

An Easy Technique for Sheep Vomeronasalectomisation

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Abstract: In mammals the vomeronasal organ (VNO) plays an important role in detection and processing of pheromones related to social and reproductive behavior. To determine in which physiological functions the VNO is implied, different blocking techniques have been used. The aim of this work was to develop a simple, fast, and minimally invasive surgical technique to eliminate completely the VNO in sheep. Ten adult crossbreed sheep were anesthetized and intubated with a tracheotube. Two incisions in the nasal mucous membrane, on the floor of the nasal cavity lateral to the VNO, and in the nasal septum dorsal to the VNO were made. The VNO was eliminated through dragging movements with a Faraebeuff separator shaped into a hook. The hemorrhage was controlled *in situ* with ferric perchlorure and cauterization with a burning cylindrical iron. Animals were slaughtered at different times after surgery, and the nose was opened to determine whether remnants of the VNO were still left. In the first slaughtered animal (one week after the surgery) remnants of the VNO were observed in a necrotic area. However, in the animals slaughtered later than 4 weeks after surgery, there were no identifiable remnants of the VNO. We concluded that this is a simple, fast and effective technique to eliminate the VNO, and may be used in experiments aimed to determine long-term effects of vomeronasalectomisation.

Key words: ovine, Jakobson organ, experimental surgery, nasal cavity, anesthesia

Introduction

In mammals, two chemosensory systems –the olfactory and the vomeronasal– play an important role in detecting and processing chemical signals (Dulac, 1997). Some of these signals are pheromones related to social and reproductive behavior, detected by the vomeronasal organ (VNO) receptor cells, the chemoreceptive organ of the accessory olfactory system. In the ovine, the VNO is a cartilaginous tube covered internally by an epithelium located bilaterally in the ventral part of the nasal septum. It is covered by the respiratory mucous membrane and it is insinuated by an elevation that indicates its location (Cohen-Tannoudji, 1984). Chemosensory stimuli gain access to the VNO through its single rostral opening, which communicates with the nasal and the oral cavities (Bland, 1989).

To determine in which physiological functions the VNO is implied, different blocking techniques, such as incision of the innervation, cauterization (Cohen-Tannoudji *et al.*, 1989) or obstruction of the routes of access (Klemm *et al.*, 1984 and Ramos *et al.*, 2004) have been used.

Incision of the VNO innervation is a risky surgical technique, whereby the frontal bone must be opened under excellent aseptic conditions, making it difficult to use in field conditions. Another limitation to this technique is that in rats it has been observed a rapid degeneration of vomeronasal receptor cells, followed by the recovery of the receptor cell population some days later after nerve transection (Yoshida-Matsuoka *et al.*, 2000 and Matsuoka *et al.*, 2002), so this should be controlled later after surgery. The incision of the main nerve in the ewe does not warrant that all innervation from the VNO is eliminated, since innumerable minor nerve fibers connect to the VNO (Bland, 1989). Although the obstruction of the entrance to the VNO is viable (Klemm *et al.*, 1984), it is difficult to ensure it if long-term effects of the VNO are being evaluated. The surgical elimination of the VNO has been used to warrant individuals free from the influence of pheromones in rodents (Meek *et al.*, 1994; Saito 1986, Saito *et al.*, 1988, 1989 and Wysocki *et al.*, 1991). In these experiments, the VNO is reached through the hard palate, and the area of the nasal septum related to the VNO is eliminated. The application of a similar technique in large mammals –such as sheep– may be very invasive and complicated. Moreover, it may require many working hours, strict aseptic conditions and has high risk of complications. Thus, the aim of this work was to develop a simple, fast, and low invasive surgical technique to eliminate completely the VNO in sheep.

Materials and Methods

Ten adult crossbreed ovines (35.8 ± 3.6 kg, mean ± SD) were anesthetized with acepromazine 1% (Acepet, Unimedical del Uruguay Ltda, Montevideo, Uruguay), diazepam 0.5% (Unicepan, Unimedical del Uruguay Ltda, Montevideo, Uruguay), ketamine 5% (Ketamin, Unimedical del Uruguay Ltda, Montevideo, Uruguay) and thiopental 2% to effect (Tiopental sódico 1 g, Laboratorio Diromax, Montevideo, Uruguay) *iv*. All sheep were positioned in left lateral decubitus recumbency. Anaesthesia was quickly induced (25-30 s) with a mix of 3 mg acepromazine, 45 mg ketamine and 14 mg diazepam by intravenous injection in the cephalic vein. Physiologic fluid was permanent infused (drip). Then, thiopental was injected *iv* to effect (100-120 mg, total dose) until the palpebral reflex was inhibited. Animals were intubated with a tracheotube (diameter =9 mm) to avoid the aspiration of fluids to the respiratory routes. Anaesthesia was maintained by additional doses of 25 mg ketamine and 2.5 mg diazepam, or thiopental to effect.

Before surgery, a solution of yodopovidone (1%) was imbibed on the nasal mucosa and the nostrils to ensure the antisepsis of the nasal cavity. Two incisions in the nasal mucous membrane were made, one on the floor of the nasal cavity lateral to the VNO, and the other in the nasal septum dorsal to the VNO (Fig. 1), entering the nose with a scalpel nº 23 blade, handle nº 4. Although there was a profuse hemorrhage, it was not controlled at this time. The caudal part of the nasal septum was located to identify the caudal part of the VNO using a hook made from a Faraebeuff separator (Fig. 2). The VNO was retired dragging with firm movements of the hook, retiring the VNO in pieces, with parts of the mucous membrane that covers it, and parts of the nasal septum.

The rostral part of the VNO and the remaining membrane were incised with a scalpel or scissors and completely extirpated. The hemorrhage was controlled using a coiled gauze soaked in ferric perchloride solution. The ventral meatus and the septum were cauterized with a burning cylindrical iron (diameter = 5 mm). The same process was repeated on the other side of the nasal cavity. The clots that remained in the nasal cavity were retired with an aspiration pump. Excessive salivation was controlled with 1 ml *iv* atropine

0.1 % (Atropina Vetcross, Portinco S.A., Montevideo, Uruguay).

After surgery, the animals were placed in sternal decubitus position, maintaining the tracheotube until the conscious recovery. To avoid aspiration problems, feeding was restricted during the first 12 h. All animals received 5 ml i/m of a combination of procainic benzilpeniciline 200000 IU, and dihidrostreptomisine 250 mg per mL (Repen, Lab Fatro S.p.A, Fedagro Ltda., Montevideo, Uruguay) every 24 h during 2 weeks. An anti-inflammatory diclofenic (Diclofenac 2.5%, Lab. Ion, Montevideo, Uruguay) dose (2.5 mL was) administered every 12 h during five days. Animals were observed frequently during the first 5 days after surgery, and at least once daily until slaughter. General status, temperature, breath frequency, evolution of injuries, and appetite were controlled until animals were slaughtered. Animals were slaughtered 1 (n=1), 2 (n=1), 3 (n=1), 4-6 (n=5), and 10 (n=1) weeks after surgery. To evaluate possible long-term effects one ewe was slaughtered 13 months after surgery. The nasal cavity was opened with three cuts to expose the VNO region, and determine the presence of VNO remnants. The parotid, mandibular and bronchial limphonodes were observed to check for possible infections.

Results

The application of the technique, since the induction of the anesthesia to the end of the aspiration of the nasal cavity took 45 minutes in the first surgery, and 20 min in the last.

The initial mixture drugs (acepromacine, diazepam and ketamine) and thiopental to effect provided anesthesia for 15-20 min. Animals manifested an acute unconsciousness and muscle relaxation immediately. Heart rate frequency increased quickly to 120, but breath frequency remained unchanged in normal values. Total doses of thiopental required during surgeries were 120-400 mg, according to duration of each surgery. Anaesthesia was characterized by muscle relaxation and profound analgesia. In effect, lack of gross purposeful movement of sheep in response to stimulation during the surgical procedures indicated an adequate analgesia and muscular relaxation. The mean side effect was an excessive salivation, which was adequately controlled with the administration of atropine. The recovery of the animals was satisfactory: 2 h after the end of the surgery all animals were up. Twelve h after surgery sheep breathed through the nose while resting, but still through the mouth when walking. During the first week after surgeries, a serosanguinolent fluid -which corresponded to necrotic tissues- landslided from the nostrils. Three weeks after surgery the liquid was dark brown. Cicatrisation of the nasal mucous membrane was completed 6-8 weeks after surgery.

In the animals slaughtered 1 and 2 weeks after surgery, remnants of the VNO were observed in a necrotic area that included the ventral meatus and the third ventral of the nasal septum. In the animals slaughtered 3-6 weeks after surgery, the necrotic area persisted, but there were no identifiable rests of the VNO (Fig. 3). In the two animals slaughtered at week 10, and 13 months after surgery, no remnants of the VNO were observed. There were no alterations of the mandibular, retropharyngeal or bronchial lymph nodes in any animal. In one ewe (slaughtered at 13 months) the edges of the narines were affected, diminishing their diameter. The constriction of narines was already important 60 days after surgery. This ewe breathed through the mouth with difficulties. Thus, local anesthesia was induced with lidocaine 2 % (Lidocaina ION; Lab. Ion, Montevideo, Uruguay), and the diameter of the nostrils was increased with an incision at the nasoincisive notch level. This incision included all the structures from the skin to the mucous membrane; the suture was made by linking the skin with the respiratory mucous. The animal breathed normally and began to eat immediately after this surgery.

Discussion

We developed an easily applicable surgical technique to extract the VNO in sheep. The developed technique is minimally invasive, simple, fast, and effective to eliminate of the VNO. There were almost no surgical complications, and post-surgery recovery of animals was very fast, without ruminal tympanism problems since the animals were up in 2 h maximum.

The anesthetic combination resulted in a quick and secure induction, and provided an excellent analgesia. Each drug -except diazepam- was used in doses lower than what is needed if each one is used alone (Lumb & Jones, 1996, Muir *et al*, 2001). The

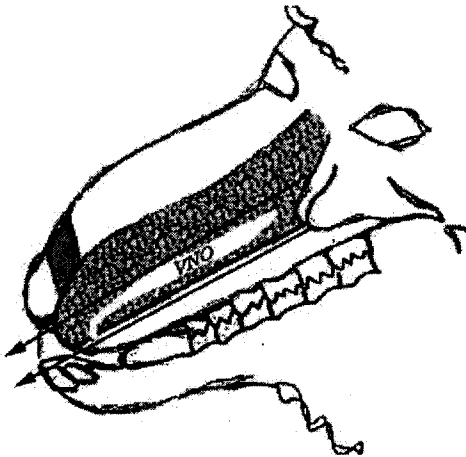


Fig. 1: Diagram showing incisions in the nasal mucous membrane: on the floor of the nasal cavity, lateral to the VNO and the nasal septum, dorsal to the VNO



Fig. 2: Diagram of the hook with one extreme in form of spear, made from a Farabeuf separator

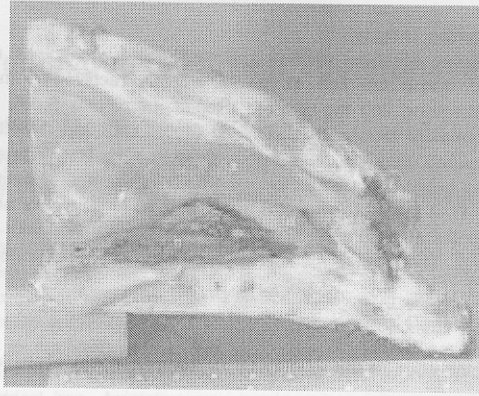


Fig. 3: Nasal cavity was opened a) nasal septum; b) the necrotic area from where the VNO was extirpated is visible; c) hard palate; d) ventral nasal concha; e) nasoincisor duct

effectiveness of the anesthetic technique was essential for the quick and easy postsurgery recovery. The hypnosis provided by thiopental low doses effectively prevented apnea, and allowed a fast recovery. Apnea was not observed in any surgery. Overall, we considered the drug combination as adequate for intravenous anesthesia of sheep.

Although there was a profuse hemorrhage, it was easily controlled with ferric perchloride and hot cauterization. The ewe in which nostrils were diminished in diameter by cicatrization did not have any more problems after surgical repair. Considering this, we recommend avoiding excessive cauterization to prevent excessive cicatrization and necrosis of the nasal septum.

The surgery did not take much time, which may also be minimized with some experience. This technique is easier to apply in sheep under experimental conditions—usually field conditions—being easier than those used in rodents, in which part of the maxilar and palatine bones should be retired (Meek *et al.*, 1994; Saito 1986, Saito *et al.*, 1988, 1989 and Wysocki *et al.*, 1991). The animals should be controlled at least during four weeks after surgery, and the time of complete recovery is between 60 and 90 days.

Conclusion

we developed an easy and minimally invasive surgical technique for sheep vomeronasalectomisation, which is quick, simple and safe to apply in field conditions.

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