much as 60-70% to the total metabolic heat produced in reindeer calves (Markussen et al. 1985).

Also, due to the high energy content in the milk, calves can support a high metabolic rate and have a rapid growth (Soppela et al. 1986).

In other study involving newborn calves laying at rest it was found that their lower critical temperature (T_{lc}) was 11ºC and the metabolic rate in the thermoneutral zone was 5.1 W/kg (Markussen et al. 1985).

Peripheral heterothermia & NHE

During the first days of life it has been shown that reindeer calves are able to regulate the skin temperature of the extremities by the counter current heat exchange mechanism (Soppela et al. 1986).

Extremities of muskoxen calves are well covered by fur to prevent excess of heat loss, but counter current heat exchange mechanism was not found on their legs (Blix et al. 1984).

Both reindeer and muskoxen calves are thus well adapted to the cold environment. However, it is not known if nasal heat exchange structures are fully developed from birth and if they are likely to make an important contribution to reduce energy costs in the newborn animal, in a similar way as in adults. Studies of NHE have only been done in adult reindeer (Johnsen et al. 1985, Langman 1985, Johnsen et al. 1986), while the role and efficiency of this mechanism in adult muskoxen or in calves of both species is not known.

1.3. Hypothesis:

The anatomical features required for efficient NHE are fully developed from birth in reindeer and muskoxen.

This hypothesis has been tested by studying and comparing the anatomy of turbinate structures in the nasal cavity of newborn and adult reindeer and muskoxen. Specifically, the following aspects have been investigated:

- gross anatomy of nasal turbinate in newborns and adults
- turbinate geometry in newborns and adults (scroll numbers, width, length, mucosal surface area)
- mucosal vasculature (density, distance to the wall epithelium, x-section surface area, type, density and size of blood vessels) and glands in newborns and adults.

2. MATERIAL AND METHODS:

2.1. Animals:

In this study a total of six reindeer (*Raginfer tarandus*) and two muskoxen (*Ovibos moschatus*) were used (table1). All of the reindeer were born and raised at the Arctic Biology research animal facility, University of Tromsø, Norway (70ºN, 19ºE). Before experiments reindeer were kept outdoors with water/snow and food (lichens and reindeer pellets - Reinfôr, Felleskjøpet, Norway) available *ad libitum*.

The two muskoxen, a calf (35-1996C) and the head of an adult (32-2002), were frozen at the animal facilities just after natural death and due to the possible access to these animals; they were included in this study.

Two of the reindeer, one newborn (5-05C) and one juvenile (R1-12), were used for anatomical studies, dissection. These two animals were frozen at the animal facilities just after death (juvenile stunned with captive bolt pistol and bled, newborn found dead).

All the animals were used for CT-scan measures, and one reindeer juvenile (R4-12) and one reindeer calf (12-10C) were used later on for histological preparations.

Table 1: Animals used for this study. Age is the age of the animal at time of death. R1-12, R4-12 and R7-12 are juvenile reindeers, 5-05C, 9-10C and 12-10C are newborn reindeer calves, 32-2002 is adult muskoxen and 35-1996C is muskoxen calf.

Animal	Sex	Age	Weight	Experiment
R1-12 Reindeer	male	6 months	60kg	CT-scan and dissection
5-05C Reindeer	female	1 day	4,535kg	CT-scan and dissection
R4-12 Reindeer	female	1 year	54,5 kg	CT-scan and histology
12-10C Reindeer	male	1 day	6,674 kg	CT-scan and histology
9-10C Reindeer	female	3 days	7,664 kg	CT-scan
R7-12 Reindeer	male	6 months	52,5 kg	CT-scan
32-2002 Muskoxen	male	10 years	310kg	CT-scan
35-1996C Muskoxen	male	1 month	15 kg	CT-scan

2.2. Gross anatomy:

2.2.1. Dissection:

The purpose of dissection was to obtain a description of the gross anatomy, a general impression of 3-D structures, for “ground-truthing” of CT-scan images and photo documentation.

Specimens:

For anatomical studies two reindeer heads, 1 juvenile (R1-12) and 1 calf (5-05C) were used. These reindeer were frozen in the Arctic Biology research animal facility just after death.

Procedures:

When reindeer were still frozen the head was cut longitudinally (fig5) along midline (nasal septum). One half was cut in cross section slices (fig6) of 1cm thickness. In this way a lateral view and cross-sectional view along the nasal cavity were obtained. An electric bone band saw (type SM280, CEG, Cardano Al Campo, Italy) was used to cut the head of the juvenile animals while a simple metal saw blade was used to section the head of the calf.

All the turbinate parts were studied and described.

Fig 5: lateral view of juvenile reindeer head (photo Acquarone).

Fig 6: Cross sections slice of juvenile reindeer head (photo Acquarone).

2.2.2. CT-scan:

The purpose of CT-scan was to obtain non-invasive anatomical description for studies of turbinate geometry. For this purpose it was necessary to also have "ground-truthing" data from the same heads, from dissections (see above). To compare the CT-scan images to what is there in reality (dissection images) in order to verify the contents on the CT-scan images.

For the CT-scan heads of 8 animals in total were used: 6 reindeer and 2 muskoxen. Some of these heads were fixated in formalin and others were frozen. The fixated animal heads were: R4-12, 9-10C and 12-10C; and the frozen ones were: R1-12, 5-05C, 32-2002 and 35-1996C.

The CT-scanner used was a Siemens Biograph 64 slice PET/CT (Munich, Germany). Doctor Sundset and his team from Tromsø University Hospital helped with the CT-scan images and the whole process related with the scan. With this scan it was obtained accurate images of the whole turbinate in different slices with a maximum resolution of 100µm.

CT-scan images were used for mucosal surface area exposed to the air stream and air space volume measurements of the whole nasal cavity. Measurements of the cross section area and cross section perimeter were taken with ImageJ program (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014), using the auto thresholding, which is a variation of the IsoData algorithm. The procedure divides the image into object and background by taking an initial threshold, and then the averages of the pixels at or below the threshold and pixels above are computed. The averages of those two values are computed, the threshold is incremented and the process is repeated until the threshold is larger than the composite average (from http://imagej.nih.gov/ij/). After, thresholding area and perimeter calculations were made using the measuring function in ImageJ. These measurements were taken in an appropriate subset of the total slices obtained from the CT-scan of each animal head.

Knowing the thickness of the individual slices (0.6 or 0.75mm) and the distance between them, it was possible by simple geometric calculation to estimate the mucosal surface area exposed to the air stream and air space volume. Measurements were taken separately in the right and left nasal passages.

In reindeer (R1-12, 9-10C and 5-05C), measurements were taken every 10 slices and the thickness of slice was 0.6mm (fig7). In R1-12 juvenile reindeer 21 slices were measured (fig7a), in reindeer calf 9-10C was not possible to calculate the whole nasal cavity because the nasal passage was filled with foreign material of the same opacity as soft tissue (fig7b) and, in reindeer calf 5-05C was not possible to obtain measurements in the middle slices from the right side because turbinate was destroyed (fig7c).

Adult muskoxen (32-2002) measurements were taken every 20 slices and the thickness of slice was 0.75mm (fig8a). 13 slices were measured in adult muskoxen (fig8a). In muskoxen calf (35-1996C) measurements were taken every 10 slices and thickness was 0.6mm (fig8b). 7 slices were measured along the nasal cavity (fig8b).

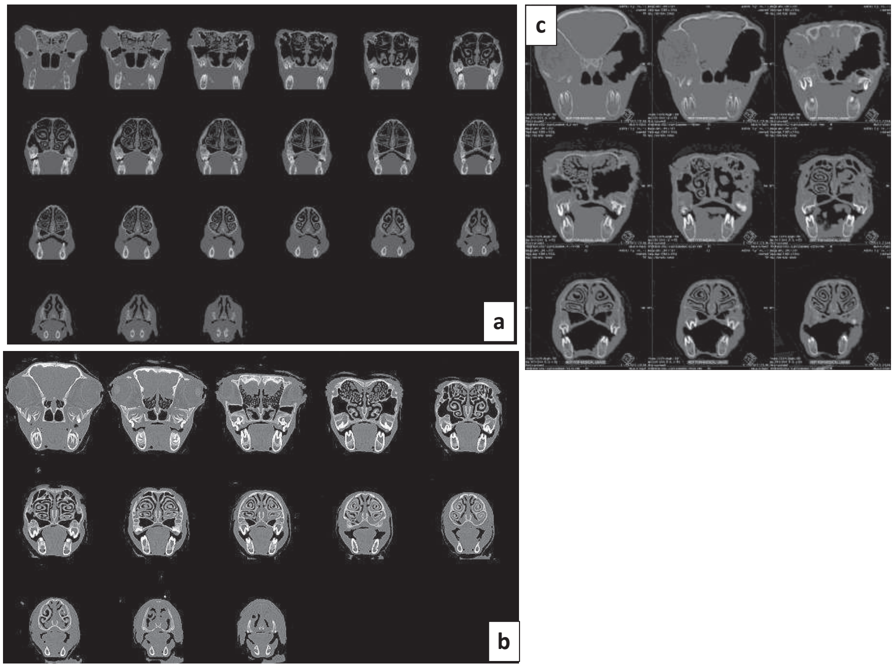

Fig7: slices measured from a: adult reindeer (R1-12), b: reindeer calf (9-10C), c: reindeer calf (5-05C) with right turbinate destroyed (images by Acquarone).

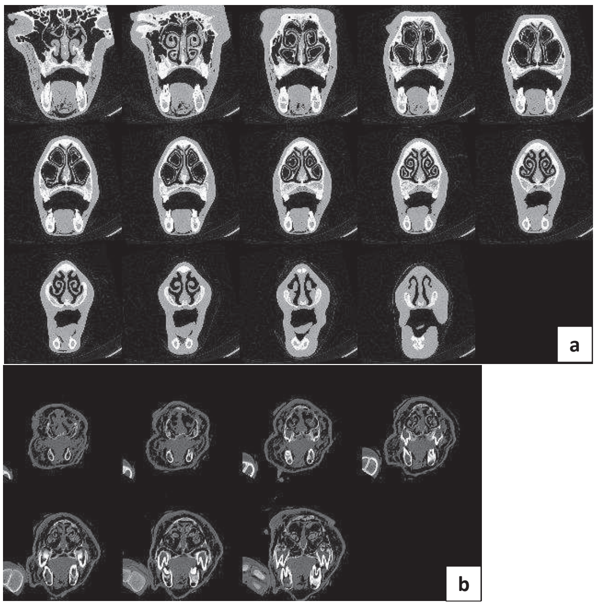

Fig8: slices measured from a: adult muskoxen (32-2002) and b: muskoxen calf (35-1996C) (images by Acquarone).

2.3. Histology:

The purpose of the histology analysis was to compare the turbinate geometry with regard to density and distribution of arteries and veins within the mucosa. Examinations were realized based on perfusion-fixed preparations.

2.3.1. Animal handling:

Animals (9-10C, 12-10C, R4-12 and R7-12) were caught in their fences. They were anaesthetized with an intramuscular injection [0,2ml for the calves and 3,5ml for the juvenile] of Rompun vet. (xylasin 20mg/ml, Bayer Health Care AG, Animal health division, Leverkusen, Germany). The animals were then immediately carried to the lab, weighed and determinate the sex.

Once in the lab, a catheter (Venflon cannula 19G/1.0mm O.D. x32mm Viggo AB, Helsingborg, Sweden) was introduced into the jugular vein. First, Heparin sodium (5000 IU/ml, LEO pharma AS Oslo, Norway) was injected [1,5ml for the calves and 3ml for the juvenile] to prevent blood clots and 5 minutes later, animal was euthanized with Pentobarbital (200mg/ml, Vitusapotek Svanen Tromsø) [2ml for the calves and 20 ml for the juvenile] which had immediate effect.

2.3.2. Fixation and preservation:

Once the animal was unconscious and without corneal reflexes, it was bled by cutting the common carotid arteries. After bleeding, the animal was decapitated and the carotid arteries were separated and cannulated.

Small catheters (Venflon cannula 22G/0.8mm O.D. x32mm Viggo AB, Helsingborg, Sweden) were used for the cannulation of both carotid arteries in reindeer calf; and bigger catheters (Venflon cannula 19G/1.0mm O.D. x32mm Viggo AB, Helsingborg, Sweden) were used for juvenile reindeer.

First, saline (0.9% NaCl solution) was perfused into the carotids to flush out all the blood from the head. The bottle containing the saline solution was suspended 130cm above the head to simulate an average blood pressure of around 95mmHg. Then, approximately 1 litre of 4% parafomaldehyde solution in phosphate buffer saline (10% Formalin, buffered) was injected into the vessels to fixate the tissues.

The head with the catheters in was then preserved in a container with the same fixative as used for perfusion until CT-scan analysis or histological studies were done.

2.3.3. Processing of sections:

For the histology studies were used 1 juvenile (R4-12) and 1 calf (12-10C) reindeer. These two reindeer were fixated as described above.

First, samples were prepared for the Department of Clinical Pathology at University Hospital of North Norway, who produced slides for light microscopy (see below).

To prepare the sample first, the skin of the front part of the head (fig9) was removed and then the head was cut longitudinally following the septum (fig10) with a metal saw blade. In the figures 9-13 it can be seen a blue colour in the tissue. It occurs because Evans blue was added to the saline to make sure that vasculature was completely flushed. Evans blue did not leave the blood vessels and did not affect histology.

Fig 9: remove skin of the reindeer calf head.

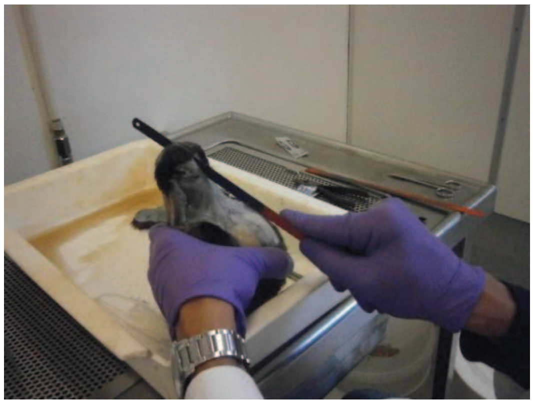

Fig 10: longitudinal cut of the head of reindeer calf

For one of the half heads (fig11) cross section slices of the nasal turbinate of 1cm width (fig12) were cut with a rotary saw (MB 0510/D1 Ø3.2mm West Germany).

Fig 11: lateral view of nasal turbinate of reindeer calf.

Fig 12: cut slices of 1cm width of calf turbinate.

The slides were kept in 4% buffered paraformaldehyde (fig13) to carry them to the engineers of the Department of Clinical Pathology at University of North Norway (DCP/UNN). The engineers prepared histological slices of the entire structure so then investigation of potential differences in vasculature/glands at different levels (different scrolls; from distal to proximal part of the nose) could be done.

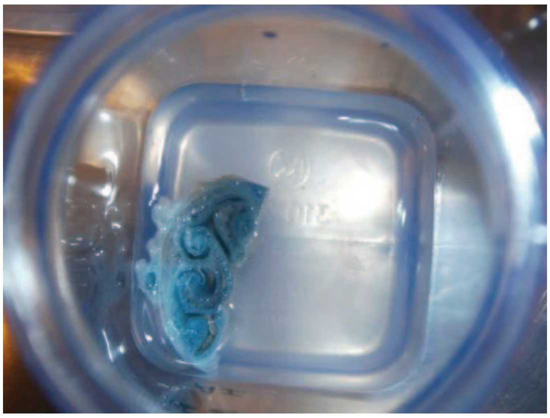

Fig13: one slice of reindeer calf in formalin

Seven slices from the reindeer calf and 13 slices from the juvenile were collected but for initial studies, all 7 slices (fig14) from the calf (12-10C A0, AI, AII, AIII, AIV, AV and AVI) and 7 (fig15) from the juvenile (R4-12 AI, AIV, AVI, AVIII, AX, AXII and AXIII) were delivered to DCP/UNN. The last 3 of the juvenile were just the turbinate, excluding the nasal wall (due to size limitations because intact histological sections could only be made of sections less than 5 cm in any direction).

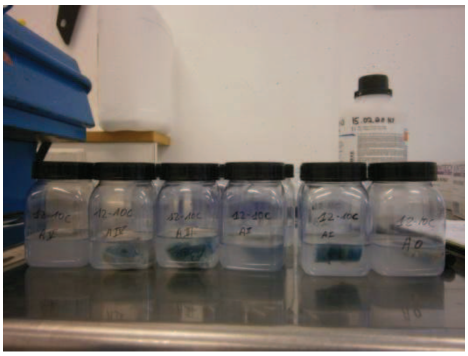

Fig 14: six of the seven slices obtained from the reindeer calf in the formalin bottles.

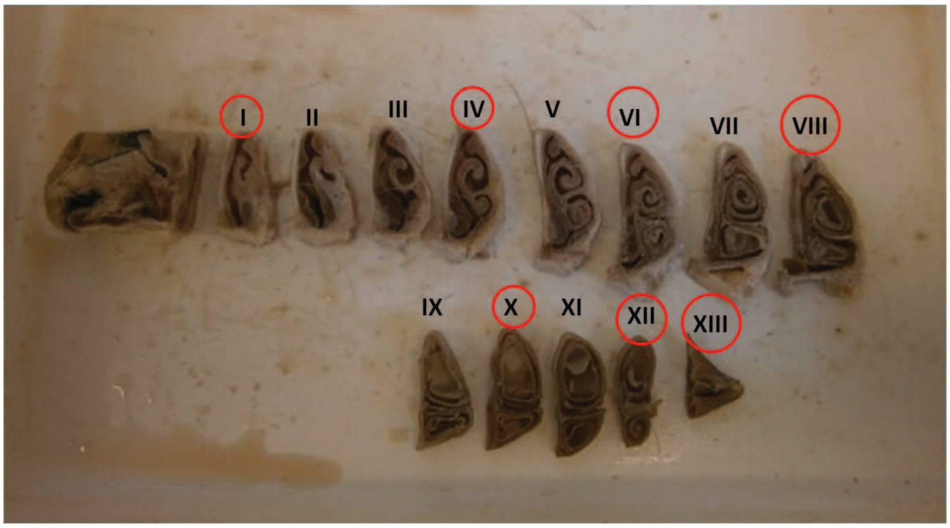

Fig 15: The 13 slices obtained from the juvenile reindeer and with red circle the 7 we choose for the histological slices.

For preparation of histological slices, the tissue slices were placed for 0.5-1 hour in a standard decalcification solution containing hydrochloric acid (Decalcinerningsvæske, Chemi-Teknik AS, Norway), to soften bone tissue. After dehydration and embedding in paraffin wax, 2 µm thick slices were prepared and stained with hematoxylin-eosin. With these histological slices light microscope was used to see the different structures in the turbinate. Different measurements were taken on the images obtained with the microscope.

2.3.4. Analysis of sections at different levels:

The first slice (12-10C A0 and R4-12 AI) and the last one (12-10C AVI and R4-12 AXIII) of both juvenile and calf (from distal and proximal part of the nose, respectively) were not used for morphometric measurements with the light microscope because the turbinate was not present.

Once the histological slices were completed, the magnifier microscope (fig16a) was used to get an overview of the structures and then the light microscope (fig16b) was used to see the structures deeper, to compare the turbinate geometry including density and distribution of vessels (arteries and veins) in the mucosa.

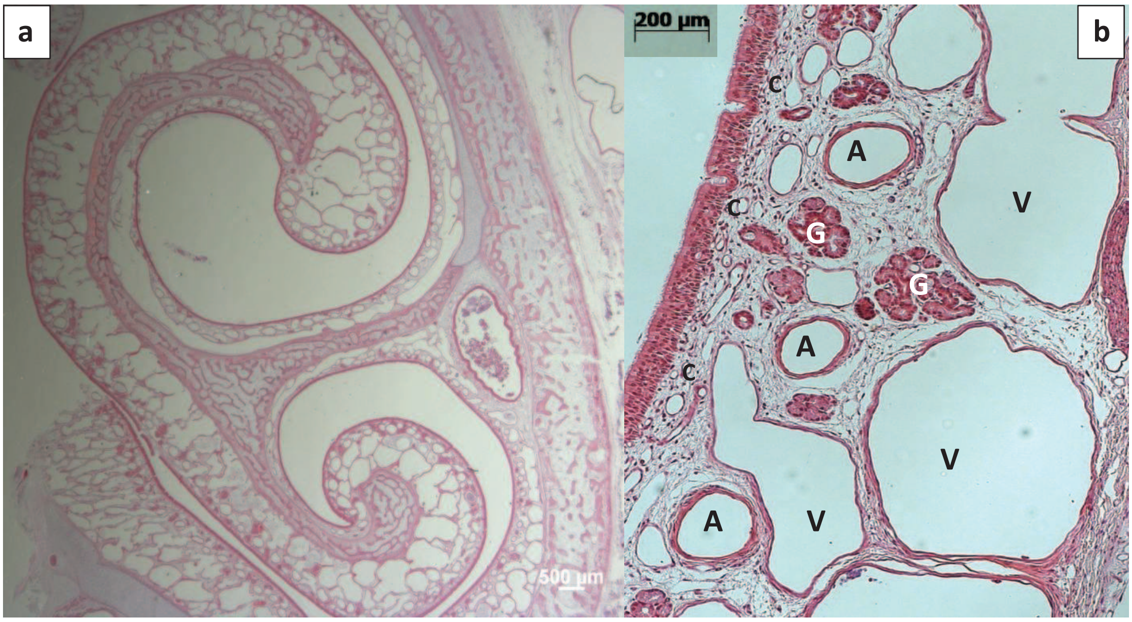

Fig 16: image of 12-10C AI with magnifier microscope (a) and image of 12-10C AI with light microscope (b).A: arteries, V: veins, G:glands, C:capillaries.

Pictures of the turbinate structures were taken with the AxioVision-Carl Zeiss microscope software (based on Release 4.8 June 2009 Göttingen, Deutschland) and Fiji (ImageJ) program (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014) was used to obtain measurements of the different structures in the pictures.

Measurements of diameter (mm), distance between the vessel and the wall of the epithelium (mm) and proportional x-section areas that were occupied by vessels (mm^2) were taken. Apart of that the densities of arteries, veins, capillaries and glands were also determined (fig16). Due to organization of structures in the turbinate it was more convenient to express such counts in relation to the length of mucosal lining investigated.

With the AxioVision-Carl Zeiss microscope software the scale on the pictures was set and images for the x-section area (fig17) measurements were prepared. For this measurement, to calculate the proportion of x-section area occupied by arteries and veins, squares of $1mm^2$ were drawn in each section of the turbinate. These squares areas were selected randomly in each section of the turbinate.

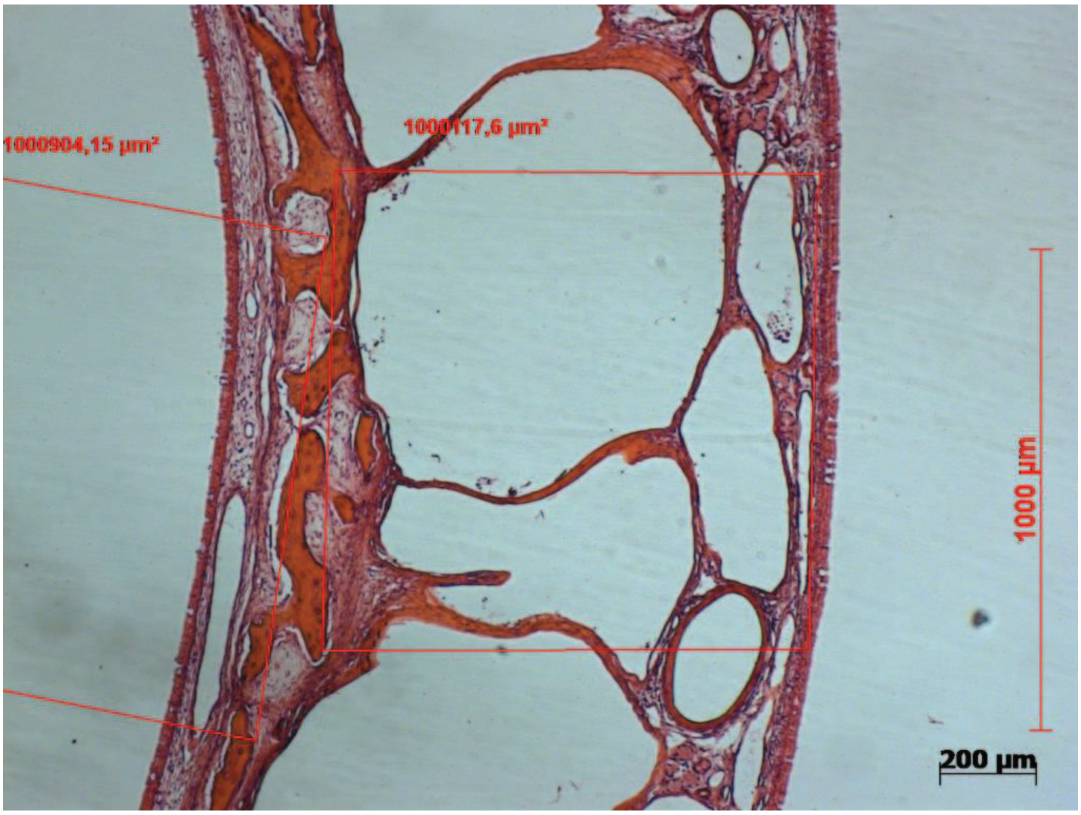

Fig 17: image of R4-12 AVI in microscope with the square of 1mm² to measure the x-section area of veins and arteries.

With ImageJ program the measurements of the diameter (mm), distance (mm) between the wall of the vessels and epithelium of the turbinate, and the x-section area (mm^2) of arteries and veins were obtained.

To realize these measurements the turbinate was divided in different sections (fig 18a, b) and labelled like T upper and lower, nasal wall U and nasal wall L, dorsal fold, U1a outer, U1b inner, and L2a outer… It was called T because it looks like a T and is the upper layer and the lower layer. The turbinate was divided in upper (U) and lower (L) scroll. Inside the scrolls it was divided in different parts, the layers of the scroll (1, 2, 3…) depending on the convolution of the scroll and then in these layers with letters (a, b, c…) the different parts of these convolutions. Apart of that every section was divided into outer or inner layer. So, for example U1a outer section is the outer layer of the first part of the first convolution in the upper scroll.

In each of these sections, 1cm long was taken to do the measurements of diameter and wall distance, and squares of $1mm^2$ were taken for the x-section data. Some of the sections were less than 1cm longer, so the measurements were taken and then, the proportion in 1cm was calculated. The number of arteries, veins, capillaries and glands was counted along each cm of mucosal lining in these sections.

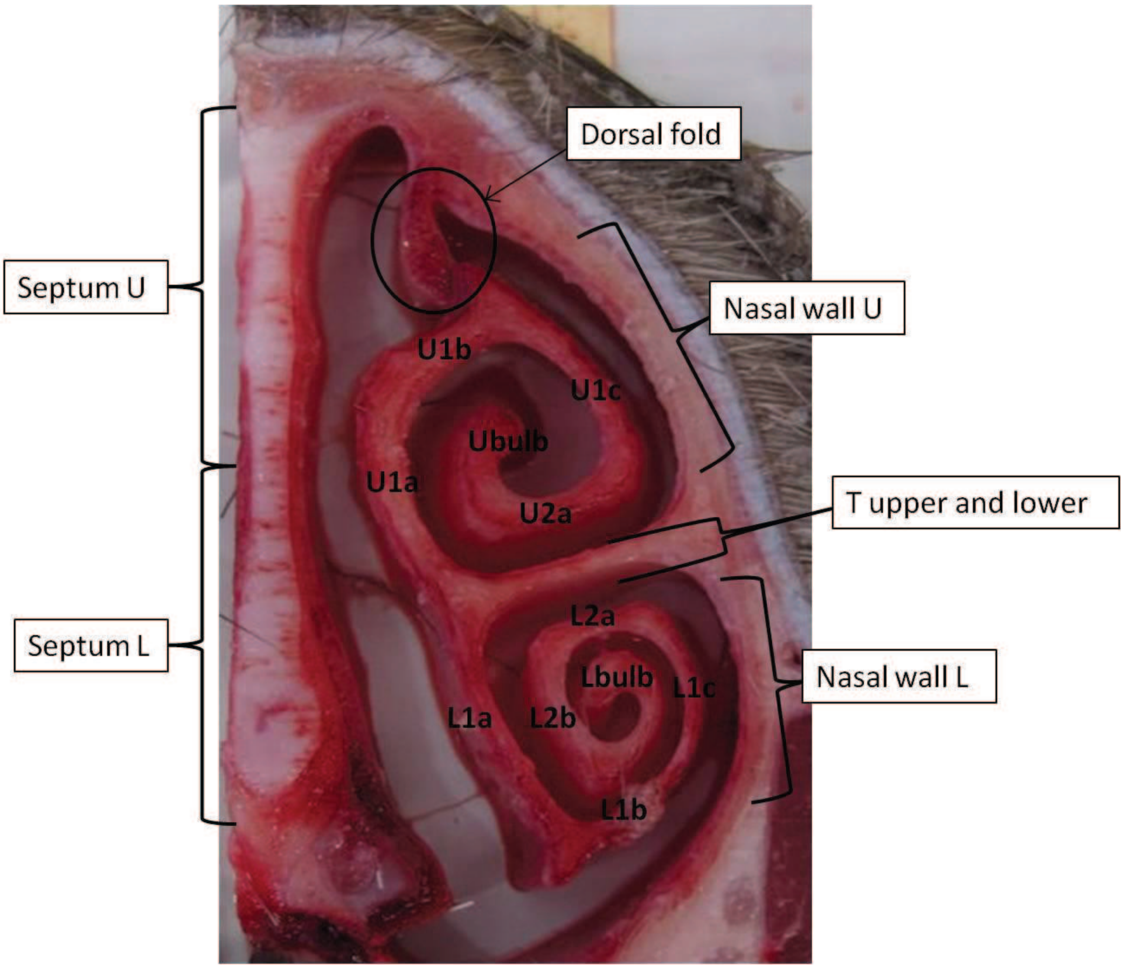

Fig 18a: labelling turbinate with all the sections which we divided the turbinate (photo by Acquarone)

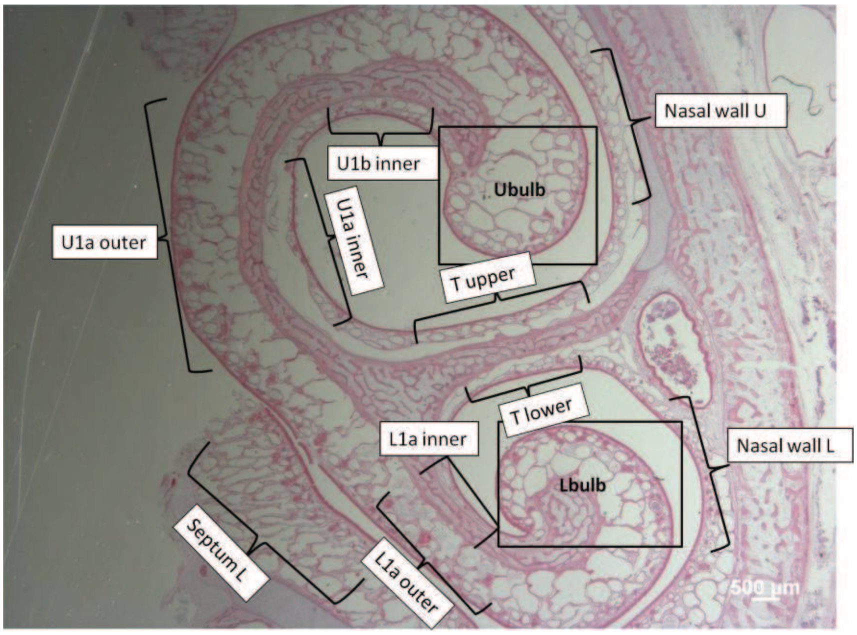

Fig 18b: sections in which was divided the turbinate in a microscopy picture.

To obtain the "diameter" the measure was taken in parallel to the epithelium wall (fig19). Normally the longest distance for the veins. Some of the veins were really long and convoluted shape so these ones were measured by hand.

To get the distance from vessels to the mucosal surface, the measure was taken perpendicular to the epithelium wall and from the middle of the wall of the vessel to the epithelium (fig20).

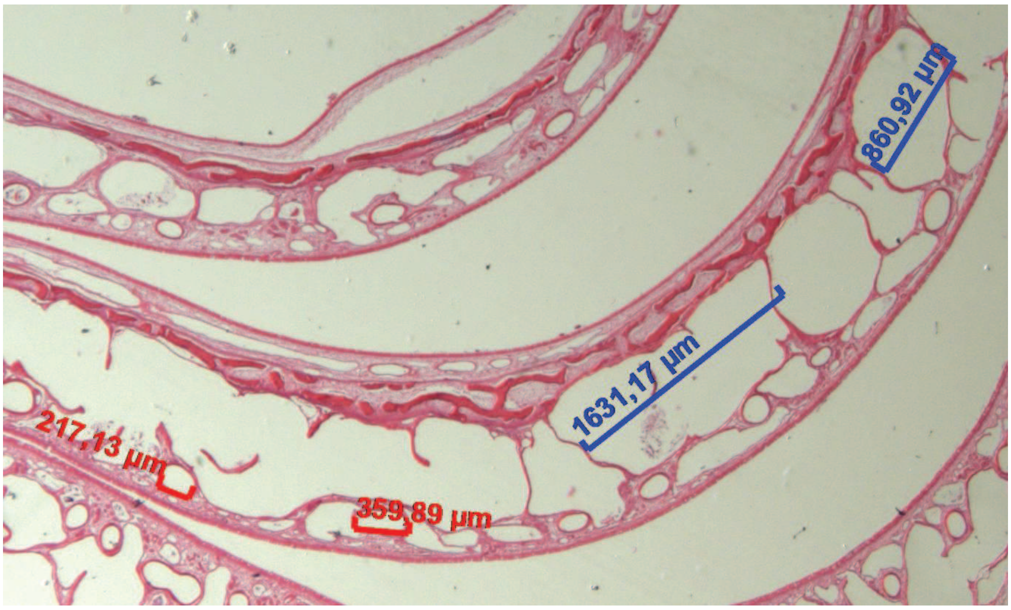

Fig 19: microscope image of R4-12 AVI lower nasal wall with some of the diameters taken in veins (blue) and arteries (red).

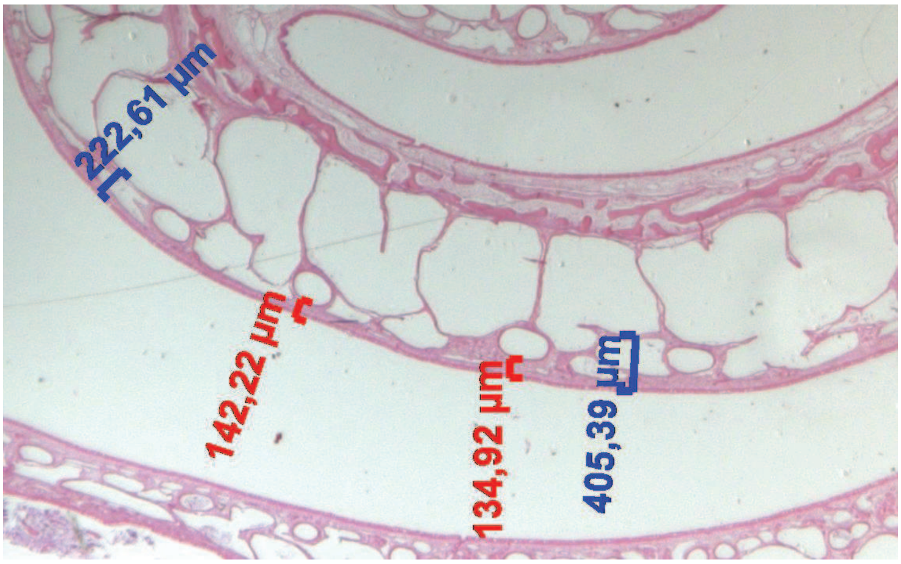

Fig 20: microscope image of R4-12 AIV lower scroll with some of the distances in veins (blue) and arteries (red).

2.3.5. Statistics:

Replicates of 30% of the measurements (around 7 replicates per slice) were taken to make sure the data were well collected. With all these data statistic and data analysis has been done. Statistical analysis and graphs were made in Microsoft Excel 2007 (Microsoft Corporation, Redmond, Washington, USA).

F-tests for equal variance were used to see if equal or not equal variance should be used for t-tests for equality of means, to compare the first values with the replicates. A p value of <0.05 was taken to indicate statistically significant differences.

I fully appreciate that n=1 for both categories of reindeer and muskoxen is a low sample size but due to limitation of availability of specimens in the case of muskoxen and due to time limitations, the studies were restricted to just one juvenile and one calf of both species.

However, many measurements have been calculated from each animal because of the different slices obtained along the nasal cavity and the different sections.

3. RESULTS:

3.1. Gross anatomy:

3.1.1. Dissection:

In the juvenile reindeer (R1-12) the nasal cavity had an extension of about 20cm, from nostrils to the pharynx (fig21a) and the reindeer calf (5-05C) has an extension of about 11cm (fig21b). The turbinate structures consist of a cartilage core covered with a well-vascularised mucosa (Johnsen et al. 1985), which branches from the lateral nasal cavity wall to fill the large surface of the nasal passages. The turbinate structure in juvenile reindeer has an extension of about 11cm (fig21a) and the reindeer calf turbinate has an extension of 5cm approximately (fig21b).

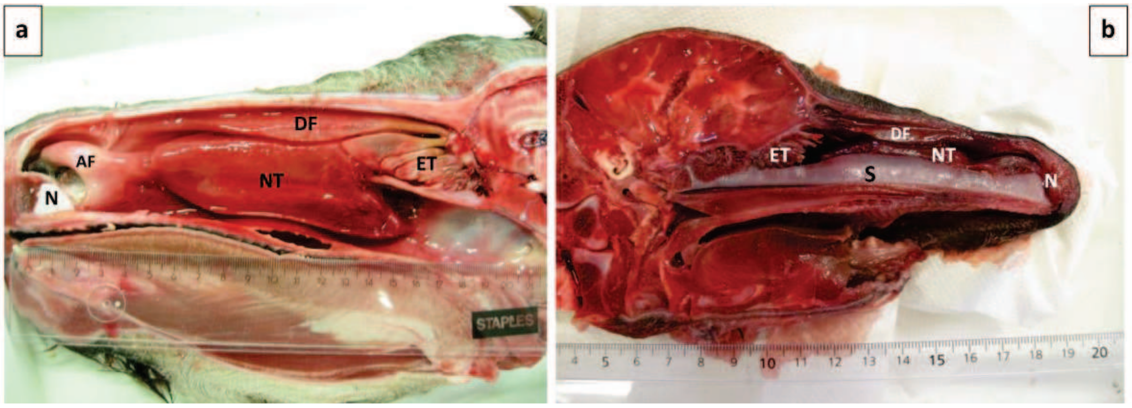

Fig21: lateral view of nasal cavity in juvenile (a) and calf (b) reindeer. N: nostrils, AF: alar fold, DF: dorsal fold, NT: nasal turbinate, ET: ethmoidal turbinate, S: septum (left photo by Acquarone).

From the lateral view of the turbinate it can be seen a rich vascularization (fig22a) along the turbinate. The turbinate was opened and cut through the different layers. In figure 22b it can be seen that the air spaces between the layers in each scroll turbinate are connected. The upper and lower scrolls are connected between them with an interconnectivity tissue (fig 22c). When the upper scroll was taken out, it was seen that the last layer, the inner compartment/space was completely closed as it can be seen in figure 22d, 22e (juvenile) and 22f (calf). It is a closed “bulb”. These results can be shown in both juvenile and reindeer calf.

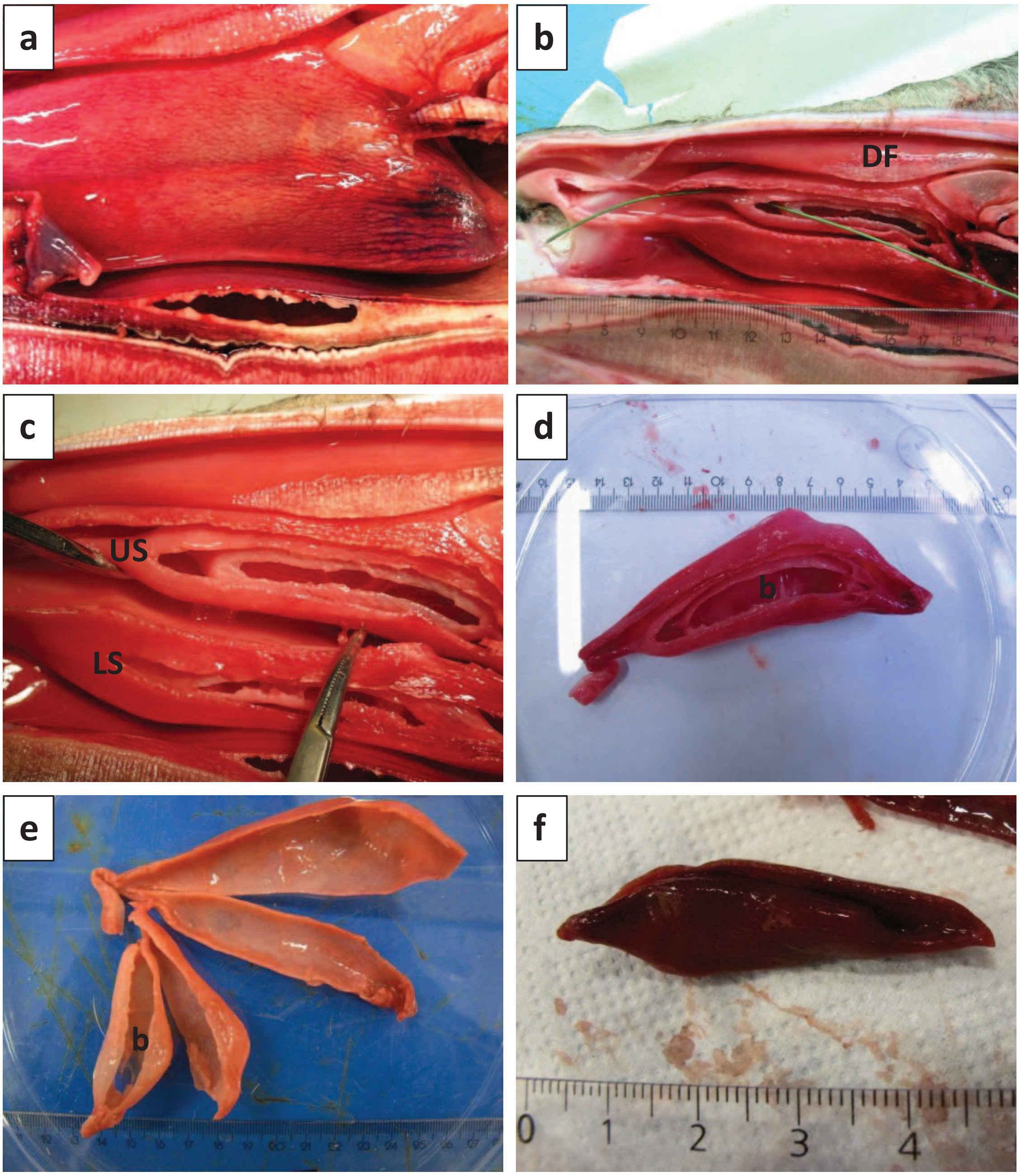

Fig22: a: well vascularised mucosa in juvenile reindeer; b: spaces between layers connected in the upper scroll (cut open) of juvenile reindeer. Checked with green cable. DF: dorsal fold; c: interconnectivity tissue between scrolls in juvenile reindeer. US: upper scroll, LS: lower scroll; d: upper scroll (cut open) of juvenile reindeer out of the nasal cavity (b: closed "bulb"); e: layers of upper scroll in juvenile reindeer (b: closed "bulb"); f: closed "bulb" of reindeer calf (photos a-e by Acquarone).

In the cross section slices from both juvenile and reindeer calf, it can be seen the same turbinate structure. In the juvenile 19 cross section slices were obtained and 11 in the calf. They were numbered from nostrils to pharynx. The double scroll turbinate is presented with the dorsal fold in the up as it can be seen in figure 23a and b. It shows that the juvenile turbinate is better structured with more space for the scrolls. In reindeer calf the space is smaller as it shows in figure 23b. The more complex structure of the turbinate can be seen in the slices from the proximal part of the nasal cavity from juvenile and reindeer calf (number 16 and 8 respectively). In the upper scroll of slice number 16 of the juvenile (fig23c) it can be seen that there is one convolution more than in the slice number 8 from the calf (fig23d).

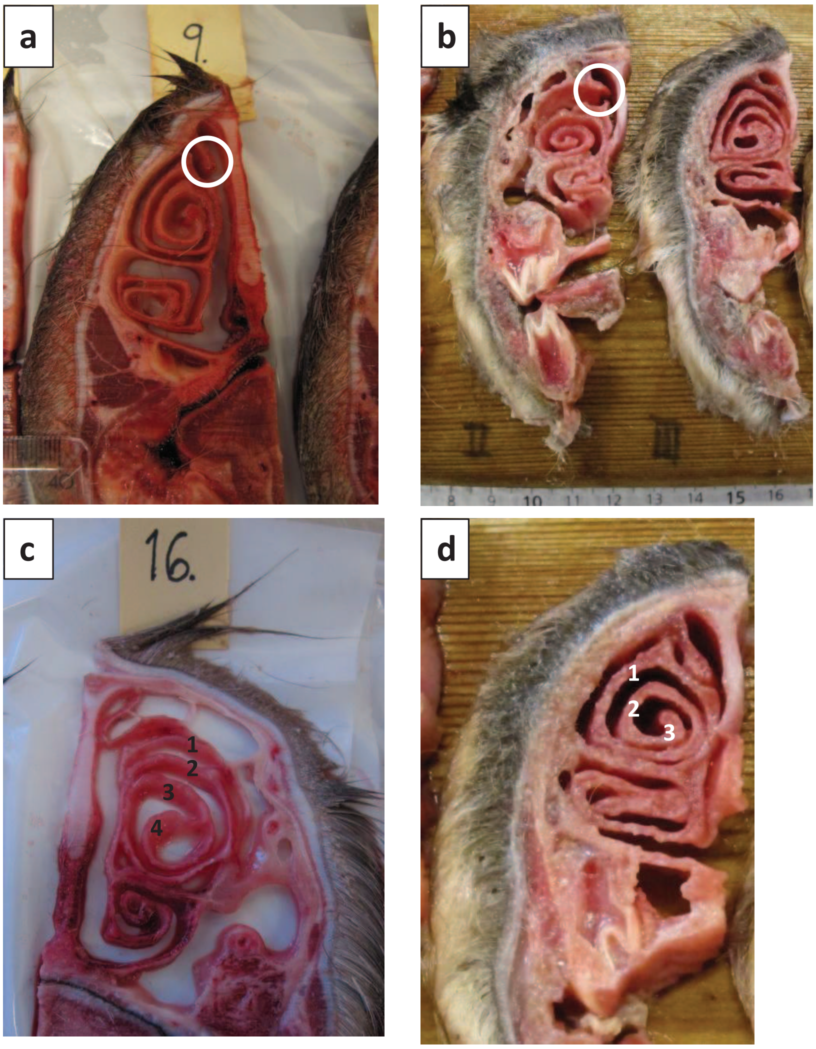

Fig23: a: cross section slice number 9 of juvenile reindeer with dorsal fold in white circle; b: cross section slices number 9 and 10 of reindeer calf with dorsal fold in white circle; c: cross section slice number 16 of juvenile reindeer with number of convolutions (1,2,3,4) in black in the upper scroll; d: cross section slice number 8 of reindeer calf with number of convolutions (1,2,3) in white in the upper scroll (photos a and c by Acquarone).

3.1.2. CT-scan:

CT-scanner image measures were kindly provided by Dr Acquarone.

In the images obtained with the CT-scanner it can be clearly seen that the turbinate structure is present in both adults and calves of both reindeer and muskoxen. The CT-scans show “undistorted” images of turbinates in contrast to the cut sections in the dissection part (fig23). The turbinate structures are visible in figures 24a (juvenile reindeer), 24b (reindeer calf), 24c (adult muskoxen) and 24d (calf muskoxen).

The images are from the middle sections of the nose, the more complex part of the turbinate. The black colour in the images is the air space. In reindeer calf (fig24b) the nasal passage is not black because it is filled with material of the same opacity as soft tissue (probably mucus). It can be seen that reindeer turbinate is more complex than the muskoxen turbinate. Reindeer have more layers, convolutions in the scrolls. The image of muskoxen (fig24c) shows also the presence of the closed “bulb”.

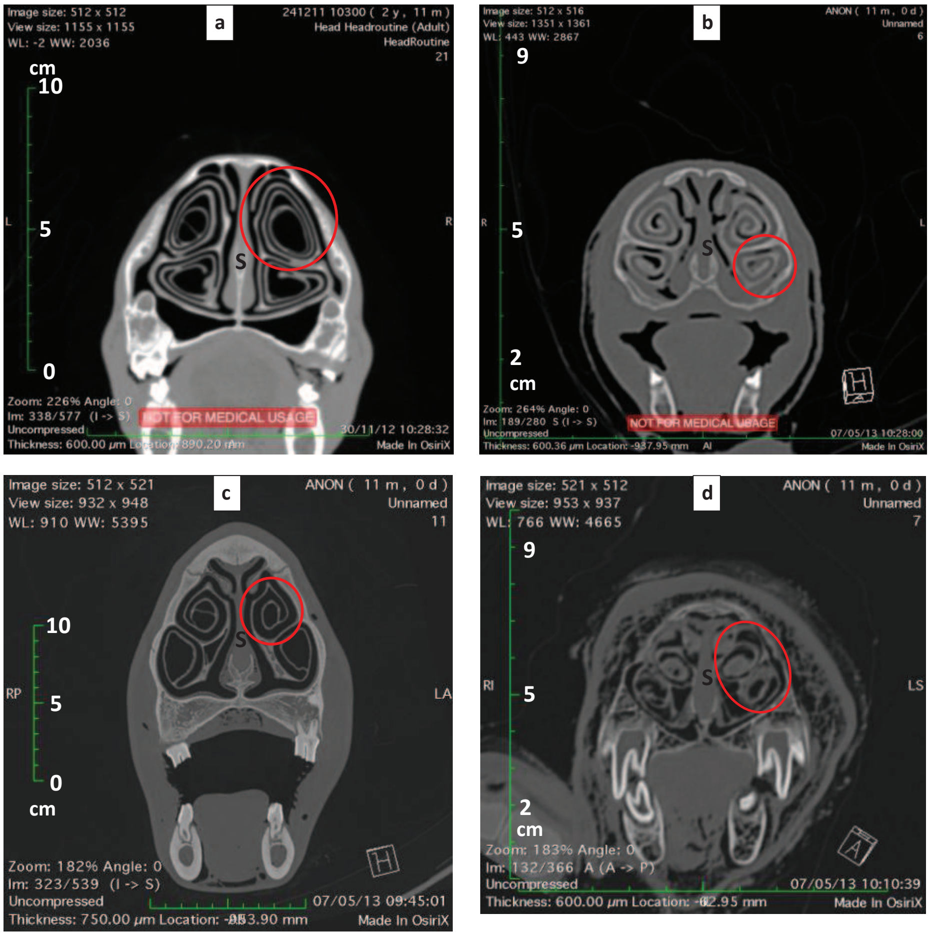

Fig24: a: CT-scan image of juvenile reindeer (R7-12) with upper scroll in red circle; b: CT-scan image of reindeer calf (9-10C) with lower scroll in red circle; c: CT-scan image of adult muskox (32-2002) with upper scroll with closed “bulb” in red circle; d: CT-scan image of calf muskox (35-1996C) with upper and lower scroll in red circle. S:septum (images by Acquarone).

It can be seen that in calves the turbinate is present but with less space for the distribution of the different layers. The muskoxen (adult and calf) are so much bigger than the reindeer (fig24).

Also, with CT-scan images the mucosal surface area exposed to air stream and air space volume of the whole nasal cavity was calculated in both reindeer and muskoxen in both calf and adult. The fact that the inside of the closed “bulb” is not exposed to air stream was taken into account to calculate the values. The mucosal surface area in juvenile reindeer (fig25a) has a curve where values in the middle proximal part of the nasal cavity are higher than the rest values. The volume of the nasal cavity in adult reindeer (fig25b) increases slowly until the middle slices of the nasal cavity when has a big increase.

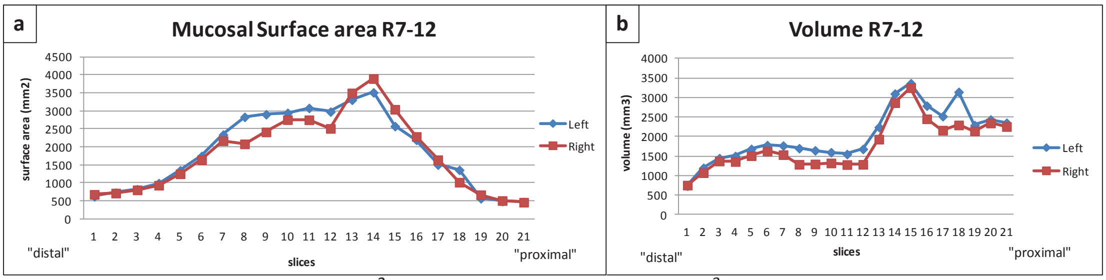

Fig25: a: mucosal surface area (mm^2) and b: air space volume (mm^3) of adult reindeer (R7-12) along the nasal cavity from distal to proximal part. Blue: left side, red: right side.

In reindeer calf 9-10C it was not possible to obtain values for the whole nasal cavity because the nasal passage was filled with some material with the same opacity as the soft tissue. But in figure 26 it can be seen that the mucosal surface area (fig26a) and the air space volume (fig26b) has the highest values in the middle part of the nasal cavity, where the measurements stop.

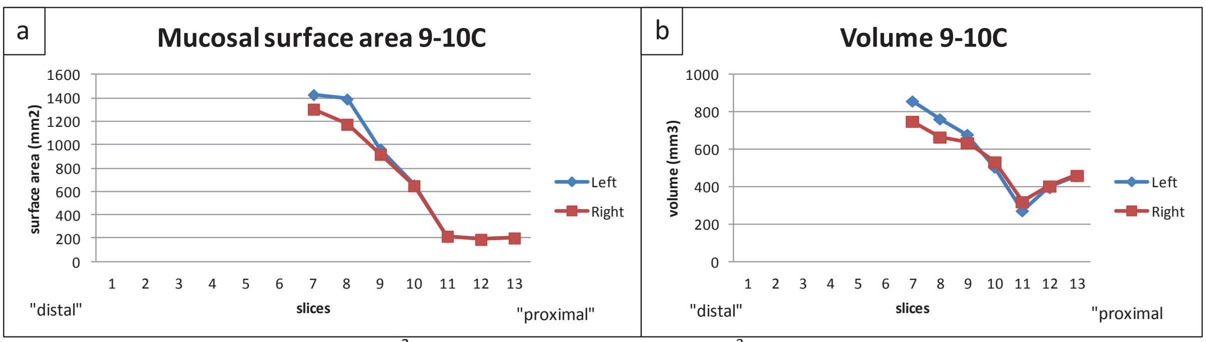

Fig26: a: mucosal surface area (mm^2) and b: air space volume (mm^3) of reindeer calf (9-10C) along the nasal cavity from distal to proximal part. Blue: left side, red: right side. No values until slice 7 because the nasal passage was filled with material of the same opacity as soft tissue.

In the other reindeer calf (5-05C) it was only possible to get measurements of the whole nasal cavity from the left side. In the right side the turbinate in the middle of the nostril was destroyed (probably scavenged by birds). In figure 27 it can be seen that in the left side the mucosal surface area (fig27a) and the volume (fig27b) have the same curvature than reindeer juvenile, higher values in the middle part of the nasal cavity.

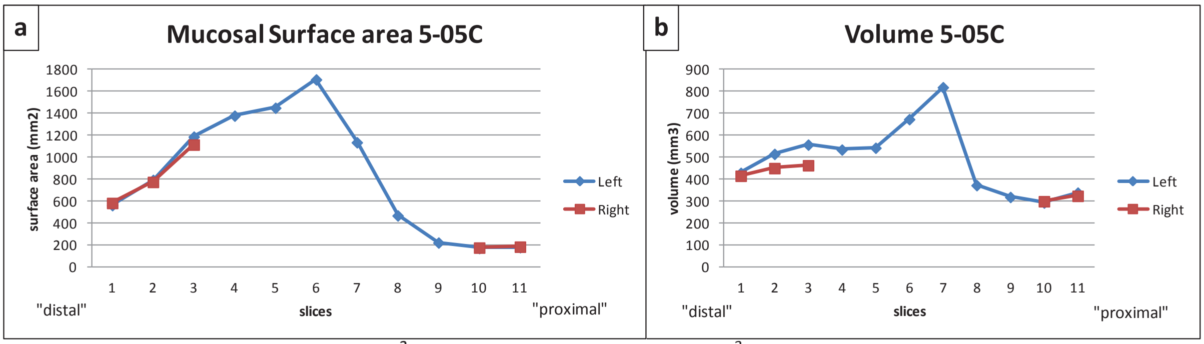

Fig27: a: mucosal surface area (mm^2) and b: air space volume (mm^3) of reindeer calf (5-05C) along the nasal cavity from distal to proximal part. Blue: left side, red: right side. Not values in the right side (red) in the middle of the nostril because turbinate was destroyed.

The mucosal surface area of the adult muskoxen (fig28a) has also an increase in the slices from the proximal part of the nasal cavity. The air space volume of the nasal cavity in the adult muskoxen (fig28b) increases from distal to proximal part.

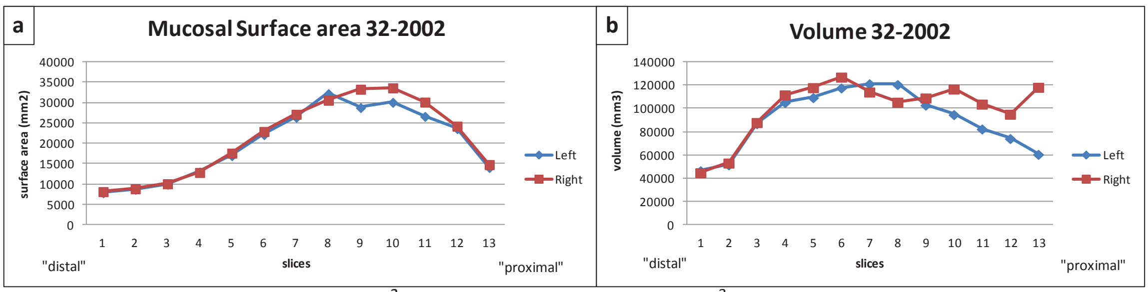

Fig28: a: mucosal surface area (mm^2) and b: mucosal volume (mm^3) of adult muskoxen (32-2002) along the nasal cavity from distal to proximal part. Blue: left side, red: right side.

In the muskoxen calf (35-1996C) the mucosal surface area (fig29a) increases along the nasal cavity, from distal to proximal part, with the highest values in the last slices. The air space volume of the nasal cavity (fig29b) is higher in the slices from the proximal part of the nasal cavity.

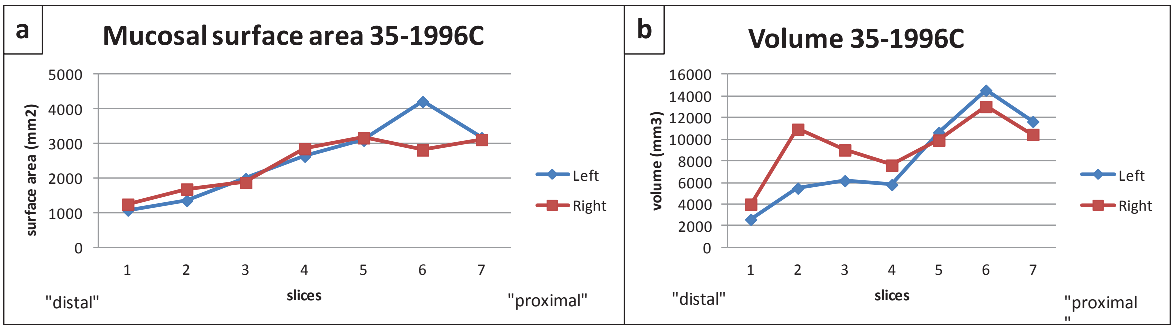

Fig29: a: mucosal surface area (mm²) and b: air space volume (mm³) of muskoxen calf (35-1996C) along the nasal cavity from distal to proximal part. Blue: left side, red: right side.

In general, the mucosal surface area along the nasal passages follow the same distribution of values, where the highest values are in the slices from the proximal part of the nasal cavity. As shown in the dissection part the most complex turbinate is in the proximal part of the nasal cavity. The volumes of the nasal cavity also follow more or less the same distribution with higher values in middle slices.

As shows table 2 the total mucosal surface area in juvenile reindeer is 772cm^2 and the total volume of the nasal cavity is 0.08 litres. The total mucosal area in reindeer calf 5-05C was calculated assuming full symmetry in both sides. So, the total mucosal area in reindeer calf 5-05C is 185cm^2 and 0.011litres the total left volume. The mucosal surface area is almost 45% bigger in juvenile reindeer than in calf (5-05C) and the volume is 80% bigger.

The total surface area of adult muskoxen is 5363cm^2 and the total volume of the nasal cavity is 2.48liters. This value is very large in comparison with adult reindeer. And the muskoxen calf is bigger than the reindeer calf (table2). The mucosal surface area is almost double in muskoxen calf than in reindeer calf (5-05C).

*Table 2: summary of the values of the mucosal surface area and air space volume of the nasal cavity from left and right sides, and the total nasal cavity. *not possible to calculate the total values because there are not measurements of the complete nasal cavity. **only values from the left side, assumed full symmetry to calculate total values.*

	Left		Right		Total	
	surface (cm2)	volume (dm3)	surface (cm2)	volume (dm3)	surface (cm2)	volume (dm3)
R7-12 reindeer juvenile	394,228	0,043	378,426	0,037	772,654	0,080
9-10C reindeer calf *						
5-05C reindeer calf **	92,687	0,005			185,373	0,011
32-2002 muskoxen adult	2611,885	1,176	2751,155	1,307	5363,040	2,484
35-1996C muskoxen calf	175,684	0,057	167,931	0,065	343,615	0,122

3.2. Histology:

The general structure of the mucosa from outer to inner layer is formed by an outmost epithelium of respiratory type, then a rich vasculature (arteries, veins and capillaries) with some glands, mostly serous and mucosal glands, and an innermost of cartilage or bone (fig30). Histology examination was only conducted in reindeer (juvenile and calf).

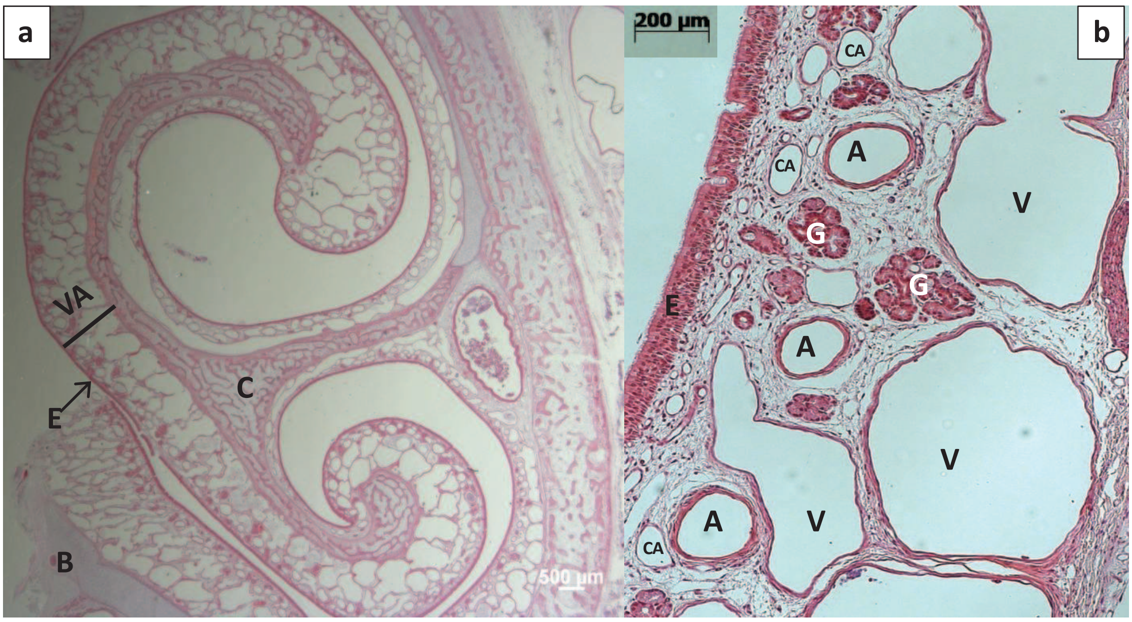

Fig30: general structure of the mucosa. E: epithelium, VA: vasculature, A: arteries, V: veins, CA: capillaries, G: glands, C: cartilage and B: bone.

3.2.1. Analysis of sections at different levels:

Along the nasal cavity, in both calf and juvenile reindeer, it can be seen that arteries and veins are in near contact (fig31 and 32). The space that veins occupy is much larger than the one occupied by the arteries, so arteries are between veins, in closer contact between them.

Figure 31 shows an overview of the full turbinate structure in reindeer calf from nostrils to pharynx. In the first histological slice, 12-10C A0, (fig31a) the turbinate is not present, but along the nasal cavity it increases in size and complexity. In slice 12-10C AIV (fig31e) the turbinate structure is the most complex along the nasal cavity and the closed "bulb" (fig33a) is present in the upper scroll.

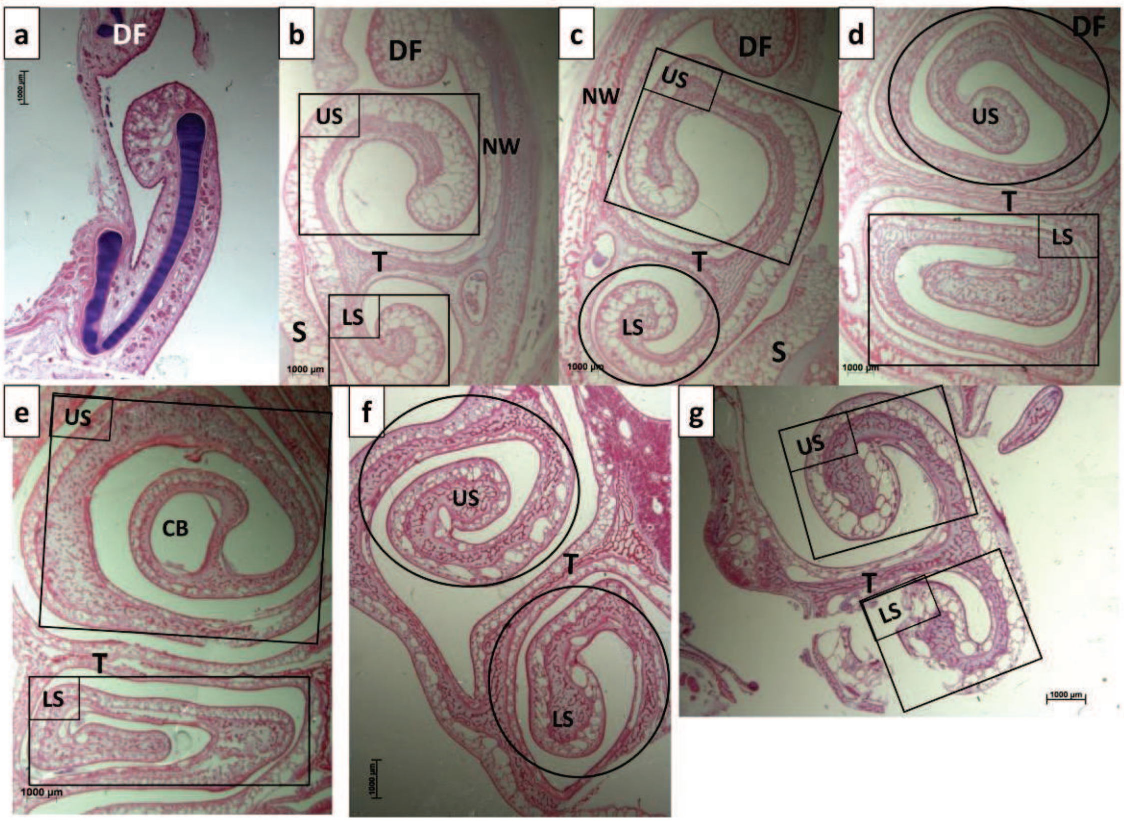

Fig31: microscopy images of nasal turbinate from reindeer calf (12-10C) from nostrils (a) to pharynx (g). a:12-10C A0, b:12-10C AI, c:12-10C AII, d:12-10C AIII, e:12-10C AIV, f:12-10C AV, g:12-10C AVI. DF: dorsal fold, S: septum, T: T part, NW: nasal wall, US: upper scroll, LS: lower scroll, CB: closed "bulb".

Figure 32 shows an overview of the full turbinate structure in juvenile reindeer, from nostrils to pharynx. In the microscopy images of the juvenile reindeer it can be seen only one of the scrolls because the turbinate was so big that with the smallest magnification it was not possible to get the whole turbinate in view. In the first histological slice, R4-12 AI, (fig32a) the turbinate is not present, but along the nasal cavity it increase in size and complexity. In slice R4-12 AVI (fig32c) the turbinate structure is at its most complex point along the nasal cavity and the closed "bulb" (fig33b) is present in the lower scroll. In the next slices the turbinate decreases in size and complexity again. And in the last slice, R4-12 AXIII, (fig32g) turbinate is not present again.

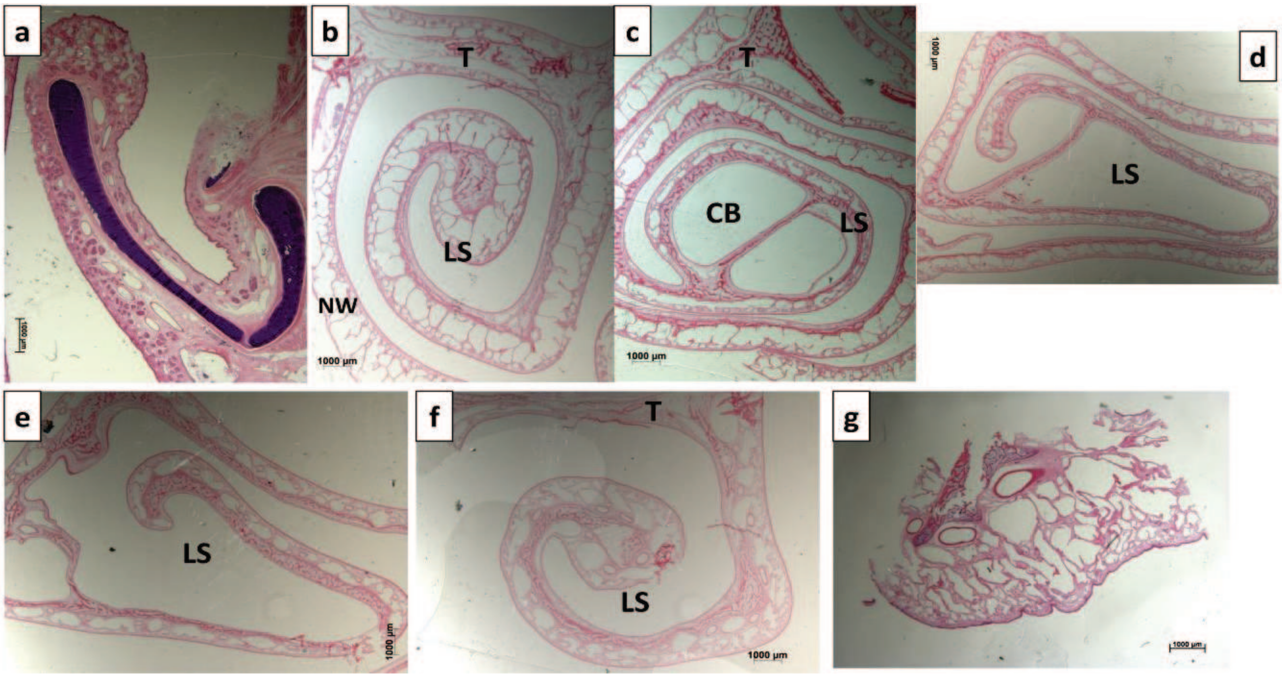

Fig32: microscopy images of nasal turbinate from juvenile reindeer (R4-12) from nostrils (a) to pharynx (g). a:R4-12 AI, b:R4-12 AIV, c:R4-12 AVI, d:R4-12 AVIII, e:R4-12 AX, f:R4-12 AXII, g:R4-12 AXIII. T: T part, NW: nasal wall, LS: lower scroll, CB: closed "bulb".

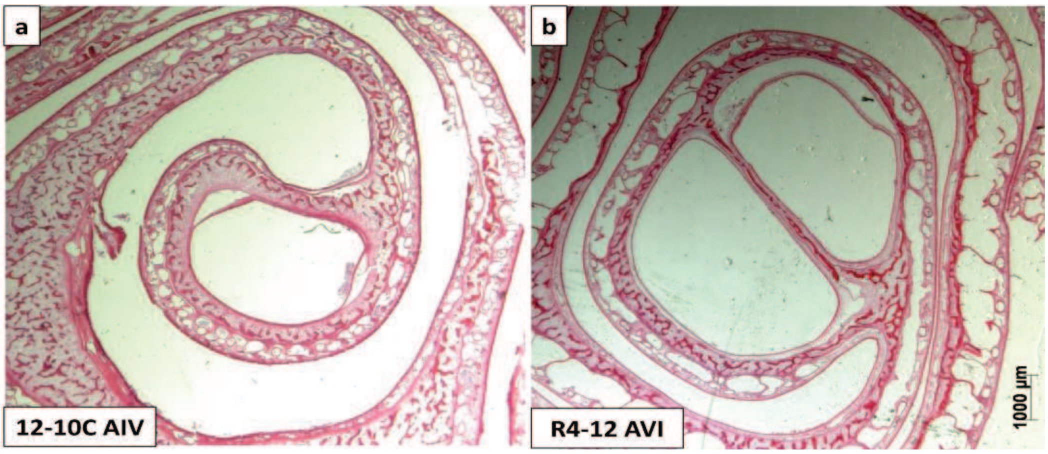

Fig 33: microscopy images of closed bulb in calf section (a) and juvenile section (b).

In the first histological slice from calf (12-10C A0) and juvenile (R4-12 AI) the turbinate was not present. However, it has been seen in the microscopy images that the amount of glands in the distal part of the nose is high (fig34) as Johnsen et al. 1985 found in their studies.

Apart from that it can be seen that in the rest of the nasal cavity (Appendix.fig1), where the turbinate is present, the number of glands (serous glands) is higher in the calf (12-10C) than in the juvenile (R4-12) reindeer (fig35).

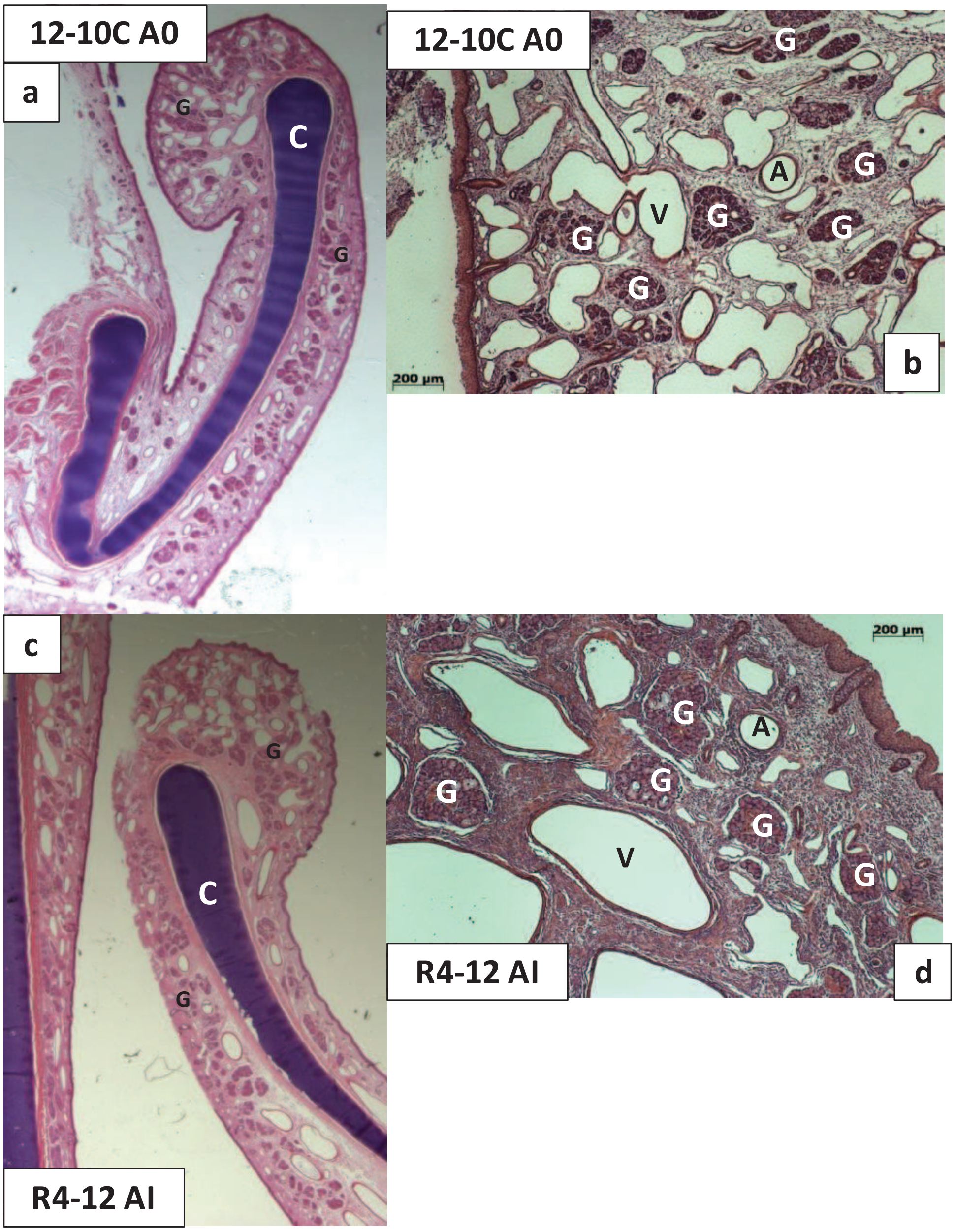

Fig34: first slice from calf (a and b) and juvenile (c and d) where it can appreciate the amount of glands (G). C: cartilage, E: epithelium, A: arteries, V: veins.

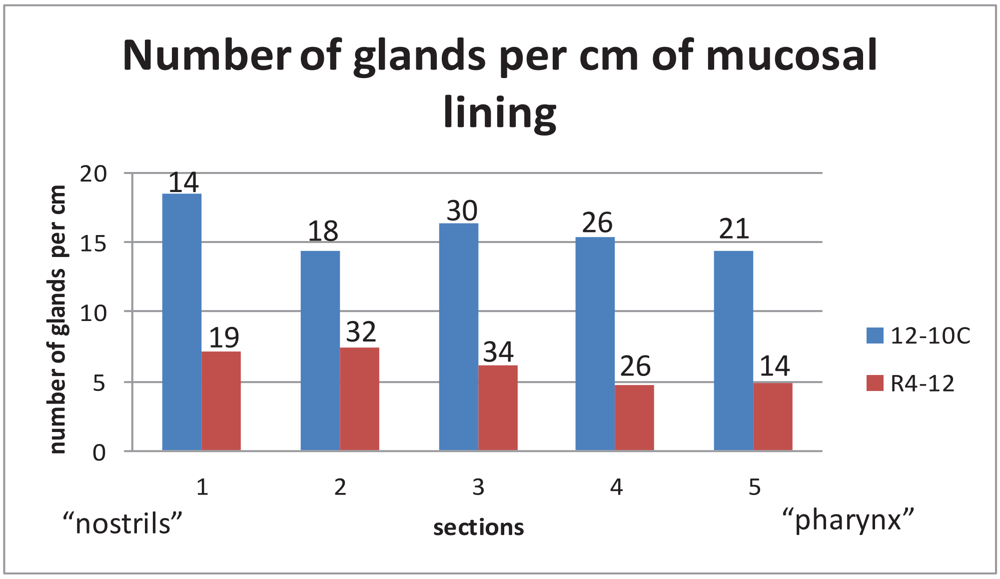

Fig35: the average number of glands per cm of mucosal lining in reindeer calf (12-10C): blue and juvenile reindeer (R4-12): red, in the turbinate along the whole nasal cavity from distal (section1) to proximal (section 5) part. Numbers above bars are "n" values.

The thickness of the turbinate lamella is very similar along the nasal cavity and in the different sections of the turbinate. The narrowest part of the turbinate is the middle sections (section 3 in fig36). It cannot see almost differences between calf and juvenile reindeer (fig36).

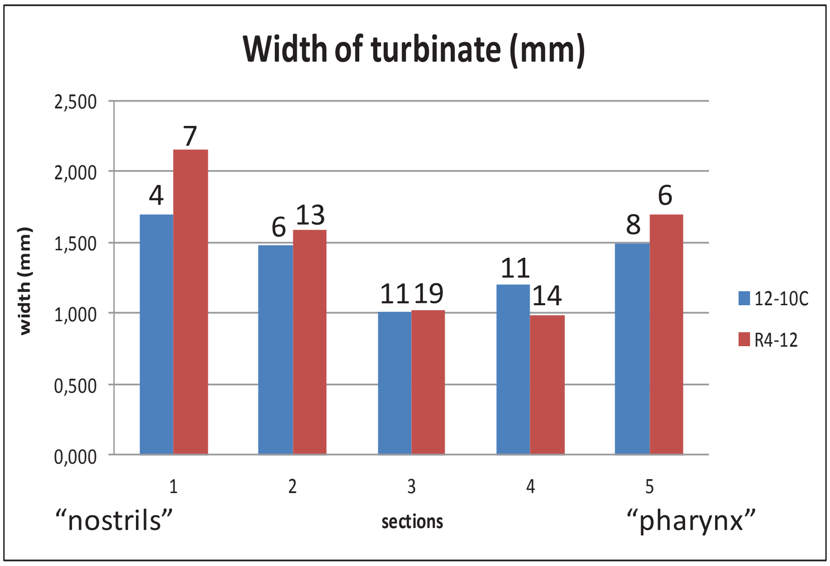

Fig36: the average of width in each section of the turbinate along the nasal cavity from distal (section 1) to proximal (section 5) part of juvenile (red) and calf (blue) reindeer. Numbers above bars are "n" values.

Figure 37 shows the differences between the outer and inner mucosal layer of the sections in the turbinate. The outer layer is much thicker than the inner. The number of vessels and the measurements of distance to the epithelium wall in the inner layers are lower in every section. The outer layer in comparison with the inner layer is extremely rich in blood vessels where veins (long with no shape determinate and thin wall) are easily distinguishable from arteries (small with round shape and thick wall).

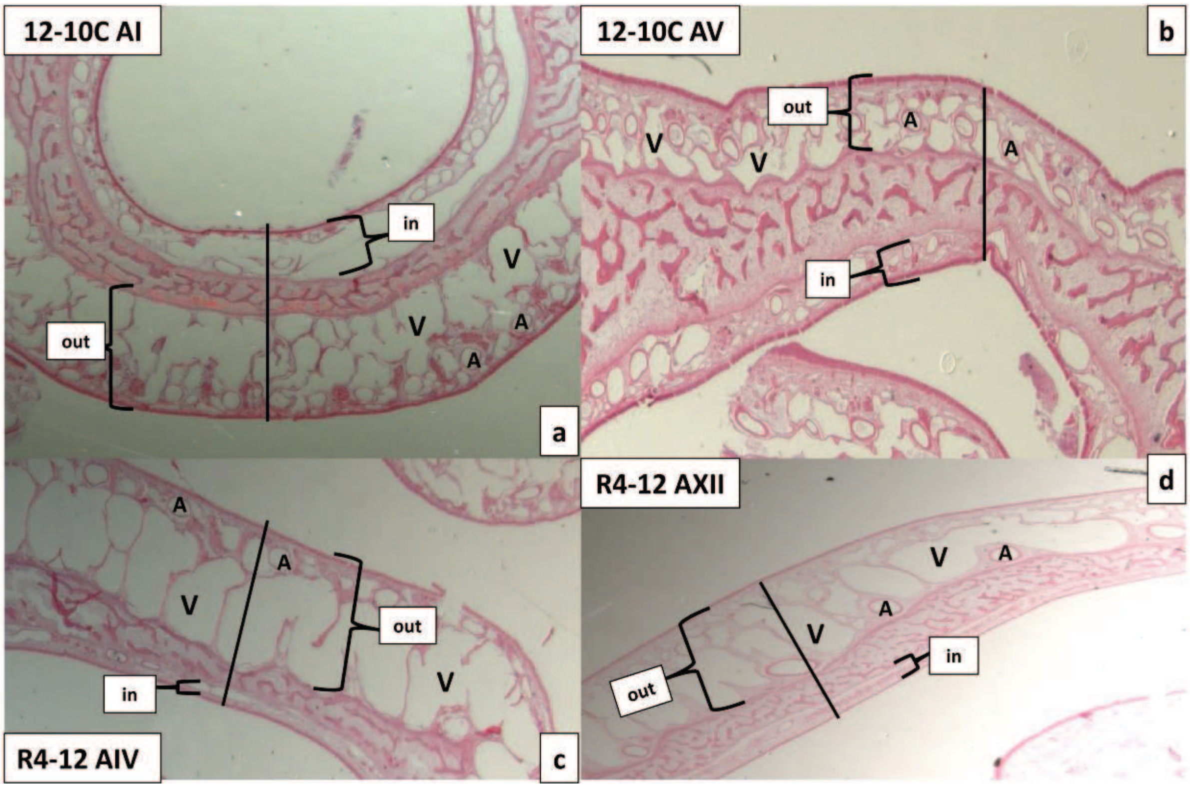

Fig37: microscopy images from reindeer calf (a and b) and reindeer juvenile (c and d) showing the width of the turbinate (black line) and the large difference between out and in layers of the section. V: veins, A: arteries.

In the study measurements of the distance between the vessels (arteries and veins) and the epithelium wall were taken to indicate the proximity of blood flow and air flow, which is relevant for assessment of efficiency of heat transfer between these.

In the reindeer calf (12-10C) the arteries move away from the epithelium along the nasal cavity, from distal to proximal part (fig38). In the proximal part of the nose (12-10C AV) the distance is highest (fig38c). The distance between the veins and the epithelium is generally larger than for arteries, and it is largest in the distal than in the proximal part of the nasal cavity (12-10C AI) (fig38d).

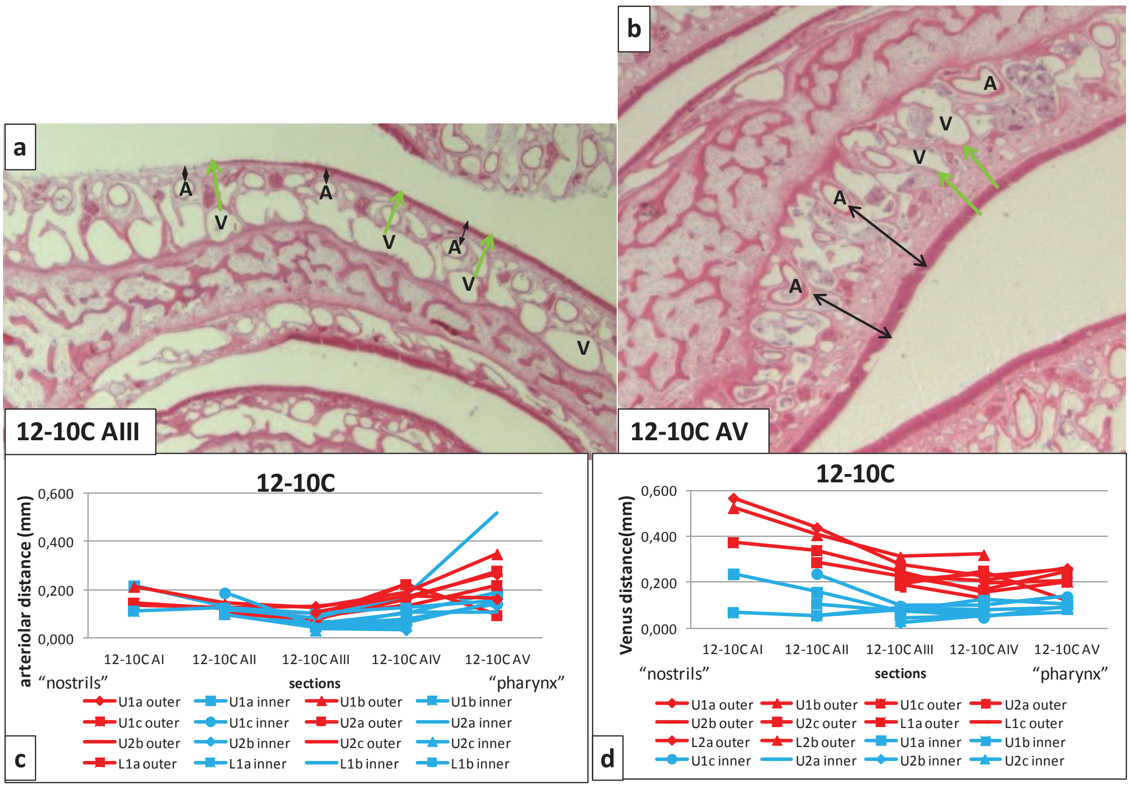

Fig38: arteriolar and vein distances from the epithelium in the reindeer calf (12-10C) in different sections of the nasal cavity. a and b: black arrows: arteriolar distances, green arrows: vein distances. V: veins, A: arteries. c: arteriolar wall distance (mm), d: venous wall distance (mm). Red lines: outer layers, blue lines: inner layers of the sections along the nasal cavity.

In juvenile reindeer (R4-12) the measurements show very similar results. The arteriolar distances from the epithelium increase in the most proximal section of the nasal cavity (R4-12 AXII) (fig39b and c). In the case of the veins it shows that in the distal section of the nasal cavity (R4-12 AIV) and in the proximal section of the nasal cavity (R4-12 AXII) the distance to the epithelium wall is somewhat larger than in the rest of the nasal cavity (fig39d).

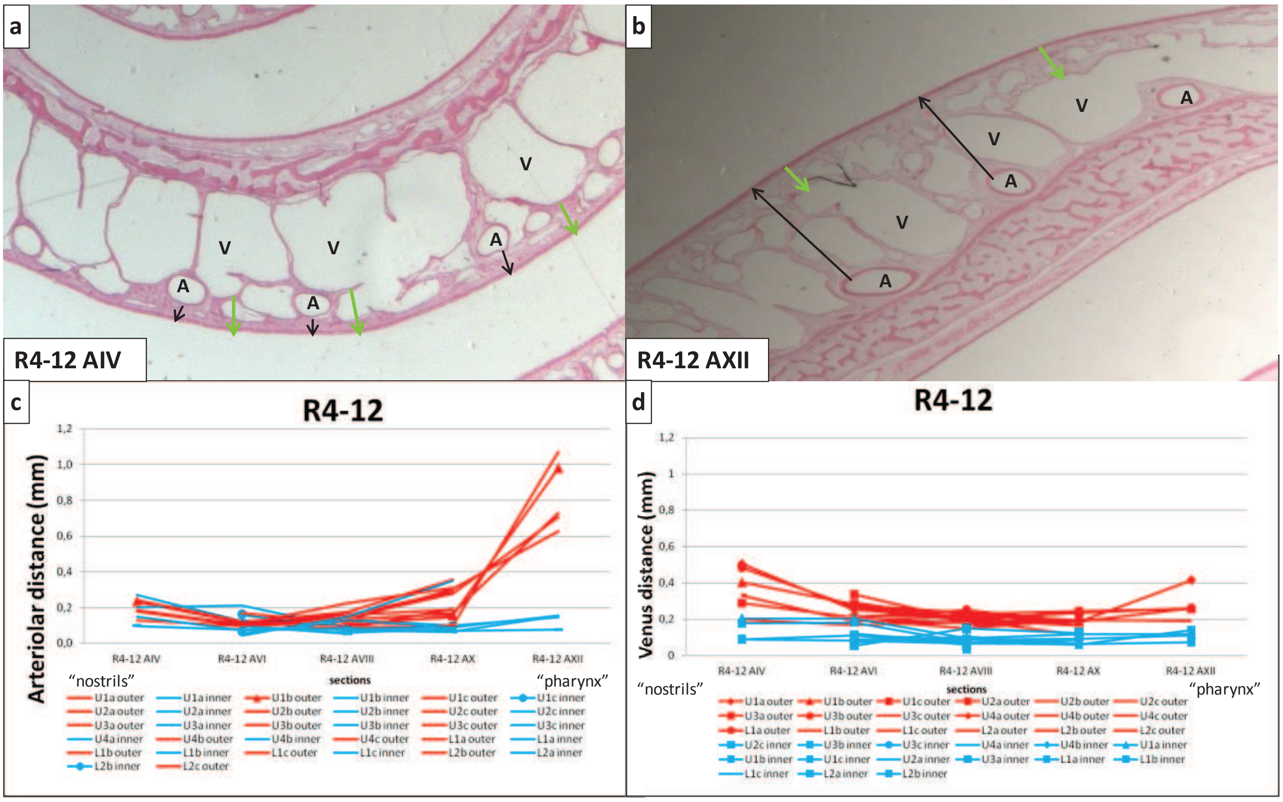

Fig39: arteriolar and vein distances from the epithelium in the juvenile reindeer (R4-12). a and b: black arrows: arteriolar distances, green arrows: vein distances. V: veins, A: arteries. c: arteriolar wall distance (mm), d: venous wall distance (mm). Red lines: outer layers, blue lines: inner layers of the sections along the nasal cavity.

Figure 40 summarizes arteriolar and venous wall distance values for both calf and juvenile reindeer. In the juvenile (red) the arteriolar wall distance is large in the proximal part of the nasal cavity (section 5). In the calf (blue) this is not so obvious. The vein graph (fig40b) shows that in both juvenile (red) and calf (blue) the veins are deeper at the distal part of the nose (section 1) and then, more superficial in the mid-sections with a small peak in the last section.

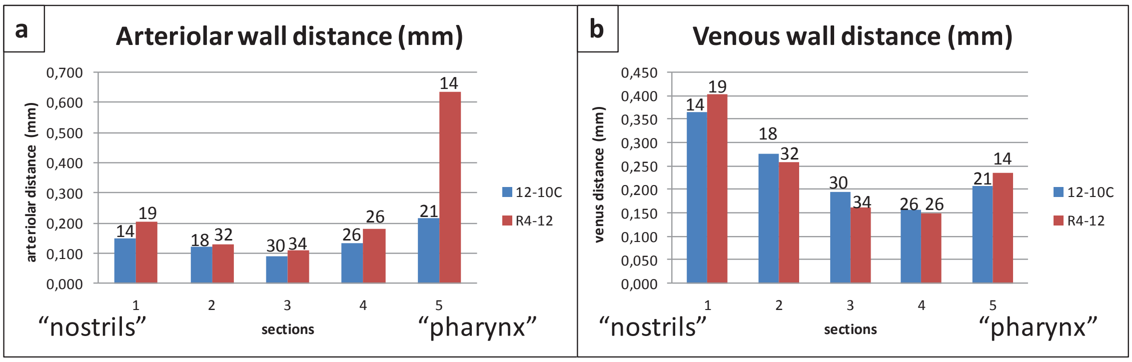

Fig40: average of arteriolar and vein distances to the epithelium wall. Red bars: juvenile (R4-12), blue bars: calf (12-10C) reindeer. Numbers above bars are "n" values.

In the study also vessels size measurements (maximum width of arteries and veins parallel to the epithelium wall called "diameter") have been taken (Appendix.fig2 and 3). In the calf (12-10C) the arteriolar diameter is very similar along the nasal cavity, from distal (1) to proximal (5) sections (fig41e). The venous diameter is larger in the first section, distal part of the nose, than in the other sections which are homogenous (fig41f).

In the juvenile reindeer (R4-12) the arteriolar diameter is larger in the proximal part of the nasal cavity (section 5) (fig41e). In the mid-sections (3 and 4) the values are smaller than in the other sections (fig41e). The venous graph (fig41f) has the same curvature. The values in the mid-sections (3 and 4) are smaller than the values in sections 1, 2 and 5 being section 5 the largest value.

In general the arteriolar and venous diameters are larger in the older than in the newborn reindeer. Also figure 41 shows that the veins are generally larger than the arteries.

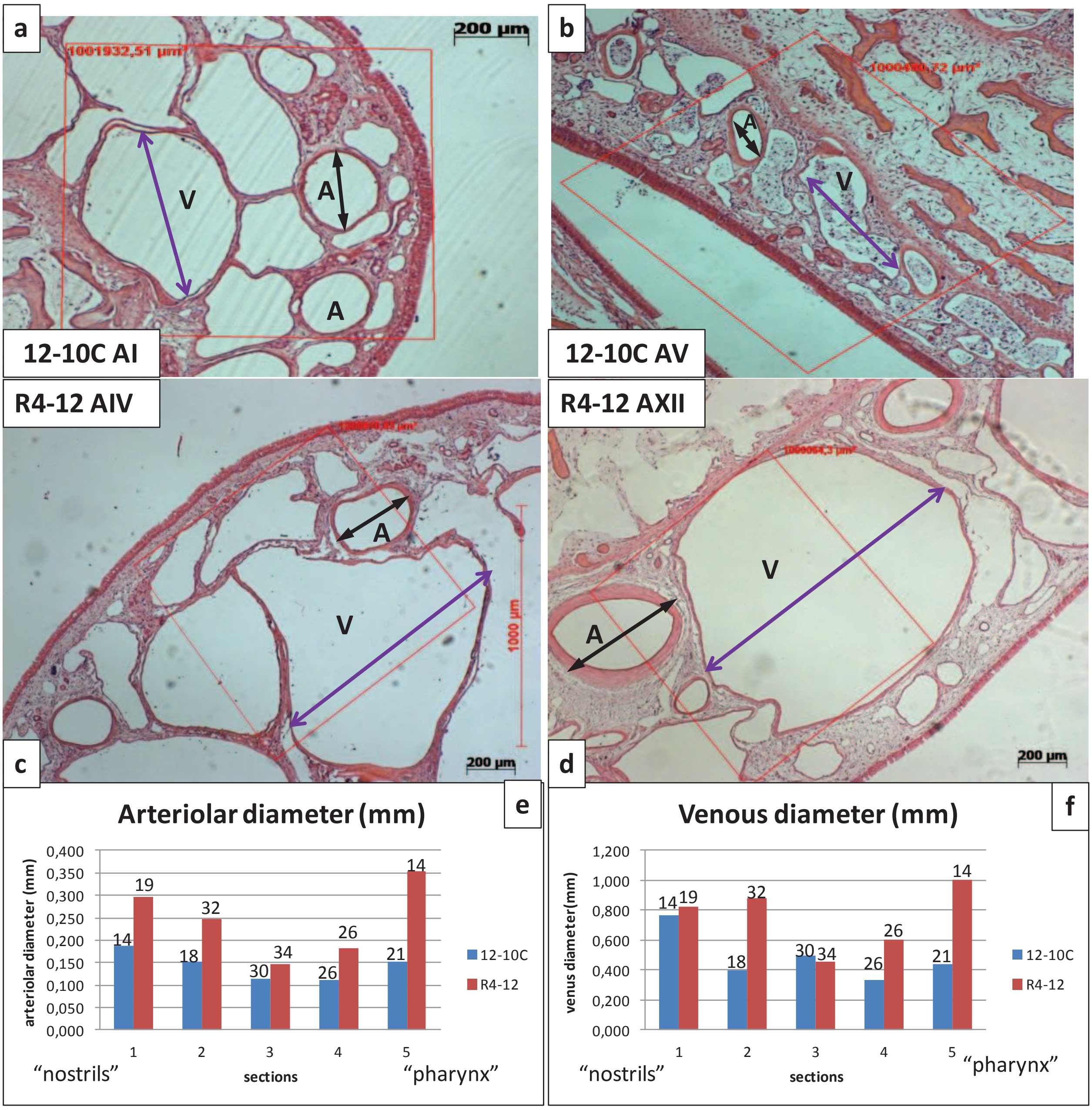

Fig41: arteriolar and venous diameters in calf (a and b) and juvenile (c and d) reindeer. Black arrows: arteriolar diameters, purple arrows: venous diameters. V: venous, A: arteries. e: average of arteriolar diameters along the nasal cavity. f: average of venous diameters along the nasal cavity. Red bars: juvenile reindeer (R4-12), blue bars: reindeer calf (12-10C). Numbers above bars are "n" values.

Arteriolar and venous proportional x-section areas were also determined in the study as an expression of the richness of vascularization. $1mm^2$ squares were taken randomly to calculate the areas in each section. Values were obtained in % of the selected total square area ($1mm^2$).

In the reindeer calf (12-10C) the arteriolar x-section values (Appendix.fig4a) decrease along the nasal cavity, from distal (section1) to proximal (section5) part of the nose (fig42e). The venous x-section values (Appendix.fig5a) decrease also, along the nasal cavity, with higher values in the distal part of the nose (sections 1 and 2) (fig42f).

In the juvenile reindeer (R4-12) the differences between arteriolar x-section values (Appendix.fig4b) along the nasal cavity are higher. The x-section areas decrease along the nasal cavity, from distal (section1) to proximal part; but in the last part of the nose (section5) there is a large increase in the arteriolar x-section area (fig42e). The venous x-section values (Appendix.fig5b) decrease also along the nasal cavity from distal to proximal part (fig42f).

In general, the space occupied by veins was some 6-10 times higher than the arteriolar values and there are almost no differences between juvenile and reindeer calf (fig42).

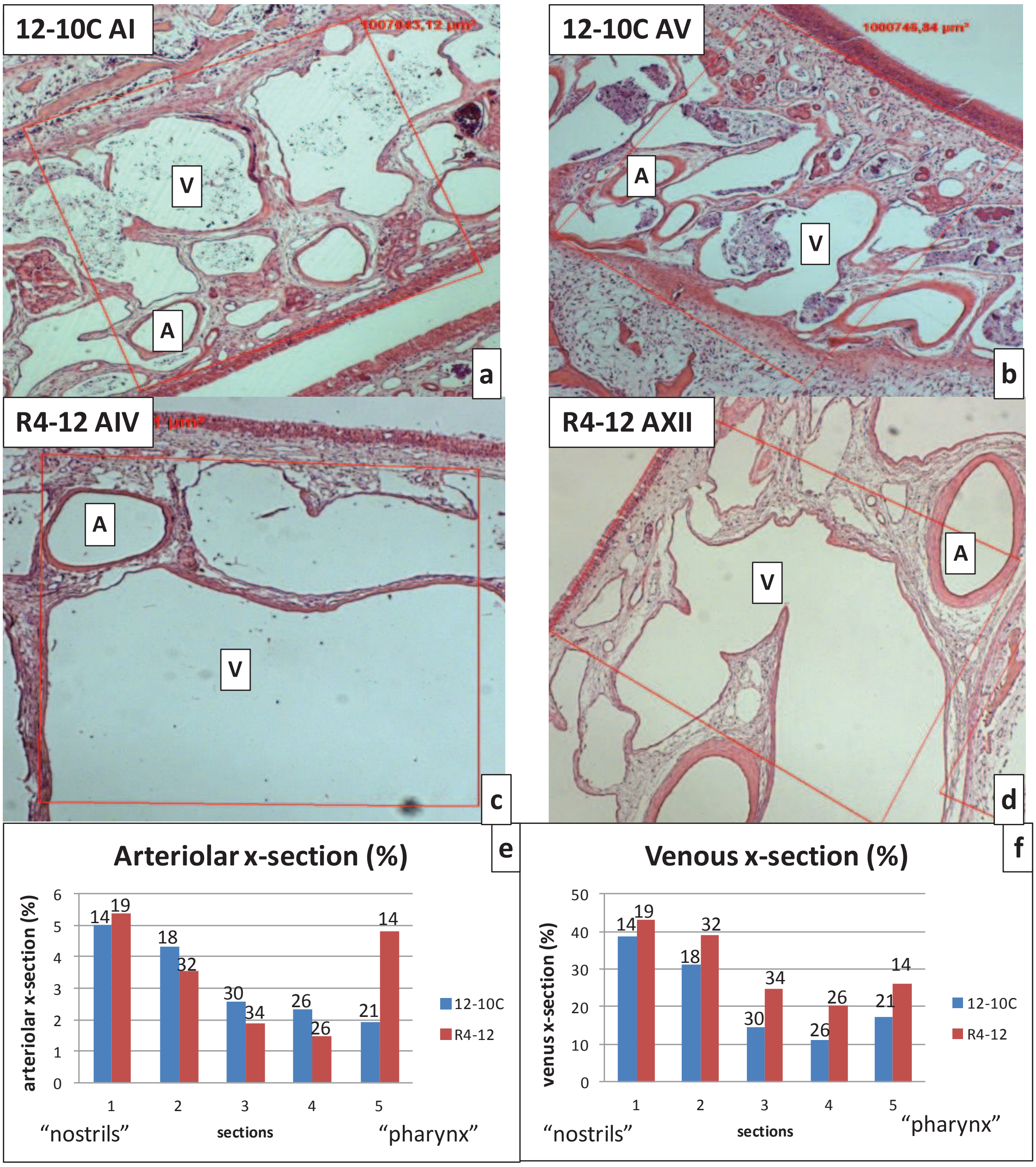

Fig 42: average of arteriolar and venous x-section values expressed in % in juvenile (R4-12) and calf (12-10C) reindeer. Red squares determine 1mm^2. A: arteries, V: venous. Numbers above bars are "n" values.

Also, counts of arteries and veins per cm were taken in the study as another measure of vascular density (Appendix.fig6 and 7). The values in figure 43 show that the density of arteries and veins in the reindeer calf (12-10C) is much higher than in the juvenile reindeer (R4-12) in any of the sections of the nasal cavity.

In general, figure 43 shows that the number of arteries is lower than the number of veins in both calf and juvenile reindeer. The values are constant along the nasal cavity, from the distal to the proximal part, with a small decrease in the vessel density (fig43).

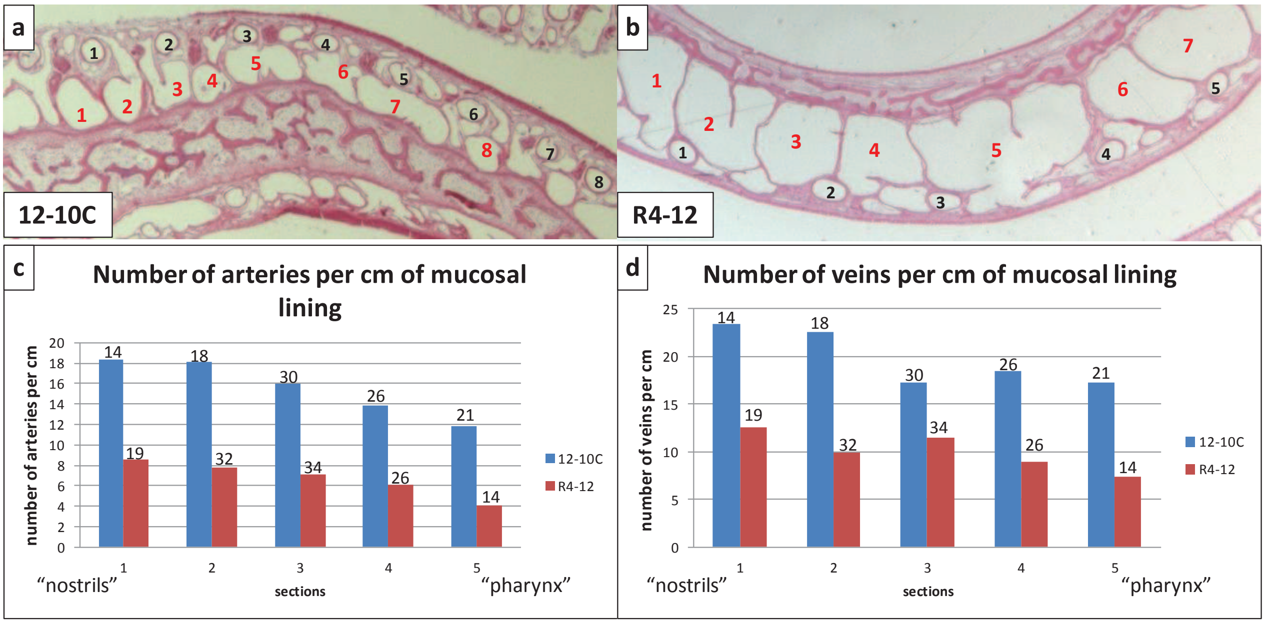

Fig 43: average number of arteries and veins per cm of mucosal lining in reindeer calf (12-10C): blue and juvenile reindeer (R4-12): red, in the turbinate along the whole nasal cavity from distal (section1) to proximal (section 5) part. Black numbers: arteries, red numbers: veins. Numbers above the bars are "n" values.

The number of capillaries per cm of mucosal lining was also measured (Appendix.fig8). Values in figure 44 shows that density of capillaries in reindeer calf (blue) is higher in the distal and proximal part of the nasal cavity, but in the middle sections (2 and 3) values are very similar.

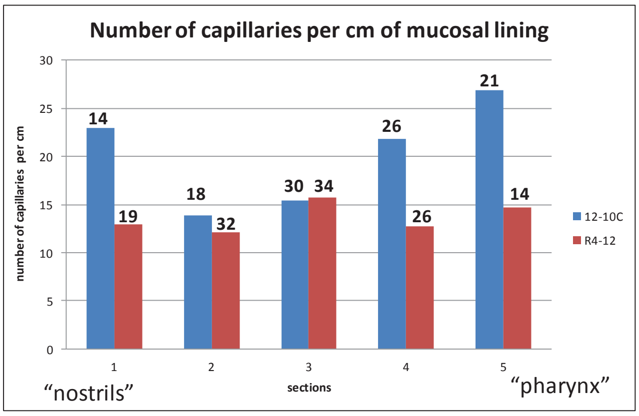

Fig 44: average number of capillaries per cm of mucosal lining in reindeer calf (12-10C): blue and juvenile reindeer (R4-12): red, in the turbinate along the whole nasal cavity from distal (section1) to proximal (section 5) part. Numbers above the bars are "n" values.

If only the wall of the nasal cavity is observed, it can be seen that arteriolar x-section in the distal part of the nose is larger in juvenile reindeer (R4-12), but in the rest of the nasal cavity the values are very similar (fig45a). The space occupied by veins is higher in juvenile reindeer than in calf (fig45b) along the nasal cavity. Figure 45 shows that venous x-section is much larger than arteriolar x-section, 10-12 times higher. The nasal wall was not present in the histological slices from the proximal part of the juvenile reindeer (R4-12), so it cannot be compared with the calf.

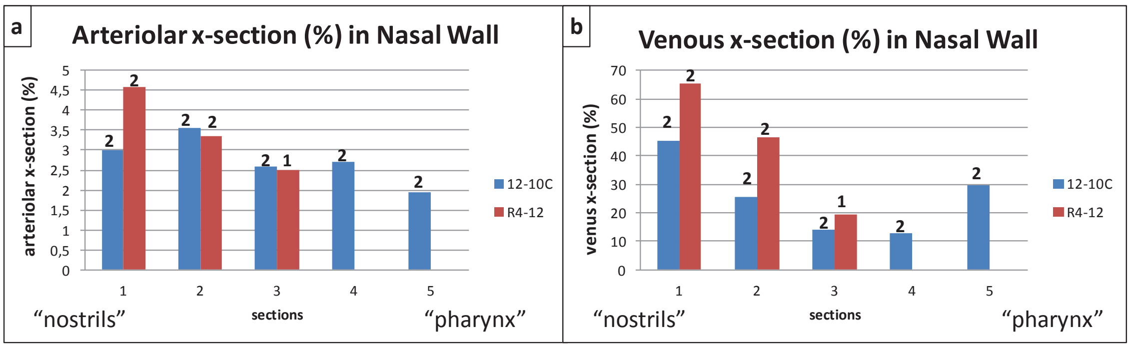

Fig 45: average of arteriolar and venous x-section values from Nasal wall expressed in % in reindeer calf (12-10C): blue and juvenile reindeer (R4-12): red, in the turbinate along the whole nasal cavity from distal (section1) to proximal (section 5) part. Numbers above the bars are "n" values.

The septum in reindeer calf was only present in preparation section number 3, and in juvenile reindeer septum was not present in the proximal slices, so the proportional x-section areas cannot be compared (fig46). But in the available material it can be seen that the space occupied by veins is much higher than the arteries space.

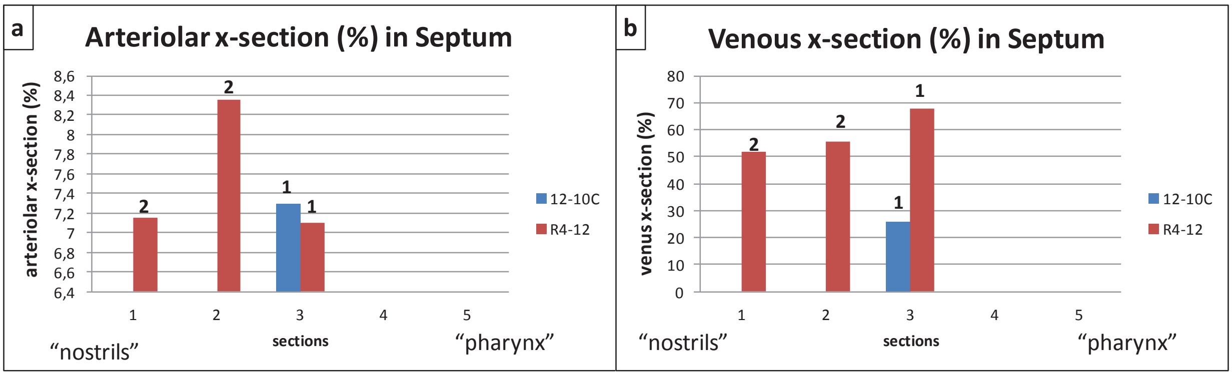

Fig 46: average of arteriolar and venous x-section values from Septum expressed in % in reindeer calf (12-10C): blue and juvenile reindeer (R4-12): red, in the turbinate along the whole nasal cavity from distal (section1) to proximal (section 5) part. Numbers above the bars are "n" values.

3.2.2. Statistics:

Replicates of measurements were taken to check if the measure method was reliable. Statistic was applied between the “real” values (called *Test* in table 3) and the replicates (called *Repl* in table 3). F-tests for equal variance were used to see if equal or not equal variance should be used for t-tests for equality of means. All the values were equal variance; therefore a t-test was used (two-sample assuming equal variances).

The t-test showed no significant difference between “*Test*” and “*Replicate*” measurements. The p values were < 0,05 for the one tail test and <0,1 in the two tails test. The test parameters and results for some of the samples are shown in table 3.

Table 3: Example of T-tests for equality of the mean assuming equal variances in different sections of the turbinate in juvenile (R4-12) and calf (12-10C) reindeer. Reindeer calf arteriolar diameter measurements, juvenile reindeer arteriolar wall distance measurements. P values in red squares.

t-Test: Two-Sample Assuming Equal Variances		
12-10C AI U1a outer	*Test*	*Repl*
Mean	0,20385714	0,16192857
Variance	0,00201475	0,00083453
Observations	14	14
Pooled Variance	0,00142464	
Hypothesized Mean Difference	0	
df	26	
t Stat	2,9390468	
P(T<=t) one-tail	0,00341029	
t Critical one-tail	1,7056179	
P(T<=t) two-tail	0,00682058	
t Critical two-tail	2,05552942	

t-Test: Two-Sample Assuming Equal Variances		
12-10C AIV nasal wall U	*Test*	*Repl*
Mean	0,24623077	0,21661538
Variance	0,00179336	0,00173642
Observations	13	13
Pooled Variance	0,00176489	
Hypothesized Mean Difference	0	
df	24	
t Stat	1,79727738	
P(T<=t) one-tail	0,04244368	
t Critical one-tail	1,71088207	
P(T<=t) two-tail	0,08488737	
t Critical two-tail	2,06389855	

t-Test: Two-Sample Assuming Equal Variances		
R4-12 AIV U1b outer	*Test*	*Repl*
Mean	0,23133333	0,27116667
Variance	0,00107867	0,00168137
Observations	6	6
Pooled Variance	0,00138002	
Hypothesized Mean Difference	0	
df	10	
t Stat	-1,85722753	
P(T<=t) one-tail	0,04646844	
t Critical one-tail	1,8124611	
P(T<=t) two-tail	0,09293689	
t Critical two-tail	2,22813884	

t-Test: Two-Sample Assuming Equal Variances		
R4-12 AVI Tupper	*Test*	*Repl*
Mean	0,1196	0,1893
Variance	0,00195604	0,00312046
Observations	10	10
Pooled Variance	0,00253825	
Hypothesized Mean Difference	0	
df	18	
t Stat	-3,09350329	
P(T<=t) one-tail	0,00313387	
t Critical one-tail	1,73406359	
P(T<=t) two-tail	0,00626774	
t Critical two-tail	2,10092204	

4. DISCUSSION:

4.1. Gross anatomy:

Juvenile and calf reindeer have the double scroll turbinate being more complex in the middle slices, with more layers (4 and 3 respectively). The calf has less space between the layers of the scrolls in the turbinate while the juvenile has more space for the distribution of the layers. In the cross section view it can be seen that juvenile reindeer has around 4cm from septum to nasal wall while the reindeer calf has only 2cm (fig24a and b). This can be just a size aspect, the turbinate is completely developed from birth but the nasal cavity is getting bigger during growing.

A non-invasive anatomy description of turbinate geometry was done using CT-scan images. In contrast to cut sections, which will be affected by the cutting process, these images gave a better impression of the positions of turbinate scrolls in relation to each other and to the nasal cavity perimeter. CT-scan was done in several reindeer (3 juveniles and 3 calves) but only in 1 adult and 1 calf muskoxen due to specimen limitations. In reindeer, it can be concluded that the structure is generally the same in both juvenile and calf. Also, in muskoxen, it can be concluded that the structure is present in both adult and newborn, at similar levels of complexity.

The discovery of the closed inner “bulb”, which is not ventilated, could affect the surface area calculation ($0.12m^2$) that Blix et al. published in 1983. Unfortunately the type of animal (age, sex, size…) which the calculation of the surface area was done is unknown. It is probable that the measurements of the mucosal surface area exposed to the air stream included the inner “bulb” surface which we now know is not ventilated, and therefore, is not involved in heat and water exchange. The measures by Blix et al (1983) could therefore be an overestimate so new measurements have been done in this study.

The total mucosal surface area in juvenile reindeer of 52.5kg is $0.077m^2$. The difference between the calculations of Blix et al. 1983 and our calculations is due to the discovery of the closed inner “bulb” in the turbinate whose inner surface was not included in our measurements; and possible also to size differences although Blix et al. did not estate the size of their animal.

The total mucosal surface area in juvenile reindeer ($772cm^2$) is higher than the reindeer calf ($185cm^2$). But, if the surface area is compared in proportion to mass, this can be different. If the surface area is calculated in relation to body mass (m^2/kg), reindeer calf has higher surface area (table4). If the surface area is calculated in relation to body mass in power of 0.75 ($m^2/kg^{0.75}$), is still higher the surface area of reindeer calf but the difference is smaller. The 0.75 power of body weight is representative of metabolic body size, and "$kg^{0.75}$" chosen as the symbol for the unit (Kleiber 1947). So if the surface area is calculated in relation to power of 0.75, the surface area is calculated in relation of metabolic body size.

If it´s calculated in relation to body mass in power of 0.67 ($m^2/kg^{0.67}$), the difference between juvenile reindeer and reindeer calf is smaller (table4). The exponent in the allometric relationship between surface area and body mass should be 0.67 (Döbeln 1966) according to the surface law. So, it may be concluded that mucosal surface areas of mature and newborn reindeer are quite comparable, if body size is taken into account.

The total surface area of the nasal cavity of adult muskoxen is 5363cm^2 and in muskoxen calf is 343cm^2. Table 4 shows a proportional surface area higher in muskoxen calf than in adult, but in relation to power of 0.75 and 0.67; the relative surface area in adult muskoxen is higher than the calf.

If the surface area of the nasal cavity of juvenile reindeer and adult muskoxen is compared it can be seen in table 4 that the relative surface area is larger in muskoxen than in reindeer, even if is compared in power of 0.75 and 0.67. If calves are compared, table 4 shows that reindeer calf has higher relative surface area than muskoxen calf.

Table4: proportional surface areas to body mass in power of 0.75 and 0.67 in juvenile reindeer (R7-12), reindeer calf (5-05C), adult muskoxen (32-2002) and muskoxen calf (35-1996C).

	Weight (kg)	Total surface area (cm^2)	cm^2/kg	$cm^2/kg^{0.75}$	$cm^2/kg^{0.67}$
R7-12 juvenile reindeer	52.5	772	14.70	39.58	54.34
5-05C reindeer calf	4.535	185	40.79	59.53	67.18
32-2002 muskoxen adult	310	5363	17.30	72.59	114.87
35-1996C muskoxen calf	15	343	22.87	45.00	55.89

In general, calves have higher metabolism and higher oxygen consumption (Vo_2) than adults. Soppela et al. 1986 calculated that the Vo_2 of newborn reindeer calves was 5.7ml/min.kg at 11ºC and highest, Vo_2= 38.8ml/min.kg at -14.5ºC, which means an increase in heat production when temperature decreases. And Luick and White 1986 described that Vo_2 in adult reindeer was 6.78ml/min.kg. Vo_2 varies inversely but not linearly with body weight.

It´s reasonable that calves have higher relative mucosal surface area than adults because they have higher ventilation and metabolic rates. Metabolic rate in the thermoneutral zone in reindeer calf is 5.1 W/kg (Markussen et al. 1985). In adult Svalbard reindeer the metabolic rate is 1.55+/-0.05 W/kg at rest in winter and in Norwegian adult reindeer is 2.05 +/- 0.15 W/kg (Nilssen et al. 1984). The increase in ventilation rates in reindeer calves at low T_a is in response to increased energy metabolism, for heat dissipating mechanisms (Soppela et al. 1986).

Apart from that, if the curvature of the mucosal surface area in adult reindeer (fig47) is compared with the temperature graph (fig48) that Johnsen et al. 1985 published in resting reindeer exposed to a low T_a, it can be seen a correlation between both of them. The most complex area in the nasal cavity is in the slices located in the proximal part of the nasal cavity, with higher surface area, and it is in that same area where the nasal mucosa temperature is lower. This section of the nasal cavity could be the most important area for the nasal heat exchange because it is in this location where the heat and water exchange between air and mucosa is most efficient.

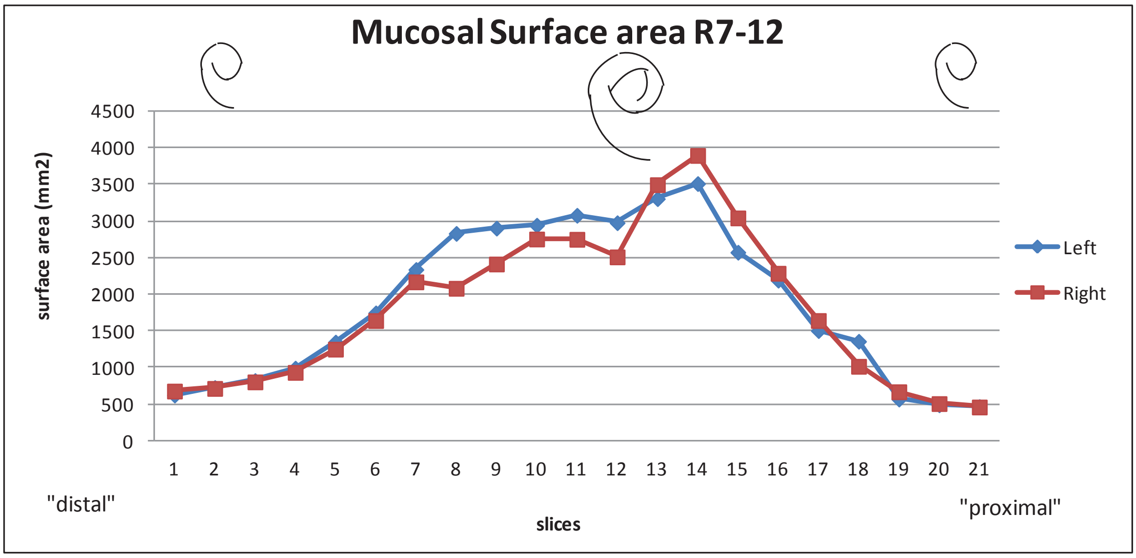

Fig47: mucosal surface area (mm^2) of adult reindeer (R7-12) along the nasal cavity from proximal to distal part with the profile of turbinate structure. Blue: left side, red: right side.

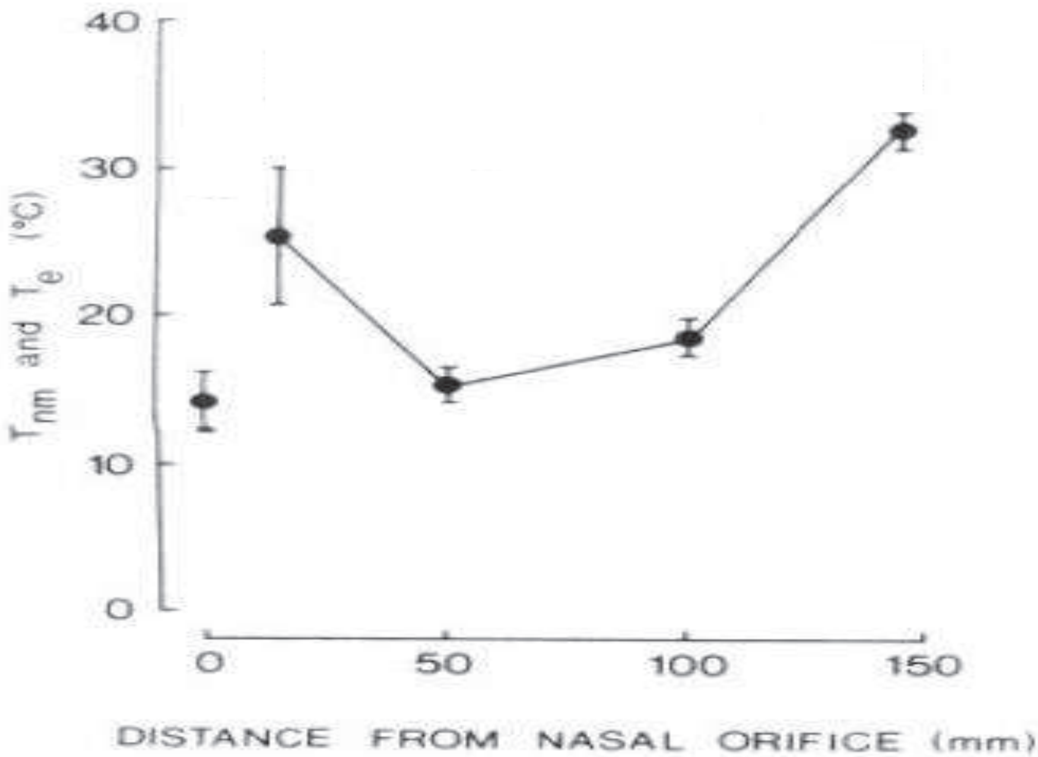

Fig48: nasal mucosal temperatures (Tnm) of resting reindeer at 3 different locations along ventral part of ventral turbinate (50, 100 and 145mm from nasal orifice) and in nasal vestibule (15mm inside nasal from nasal orifice), at ambient temperature of -30ºC in winter (n=10). Symbols represent means +/-SD (modified from Johnsen et al. 1985).

4.2. Histology:

The purpose of doing histological analysis was to compare the turbinate structure regard to density and distribution of the vessels between newborns and adults. Since, in theory, scroll arrangements and complexity could be similar in calf vs. juvenile, while internal organization was not. Due to time limitations only 1 juvenile and 1 reindeer calf have been used. A lot of time was spent to produce the microscopy images which were then measured using the ImageJ program.

The microscopy images confirmed the existence of the closed bulb in the turbinates (fig33) of both calf and juvenile.

At the beginning of the nose, in the distal part, there is a higher density of serous glands in both juvenile and calf (fig34), than in the other parts of the nasal cavity. As Johnsen et al. 1985 showed in their studies, the tissue around the nasal orifice is outside of the nasal turbinate area, so it´s independent of the effect of the turbinate. Probably, the function of the mucous glands is related to the control of passage of water through the epithelium wall (Johnsen 1988). Much of the water required for humidification of the inspired air is probably derived from nasal glands secretions (Negus 1958).

Some glands can be seen also along the turbinate, but there are more of them in the calf than in the juvenile reindeer (fig35). Small animals have high mass-specific metabolic rate (Schmidt-Nielsen 1984) so they have a high breath frequency. Reindeer calves breathe more often than adults, due to high metabolic rate and glands are well developed in mammals which pant (Bojsen-Møller 1964). So, the higher amount of glands in reindeer calf could be due to high metabolic rate.

There is a large difference in the thickness of the inner and outer layers of the turbinate. The outer layers are much thicker than the inner ones (fig37) and there is a high vessel density in the outer layers. This could be just the effect of ontogeny. The turbinate most likely starts growing from the "T part" and the layers of the scroll grow later. The air contact in the outer layers is good enough to perform efficient NHE, so it may not be necessary to have dense vasculature also in the inner layers. The exchange of heat and water presumably mainly happens along the outer part of the turbinate.

Measurements of the distance between the vessels (arteries and veins) and the epithelium were taken to indicate the proximity of blood flow and air flow, e.g. for assessment of efficiency of heat transfer between these. The arteries are closer from the nasal epithelium in the beginning of the nasal cavity, the distal part close to the nostrils (fig40), possibly to prevent freezing of tissue at sub-zero ambient temperatures. The blood goes fast in the arteries. At the proximal part of the turbinate the arteries branch from the main artery (sphenopalatine artery) and that is why they are farther away from the nasal wall epithelium (fig40a) and also to some extent larger-sized (fig41e).

On the other side, in the distal part of the nasal cavity the veins are farther from the epithelium (fig40). This may be due to the venous blood runs slowly because the venous space is bigger. If the veins were closer to the air stream in the distal part of the nose could be a possible risk of freezing in the tissues. In the proximal part of the nasal cavity the veins are not so far away from the epithelium. This may be due to the arteries are farther from the epithelium at this level.

Similarly, at the pharyngeal end of the nasal cavity, in the proximal part, the arteriolar diameter of the juvenile reindeer increases (fig41) because they have recently branched from the sphenopalatine artery. The diameter of veins is higher in the distal part of the nasal cavity than in the proximal part (fig41). The size of the vessels is generally higher in juvenile reindeer than in calves. That could be due to size difference. The juvenile reindeer is bigger than the calf so the diameter of the vessels is larger too.

In the x-section area measurements of vessels, only 1mm^2 was used due to size limitations with the histological images. Ideally, larger squares (e.g. of 1cm^2) should have been used, which would have been much more representative, but this was not possible with the available equipment. Arteriolar and venous x-section areas, as an expression of the richness of vascularization, also depended on how well distended (perfusion-fixed) the structures were. Based on the appearance of the vessels in the histology figures, perfusion fixation seems to have been successful.

In the reindeer calf (12-10C) the arteriolar and venous x-section values decrease from distal (section1) to proximal (section5) part of the nose (fig42), with higher values in the distal part of the nose (sections 1 and 2).

In the juvenile reindeer (R4-12) the x-section areas decrease along the nasal cavity, from distal (section1) to proximal part; but in the last part of the nose (section5) there is a large increase in the arteriolar x-section area (fig42). The arteries are bigger presumably because they have been recently branched from the sphenopalatine artery.

In general, the venous x-section values are much higher than the arteriolar values (6-10 times) and there are almost no differences between juvenile and reindeer calf (fig42). The reason why venous space is much higher than arteriolar space is unknown, but Olsson (2011) mentioned that in reindeer legs the veins were at least 5 times bigger than the arteries.

Finally, the density of arteries and veins (number per cm) is much higher in calves than in juveniles (fig43). The higher density of vessels in calves could be due to the high number of glands, to ensure the proper functioning of the nasal glands, but it could also be due to size differences. From birth the density of vessels could be fixed but while growing the distance between these vessels become larger. So, when it is measured 1cm of the mucosal lining, it is counted higher number of vessels in the calf than in the juvenile. Also the number of veins is higher than the number of arteries, and the density of capillaries (number per cm) is higher also in calf than in juvenile (fig44), may be for the same size reason.

Along the nasal cavity, the arteries and veins at different levels (sections) are really close between them (0.04-0.15mm). This proximity could be in relation to the efficiency of vascular heat exchange between the vessels. The efficient nasal heat exchange in the cold depends partly on the distance between vessels.

The mucosa is cooled during inspiration and, still remains cool when the warm expired air passes again over the mucosa some seconds later. This can happen due to the arteries and veins can exchange efficiently the heat. As observed in figure 48 the arterial blood enters at 35ºC in the proximal part of the nose while the temperature in the distal part may be 15ºC.

The more effective heat exchange is due to the closer countercurrent blood vessels are to each other and the larger the area of heat transfer is (Olsson 2011).

The statistics of the replicate measurements suggest that measurements and data are reliable (table3), the method used to obtain the measurements was correct.

Maybe the use of only 1 juvenile and 1 calf could be not be considered representative enough for general studies but if other animals were been taken it is highly likely that the result would be similar because anatomy usually does not vary much between members of the same species. And looking into the CT-scan images of the other individuals the same structure can be seen in all of them, so probably the histology would not be different among them.

5. CONCLUSION:

This study has shown that reindeer and muskoxen calves have the similar complexity in the turbinate structure as in older animals. The calves have similar structures to the adult but smaller.

The CT-scan images show that well developed nasal turbinates are present in both reindeer and muskoxen, in both calves and juveniles/adults. And the total exposed surface area in juvenile reindeer $0.077m^2$ is higher than the area calculated by Blix et al. 1983, presumably because these authors assumed that the closed inner "bulb" was ventilated. In proportion of weight, the reindeer calf has a higher relative mucosal surface area than the juvenile reindeer due to higher metabolic rate and Vo_2. In power of 0.75 muskoxen adult has higher surface area than the muskoxen calf; and if muskoxen and reindeer are compared, it can be concluded that adult muskoxen has higher relative surface area than juvenile reindeer. If calves are compared, reindeer calf has higher relative surface area than muskoxen calf.

The histological examinations of reindeer suggest that the turbinate structure is present and similarly arranged in both calves and adults. The general structure is the same in calves and adults and the distribution of vessels and glands in the different layers also, although the older animal has more layers in the scrolls. Juvenile reindeer has four layers, while calf has three layers in the most complex part of the turbinate.

In calf the size of vessels is smaller than in juvenile, but the density of vessels is higher. Small animals have higher metabolism and hence respiratory minute volume, which may explain why the calf has higher amount of glands and higher vessel density to control and ensure the functioning of these glands.

In the proximal part of the nasal cavity, in both reindeer juveniles and calves, the arteries are farther from the epithelium and they are bigger due to they have recently branched from the sphenopalatine artery.

In both reindeer juveniles and calves the most complex structure in the nasal cavity is in the middle part, where the surface area is bigger. It can be conclude that this part of the nasal cavity is important for the efficient nasal heat exchange for both adult and calves.

Although there are some small differences between the sizes and densities of the vessels in the turbinate of calves and juveniles, it can be conclude that calves of both species have present the anatomical structures required for efficient NHE from birth.

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Appendix:

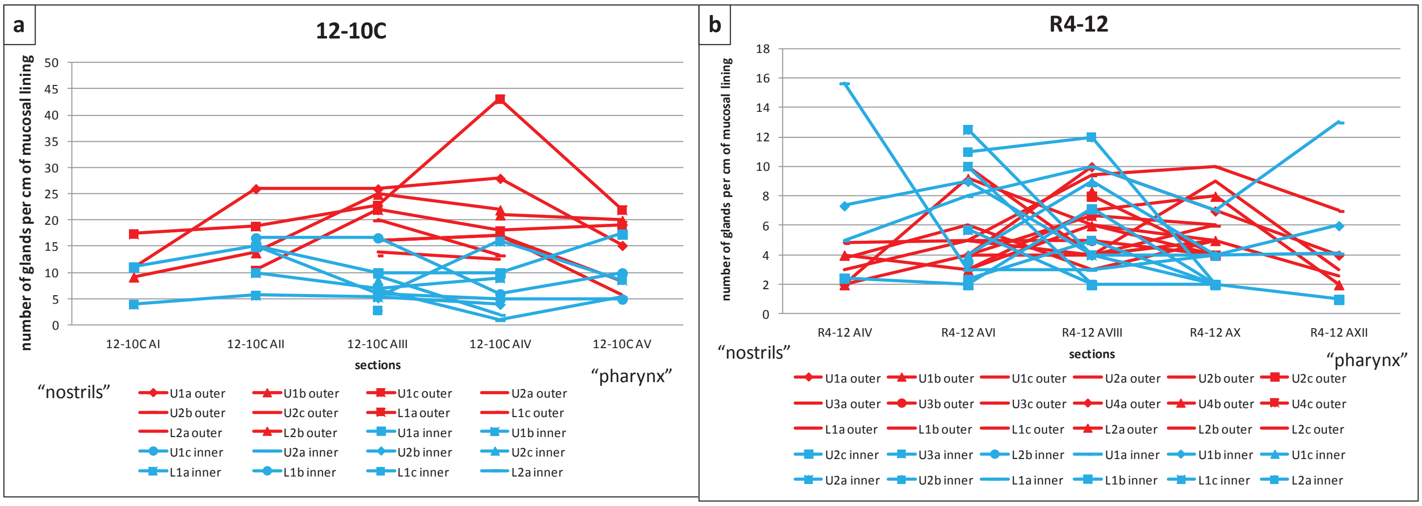

Fig1: number of glands per cm of mucosal lining in reindeer calf (12-10C) and in juvenile reindeer (R4-12) in the turbinate along the nasal cavity from distal to proximal part. Red lines: outer layers and blue lines: inner layers.

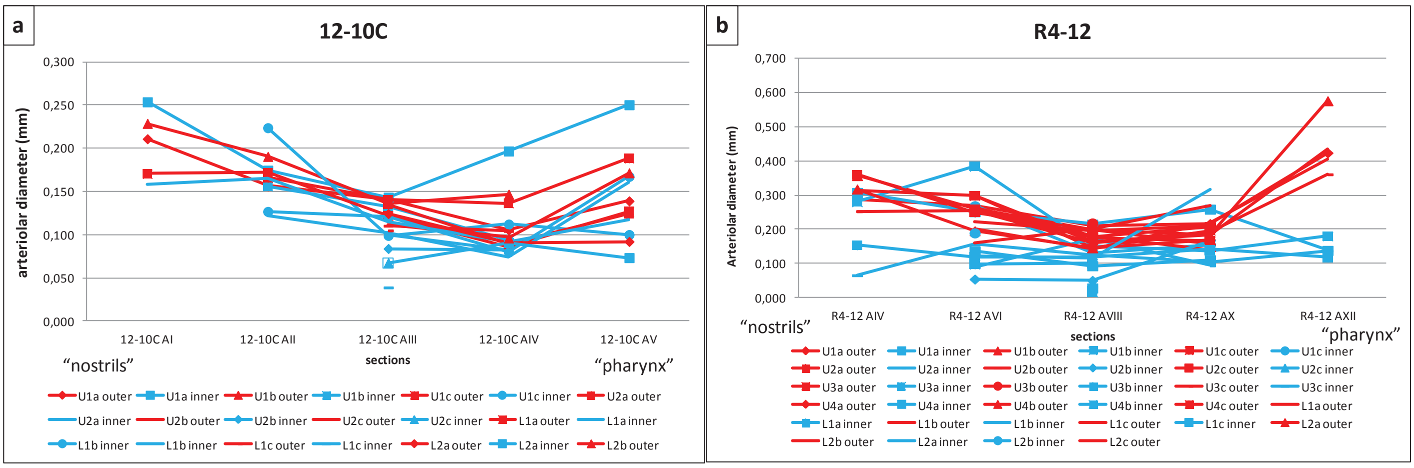

Fig2: arteriolar diameters in reindeer calf (12-10C) and in juvenile reindeer (R4-12) in the turbinate along the nasal cavity from distal to proximal part. Red lines: outer layers and blue lines: inner layers.

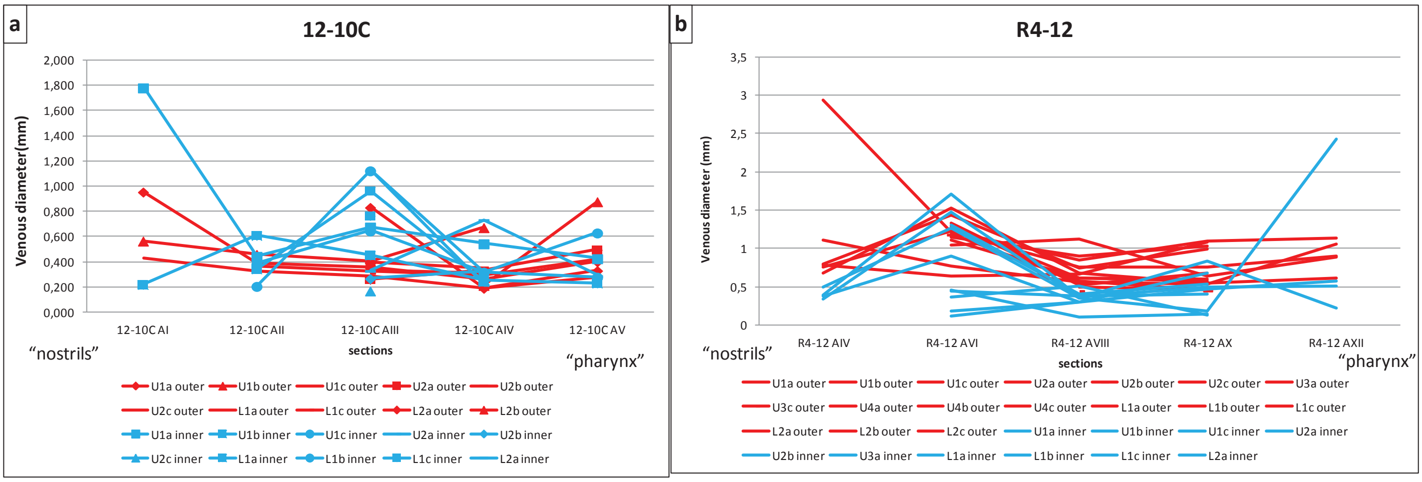

Fig3: venous diameters in reindeer calf (12-10C) and in juvenile reindeer (R4-12) in the turbinate along the nasal cavity from distal to proximal part. Red lines: outer layers and blue lines: inner layers.

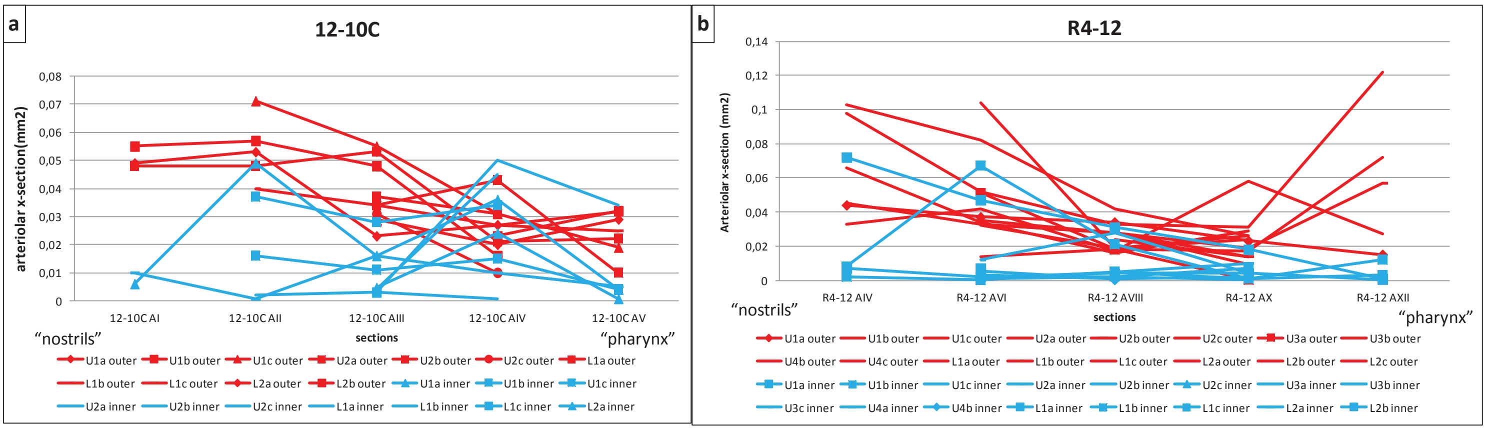

Fig4: arteriolar x-section areas in reindeer calf (12-10C) and in juvenile reindeer (R4-12) in the turbinate along the nasal cavity from distal to proximal part. Red lines: outer layers and blue lines: inner layers.

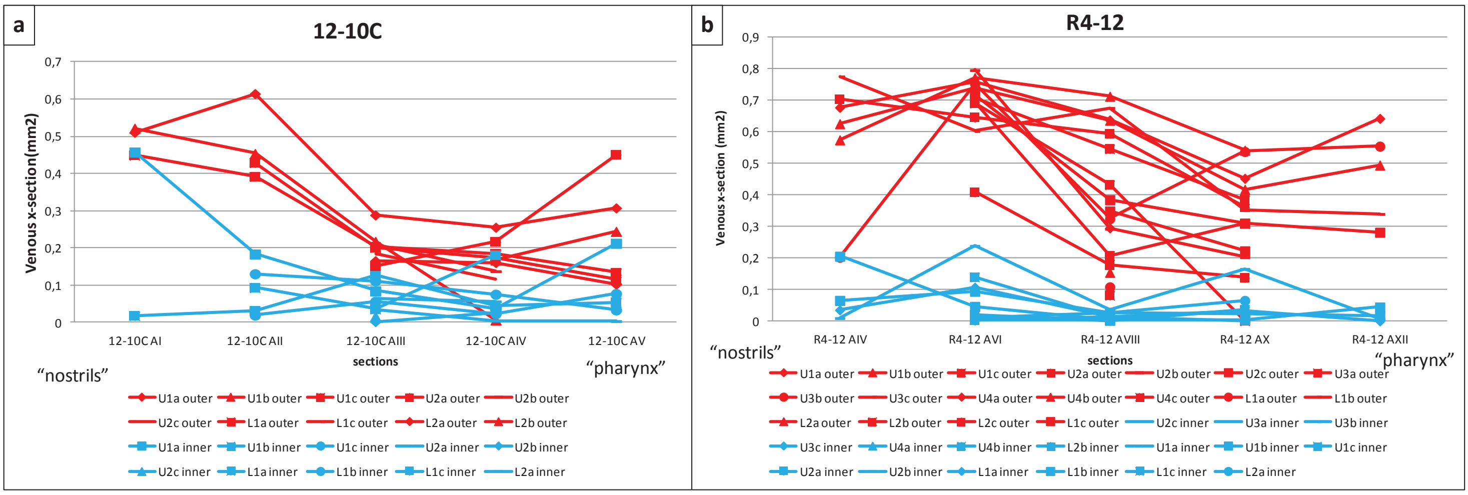

Fig5: venous x-section areas in reindeer calf (12-10C) and in juvenile reindeer (R4-12) in the turbinate along the nasal cavity from distal to proximal part. Red lines: outer layers and blue lines: inner layers.

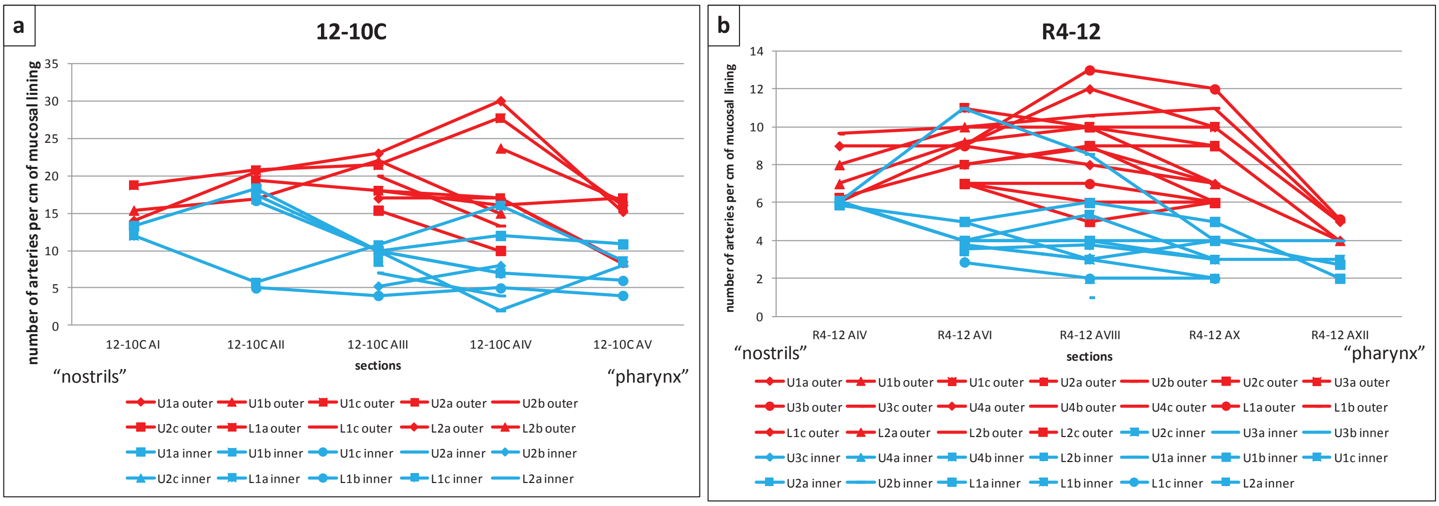

Fig6: number of arteries per cm of mucosal lining in reindeer calf (12-10C) and in juvenile reindeer (R4-12) in the turbinate along the nasal cavity from distal to proximal part. Red lines: outer layers and blue lines: inner layers.

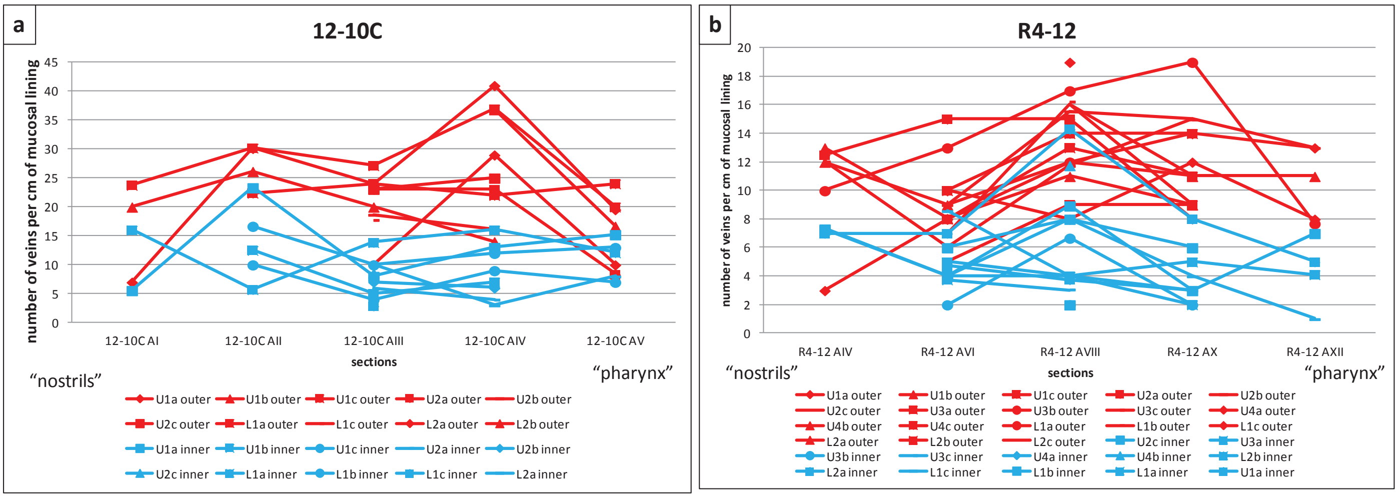

Fig7: number of veins per cm of mucosal lining in reindeer calf (12-10C) and in juvenile reindeer (R4-12) in the turbinate along the nasal cavity from distal to proximal part. Red lines: outer layers and blue lines: inner layers.

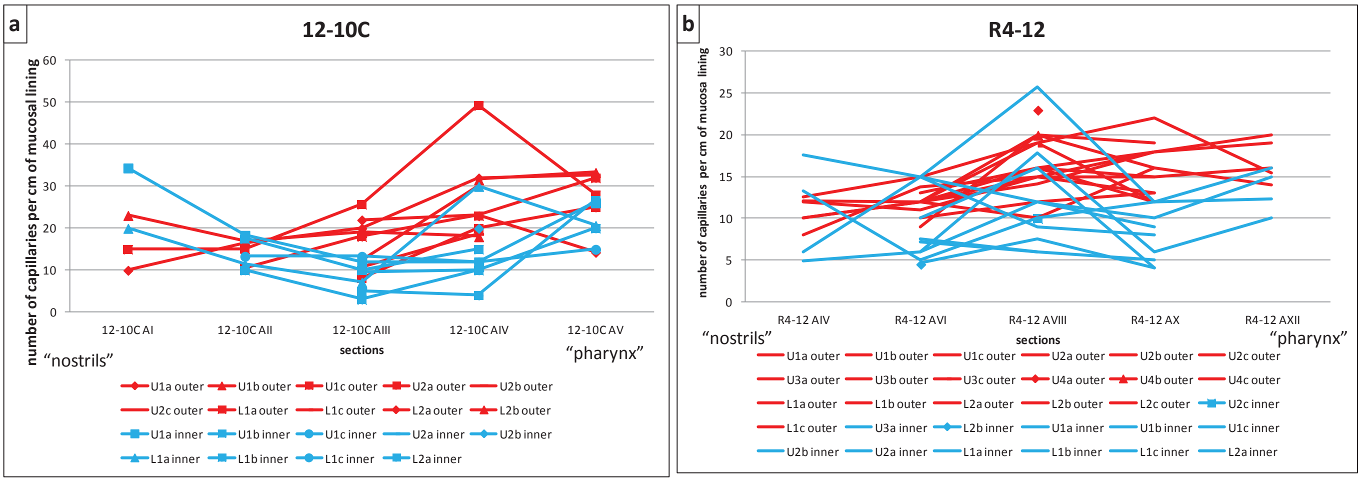

Fig8: number of capillaries per cm of mucosal lining in reindeer calf (12-10C) and in juvenile reindeer (R4-12) in the turbinate along the nasal cavity from distal to proximal part. Red lines: outer layers and blue lines: inner layers.