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**[Ultrasound-Guided Transversus Abdominis Plane Block in sheep: A
Descriptive cadaveric study of a Three-Point Injection Technique]**

Thesis

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1. INTRODUCTION AND HYPOTHESIS

Regional anaesthesia is employed as part of a multimodal anaesthesia regimen and aims to provide adequate analgesia, reducing the requirement for systemic anaesthetic drugs (such as opioids), thus avoiding their possible side effects, and contributing to overall postoperative patient comfort (Campoy and Read 2013).

Surgical procedures involving an incision in the abdominal wall are considered very painful and regional anaesthesia is often necessary to provide sensory blockage of the nerves supplying the abdominal wall (Grubb and Lobprise 2020). A relatively novel approach to providing sensory analgesia to the ventral and lateral abdominal wall is the transversus abdominis plane (TAP) block. This involves injecting of local anaesthetic within the neurovascular plane between the transversus abdominis and internal oblique abdominal muscles, targeting the ventral branches of spinal nerves supplying the abdominal wall and the parietal peritoneum (Portela et al. 2014).

The TAP block was first described in human medicine by Rafi in 2001 as a blind technique, using only anatomical landmarks to inject local anaesthetic into the TAP space (Rafi 2001). A later developed ultrasound-guided approach to this block increased the precision of its administration and helped reduce possible complications and risks of inadvertent needle placement (Hebbard et al. 2007).

The first TAP block in veterinary medicine was performed in a Canadian lynx in 2010 as an alternative approach to providing analgesia of the abdominal wall to wild and hard-to-handle animals (Schroeder et al. 2010). Over the past decade, extensive research and experimental trials were performed in different animal species and the efficacy of the TAP block for clinical use was confirmed. Numerous cadaveric studies performed in different species aimed to develop the adequate TAP block technique in accordance with the differences in the anatomical structure of their abdominal wall (Johnson et al. 2017; Baldo et al. 2018; Drożdżyńska et al. 2017; Freitag et al. 2021; Calice et al. 2021; Di Bella et al. 2021; Mirra et al. 2018; Otero et al. 2021).

However, to this date no studies on the use of TAP block in sheep have been published, and this thesis was developed to describe an ultrasound-guided TAP block in sheep cadavers using a three-point injection technique.

The hypothesis of this work is that an ultrasound-guided three-point injection technique of the TAP block will result in sufficient spread of methylene blue solution within the TAP space to stain the nerve branches supplying the ventrolateral abdominal wall of sheep, demonstrating its potential for effective clinical use in perioperative pain management.

2. LITERATURE REVIEW

2.1 Regional anaesthesia

As opposed to general anaesthesia, regional anaesthesia can be used to relieve pain without affecting a patient's consciousness level. It involves injecting of local anaesthetic into an interfascial space which contains the target nerve, resulting in sensory and/or motor block of peripheral nerves to relieve or avoid pain (Jinlei Li et al. 2019). Regional anaesthesia is used as part of a multimodal analgesia regime and its clinical significance is reflected in improving pain control, postoperative recovery and patient comfort, as well as reducing use of general anaesthetic drugs and decreasing the risk of their adverse effects (such as cardiovascular and respiratory complications, postoperative nausea etc.) (Johns et al. 2012).

Regional anaesthesia is widely used in human medicine and has recently become widespread amongst veterinarians, where techniques used on humans have successfully been adapted to use in various animal species. A deep understanding of relevant anatomical regions and nerve structure is necessary for successful and safe performance of loco-regional anaesthesia procedures in different species (Campoy and Read 2013).

2.2 Local anaesthetic drugs

Local anaesthetics are known as the only class of drugs that can completely prevent perception of a painful stimulus. Their first use was reported in the late 19th century and has since then become increasingly widespread and commonly used in both human and veterinary medicine. Local anaesthetics are regularly used for neuraxial anaesthesia, peripheral nerve blocks as well as intravenous infusions (Barletta and Reed 2019).

Local anaesthetic agents interrupt the transmission of nerve electrical impulse by blocking sodium channels found in the plasma membrane of nerve cells and preventing entrance of sodium ions into the cell (Földi et al. 2021).

Higher lipid solubility of local anaesthetic agents results in their slower absorption from the site of administration, having a lower maximum plasma concentration and longer time to reach the maximum plasma concentration. Vascularity of the injection site also determines the absorption

of local anaesthetic, i.e. if the injection site is highly vascularised, the maximum plasma concentration will be higher and the time to reach this concentration will be shorter (Barletta and Reed 2019). The combination of local anaesthetic agent with an adjuvant (such as epinephrine) can be used to slow down the systemic absorption of the local anaesthetic and prolong its effect (Michael J. Cousins and Phillip O. Bridenbaugh 2008).

According to their structure, local anaesthetics can be divided into two categories: aminoamides and aminoesters, depending on the intermediate carbon chain component (aminoamide or aminoester) which binds a hydrophilic to a lipophilic element. Aminoamides such as lidocaine, bupivacaine and ropivacaine are metabolised in the liver, and due to their longer duration of action are more commonly used in veterinary medicine. On the other hand, aminoesters such as procaine and tetracaine are metabolised by plasma cholinesterase, and are often unstable and thus not favoured in veterinary medicine for loco-regional blocks (Yi Lin, Spencer S. Liu 2017).

2.3 Toxicity and complications

A high plasma concentration of local anaesthetics can be a result of inadvertent intravascular injection or overdose and can lead to neurotoxicity or cardiotoxicity. Signs of central nervous system toxicity usually appear before those of the cardiovascular system, and this occurs when lipid-soluble local anaesthetic agents pass the blood-brain barriers. This causes abnormal excitation of neuronal pathways and leads to tremors or seizures. Further increase in plasma concentration levels can result in cardiovascular signs of toxicity, such as hypotension, bradycardia, ventricular dysrhythmias, or cardiovascular collapse. Bupivacaine, levobupivacaine and ropivacaine are lipid-soluble anaesthetic agents, and are considered more potent and toxic (Campoy and Read 2013). It is therefore of great importance to calculate doses appropriately and have trained professionals perform local anaesthetic blocks to minimise as much as possible such undesired consequences.

2.4 The Transversus Abdominis Plane (TAP) Block

The TAP (transversus abdominis plane) block is a regional anaesthesia technique used as part of a multimodal analgesia regimen and known to effectively reduce postoperative pain in the abdominal wall following surgeries in the abdominal region (Tsai et al. 2017).

The abdominal wall is comprised of three muscle layers: the external oblique, the internal oblique, and the transversus abdominis muscles. The parietal peritoneum, muscles and skin of the ventral abdominal wall are innervated by the ventral branches of the caudal thoracic and cranial lumbar nerves. These segmental branches emerge from their respective intervertebral foramina, run over the transverse processes, and penetrate the muscles of the abdominal wall, running ventrally within the fascial plane between the transversus abdominis and internal oblique muscles (Luis Campoy 2013; König and Liebich 2007; Campoy and Read 2013).

The TAP block is performed by injecting a local anaesthetic agent within the neurovascular plane of the abdominal musculature, between the internal oblique and transversus abdominis muscles, targeting the nerves that supply the abdominal wall. This technique was initially described for use in human medicine by Rafi in 2001 as a novel approach in sensory analgesia and involved injection of local anaesthetic using only anatomical landmarks to reach the transversus abdominis plane (Rafi 2001). The TAP block approach was later developed and improved with the use of ultrasound which allowed more accurate needle placement and administration of the local anaesthetic (Hebbard et al. 2007; McDonnell et al. 2007).

Today the TAP block is used widely and is considered a very important element of post-operative pain management in humans undergoing abdominal surgery and is also becoming increasingly popular in veterinary medicine.

There are several common ultrasound-guided TAP block approaches, applied according to the desired anatomical region and nerve involvement:

The ***lateral approach*** implies the positioning of the ultrasound transducer midway between the costal arch and the iliac crest and injecting local anaesthetic into the TAP space between the internal oblique and the transversus abdominis muscles, with the needle inserted in plane (Tsai et al. 2017). It was demonstrated that the lateral TAP block approach is more suitable for

abdominal surgeries only below the umbilicus because of the limited extent of nerve block (Shibata et al. 2007).

A similar approach is the *posterior TAP block*, in which the ultrasound probe is positioned midway between the costal arch and iliac crest, but more posteriorly in order to visualise the edge of the transversus abdominis muscle and its aponeurosis. The local anaesthetic is injected near the quadratus lumborum, superficial to the transversus abdominis aponeurosis (Tsai et al. 2017). It has been suggested that this approach results in wider spread of local anaesthetic and improves analgetic efficacy as well as duration compared to the lateral approach (Carney et al. 2011).

The *subcostal approach* was described in human medicine as a means of blocking the nerve branches of T9 to T12 to provide analgesia for supraumbilical abdominal surgeries (Hebbard 2008). This approach involves the deposition of a local anaesthetic agent into the TAP space between the rectus abdominis and transversus abdominis muscles, with the transducer positioned caudal and parallel to the costal margin (Soliz et al. 2017) and the needle inserted in plane (Tsai et al. 2017). A study conducted on canine cadavers showed successful staining of the T9 to T13 nerves and suggested the potential use of the subcostal approach for surgeries in the cranial part of the abdomen, such as cholecystectomies, splenectomies and gastrotomies (Drożdżyńska et al. 2017).

Further research on the TAP block in veterinary medicine showed that a combination of the lateral and subcostal approaches results in wider spread of local anaesthetic within the TAP space. In a study conducted on calf cadavers (Mirra et al. 2018), a two-point injection technique combining the subcostal and lateral TAP blocks resulted in successful staining of the sensory nerves supplying the ventral abdominal wall, suggesting the analgesic potential for surgeries involving incisions in the ventral abdominal midline.

A three-point injection technique in cat cadavers, using a combination of the subcostal, retrocostal (perpendicular to the midline and caudal to the last rib) and preiliac (cranial to the iliac crest) approaches showed a broader distribution of local anaesthetic solution around the T10-L2 nerves following the three-point injection technique, while a two-point injection technique (subcostal and preiliac) resulted in inadequate staining of T13 and L1 (Otero et al. 2021).

The volume of local anaesthetic injected is another factor which could affect spread within the transversus abdominis plane. In one study on canine cadavers, increases in the volume injected using a one-point injection approach resulted in a significantly wider cranio-caudal spread of local anaesthetic/dye solution within the TAP space (Bruggink et al. 2012). Another study on canine cadavers showed no significant difference in spread of solution with an increase in injected volume (Zoff et al. 2017a).

However, further research on spread of local anaesthetic within the TAP emphasises that a multiple injection technique results in a more effective distribution of injectate rather than increasing the injected volume, as the injectate will rather pool than spread around the site of injection (Zoff et al. 2017a; Mirra et al. 2018; Küls et al. 2020).

Complications associated with the TAP block are rare. Considering that the block does not target a specific nerve, but rather a whole region, neurological injury is not common, but can occur nevertheless because of hematoma, needle trauma or local infection (Hammi and Ahn 2022). Inadvertent needle placement can result in intraperitoneal injection, visceral and organ trauma or vascular injury (Frigon et al. 2006; Farooq and Carey 2008). One report has also described transient bilateral femoral nerve palsy following a TAP block performed in a woman after a caesarean section, likely as a result of a large volume of local anaesthetic used (Salaria et al. 2017). Local anaesthetic systemic toxicity is also rare but can be a consequence of intravascular injection of local anaesthetic (Hammi and Ahn 2022). The implementation of ultrasound guidance when performing TAP blocks has helped minimize such complications and increase the success rate.

2.5 TAP block in human medicine

The TAP block was first described and used in human medicine by Rafi in 2001 as a new approach to an abdominal field block. This technique included locating the "lumbar triangle of Petit" as the only anatomical landmark where the internal oblique muscle could be reached directly and injecting local anaesthetic into the neurovascular plane between the internal oblique and the transversus abdominis muscles, which blocks the lower intercostal nerves as well as the

iliohypogastric and the ilioinguinal nerves that cross between the subcostal margin and the iliac crest (Rafi 2001).

In 2007 McDonnell and colleagues conducted one of the first studies describing the TAP block in human medicine and its efficacy in reducing post-operative pain following large bowel resection, as opposed to standard methods of post-operative analgesia. The TAP block was performed blindly, using anatomical landmarks on the abdomen (“lumbar triangle of Petit”), with bilateral injection of 20 ml of 0.375 % levobupivacaine. A post-operative assessment of patients was carried out over a 24-hour period, and it was concluded that patients receiving the TAP block had much lower pain scores and hence, a lower requirement for morphine analgesia, compared to patients receiving standard care analgesic drugs (McDonnell et al. 2007).

The efficacy of the TAP block was also demonstrated in a study in patients undergoing total abdominal hysterectomy, a surgical procedure that is associated with significant post-operative pain. The analgesic efficacy of the TAP block was evaluated in a randomized, controlled, double-blinded clinical trial, where a group of 24 females received the TAP block with 1.5 mg/kg ropivacaine and another group of 26 females received a placebo with saline. Post-operative pain was assessed over 48 hours, and it was concluded that the patients who received the TAP block had lower visual analogue pain scores and less morphine requirements compared to those who received a placebo block, while no complications were associated with the TAP block (Carney et al. 2008).

The initially applied blind, landmark-guided technique included locating the “lumbar triangle of Petit” and advancing a blunt needle into the TAP space. Correct placement of the needle within the transversus abdominis plane was assumed by feeling a 'pop' or 'sensation of giving way', accounting for disputes amongst authors. Rafi suggested that a single pop indicated that the needle was in the correct plane and a second pop meant that it had pierced the transversus abdominis muscle. On the other hand, McDonnell et al. argued that the first pop indicated passing of the needle through the external oblique muscle and that the second pop indicated entry of the needle into the TAP space. Because of the uncertainty regarding correct needle placement, identification of the TAP and the risk of potential peritoneal perforation and organ damage (Farooq and Carey 2008), the blind, landmark-guided technique is not recommended any longer as a standard procedure for the TAP block. This claim is supported by a study

conducted on 36 patients receiving a bilateral TAP block, where results showed that a double-pop sensation technique resulted in correct needle placement in only 24 % of the cases and perforation of the peritoneum could be observed in 18 % of the cases (McDermott et al. 2012). Furthermore, locating the TAP space through the “lumbar triangle of Petit” was also difficult in elderly or obese patients.

To overcome the mentioned obstacles and risks when performing the TAP block, an ultrasound-guided approach was introduced in 2007, allowing a more reliable visualisation of the muscle layers and more accurate needle placement in the TAP space before injection of local anaesthetic agent (Hebbard et al. 2007). This approach is considered today as the gold standard for TAP block administration, both in human and in veterinary medicine.

El-Dawlatly et al. were one of the first to describe the ultrasound-guided approach to the TAP block in a study conducted in 2009. The TAP block performed using ultrasound resulted in successful visualisation of the relevant anatomy, detection and exact placement of the needle, and visualization of the spread of local anaesthetic in all the cases where a TAP block was performed. The study also concluded that patients undergoing laparoscopic cholecystectomy under standard anaesthesia together with ultrasound-guided TAP block had a substantially reduced requirement for opioids compared to the patients under just standard anaesthesia (El-Dawlatly et al. 2009).

Further research on ultrasound-guided TAP block techniques resulted in development of different approaches, aiming to evaluate the extent of sensory blockade of the ventrolateral abdominal wall, as nerve involvement and the pattern of spread of local anaesthetic depends on the site of injection (Barrington et al. 2009; Carney et al. 2011).

A comprehensive review of the anatomy and TAP block techniques in human medicine was published by Tsai et al. in 2017, highlighting the advancement in TAP block techniques that use several different approaches for ultrasound-guided TAP block, which is considered today as the gold standard in performing TAP blocks. In this review, the authors insisted on the importance of classifying different TAP block approaches for evaluation of their varying analgesic effects and block duration. To ensure the efficacy and consistency of the TAP block, the different approaches were categorized in a nomenclature system of four groups based on

the spinal nerves involved: subcostal, lateral, posterior, and oblique subcostal TAP blocks, allowing precise application and better analgesic effects (Tsai et al. 2017).

2.6 TAP block in animals

The first TAP block in veterinary medicine was described by Schroeder et al. in 2010 on a Canadian lynx undergoing exploratory laparotomy for removal of a foreign body. It was hypothesised that the TAP block would provide long (8-10 hours) intra- and postoperative analgesia, demonstrating the potential benefit in veterinary medicine, particularly with nondomestic animals. A one-point injection technique using an ultrasound-guided approach was performed bilaterally and the needle was placed midway between the iliac crest and the last rib, approximately 3 cm lateral to the ventral midline. First a test dose of 1 ml 0.125 % bupivacaine was injected to confirm correct placement of the needle, then an additional 4 ml were injected. The total dosage of bupivacaine was 1.04 mg/kg which did not exceed the recommended dosage for cats, and no cardiovascular or respiratory changes were observed after the procedure. Intraoperative anaesthesia and recovery were smooth, and first signs of aggression were reported 10-12 hours following the TAP block. Some limitations to this study included use of other anaesthetic drugs, difficulty in handling of the lynx as well as restricted direct post-operative observation. However, it still demonstrated a great potential for the use of the TAP block in exotic animals (Schroeder et al. 2010).

2.6.1 TAP block in canines

Following the study of the TAP block in a Canadian lynx, Schroeder et al. further investigated the efficacy of the TAP block, this time in canine cadavers, with the aim to evaluate the spread of injected local anaesthetic within the transversus abdominis plane, demonstrating the potential efficacy of this block for use in canine abdominal surgeries.

The transversus abdominis plane was visualised using ultrasound and the TAP block was performed bilaterally on unpreserved dog cadavers. A volume of 10 ml of bupivacaine/methylene blue solution was injected in each hemiabdomen, and dissection of the

abdominal wall was carried out between 15 and 55 minutes following the block to evaluate the spread of injectate within the transversus abdominis plane. Results showed that “the segmental branches of T11, T12, T13, L1, L2 and L3 were stained in 20 %, 60 %, 100 %, 100 %, 90 % and 30 % of injections, respectively” (Schroeder et al. 2011). Branches of T12-L2 were adequately stained, while spread of the injectate to T11 and L3 (in 20 % and 30 % of the injections, respectively) also showed the potential for regional anaesthesia in the cranial and caudal limits of the abdomen. The results of this study demonstrated the clinical potential of the TAP block for use in veterinary medicine (Schroeder et al. 2011).

In 2014 Portela and colleagues conducted a study to evaluate the efficacy of the TAP block in female dogs undergoing regional or radical unilateral mastectomy, where an ultrasound-guided two-point injection technique was performed. The authors aimed to investigate the efficacy of the TAP block alone in regional mastectomy or in combination with intercostal nerve blocks for radical mastectomy. The TAP block was performed by injecting 0.25 % bupivacaine diluted 1:1 with NaCl at a dosage of 0.3-0.35 ml/kg in each TAP block point – one cranial to the iliac crest and the other caudal to the last rib. This two-point injection technique was performed to try and improve the blocking of T11 and L3, which were blocked in only 20 % and 30 % of the cases in the study conducted by Schroeder et al. in 2011. No complications were observed in any of the dogs, recovery from anaesthesia was smooth and pain scores were low during 120 minutes of post-operative observations. Results showed possible efficacy of the TAP block in providing regional anaesthesia and analgesia of the abdominal wall in cases of unilateral mastectomy, whereas a combination with intercostal blocks is necessary in cases of radical unilateral mastectomies (Portela et al. 2014).

Another two-point ultrasound guided TAP block was performed in a canine cadaver model by Johnson et al. in 2018. The study aimed to investigate the consistency of local anaesthetic spread to nerve segments T11, T12, T13, L1, L2 and L3 using a two-point injection technique. The TAP block was performed bilaterally in five canine cadavers, with the first point of injection caudal to the last rib and the second injection cranial to the iliac crest. The total volume injected per hemiabdomen was 0.6 ml/kg. The abdominal walls were dissected 10 minutes after the injections and any nerve segments stained for a minimum of 1 cm were recorded. Results showed that “branches of T12, T13, L1, L2 and L3 were adequately stained in 30 %, 100 %, 100 %, 100 % and 100 % of injections, respectively” (Johnson et al. 2018).

100 %, 90 % and 90 % of injections, respectively” (Johnson et al. 2018). T11 was not stained in any of the hemiabdomens. The authors suggested that further research should be conducted to evaluate whether a larger volume of injectate would result in greater spread of dye in segments T11 and T12 (Johnson et al. 2018).

In 2017 Drożdżyńska et al. performed an ultrasound-guided subcostal oblique TAP block in canine cadavers, aiming to evaluate the spread of methylene blue using a three-point injection technique. With the transducer positioned parallel to the costal arch and oblique to the midline, the needle was placed within the transversus abdominis plane, and the first injection was performed with 3.3 ml of methylene blue solution. A fluid pocket could be observed sonographically and was followed caudolaterally so that its edge served as the second point of injection. Another 3.3 ml were injected, and this procedure was repeated a third time (a total volume of 10 ml per hemiabdomen). Dissection was performed 20 minutes following the injections and the following results were obtained: T9, T10, T11, T12, T13, L1 and L2 were stained in 72 %, 95 %, 100 %, 95 %, 61 %, 33 % and 11 % of the cases, respectively. These results showed that this approach to the TAP block adequately stained the nerve segments of the cranial abdominal wall, but further research was necessary to evaluate its clinical use. The authors pointed out that the lower rates of injectate spread in segments L1 and L2 were possibly due to a low injectate volume (Drożdżyńska et al. 2017). However, they explained that the volume used in this study was based on the study conducted by Schroeder et al. in 2011 (Schroeder et al. 2011) and that the volume used in a clinical context would have to be considered carefully, particularly regarding local anaesthetic toxicity.

2.6.2 TAP block in equines

One of the first studies on the TAP block in equines was performed by Baldo et al. in 2018. This was a preliminary anatomical study designed to evaluate a single-point TAP block technique in horses. The TAP blocks were performed in four adult pony cadavers positioned in dorsal recumbency and a one-point injection technique was used, with needle placement midway between the iliac crest and the last rib. A volume of 0.5 ml/kg of a 1:1 methylene blue and 0.5 % bupivacaine solution was injected per hemiabdomen. Three hours after the injections,

dissection of the abdominal walls showed staining of the ventral branches of T15, T16, T17, T18, L1 and L2 in 37.5 %, 75 %, 100 %, 100 %, 100 % and 87.5 % of the cases, respectively. It was concluded that this approach to the TAP block provided adequate staining in the caudal abdominal wall, but not to the ventral cranial abdomen, assuming that this approach would be insufficient for midline laparotomy surgeries in horses (Baldo et al. 2018).

The TAP block using a three-point injection technique in equines was first described and developed in a study by Küls et al. in 2020. The first phase of the study was performed on pony cadavers to determine the ideal anatomical landmarks for injection as well as the volume of local anaesthetic required for adequate staining of nerves which supply the ventrolateral abdominal wall. A three-point injection technique resulted in adequate staining of all nerve segments from T8 to T18, as opposed to a two-point injection technique which did not show staining of T12, T13, T14 and T15. Based on the obtained results from the cadaver study, the ultrasound-guided three-point injection TAP block was then performed in standing Shetland ponies. In this prospective, self-controlled, blinded trial the block was performed bilaterally using 0.1 ml/kg of either 0.125 % bupivacaine or saline solution per injection site. A cutaneous pinprick method was carried out over a period of four hours to evaluate the efficacy of the TAP block and was considered positive or negative, depending on whether a panniculus reflex was present or not. This test showed a significant difference in the response between the bupivacaine- and the saline-treated hemiabdomens. The results demonstrated that the TAP block performed using the described technique “successfully desensitized the dermatomes of the ventral abdomen of ponies submitted to pinprick tests for a duration of approximately 120 minutes” (Küls et al. 2020). Although the study shows great potential for the use of TAP block in horses, further research is required to evaluate its analgesic efficacy for horses undergoing abdominal surgery (Küls et al. 2020).

A modified subcostal TAP block approach was described by Freitag et al. in 2021, and the spread of methylene blue solution was evaluated using this ultrasound-guided approach in horse cadavers in lateral or dorsal recumbency (Freitag et al. 2021). They hypothesised that this method would allow staining of the ventral branches of T10 to T18 and that a lateral or dorsal recumbency position would result in different spread of the dye solution within the transversus abdominis plane. One horse cadaver was used to evaluate the standard TAP block approach

(one injection midway between iliac crest and last rib), and results showed staining of only T16-T18. Six additional horse cadavers were used for the modified subcostal TAP block, where a two-point injection technique was performed, with three cadavers in lateral recumbency and four cadavers in dorsal recumbency. A volume of 0.06-0.08 ml/kg of dye solution was injected per point – the first injection was midway between xiphoid cartilage and umbilicus scar and the second was injected between the middle and caudal third of the caudal abdominal half. Results showed staining of T9-T17 in at least 50 % of the hemiabdomens in lateral recumbency and staining of T13-T18 in at least 50 % of the hemiabdomens in dorsal recumbency. The modified subcostal TAP block approach exhibited the potential to stain more nerves supplying the ventral abdomen than the standard TAP block approach, and similar results were obtained in the study by Küls et al. in 2020 using a three-point injection technique in horse cadavers.

2.6.3 TAP block in pigs

The ultrasound-guided two-point TAP block approach was also demonstrated on pig cadavers in a pilot study conducted by Calice et al. in 2021. An anatomical study was performed first to identify suitable anatomical landmarks and showed that there was a varying number of thoracic and lumbar vertebrae, and that the ventral branches of spinal nerves could be found between different muscle layers. The TAP block was then carried out using the caudal retrocostal and lateral approach, and the subsequent dissection showed that the caudal retrocostal TAP block stained the 4th, 3rd and 2nd last thoracic nerves in three, seven and eight of eight hemiabdomens, respectively, while the lateral TAP block resulted in staining of the last thoracic, L1 and L2 nerves in seven, eight and five of eight hemiabdomens, respectively. The authors concluded that combining these TAP block approaches shows a potential for desensitizing the nerve branches that innervate the periumbilical and caudal abdominal wall (Calice et al. 2021).

Another, broader pilot study to describe different ultrasound-guided TAP block approaches in pigs was conducted by Michielsen in 2021. This study was divided in several phases: preliminary anatomical description, a pilot study, a two-point and a three-point TAP block approaches. The pilot study was performed with the aim to determine the most suitable injection points – a cranial injection was made at two thirds of the distance between the xiphoid process

and the iliac crest, and a caudal injection was made ventral to the last rib (Michielsen et al. 2021). The two-point injection technique was then performed on ten hemiabdomens and revealed the following results of the correctly placed injections: staining of T12, T13, T14, T15, T16, L1, L2 and L3 in 29 %, 29 %, 73 %, 75 %, 58 %, 100 %, 88 % and 23 % of the injections, respectively. The three-point injection technique was performed in an additional ten hemiabdomens, using a third point of injection halfway between the cranial and caudal points used in the two-injection approach. This resulted in staining of T12, T13, T14, T15, T16, L1 and L2 in 45 %, 100 %, 45 %, 78 %, 78 %, 75 % and 84 % of the correctly placed injections, respectively. No staining was observed in L3.

The study confirmed a moderate success rate for successful staining of nerves in both two-point and three-point approaches in pig cadavers. The extent of successful staining using the three-point approach was not significantly better than that of the two-point injection technique. However further research is necessary in clinical trials to assess the efficacy of the TAP block in pigs.

2.6.4 TAP block in bovines

Zoff et al. performed the TAP block in two calves undergoing surgery for umbilical hernia repair. The calves were anaesthetised using standard anaesthetic protocols and a one-point injection TAP block was performed bilaterally with ultrasound guidance in dorsal recumbency. A total of 0.3 ml/kg was injected per side. One of the calves required rescue analgesia and there was no reduction in isoflurane requirements in either of the surgeries. A possible limitation to the study could be the recumbency position as already reported in a study mentioned previously (Freitag et al. 2021), but also the short time (10 minutes for the first calf and five minutes for the second calf) between block completion and first incision, which might not have been enough for adequate spread of local anaesthetic within the neurovascular plane (Zoff et al. 2017b). The case report highlights possible advantages of the TAP block in comparison to the paravertebral block or line block and indicates the potential for use of the TAP block in calves undergoing abdominal surgery. However, further research is warranted to evaluate its true clinical efficacy.

A cadaveric study was also conducted on calves using ultrasound-guided lateral and subcostal transversus abdominis plane block approaches by Mirra et al. in 2018. This prospective, descriptive, anatomical study aimed to describe the TAP block in calf cadavers, to compare two injection volumes and to assess possible unwanted solution spread. A total of 24 lateral and twelve subcostal TAP blocks were performed in calf cadavers and for each approach two different volumes (0.2 or 0.4 ml/kg) of toluidine blue with contrast medium were injected. Dissection revealed the extent of nerve staining and contrast-enhanced computer tomography was done to assess the spread of injectate. The TAP block lateral approach resulted in staining of ventral branches of T13, L1 and L2 in 58 %, 92 % and 25 % of the cases using 0.2 ml/kg and 75 %, 83 % and 25 % of the cases using 0.4 ml/kg. On the other hand, the subcostal approach resulted in staining of T8, T9, T10, T11 and T12 in 67 %, 83 %, 67 %, 67 % and 50 % of the cases using 0.2 ml/kg and in 83 %, 100 %, 83 %, 83 % and 50 % of the cases using 0.4 ml/kg of injectate volume. There were no significant differences in the staining of nerves with different injectate volumes. This study concludes that a combination of the lateral and subcostal approaches is necessary, but still does not provide adequate blockage of all nerves supplying the ventral abdominal wall (Mirra et al. 2018).

2.6.5 TAP block in other species

A 2018 controlled, randomized, perspective, blinded clinical study by Skouropoulou et al. aimed to evaluate the efficacy of the TAP block in cats undergoing ovarioectomy. The TAP block was administered bilaterally as a single injection as part of a multimodal analgesic protocol. A total of 0.2 ml/kg of 0.5 % bupivacaine diluted in a total volume of 1.5 ml 2 % lidocaine was injected per site in ten cats in the TAP group, and another ten cats in the control group were injected with saline. Pain scoring was carried out before premedication and at regular intervals over 24 hours after extubating. Two cats in the control group required rescue analgesia during surgery and all cats in the control group were treated with methadone within 24 hours as pain scores were higher. None of the cats in the TAP group required methadone within 24 hours post extubation. The authors demonstrated the effectiveness of a single-point TAP block as part of a multimodal analgesic protocol in providing perioperative analgesia in cats undergoing ovarioectomy (Skouropoulou et al. 2018).

Otero et al. conducted a prospective anatomical study in 2021 on the TAP block in cat cadavers. The first part of the study included a sonographic anatomical study of suitable landmarks and injection points on live cats, followed by a comparison of the TAP block using two- and three-point injection approaches on cat cadavers. The two-point injection approach was performed by injecting 0.25 ml/kg of methylene blue and lidocaine solution in the subcostal (caudal and parallel to the costal arch) and the preiliac (cranial to the iliac crest) regions. The three-point injection approach was carried out in the subcostal, retrocostal and preiliac regions (retrocostal being caudal to the last rib) and each point was injected with 0.16 ml/kg of the solution. Following an anatomical dissection, results showed staining of the ventral branches of T10, T11, T12, T13, L1 and L2 in 57.1 %, 100.0 %, 85.7 %, 28.6 %, 42.9 % and 85.7 % of the cases, respectively, using a two-point injection approach, and in 66.7 %, 100.0 %, 100.0 %, 100.0 %, 88.9 % and 100.0 % of the cases, respectively, using a three-point injection approach. The authors concluded that a more effective distribution of local anaesthetic could be observed using a three-point TAP block approach in cat cadavers (Otero et al. 2021).

The transversus abdominis plane block was also investigated in rabbits in a cadaveric study by Di Bella et al. in 2021. The ultrasound-guided block was performed using a single-injection approach (halfway between the last rib and iliac crest) and a total of 1 ml/kg of methylene blue and lidocaine solution was injected in each hemiabdomen (n = 34). The following results were obtained: the ventral nerve branches of T11, T12, L1, L2, L3 and L4 were stained in 52 %, 75 %, 95 %, 100 %, 60 % and 40 % of the cases, respectively. Taking into consideration that rabbits are prone to high levels of stress when undergoing painful procedures and thus have special anaesthetic requirements, this study showed a great potential for the use of the TAP block in these animals. For surgeries requiring larger incisions, however, the single-injection approach would not be sufficient to block the nerves innervating the cranial abdominal wall. In this case a two-point approach would represent a more feasible option (Di Bella et al. 2021).

3. CADAVERIC STUDY OF A THREE-POINT INJECTION TECHNIQUE IN SHEEP

3.1 Materials and methods

3.1.1 Study animals

Seven adult experimental sheep cadavers were used in this study. They weighed between 77-88kg and were euthanised shortly prior to the experiment for reasons unrelated to the present study. An 18 G needle was placed transdermally into the rumen to avoid compression of the abdominal wall caused by ruminal tympany (as compression of the abdominal wall could possibly affect the spread of injectate).

3.1.2 Anatomical study

Four hemiabdomens from two sheep cadavers were used to investigate the anatomy and topography of the abdominal wall of the sheep, including the abdominal muscles and the nerve branches supplying the ventral abdominal wall.

The animal cadaver was placed in lateral recumbency, and an incision was made to expose a large rectangular area, extending from the spine to the ventral midline, and from the fifth rib to the tuber coxae. The skin was removed together with the cutaneous muscle, followed by the identification and separation of each abdominal muscle layer. The relevant muscles were traced to their origin and insertion sites, remarking any noticeable anatomical differences to other species. After removing the internal oblique abdominal muscle and exposing the transversus abdominis plane, the nerves supplying the abdominal ventral midline were identified. The abdominal wall structures were then further studied to determine the most appropriate anatomical points for placement of the ultrasound probe and for injection of the solution into the TAP space.

3.1.3 TAP block injections and dissection

Following the anatomical study and identification of the most appropriate points for injections, the ultrasound-guided three-point injection TAP block was performed in five sheep cadavers (ten hemiabdomens).

The ultrasound machine used for this study was MicroMaxx (SonoSite, Washington, USA) with a 38 mm wide linear transducer to visualize the relevant sonographic structures (external oblique, internal oblique, transversus, and rectus abdominis muscles, and peritoneum) as well as the needle pathway.

Three injections were administered in each hemiabdomen using a 21 G, 110 mm long echogenic needle (SonoTAP® II, Pajunk, Austria). A volume of 0.2 ml/kg of 1:1 methylene blue and 2 % lidocaine solution (Xylanaest® purum 2 %, Gebro Pharm GmbH, Austria) was used for each injection, i.e., a total volume of 1.2 ml/kg per cadaver. The cadavers were placed in lateral recumbency, the abdomen was clipped and cleaned, and the skin was prepped with alcohol and ultrasound gel. For each injection, the needle was inserted at an angle of 30° to the skin surface, beneath the transducer using an in-plane approach, to ensure direct visualization of the needle pathway through the layers of the abdominal wall until the TAP space is reached.

If encountering difficulty identifying the TAP space, the rectus abdominis muscle was considered a useful point of orientation. As determined in the anatomical study, the transversus abdominis muscle lies lateral and posterior to the rectus abdominis muscle, while the external oblique muscle lies anterior to the rectus abdominis muscle. Between the transversus abdominis and the external oblique muscle lies the aponeurosis of the internal oblique muscle.

Prior to injecting the total volume of the methylene blue solution into the TAP space, a test volume of 2 ml was first injected to confirm correct needle placement, and this was visualized as a fluid pocket which indicates separation of two fascial layers through hydrodissection (*see Figures 1b, 2b and 3b*). On the other hand, intramuscular injection of the test volume would show inconsistent spread of the solution and thickening of the muscle. If the needle was placed incorrectly, it was withdrawn slightly and repositioned. Following confirmation of correct needle placement, the rest of the volume was injected into the TAP space.

The first injection was performed using the lateral TAP block approach. The ultrasound probe was positioned in a longitudinal orientation midway between the last rib and the iliac crest, about 5 cm ventral to the spinal processes of the lumbar vertebrae (*see Figure 1a*). The needle was inserted at the cranial end of the transducer and advanced in a caudomedial direction to reach the TAP space.

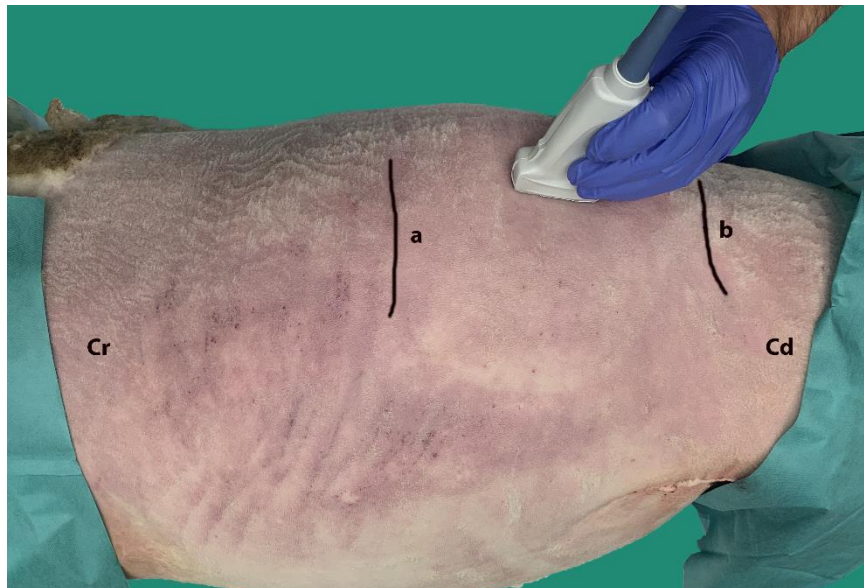


Figure 1a - lateral TAP block approach. a: line depicting position of last rib, b: line depicting position of iliac crest, Cr: cranial, Cd: caudal. Transducer positioned in a longitudinal orientation midway between the last rib and the iliac crest.

For the second injection, a caudal subcostal oblique approach was used, and the ultrasound probe was positioned parallel and 2 cm caudal to the costal arch, approximately midway between the xiphoid process and the spinous processes (*see Figure 2a*). The needle was introduced at the caudal end of the transducer and advanced in a cranioventral direction until the TAP space was reached.

The third injection was performed using the cranial subcostal oblique approach. The transducer was placed midway between the xiphoid process and the second injection point (caudal subcostal oblique approach), and the needle was inserted at the cranial end of the transducer in a caudodorsal direction.

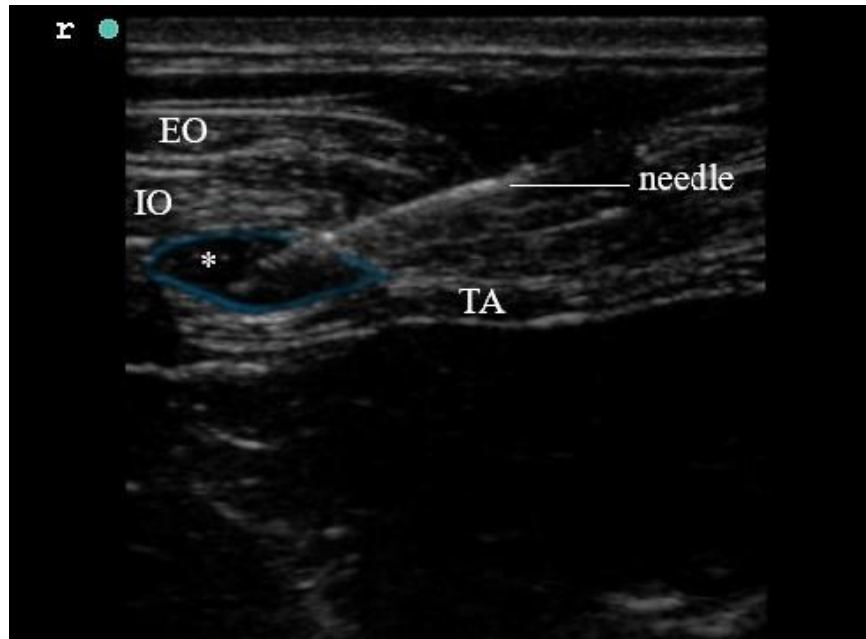


Figure 1b – sonographic image of the lateral TAP block approach. EO: external oblique abdominal muscle, IO: internal oblique abdominal muscle, TA: transversus abdominis muscle, *: needle placement within the TAP space and hydrodissection of muscle layers (marked blue) following injection of methylene blue solution

The cadaver was then placed in the other lateral recumbency, and the same procedure was repeated on the contralateral side.

Dissection of the sheep cadavers began approximately 20 minutes after the last injection, following the order in which the hemiabdomens were injected. The cadavers were positioned in lateral recumbency, and an incision was made to expose a large rectangular area, extending from the spine to the ventral midline, and from the fifth rib to the tuber coxae. The skin and each abdominal muscle layer were removed separately to reveal the transversus abdominis plane and the nerves innervating the ventrolateral abdominal wall. The branches of T8 to L2 nerves were identified and level of staining was observed and assessed by two participants of this study independently. Those nerve segments that had been stained for a minimum of 10 mm along their long axis were considered successfully stained.

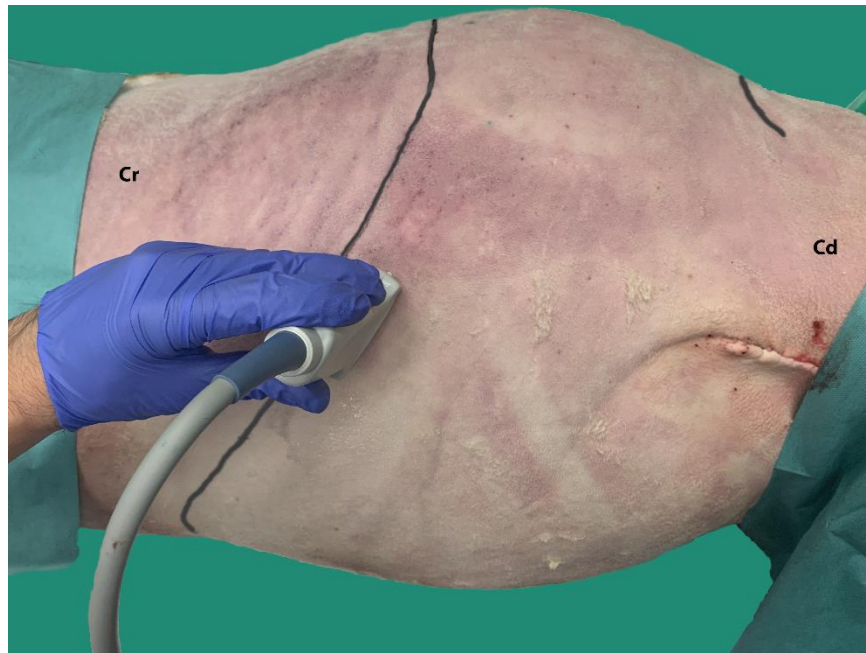


Figure 2a – caudal subcostal oblique approach. Transducer positioned parallel and 2 cm caudal to the costal arch, approximately midway between the xiphoid process and the spinous processes.

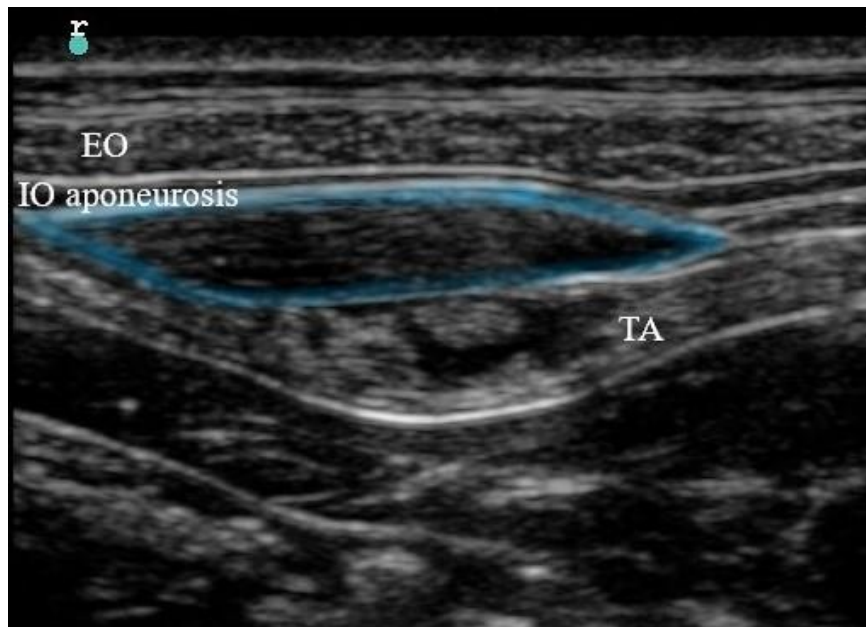


Figure 2b – sonographic image of the caudal subcostal oblique approach. EO: external oblique abdominal muscle, IO aponeurosis: aponeurosis of internal oblique abdominal muscle, TA: transversus abdominis muscle. Hydrodissection of muscle layers following injection of methylene blue solution into the TAP space (marked blue).

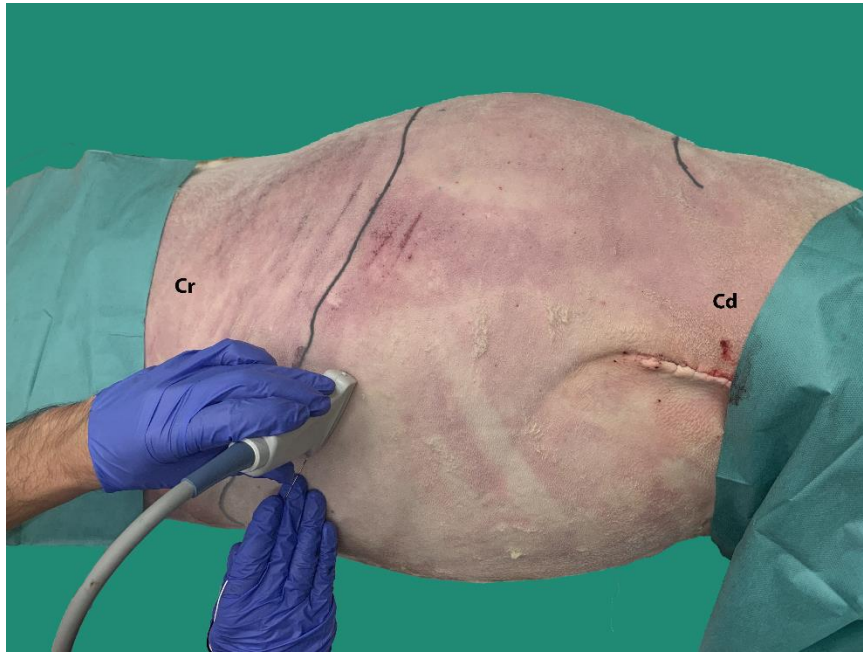


Figure 3a – cranial subcostal oblique approach. Transducer positioned midway between the xiphoid process and the second injection point.

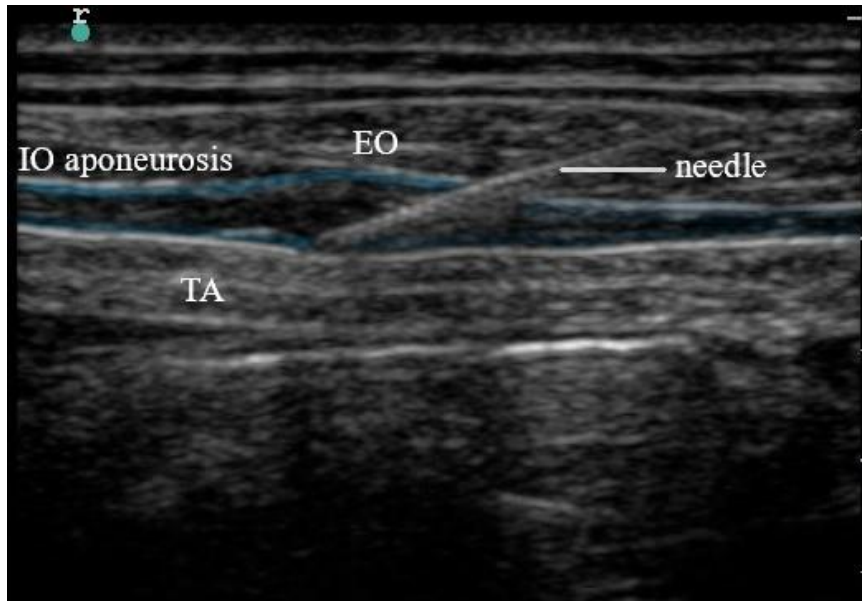


Figure 3b – sonographic image of cranial subcostal oblique approach. EO: external oblique abdominal muscle, IO aponeurosis: aponeurosis of internal oblique abdominal muscle, TA: transversus abdominis muscle. Hydrodissection of muscle layers following injection of methylene blue solution into the TAP space (marked blue).

3.2 Results

3.2.1 Anatomical study

Dissection of two cadavers (four hemiabdomens) revealed the anatomical structure of the abdominal wall of the sheep. The four muscles of the abdominal wall, including their origin and insertion sites, were identified. The external oblique muscle is the first abdominal muscle to be seen following removal of the skin and the cutaneous trunci muscle. It originates from the outer edges of the last ribs, partly attaching to the tuber coxae and mostly running caudoventrally to attach to the linea alba (*see Figure 4*). The internal oblique muscle is the second abdominal muscle, running from the tuber coxae, transverse processes of lumbar vertebrae and the thoracolumbar fascia to the last rib and linea alba. Its fleshy part becomes a wide aponeurosis along the lateral border of the rectus abdominis muscle (*see Figure 5*). The third muscle observed is the transversus abdominis muscle which lies deep to the internal oblique muscle. It originates from the inner rib cartilages and the lumbar transverse processes, runs transversely, and inserts into the linea alba (*see Figure 6*). The last abdominal muscle is the rectus abdominis muscle and runs longitudinally along the ventral abdominal wall. It originates from the rib cartilages and sternum and inserts into the prepubic tendon. It is surrounded by the aponeuroses of the other abdominal muscles in the so-called rectus sheath. A substantial amount of fat was found between the muscle layers of the abdominal wall in the sheep used for the anatomical study.

Between the fascial layers of the transversus abdominis muscle and the internal oblique muscle is a neurovascular plane which exposes the ventral branches of the spinal nerves T8-L2 which innervate the ventrolateral abdominal wall (*see Figure 6*). In only one of the four hemiabdomens it was observed that the ventral branch of T8 runs along the costal arch in a caudoventral direction, indicating that it potentially also contributes to the innervation of the abdominal wall. Conversely, in three hemiabdomens the T8 ventral branch showed a cranial course of direction, thus suggesting its unlikely involvement in the innervation of the abdominal wall.

The anatomical landmarks suitable for transducer positioning and injection points were determined according to the distribution and location of the targeted nerve branches, with the

aim that the three-point injection technique would result in a wide spread of dye solution within nerve branches in the cranial, central, and caudal parts of the abdominal wall.

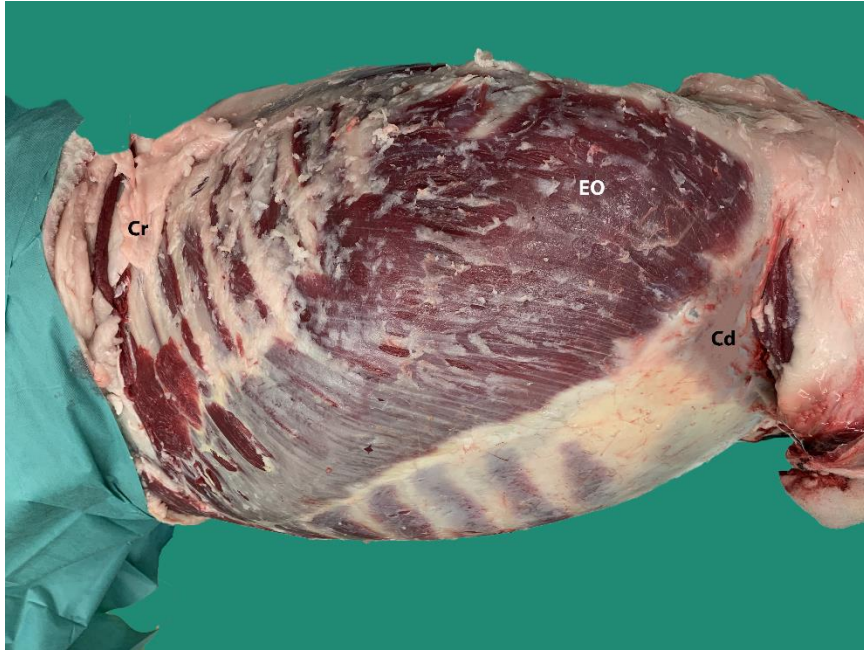


Figure 4 – EO: external oblique abdominal muscle. Origin: outer edges of last ribs. Insertion: tuber coxae, linea alba



Figure 5 – IO: internal oblique abdominal muscle. Origin: tuber coxae, transverse processes of lumbar vertebrae and the thoracolumbar fascia. Insertion: last rib, linea alba



Figure 6 – TAM: transversus abdominis muscle. Transversus abdominis plane showing ventral branches of thoracic nerves T9-T13 and lumbar nerves L1 and L2.

3.2.2 TAP Block

Ultrasound-guided TAP injections were performed in five sheep cadavers (ten hemiabdomens). All relevant structures were easily identified when scanning the abdominal wall and corresponded to the results obtained in the anatomical study. Sonographic images obtained when performing all 30 injections enabled excellent visualization of the needle (tip and entire shaft) and its pathway to reach the TAP space (*see Figures 1b, 2b and 3b*).

Following dissection, assessment of dye spread within the nerve branches of T8 to L2 was performed by two participants of the study, separately. Only those nerves showing dye solution spread for a minimum of 10 mm in their length were considered successfully stained.

<i>Sheep</i>	<i>Weight (kg)</i>	<i>Hemiabdomen</i>	<i>Volume of injectate (ml)</i>	<i>Nerves stained >10 mm</i>
<i>1</i>	<i>77</i>	<i>Right</i>	<i>3 x 15</i>	<i>T9-L2</i>
		<i>Left</i>	<i>3 x 15</i>	<i>T8-L2</i>
<i>2</i>	<i>88</i>	<i>Right</i>	<i>3 x 17</i>	<i>T9-L2</i>
		<i>Left</i>	<i>3 x 17</i>	<i>T9-L2</i>
<i>3</i>	<i>86</i>	<i>Right</i>	<i>3 x 17</i>	<i>T9-L2</i>
		<i>Left</i>	<i>3 x 17</i>	<i>T10-L2</i>
<i>4</i>	<i>88</i>	<i>Right</i>	<i>3 x 17</i>	<i>T9-L2</i>
		<i>Left</i>	<i>3 x 17</i>	<i>T9-T13</i>
<i>5</i>	<i>80</i>	<i>Right</i>	<i>3 x 16</i>	<i>T8-T12; L2</i>
		<i>Left</i>	<i>3 x 16</i>	<i>T9-L2</i>

Table 1 Summary of results obtained following a three-point injection TAP block

The most cranial nerve branch that was successfully stained was the T8 in two hemiabdomens, T9 in seven hemiabdomens and T10 in one hemiabdomen. The most caudal spread of dye was observed at the level of L2 in eight of ten hemiabdomens. Branches of T8, T9, T10, T11, T12, T13, L1 and L2 were adequately stained in 20 %, 90 %, 100 %, 100 %, 100 %, 90 %, 80 % and 90 % of the cases, respectively (see Table 1, Figures 7 and 8).

In one hemiabdomen, dye was partly found between the external oblique and internal oblique abdominal muscles and resulted in staining of T9 to T13 branches, but not L1 and L2. In another hemiabdomen, the dye was found between the transversus abdominis muscle and the transverse fascia (which lies deep to the transversus abdominis muscle and superficial to the parietal peritoneum), outside of the TAP space. Branches of T13 and L1 were not successfully stained in this case. Both errors were associated with the first injection (lateral approach).

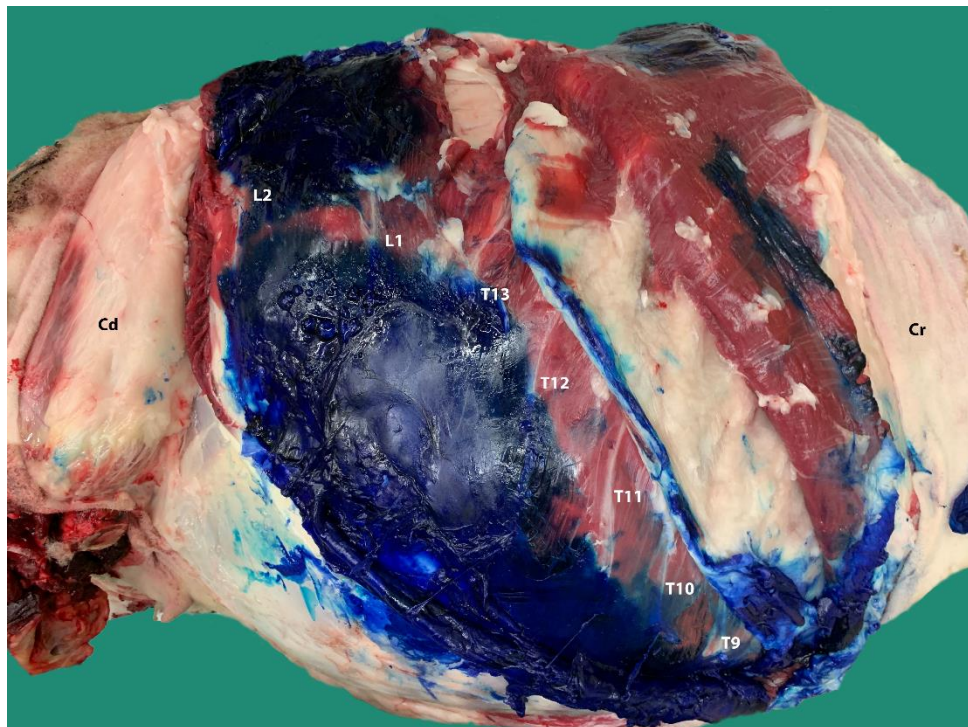


Figure 7 – Dissection following three-point injection TAP block showing adequate dye spread within nerve branches of T9 to L2.

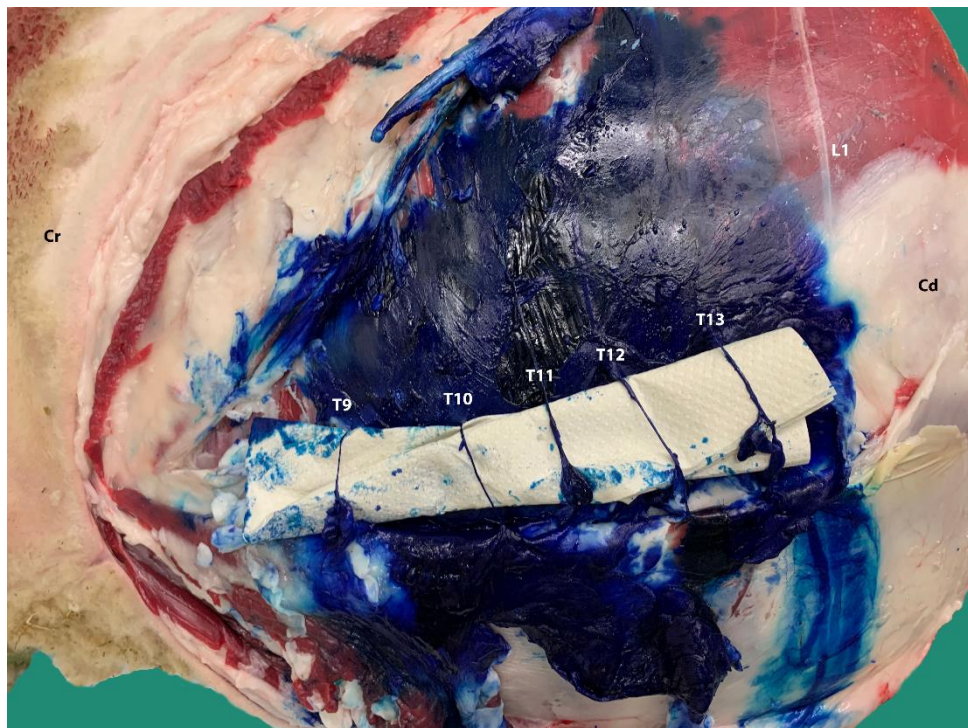


Figure 8 – Dissection following three-point injection TAP block showing clearly the isolated and adequately stained nerve branches of T9 to T13.

4. DISCUSSION

The transversus abdominis plane block is a regional anaesthesia technique rapidly gaining popularity in clinical use as part of a multimodal anaesthesia regime, both in human and veterinary medicine. It provides effective perioperative analgesia to patients undergoing surgeries involving incision in the ventrolateral abdominal wall, contributing to a decreased requirement for other anaesthetic drugs, such as opioids, known to cause various unwanted side effects. Initially described as a blind technique (Rafi 2001), the TAP block was later developed with the help of ultrasound (Hebbard 2008), which allowed direct visualisation of relevant anatomical structures and safer, more precise injection of local anaesthetic within the transversus abdominis neurovascular plane. In veterinary medicine, numerous studies have been conducted on different animal species. The variation in anatomical structure of the abdominal wall amongst species has initiated the development of various approaches and techniques of the TAP block to achieve effective desensitization of the ventrolateral abdominal wall.

However, no studies on the TAP block in sheep have been published to date and the present study aimed to identify the nerve branches which supply the ventrolateral abdominal wall, to describe an adequate ultrasound-guided TAP block technique for sheep and to evaluate the spread of methylene blue solution and nerve staining within the TAP space.

The study showed that a bilateral three-point injection technique, using one lateral and two subcostal approaches resulted in successful staining of ventral branches of T9, T10, T11, T12, T13, L1 and L2 in 90 %, 100 %, 100 %, 100 %, 90 %, 80 % and 90 % of the cases, respectively, suggesting the potential analgesic efficacy of the TAP block for surgeries involving the ventrolateral abdomen, especially when long incisions are required. Previously conducted research in other species has shown that combining multiple injection points instead of increasing the injected volume resulted in a wider cranio-caudal spread of injectate (Mirra et al. 2018; Küls et al. 2020; Johnson et al. 2018). Based on the anatomical study and findings that the ventral nerve branches of T8 to L2 run over the transversus abdominis muscle and hence innervate the ventrolateral abdominal wall of the sheep, it was assumed that a three-point injection technique would result in the broadest cranio-caudal spread of the injectate.

The involvement of the ventral branch of the T8 nerve in the innervation of the cranial part of the abdominal wall of sheep remains questionable. In the anatomical study only one of the four hemiabdomens used showed the T8 running caudoventrally towards the abdominal midline, whereas in three hemiabdomens the ventral branch of the T8 showed a cranial course of direction. The TAP block study resulted in staining of the T8 in only 20 % of the cases, suggesting that its involvement is variable amongst sheep. Further research is necessary to investigate whether blocking this nerve would desensitize the most cranial aspect of the abdominal wall in living sheep.

No difficulties were encountered when performing any of the injections as sonographic images of the relevant structures corresponded to the findings of the previously conducted anatomical study and visualisation of the TAP space and needle trajectory were deemed excellent. For the sake of consistency, all blocks were performed by the same veterinarian experienced in regional anaesthesia techniques. Although not recorded in this study, performing all three injections per hemiabdomen did not appear to be excessively time consuming.

The first injection was performed using the lateral approach, with the transducer positioned halfway between the last rib and the iliac crest. No anatomical differences were observed in this region compared to other species. The three layers of muscle were easily identified using ultrasound and the TAP space was located between the internal oblique and the transversus abdominis muscles. Following dissection, in two hemiabdomens the injectate was found outside of the TAP space. In one of the cases, dye was partly found between the external oblique and internal oblique muscles, resulting in staining of T9-T13, but not of L1 and L2. This could have been a consequence of the needle tip slipping outside of the TAP space during injection or leakage of injectate following withdrawal of the needle. The portion of dye that was correctly injected into the TAP space was found exactly between the L1 and L2 ventral branches, but that volume was not sufficient to reach and stain them. In another hemiabdomen, a moderate amount of dye was found outside of the TAP space between the transversus abdominis muscle and the transverse fascia, resulting in unsuccessful staining of T13 and L1. This error likely occurred due to human error and incorrect placement of the needle, just outside of the transversus abdominis plane.

The second and third injections were performed using the caudal and cranial subcostal oblique approaches, respectively. In this region the structure of the abdominal wall of sheep differs to that of other species. In sheep, the TAP space lies between the broad aponeurosis of the internal oblique abdominal muscle and the transversus abdominis muscle, whereas for example, in calves, horses and cats it is found between the rectus abdominis and transversus abdominis muscles (Mirra et al. 2018; Freitag et al. 2021; Otero et al. 2021). As observed in the anatomical study, a substantial amount of fat was present in this region between the fascia transversa abdominis and the peritoneum, which could possibly result in misinterpretation of the muscle layers and incorrect needle placement.

The present study was performed on freshly euthanised sheep, which most closely mimics live animal tissue, as opposed to frozen or preserved specimens. About 30 minutes prior to the TAP injections, an 18 G needle was placed transdermally into the rumen to avoid compression of the abdominal wall caused by ruminal tympany. Following the TAP injections, the cadavers were left in lateral recumbency and dissected after 20 minutes, which does not entirely simulate a clinical setting and could possibly have affected the spread of injectate. Some cases revealed pooling of dye solution rather than spreading within the TAP space, which would likely not occur *in vivo* as the solution would continue to spread within the plane as a consequence of muscle contraction and the biomechanical properties of fascia, allowing active transport of local anaesthetic within the fascial planes (Elsharkawy et al. 2019). In addition to this, a large layer of fat was found between the muscle layers, which could also affect the spread of injectate. Therefore, it is necessary to further investigate the spread of injectate in living sheep to determine whether, for example, a two-point injection technique would be sufficient to obtain the same extent of nerve staining as a three-point injection technique.

The total volume of injectate (1:1 solution of methylene blue and 2 % lidocaine) used in this study was 1,2 ml/kg per cadaver, or 0,2 ml/kg per injection site. This was based on volumes used in previous studies (Johnson et al. 2018). The number of test injections was not recorded in this study but should be considered when determining the optimal volume, as multiple tests could waste a significant amount of the total volume injected. The results obtained indicate, however, that the injected volume was sufficient to stain all targeted nerves. Further research is warranted to evaluate whether a smaller volume would also provide satisfactory results. This is

especially important for *in vivo* studies to avoid possible adverse effects such as dorsal spread of local anaesthetic which could migrate into the paravertebral or epidural space, potentially resulting in paralysis of the femoral nerve (Baldo et al. 2018). Furthermore, viscosity of the injectate is another factor which could possibly affect spread of solution within the TAP space, but this was not measured in this study.

5. CONCLUSION

The ultrasound-guided three-point injection transversus abdominis plane block conducted in sheep cadavers showed successful staining of the ventral branches of nerves innervating the ventrolateral abdominal wall. The ultrasound-guided approach allows direct visualization of the abdominal wall and its structures, as well as safe and rather precise injection of local anaesthetic into the TAP space. The technique is not difficult to perform in sheep and the results obtained suggest the potential of the TAP block in providing excellent sensory blockage to a large extent of the abdominal wall, following a combination of the lateral and subcostal oblique approaches. Desensitization of the most cranial part of the abdomen remains questionable and the involvement of the T8 nerve should be investigated in living sheep to confirm whether the described approach would suffice in providing adequate analgesia to this part of the abdomen. Further research should be conducted on sheep to determine the adequate volume and concentration of local anaesthetic necessary to provide effective analgesia of the ventrolateral abdominal wall and to evaluate the perioperative analgesic efficacy of the TAP block *in vivo*.

6. SUMMARY

Development in veterinary anaesthesia and analgesia has led to prioritization of perioperative patient comfort. Surgeries involving an incision in the abdominal wall are considered very painful, so use of opioid drugs and/or loco-regional anaesthetic techniques is often implemented for such procedures. The transversus abdominis plane (TAP) block was developed to provide sensory analgesia to the ventral and lateral abdominal wall. It is a technique involving injection of local anaesthetic into the neurovascular plane between the transversus abdominis and internal oblique abdominal muscles, aiming to desensitize the ventral branches of the thoracic and lumbar nerves involved in the innervation of the ventrolateral abdominal wall, thus contributing to overall reduction in opioid use for perioperative pain management.

Numerous studies on the TAP block have been conducted both in human medicine and in several species in veterinary medicine, however no research to date has been published on the TAP block in sheep, animals often used as experimental research models. Therefore, the objective of this thesis was to describe an ultrasound-guided three-point TAP block approach in this species. The study was conducted on sheep cadavers and a three-point injection technique was performed, following an anatomical study used to determine appropriate injection points.

The results obtained in the study showed adequate dye dispersion and staining of the thoracic nerve branches of T9, T10, T11, T12 and T13 and lumbar nerve branches of L1 and L2. These results suggest a great potential for the use of TAP block in sheep to provide excellent sensory blockage to a large area of the abdominal wall following a combination of the three injection points.

This study represents a remarkable first step into the development of the TAP block in sheep, however further research is warranted to determine the adequate settings for performing this technique in living sheep and to evaluate its perioperative analgesic efficacy for clinical use.

7. ZUSAMMENFASSUNG

Die Weiterentwicklung im Bereich der veterinärmedizinischen Anästhesie und Analgesie hat dazu geführt, dass dem perioperativen Komfort des Patienten eine Priorität gegeben wird. Chirurgische Eingriffe, bei denen eine Inzision durch die Bauchdecke gemacht wird, gelten als sehr schmerzhaft, und für solche Eingriffe werden häufig Opioide und/oder Lokoregionalanästhesie eingesetzt. Der transversus abdominis plane (TAP)-Block wurde deswegen entwickelt, um eine sensorische Analgesie der ventralen und lateralen Bauchwand bereitzustellen. Es handelt sich um eine Technik, bei der ein Lokalanästhetikum in die neurovaskuläre Ebene zwischen dem *Musculus transversus abdominis* und den inneren schrägen Bauchmuskeln injiziert wird, um die ventralen Äste der Brust- und Lendennerven zu desensibilisieren, die an der Innervation der ventrolateralen Bauchdecke beteiligt sind. Somit führt das zu einer Gesamtreduktion in der Anwendung von Opioiden für perioperative Schmerzbehandlung.

Zahlreiche Studien zum TAP-Block wurden sowohl in der Humanmedizin als auch bei mehreren Tierarten in der Veterinärmedizin durchgeführt. Jedoch wurde bisher keine Forschung zum Thema “TAP-Block bei Schafen“ veröffentlicht – eine Tierart, die häufig als experimentelles Forschungsmodell eingesetzt wird. Daher war das Ziel dieser Arbeit, einen ultraschallgeführten Dreipunkt-TAP-Block-Ansatz bei dieser Tierart zu beschreiben. Die Studie wurde an Schafkadavern durch eine Dreipunkt-Injektionstechnik mithilfe einer anatomischen Studie zur Bestimmung geeigneter Injektionspunkte durchgeführt.

Die in der Studie erhaltenen Ergebnisse zeigten eine angemessene Farbstoffverteilung, Färbung der Brustnervenäste von T9, T10, T11, T12 und T13 und der Lendennervenäste von L1 und L2. Diese Ergebnisse deuten auf ein großes Potenzial in der Verwendung von TAP-Blocks bei Schafen hin, die mit einer Kombination der drei Injektionspunkte eine hervorragende Desensibilisierung im großen Bereich der Bauchdecke ermöglichen.

Diese Studie stellt einen bemerkenswerten ersten Schritt in der Entwicklung des TAP-Blocks bei Schafen dar. Jedoch sind weitere Forschungsarbeiten gerechtfertigt, um die angemessenen Bedingungen für die Durchführung dieser Technik bei lebenden Schafen zu bestimmen und ihre perioperative analgetische Wirksamkeit für eine klinische Anwendung zu bewerten.

8. LIST OF ABBREVIATIONS

Cd:	caudal
Cr:	cranial
EO:	external oblique (abdominal muscle)
IO:	internal oblique (abdominal muscle)
TAM:	transversus abdominis (muscle)
TAP:	transversus abdominis plane

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