Comparative Evaluation of Conventional Anatomical Landmark Guided, Perineural Ultrasound Guided and Perivascular Colour Doppler Guided Brachial Plexus Block In Sheep

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Abstract: Thirty six adult sheep irrespective of sex, randomly allotted to six groups i.e., A, B, C, D, E & F with six animals each formed the subjects of study with the objectives to identify the window most feasible and an alternative site to conventional site for localization of brachial plexus in adult sheep and its subsequent blockade under ultrasound guidance; to compare the perivascular brachial plexus blockade (BPB) using ultrasound guidance and perivascular BPB using color doppler and to ascertain possible dose regimen variation and anesthetic effect with and without ultrasound guidance. All the animals received 0.75% ropivacaine hydrochloride @ 3mg/kgBW. Perineural ultrasound guided BPB was performed in animals of group A & B, in which group A animals received ropivacaine whereas group B animals received additional 4mg isoflupredone, perivascular colour doppler guided BPB was performed in groups E & F, in which received ropivacaine and ropivacaine plus 4mg isoflupredone respectively. BPB in groups C & D was performed using landmark approach using ropivacainein group C and additional 4mg isoflupredone in group D. We identified the ultrasound scanning window. The perivascular and perineural BPB allowed a feasible and accurate access to BP and significantly shortened the onset and prolonged the duration of anesthesia as compared to conventional technique of BPB. By incorporating 4mg isoflupredone, ropivacaine dose was reduced by 15mg. Therefore, it could be concluded that this anesthetic regimen can provide a very good anesthesia for forelimb surgery upto four hours duration in sheep.

Keywords: Brachial plexus, Colour Doppler, Sheep, Ultrasound

I. INTRODUCTION

In ruminants, surgical procedures are commonly performed under sedation and local or regional anesthesia and are preferred over general anesthesia in these animals because they produce minimal cardiopulmonary alterations, require limited amount of equipment, minimize veterinary supervision, lower the cost of the procedure [35], facilitate clinical work with the animal in the standing position, while providing analgesia with minimal adverse effects [32] and avoid other known complications of general anesthesia. However, disadvantages may include the difficulty in identifying anatomic landmarks, variability in anatomic pathways of peripheral nerves, risk of penetrating into other structures or the large volume of local anesthetic. The variable anatomy between individuals may lead to poor success rates for specific peripheral nerve blocks[34,28].To overcome such difficulties, ultrasound and peripheral nerve stimulation guided techniques of local anesthesia have been started in animals. For the past two decades, the electrical nerve stimulator has been the gold standard for nerve localization in regional anesthesia [39,6,9,11] but the nerve stimulation may not always elicit a motor response and doesn’t guarantee success [26]. However, with recent developments in high-frequency imaging, the use of ultrasound technology has significantly increased for nerve localization [19,12,25,29]. The ultrasound guided technique offers reported additional advantages, including avoidance of intraneuronal/intravascular injection, faster onset times, improved block quality, decreased pain from muscular contractions, prolonged postoperative analgesia, and decreased need
for rescue analgesics [27,22,15,24]. Local anesthetics alone provide analgesia for not more than 4-8 hrs., so various additives like opioids,[23] clonidine[20],hylase etc. were added to local anesthetics, but the results are either inconclusive or associated with side effects[17]. Steroids have powerful anti-inflammatory as well as analgesic property. Perineural injection of steroids is reported to influence post operative analgesia; hence injection isoflurupredone acetate along with ropivacaine, a long acting local anesthetic agent was used in the present study. Steroids relieve pain by reducing inflammation and blocking transmission of nociceptive C-fibres and by suppressing ectopic neural discharge [2].

Brachial plexus block, as a regional analgesic technique, is used in veterinary patients to provide analgesia for animals undergoing surgery of the distal forelimb. The brachial plexus block in sheep involves desensitization of the ventral roots of the sixth, seventh, and eighth cervical nerves (C6, C7, C8) as well as the first and second thoracic nerves (T1, T2) as they pass together over the lateral aspect of the middle third of the first rib [30]. There are only few reports of its clinical use in animals. This block is likely to be most useful in calves, sheep, or goats in a clinical or research setting for procedures on the thoracic limb under general anesthesia as far the frequency of the operations in the areas of limb supplied by brachial plexus is concerned. A brachial plexus block has been described in cattle [35] and sheep [8, 13] to provide analgesia of the thoracic limb distal to and including the elbow. Brachial Plexus Block was first described by Tufvesson (1951) as a means of providing anesthesia of the forelimb in dogs [37]. Abramowitz and Cohen (1981) described the first use of doppler ultrasound to identify the axillary artery, thereby aiding the performance of axillary plexus block for upper limb surgery[1]. But it was the use of B-mode ultrasound (Ting and Sivagnanaratham, 1989) for axillary block performance that heralded the era of ultrasound-guided peripheral nerve block[36]. Sheppard et al. (1998) evaluated the ability of ultrasound to visualize components of the brachial plexus, using MRI as a guide to background anatomy. They described the plexus as having a hypoechoic appearance within hyperechoic rims which were tubular on longitudinal scans and oval to round on transverse scan they also felt that color doppler was essential to prevent the confusion of nerves with small blood vessels [33]. Yang et al. (1998) studied anatomy of the brachial plexus under ultrasound and subsequently used it to guide the placement of catheters for interscalene and supraclavicular blocks for an arm surgery in humans [38]. Guilherme and Benigni (2008) described a technique for ultrasound of the brachial plexus and major nerves of canine thoracic limb by positioning the probe in a parasagittal plane between the sternum and the shoulder with the ultrasound beam directed caudally, a transverse image of the brachial plexus close to the origin of the axillary artery and vein was obtained [18]. Christophe et al. (2009) studied the ultrasound guided axillary brachial plexus block technique in humans. The pulsating axillary artery was visualized and the transducer moved to locate the individual nerves around the artery. The nerves at this level have a honeycomb appearance, but their locations relative to the artery are variable[5]. Eichenberger et al. (2009) stated that contrary to indirect methods of nerve localization (e.g., nerve or transcutaneous stimulation, pure landmark-based techniques etc); ultrasonography facilitates not only the deposition of the local anesthetic solution near the nerve but helps to distribute the injected solution optimally around the nerve. Ultrasound guidance may therefore allow smaller volumes of local anesthesia to achieve similar results [7]. O'Donnell et al. (2009) performed a randomized, controlled trial comparing low-dose ultrasound-guided axillary block with general anesthesia evaluating anesthetic and perioperative analgesic outcomes and concluded that ultrasound-guided axillary brachial plexus block with 20 ml local anesthetic mixture provided satisfactory anesthesia and superior analgesia after upper limb trauma surgery when compared with general anesthesia [31]. Campoy et al. (2010) described an ultrasound guided technique for axillary brachial plexus, femoral and sciatic nerves in dog. Location of the transducer in the axilla produced images of the axillary blood vessels. Axillary artery was identified by its characteristic anechoic pulsatile ultrasound image. Three rounded hyperechoic structures were observed dorsal and close to the axillary vessels presumed to be the C7, C8, and T1 roots of the brachial plexus [3]. According to Carter and Bhat(2011), ultrasound guided inter scalene block target the roots and proximal trunks of the brachial plexus as they are sandwiched between the anterior and the middle scalene muscle [4]. An interventional ultrasound technique to increase the safety of surgical treatment of the calf forelimb was tested by Iwamoto et al. (2012). They evaluated the brachial plexus using ultrasonography and then injected 2% lidocaine under ultrasound guidance. The results suggest the clinical feasibility of ultrasound-guided brachial plexus block in bovine medicine[21]. According to Gofeld et al. (2013), ultrasonography is an alternative imaging method for localization and examination of peripheral nerves and allows serial examinations over almost any anatomical region so that a long length of a nerve can be studied under dynamic conditions where changes in body position or orientation may influence the nerve’s location[16]. Fonseca et al. (2015) stated that ultrasound guidance is an emerging aspect of regional anesthesia that has the potential to optimize local delivery and distribution of anesthetic agents in rabbits, thereby reducing the amounts of these agents that must be administered. They recommend that this technique be integrated into multimodal approaches to pain management in rabbits undergoing thoracic limb surgery [10]. Ghadirian et al., 2016 did the comparison of lidocaine, lidocaine–morphine, lidocaine-tramadol or bupivacaine for neural blockade of the brachial plexus in fat-tailed lambs and concluded that the addition of morphine or tramadol to lidocaine did not affect the duration of antinociception of lidocaine for brachial plexus block in fat-tailed lambs [14].
Keeping the scenario in view the present study was planned with the following objectives:

A. To identify the window most feasible and an alternative site to conventional site for localization of brachial plexus in adult sheep and its subsequent blockade under ultrasonographic guidance.
B. To compare the perineural brachial plexus blockade using ultrasound guidance and perivascular brachial plexus blockade using color doppler.
C. To ascertain the possible dose regimen variation and anesthetic effect with and without ultrasound guidance.

II. MATERIALS AND METHODS

The study was conducted at Mountain Research Station for Sheep and Goat (MRSG) / Division of VSR, F.V.Sc& AH, SKUAST - Kashmir.

A. Animals of the study

The study was conducted on 36 adult sheep irrespective of sex in the age group of six months to one year. The animals were housed under similar management conditions at the research centre since birth. The animals were divided randomly in six groups viz. Group A, B, C, D, E and F comprising six animals each.

B. Instrumentation

An ultrasound system TELEMED CAB with a 5-10MHz linear transducer was used for ultrasound guided brachial plexus per neural and per vascular block in sheep in lateral recumbence (Fig.1).

C. Preparation of the patient

Before administering any drug, the sheep were subjected to overnight fasting for 12 hours. All animals underwent antisepsis of the appendages prior to brachial plexus block.

D. Technique used for brachial plexus block

1) Brachial plexus block in Group A animals: The scapula and the area around the scapular region was cleaned, shaved, surgically scrubbed and prepared aseptically (Fig. 2). The animals were restrained in lateral recumbence which was followed by application of copious gel over the prepared site. After standardization of procedure from different possible angles and borders of scapula and anatomical area in vicinity the window was identified. The exact area where from the brachial plexus was visible was by placing the transducer along the medial aspect of scapula over the triceps and latissimusdorsi muscle (Fig. 3). The axillary lymph node was identified and the needle was inserted under the guidance of ultrasound scanner. The needle was slowly pushed forward above the level of axillary lymph node so that the bevel of the needle was nearer to the plexus close to the radial nerve and the anesthetic agent i.e. 0.75% ropivacaine hydrochloride was injected and its spread around the brachial plexus was clearly monitored on the screen of the ultra sound scanner. Ultrasonography was performed by using TELEMED CAB with a 5-10MHz linear transducer. 0.75% ropivacaine hydrochloride@ 3mg/kg b.wt. was deposited near the brachial plexus slowly in installments(Fig.4). The deposition of the anesthetic agent at brachial plexus was monitored on USG screen.
2) **Brachial plexus block in Group B animals:** In Group B, the procedure performed was same as that in group A. Ultrasonography was performed by using TELEMED CAB with a 5-10MHz linear transducer. The animals were subjected to brachial plexus blockade using a combination of 0.75% ropivacaine hydrochloride@ 3mg/kg b.wt. and 2ml (4mg) isoflupredone acetate. However addition of 2 ml (4 mg) isoflupredone acetate replaced 2 ml (15 mg) of ropivacaine hydrochloride, thereby reducing the dose of latter by 15 mg.

3) **Brachial plexus block in Group C animals:** The group C included the sheep in which brachial plexus was blocked without the use of ultrasound using 0.75% ropivacaine hydrochloride @ 3mg/kg b.wt. After appropriate patient positioning in lateral recumbence and strict aseptic and antiseptic precautions, the conventional landmarks were determined by pulling the forelimbs caudally (neck axis, 110-120°). At the base of the neck, the surface landmarks could be determined by palpation and examination of external jugular vein, cervical esophagus, and forward trachea; clavicle, transverse and descending pectoral muscles at the bottom; and scalenusventralis, dorsalis muscles, and cervical vertebral column at the top. The area 10 cm cranial to the acromion process of the scapula and axillary lymph node was surgically scrubbed, shaved and 3 ml of local anesthetic solution was infiltrated to form insensitive wheel (Fig. 5). A 16 cm, 18-gauge needle, was inserted through the desensitized area.
and pushed horizontally till it struck the lateral surface of the first rib, where approximately half the calculated dose of a 0.75% ropivacaine hydrochloride solution @ 3mg/kg b.wt. was injected. The needle was then withdrawn and then its tip was redirected 3 cm more distal to the first injection site, where the other half of the anesthetic was deposited. Care was exercised that no air, blood, or cerebrospinal fluid was withdrawn upon needle aspiration.

Fig. 5: Site for anesthetic deposition in conventional brachial plexus block

4) **Brachial plexus block in Group D animals:** In Group D, the animals were restrained in lateral recumbancy, 0.75% ropivacaine hydrochloride @ 3mg/kg b.wt. and 2ml (4mg) of isoflupredone was administered by using 16 cm 18 gauge needle as in group A in the similar manner as in group C animals. However addition of 2 ml (4 mg) isoflupredone acetate replaced 2 ml (15 mg) of ropivacaine hydrochloride, thereby reducing the dose of latter by 15 mg.

5) **Brachial plexus block in Group E animals:** In this group of animals, brachial plexus was blocked by using perivascular ultrasound guided block technique. The area on the caudal border and distal aspect of scapula was surgically scrubbed and prepared aseptically. Animals were restrained in lateral recumbancy and copius gel was applied on the prepared part. Ultrasonography was performed by using TELEMED CAB by placing 5-10MHz linear transducer on the triceps brachial muscle. The axillary area was then scanned with the transducer orientated in a parasagittal plane, the transducer was glided, rotated or tilted until an optimal short axis (transverse) view of the axillary vessels (axillary vein, axillary artery) was obtained. The axillary artery was identified by its characteristic anechoic pulsatile ultrasound image (Fig. 6). After confirming that blood could not be aspirated and that there was minimal resistance to injection, calculated dose of 0.75% ropivacaine hydrochloride @ 3mg/kg BW was deposited around the artery(Fig. 7).

Fig. 6: Colour Doppler showing needle insertion near axillary artery.

6) **Brachial plexus block in Group F animals:** In Group F, the animals were restrained in lateral recumbancy and the procedure performed for ultrasound scanning was same as in group E. Ultrasonography was performed by using TELEMED CAB with a
5-10MHz linear transducer. The axillary artery was identified by using the color doppler. Calculated dose of 0.75% ropivacaine hydrochloride @ 3mg/kg b.wt. and 2ml (4mg) isoflupredone acetate was deposited around the axillary artery. However addition of 2 ml (4 mg) isoflupredone acetate replaced 2 ml (15 mg) of ropivacaine hydrochloride, thereby reducing the dose of latter by 15 mg.

Fig. 7: Deposition of anesthetic agent around axillary artery in transverse scan.

After the deposition of anesthetic agent the needle was withdrawn slowly, and isopropyl alcohol swab was kept in position for two minutes. The effect of the anesthesia was monitored for 360 minutes and parameters were recorded as under:

7) Assessment of sensory blockade: Sensory blockade of the musculocutaneous, median, radial and ulnar nerves was assessed in the corresponding dermatome areas (Fig. 8). After the completion of the block procedure, sensory onset was considered when there was dull sensation to pin prick along the distribution of any of the above-mentioned nerves. The duration of sensory block was defined as the time interval between the end of anesthetic administration and the complete resolution of anesthesia on all nerves.

Fig. 8: Schematic diagram showing sensory distribution of the major nerves from the brachial plexus and corresponding pinprick points (red asterisk) while evaluating the sensitive effect [21].

Sensory blockade of musculocutaneous, median, radial and ulnar nerves was graded according to three-point numerical rating scale (NRS) using pin prick test:
Grade-0: Sharp pin sensation felt
Grade-1: Analgesia (dull sensation felt)
Grade-2: Anesthesia (no sensation felt)

8) Assessment of motor blockade: Motor blockade was assessed based on the degree of abnormal gait while walking and by observing abnormal clinical signs (Fig.s 9, 10, 11 and 12). The scoring was done on a three-point numerical rating scale (NRS) as follows:
Grade-0: Normal motor function/normal gait while walking and no abnormal sign while standing.
Grade-1: Animal can walk while bearing mild to moderate weight and no abnormal sign while standing.
Grade-2: Complete motor blockade with inability to bear weight.
Onset of motor blockade was considered when there was Grade 1 motor blockade after completion of block procedure. Peak motor block was considered at Grade 2 motor blockade. The duration of motor block was defined as the time interval between the end of local anesthetic administration and the recovery of complete motor function of the forelimb. Motor and sensory blockade and vitals of the animals were noted after every 15 minutes up to 240 minutes and thereafter at 360 minutes of the block. The block was considered as failed when analgesia to pin prick was not elicited at the site of surgical incision even after 30 minutes of drug administration. In all the six groups, the block onset, duration and recovery were monitored by application of neuromuscular stimulator after calibration of the requisite frequency. The onset and duration of sensory block, motor block and complications were noted at 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 165, 180, 210 and 240 minutes and at 360 minutes post induction of nerve blocks.

Fig. 9: Abnormal posture attained by the animal immediately after brachial plexus blockade.

Fig. 10: Flexion of fetlock joint after brachial plexus block.

Fig. 11: Flexion of knee joint after brachial plexus block.
9) **Statistical analysis:** The data was statistically analyzed by Duncan’s Multiple Range Test using software SPSS 20 and results were presented as mean ± SD. Statistical significance was defined as $P < 0.05$ and inferences were drawn.

### III. RESULTS AND DISCUSSION

The results of the present study are detailed under following headings:

**A. Group A (n=6): Brachial plexus blockade under ultrasound guidance**

The site selected was near the caudal border and on the distal aspect of scapular area with the transducer placed on the long head of triceps muscle. The axillary lymph node was identified as landmark under USG scanning (Fig. 13, Fig. 14). A 5 inch 16 gauge needle was directed between thoracic wall and scapula through triceps above the level of axillary lymph node.

![Fig. 13: Sonogram showing brachial plexus and axillary lymph node.](image1)

![Fig. 14: Sonogram showing brachial plexus bathed in local anesthetic solution.](image2)
The first nerve to get anesthetized and desensitized was radial nerve at 5 min (0.50±0.55) post injection (Fig. 21). The animals of this group showed inability to extend the segments distal to the radial nerve with resultant development of dropped elbow and overextension of the limb without abduction of shoulder which marked the initiation of anesthetic effect. The results increased significantly (p<0.05) to 1.67±0.52 at 15 minutes and maximum effects were pronounced at 30 minutes (1.83±0.41) which continued up to 105 minutes and thereafter non-significantly (p>0.05) declined to 0.67±0.52 at 240 minutes. At 360 minutes post-injection, animal resumed normal gait and anesthetic effect was over (Fig. 16).

Blockade of radial nerve followed the anesthetic effect development in ulnar nerve which was desensitized at 10 minutes post injection, however the degree of desensitization was comparatively lesser (0.50±0.55) compared to radial nerve (1.33±0.52) in the same group at 10 minutes. The effect significantly (p<0.05) increased at 15 minutes (1.50±0.55) and reached maximum at 30 minutes post injection (1.83±0.41). The reflexes in anesthetized area remained steady up to 75 minutes post injection. The effect non-significantly (p>0.05) decreased at 90 minutes (1.50±0.84), 120 minutes (1.33±1.03), 150 minutes (1.17±0.98) and 180 minutes (0.67±0.82) and lasted up to 210 minutes (0.50±0.55) post injection and thereafter no effect in the region could be noted (Fig. 17).

Pinpricking revealed desensitization of the median nerve was similar to that of ulnar nerve, it started at 10 minutes (0.17±0.41) and the effect increased significantly (p<0.05) at 15 minutes (1.17±0.75). The effect of anesthesia remained steadily non-significant from 30 minutes to 75 minutes post injection (1.83±0.41); thereafter the anesthetic effect decreased non-significantly (p>0.05) at 90 minutes (1.50±0.84), 105 minutes (1.33±1.03), 150 minutes (1.17±0.98) and the effect lasted up to 210 minutes (0.50±0.55) post injection beyond which no effect was seen in the animals of this group (Fig. 18).

The desensitization of the medial aspect in the distal third of humerus marked the desensitization of musculocutaneous nerve which started at 10 minutes (1.00±0.63). It non-significantly (p>0.05) increased at 15 minutes post injection interval (1.67±0.52). The effect increased to 1.83±0.41 at 30 minutes and continued up to 90 minutes post injection, thereafter, it non-significantly (p>0.05) decreased at 105 and 120 minutes (1.67±0.82 and 1.5±0.84) respectively. The effect further decreased non-significantly (p>0.05) to 1.33±0.82 at 165 minutes, 1.17±0.98 at 180 minutes, 1.00±0.89 at 210 minutes and significantly (p<0.05) to 0.33±0.52 at 240 minutes. The anesthetic effect of musculocutaneous nerve got abolished after 240 minutes post injection (Fig. 19).

The signs of motor block namely, walking while bearing mild weight, abnormal posture, flexion of fetlock joint appeared at the 5th minute post injection (0.67±0.52) in the animals of group A. The effect significantly (p<0.05) was much pronounced clinically at 10 minutes (1.50±0.55) and the effect remained higher from 15 to 120 minutes (1.83±0.41), though non-significant (p>0.05). Thereafter, it non-significantly (p>0.05) decreased to 1.67±0.82, 1.50±0.84 and 1.17±0.98 at 135, 165 and 210 minutes post injection respectively (Fig. 20).

The duration of motor block and anesthesia for radial nerve was 235 minutes followed by anesthesia of musculocutaneous nerve (230 minutes) and ulnar and median nerves (200 minutes). The anesthesia was completely abolished at 360 minutes in this group (Fig. 6). The application of transcutaneous electrical nerve stimulator at the calibrated frequency for 0.9 seconds revealed no response during the phase of anesthesia. However, the animals of this group responded to the stimulation by transcutaneous electrical nerve stimulation at the preset frequency for 0.9 seconds which confirmed the return of reflexes after 360 minutes post injection.

B. Group B (n=6): Brachial plexus blockade under ultrasound guidance
(Combination of 0.75% ropivacaine hydrochloride and injection isoflupredone acetate)

In the animals of group B, among the nerves emerging from the brachial plexus, the radial nerve and musculocutaneous nerves were the first to get desensitized which was revealed by the signs resembling radial paralysis type of symptoms by the animals and desensitization of the medial aspect in the distal third of humerus; however the radial nerve desensitization was more pronounced (Fig. 22). The onset of effect of the radial nerve was noted at 5 minutes post injection (0.83±0.75). The depth of desensitization significantly (p<0.05) increased at 15 minutes post injection (1.83±0.41). Up to 210 minutes post injection remained almost unchanged which followed abrupt and significant (p<0.05) decline (0.83±0.75) at 240 minutes post injection. The anesthetic effect further declined significantly (p<0.05) and mild effect was seen at 360 minutes post injection (0.33±0.52). On comparison of the anesthetic effect at various intervals in the groups A and B, although the radial nerve showed anesthetic effect at 5 minutes post injection in both the groups, however the effect was significantly (p<0.05) more in group B animals than in the animals of group A as the number of animals showing onset of sensory blockade in group B was more than in group A. Similarly, the level of anesthesia was non-significantly (p>0.05) higher in the radial nerve of group B, than group A at maximum hours of observation during the period of study (Fig. 16).

The anesthetic effect of the ulnar nerve was evident at 10 minutes post injection (0.33±0.52) which increased significantly (p<0.05) at 15 minutes (1.00±0.63). After that the effect increased non-significantly (p>0.05) and remained constant from 30 to 165 minutes.
(1.83±0.41) post injection period. This followed a non-significantly (p>0.05) declined trend from 180 minutes (1.50±0.55) to 210 minutes (1.00±0.63) and significantly (p<0.05) declined trend at 240 minutes (0.33±0.52) respectively. The anesthetic effect ended thereafter and at 360 minute post injection the animals were free from the effect of anesthesia as far the ulnar nerve is concerned. On comparative basis the anesthetic effect of ulnar nerve, although followed the same trend as far the duration is concerned yet the degree of desensitization was lower at most of the hours of the observation during the course of study (Fig. 17).

As far the duration of anesthesia is concerned, median nerve desensitization in the animal of group B followed a similar pattern, desensitized at 10 and 15 minutes post injection with mean values of 0.17±0.41 and 0.50±0.55 respectively. The anesthetic effect increased non-significantly (p>0.05) at 20 minutes (1.17±0.75) and 30 minutes (1.50±0.84) post injection period followed by a non-significant (p>0.05) and constant increase at 45 minutes post injection (1.67±0.82) which continued up to 180 minutes. The anesthetic effect significantly (p<0.05) dropped to 0.50±0.55 at 210 minutes and the anesthesia lasted thereafter so that towards the terminal period of observation i.e., 360 minutes post injection, the animal did not reveal any anesthetic effect (Fig. 18).

The muscularcutaneous nerve showed desensitization at 5 minutes (0.17±0.41) and the effect increased significantly (p<0.05) to 0.83±0.75 at 10 minutes and non-significantly (p>0.05) to 1.17±0.75 at 15 minutes respectively. The effect of anesthesia was however maximum from 20 to 165 minutes (1.83±0.41) and thereafter followed a non-significant (p>0.05) decline from 180 to 240 minutes post injection and no effect of anesthesia could be detected at 360 minutes. As far the degree of desensitization at 30 to 90 minutes is concerned both the groups A and B followed the same trends and the degree of depth (1.83±0.41), however the effect was rather less at 240 minutes in the muscularcutaneous nerve of group A (0.33±0.52) than in the animals of group B at the same hour (0.83±0.75) (Fig. 19).

Motor block could not be perceived in the animals of group B at 5 minutes post injection. It appeared at 10 minutes (1.17±0.41), significantly (p<0.05) increased at 15 minutes post injection (1.83±0.41) and remained steady up to 240 minutes. The depth followed a significantly (p<0.05) declining trend and decreased to 0.33±0.52 at 360 minutes post injections, so much so that the motor block did not abolish by 360 minute of observation. On comparing the duration of sensory and motor blockade in group B animals, the duration of sensory blockade was more and radial nerve was desensitized for maximum duration (355 minutes) followed by muscularcutaneous nerve (235 minutes), ulnar nerve (230 minutes) and finally the median nerve (200 minutes). However, the duration of motor effect was 350 minutes. As far the comparative degree of the anesthetic effect in the motor block in group A and B goes, both the groups showed similar depth and the pattern from 15 to 120 minutes post injection (Fig. 20).

C. Group C (n=6): Brachial plexus blockade without ultrasound using anatomical landmarks

The brachial plexus block was undertaken in conventional manner at the site 10 centimeter cranial to acromian of scapula (Plate 15).

The onset of anesthesia initiated with radial nerve desensitization at 15 minute post injection (0.67±0.82) (Fig. 23). The effect non-significantly (p>0.05) increased to 1.17±0.75 at 20 minutes and 1.50±0.55 at 30 minutes. It non-significantly (p>0.05) increased at 45 minutes and remained constant up to 75 minutes (1.67±0.52). This followed a non-significant (p>0.05) decline in anesthetic effect to 1.50±0.84, 1.17±0.98 and 0.83±0.75 at 90, 120 and 180 minutes respectively. The effect remained sluggish thereafter however, it could not be detected at all at 240 minutes and onwards (Fig. 16). When we compared induction of anesthesia between groups A, B and C, the animals of group C showed induction later (at minute 15) than was shown by group A and group B animals.
Musculocutaneous nerve desensitization started at 10 minutes post injection (0.33±0.52), it non-significantly (p>0.05) lower in this group at 20 minutes and 30 minutes (1.17±0.09 and 1.32±0.03 respectively) compared to the depth of anesthesia of group A at 20 and 30 minutes (1.67±0.52 and 1.83±0.41 respectively) and group B at 20 and 30 minutes (1.83±0.41 and 1.83±0.41). The duration of anesthesia was significantly lower in this group (195 minutes) and the anesthetic effect was abolished at 210 minutes onwards; however the anesthetic effect lasted up to 240 minutes and 360 minutes in animals of groups A and B respectively.

In the animals of group C the ulnar and median nerve blockade was observed at 15 minutes following injection of anesthesia. In both of these nerves, the effect non-significantly (p>0.05) increased at 45 minutes and reached maximum value of 1.50±0.84 for ulnar and 1.33±0.82 for median nerves at this interval of duration of study. Thereafter it non-significantly (p>0.05) declined at 75 minutes (1.33±1.03) and at 90 minutes (1.17±0.98) for ulnar and median nerves respectively. The anesthesia lasted till 210 minutes for both the nerves in this group and thereafter at no interval of observation the anesthetic effect was detected on comparative basis. The duration of anesthesia for ulnar and median nerve lasted for 195 minutes only which was less than the duration of anesthesia observed for the animals of group A (200 minutes for both ulnar and median nerves) and B (230 minutes for ulnar and 200 minutes for median nerve) respectively (Fig. 17, Fig. 18).

Musculocutaneous nerve desensitization started at 15 minute (0.17±0.41) and the effect was lower than in the animals of all other groups at this interval during the course of study. The peak effect of musculocutaneous nerve desensitization was recorded at 45 minutes post injection (1.67±0.52). The effect non-significantly (p>0.05) decreased to 1.50±0.84 at 75 minutes and 1.33±1.03 at 120 minutes; and then further non-significantly (p>0.05) decreased to 1.17±0.98, 0.83±0.75, 0.50±0.55, 0.17±0.41 at 135, 180, 210 and 240 minutes respectively. No anesthetic effect of musculocutaneous nerve was recorded 360 minutes of anesthetic injection in this group (Fig. 19).

The signs of motor block appeared at 15 minute with the mean value of 0.83±0.75. It showed peak effect at 45 to 105 minute (1.83±0.41); thereafter the effect showed a non-significantly (p>0.05) declined trend with the mean values 1.50±0.84, 1.33±1.03, 1.00±0.89 and 0.83±0.75 at 120, 150, 180 and 210 minutes respectively. The effect of motor block could not be detected up to 240 minutes and beyond of anesthetic administration. The duration of anesthesia was lower (195 minutes) in this group than groups A and B (235 and 350 minutes) respectively (Fig. 20).

D. Group D (n=6): Brachial plexus blockade without ultrasound using anatomical landmarks

(Combination of 0.75% ropivacaine hydrochloride and injection isoflupredone acetate) In the animals of this group, motor blockade was pronounced earlier than sensory blockade (Fig. 24). The onset of desensitization of the radial nerve was non-significantly (p>0.05) delayed (at minute 10) compared to the animals of group B (at minute 5) in which the same combination was used and the onset of radial nerve desensitization was recorded at 10 minute and lasted till 240 minutes. The anesthetic effect for the radial nerve in this group revealed a declining pattern from 150 minute onward first non-significantly (p>0.05) up to 180 minutes and then significantly (p<0.05) up to 240 minutes to the extent that no desensitization was detected at any hour beyond that period so much so that the animal showed normal reflexes at termination hour of study i.e., 360 minutes post injection. Comparing the duration of anesthesia of radial nerve between this group and group B animals, the duration was shorter in animals of this group i.e., group D (230 minutes) compared to 355 minutes in the animals of group B (Fig. 16).

On a comparative basis table 2 and table 3 reveals that the desensitization of the ulnar and median nerve as marked by loss of sensation in the areas supplied followed a similar pattern in both the nerves in group D animals. Indication of desensitization occurred at 15 minutes and attained peak effect at 60 minutes (mean value 1.83±0.41) in both the nerves in this group. The effect attained the same level up to 165 minutes followed by a non-significant (p>0.05) decline at 180 minutes to 1.33±0.82 for ulnar and 1.17±0.41 for median nerve respectively. In both the nerves the effect significantly (p<0.05) decreased up to 210 minutes. In ulnar nerve, anesthesia effect disappeared after 240 minutes i.e., 0.50±0.55 and in median nerve the effect disappeared after 210 minutes i.e., 0.17±0.41. Comparing with the radial nerve blockade of the animals in the same group, the onset of anesthesia for radial nerve was much earlier (at minute 10) than the ulnar nerve and median nerve and the duration of anesthesia of ulnar nerve (225 minutes) and median nerve (195 minutes) was shorter as compared to radial nerve blockade in the animals of group D (230 minutes) (Fig. 17, Fig. 18).

Musculocutaneous nerve desensitization started at 10 minutes post injection (0.33±0.52), it non-significantly (p>0.05) increased from 15 minutes (0.67±0.52) to 45 minutes (1.83±0.41) thereafter it remained steady up to 165 minutes post injection.
desensitization non-significantly (p>0.05) decreased to 1.50±0.84 at 180 minutes, 1.33±0.82 at 210 minutes and significantly (p<0.05) to 0.33±0.52 at 240 minutes. After 240 minutes, the anesthetic effect was totally over (Fig. 19).

Motor blockade as revealed by the loss of weight bearing on the limb of which brachial plexus was blocked was appreciated at 5 minutes post injection (0.17±0.41), which significantly (p<0.05) increased from 15-20 minutes then non-significantly (p>0.05) reached maximum at 30 minutes and continued for 180 minutes with a mean value (1.83±0.41) beyond which the motor block effect got reduced non-significantly (p>0.05) at 210 minutes (1.67±0.82) and then significantly (p<0.05) towards the terminal periods of observation with the mean value of 0.67±0.52 at 240 minutes post injection. As far the duration of motor block in the animals of group D (235 minutes) is concerned, it is comparable to the animals of group A, E and F however, it is lesser than the animals of groups B (350 minutes) but greater than group C (195 minutes) (Fig. 20).

E. Group E (n=6): Ultrasound guided perivascular brachial plexus blockade

Fig. 25 compares the variation in sensory and motor responses at different time intervals in animals of group E. The onset of sensory analgesia was observed at 5 minutes post injection, remarkably noted by the dropped elbow like signs indicating blockade of radial nerve. The levels of desensitization of radial nerve were rather mild (0.50±0.55) like groups A and F. When compared with the radial nerve desensitization of groups A, B and F, the effect deepened with the passage of time and continued to remain constant from 15 to 90 minutes (1.83±0.41); the anesthetic effect started decreasing non-significantly (p>0.05) at 105 minutes (1.67±0.82) and at 150 minutes (1.50±0.84). The radial nerve was free from the effect of the anesthetic effect after 240 minutes (Fig. 16).

In this group of animals blockade of ulnar nerve followed a similar pattern with the onset with mild effect at 5 minutes (0.17±0.41); peak effect between 30 to 75 minutes (1.83±0.41). The effect decreased non significantly (p>0.05) to 1.67±0.82 at 90 minutes, 1.50±0.84 at 135 minutes, 1.17±0.75 at 165 minutes, 1.00±0.63 at 180 minutes and the effect lasted at 240 minute (0.67±0.52) (Fig. 17).

Sensory anesthesia in median nerve of group E animals showed onset at 10 minutes post injection (0.83±0.41). The effect non-significantly (p>0.05) varied at 15 and 20 minutes post injection with mean values of 1.33±0.82 and 1.67±0.82 respectively. The peak effect was observed at 30 minutes (1.83±0.41) which lasted till 90 minutes, after that the effect got decreased non-significantly (p>0.05) and lasted for about 210 minutes and no effect could be detected beyond that hour of observation (Fig. 18).

animals of group E, the musculocutaneous nerve desensitization showed effect with mean value 0.50±0.55 at 5 minutes post injection. The depth remained highest only between 20 to 75 minutes (1.83±0.41) and the anesthesia lasted up to 240 minutes. When compared with other groups, the musculocutaneous nerve remained desensitized for a longer duration (235 minutes) compared the animals of Group A, D (230 minutes) and C (225 minutes) but comparable to group B (Fig. 19).

The onset of motor blockade initiated in the animals of group E at 5 minutes which significantly (p<0.05) increased at 10 minutes and continued increasing non-significantly (p>0.05) till 165 minutes (1.83±0.41); the anesthetic effect slowly decreased non-significantly (p>0.05) and showed a mean value 1.50±0.84 and 1.00±0.63 at 180 and 210 minutes respectively. The effect lasted up to 240 minutes. The onset of signs of motor block in this group was earlier compared with the animals of group B, C and D (Fig. 20).

F. Group F (n=6): Ultrasound guided perivascular brachial plexus block (Combination of 0.75% ropivacaine hydrochloride and injection isoflupredone acetate)

Ultrasound guided perivascular blockade in the animals of Group F revealed the onset of sensory and motor blockade both at 5 minutes (Fig. 26). Onset of radial nerve anesthesia was at 5 minutes post injection with the mean value 0.50±0.55, the effect non-significantly (p>0.05) increased up to 20 minutes and continued to remain non-significantly (p>0.05) higher at 30 to 120 minutes (1.83±0.41). The effect was over at 240 minutes. The duration of anesthetic effect was similar to the animals of groups A and E. No anesthetic effect of radial nerve was noted beyond 240 minutes (Fig. 16).

Similar trend was followed by the anesthetic effect on ulnar and median nerve with the onset at 5 minutes and duration of anesthesia up to 235 minutes with the peak effect at 45 minutes (ulnar) and 20 minutes (median). The onset of anesthesia for ulnar and median nerve was non-significantly (p>0.05) earlier when compared to the animals of groups C and D (Fig. 17, Fig. 18).

The musculocutaneous nerve was desensitized at 5 minute with a mean value 0.33±0.52. The peak effect of the anesthesia was recorded between 45 and 75 minutes (1.83±0.41). After 210 minutes, the effect was non-significantly (p>0.05) decreased up to 360 minutes (0.33±0.52). When compared with the onset and duration of anesthesia, with the animals of other groups the mean value 0.33±0.52 at 5 minutes was only recorded in this group and with the mean value 0.50±0.55 in the animals of group E, 0.17±0.41 in
the animals of group B. The duration of anesthetic effect was more (355 minutes) in animals of this group compared to the animals of groups A, D (230 minutes), B, E (235 minutes) and C (225 minutes) (Fig. 19).

In the animals of Group F, signs of motor blockade were marked with a mean value 0.67±0.52 at 5 minutes post injection. The effect increased significantly (p<0.05) at 10 minutes (1.67±0.52) and the peak motor block was recorded from 20-180 minutes (1.83±0.41). The motor block effect continued till 240 minutes post injection with a mean value of 1.67±0.82. Thereafter no motor blockade was detected and animals walked with normal weight bearing (Fig. 20).

**Fig. 16:** Showing score of anesthetic effect for radial nerve in different groups at different observational intervals.

**Fig. 17:** Showing score of anesthetic effect for ulnar nerve in different groups at different observational intervals.
Fig. 18: Showing score of anesthetic effect for median nerve in different groups at different observational intervals.

Fig. 19: Showing score of anesthetic effect for musculocutaneous nerve in different groups at different observational intervals.

Fig. 20: Showing score of anesthetic effect for motor blockade in different groups at different observational intervals.
Fig. 21: Desensitization pattern of different nerves and variation in motor response at different time intervals in animals of group A.

Fig. 22: Desensitization pattern of different nerves and variation in motor response at different time intervals in animals of group B.

Fig. 23: Desensitization pattern of different nerves and variation in motor response at different time intervals in animals of group C.
Fig. 24: Desensitization pattern of different nerves and variation in motor response at different time intervals in animals of group D.

Fig. 25: Desensitization pattern of different nerves and variation in motor response at different time intervals in animals of group E.

Fig. 26: Desensitization pattern of different nerves and variation in motor response at different time intervals in animals of group F.
In this study 0.75% ropivacaine hydrochloride was used at the dose rate of 3mg per kg body weight based on the pilot trials conducted prior to study. By incorporating a corticosteroid isoflupredone, we were able to further decrease the dose of anesthesia as well as increase the duration of postoperative analgesia.

The overall results of the present study led to the following conclusions:

A. The identified ultra scanning window allowed a feasible and accurate access to brachial plexus in the sheep and significantly shortened the onset and prolonged the block duration thereby decreasing the dose of anesthesia when compared with traditional brachial plexus block.

B. Perivascular brachial plexus blockade using color doppler significantly shortened the block onset time and prolonged the block duration when compared with the perineural brachial plexus blockade using ultrasound guidance.

C. In case of long acting anesthesia by incorporating 2ml of isoflupredone, we have been in a position to reduce the dose of ropivacaine by 15mg per animal. However, it has produced early onset and prolonged duration of anesthesia. Therefore, it could be concluded that this anesthetic regimen when used in brachial plexus block in sheep can form a very good anesthesia for forelimb surgery of four hours duration. It can be concluded that, ultrasound guidance enables safe, reliable and successful nerve block in a short time and prolongs the block duration when compared with conventional brachial plexus block. This technique is accurate, feasible, reproducible, safe and not time-consuming. All animals demonstrated a complete recovery, and no sequelae were recorded. Moreover, absence of any apparent sign of cardiac or central nervous system toxicities may be attributed to the efficacy of brachial plexus block. This anesthesia protocol is therefore recommended for long duration surgeries of the forelimb in sheep. Clinical studies are needed to definitively demonstrate its clinical benefits.

REFERENCES


