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Ultrasound-guided femoral and obturator nerves block in the psoas compartment in dogs: anatomical and clinical study

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Abstract

Objective To describe and evaluate the clinical usefulness, of an ultrasound-guided approach to the femoral (FN) and obturator nerves (ON), in the iliopsoas compartment.

Study design Anatomical research and randomized, prospective, blinded clinical study.

Animals Six dogs cadavers, for the anatomic phase, and twenty client-owned dogs anaesthetized for elective tibial plateau levelling osteotomy (TPLO) surgery, for the clinical phase.

Methods

The study was divided in two phases. Phase-1: gross anatomy of the lumbar plexus was studied. Ultrasound-guided (US-guided) approach, involving simultaneously femoral nerve (FN) and obturator nerve (ON) within the iliopsoas muscle (IPM), was designed. The accuracy was established by the injection of 0.1 mL kg^{-1} of new methylene blue solution around the nerves and its spread was evaluated by dissection and cryosections. Phase-2: twenty client-owned dogs anaesthetized for TPLO surgery randomly assigned to 2 treatment groups were enrolled: Ten dogs received ropivacaine 0.3% (R3 Group) and ten dogs received ropivacaine 0.5% (R5 Group) for both groups a volume of 0.1 mL kg^{-1}

was injected for each target point, FN-ON and sciatic nerve (ScN). Postoperative pain was evaluated using a Glasgow composite pain scale until the first rescue analgesia requirement (methadone 0.2 mg kg^{-1} IV).

Results

Phase1: Individual variability was found regarding the localization of the target US image. The needle was inserted using an in-plane technique, the nerves were stained >4 cm in 6/6 cases. No abdominal and epidural spread were registered. *Phase2:* US-guided FN-ON block resulted in an adequate intra-operative analgesia in 17/20 dogs postponing the first rescue analgesia request at least 5 hours after the surgery.

Conclusion and clinical relevance

Performing US-guided FN-ON block in iliopsoas using an in plane technique may provide adequate perioperative analgesia capable to minimise response to the surgical stimulation during TPLO surgery in dogs.

Keywords: Ultrasound, loco regional anaesthesia, femoral nerve, obturator nerve, dog.

Introduction

Regional anaesthesia is widely used in human medicine in order to avoid general anaesthesia and associated risks, to improve intra-operative analgesia and to increase post-operatively patient comfort (Roberts 2006).

Introduction of ultrasound technology to perform peripheral nerve blocks (PNB) improved the accuracy of localization of the target nerves (Mogicato et al. 2015) and the exact placement and spread of LA (local anaesthetic), additionally the needle can be manipulated under real-time (Marhofer et al. 2005). If the LA is administered accurately, a low dose can be used, reducing the likelihood of systemic toxicity (Liu et al. 2014). This could be important in patients with renal or hepatic insufficiency, elderly patients or when more than one simultaneous local anaesthesia is required. Accurate placement of LA has a faster onset, a more predictable duration and overall improvement of block quality (Marhofer et al. 1998; Marhofer et al. 2005).

Successful PNB technique requires excellent anatomical knowledge to establish anatomical landmarks, puncture sites, direction and depth of needle insertion and to avoid relevant structures (Gurney & Lecce 2014).

Regarding the sensory innervation of the pelvic limb this is provided by lumbosacral plexus (L3–S2) which can be divided in two parts: lumbar plexus and sacral plexus. Lumbar plexus includes: lateral femoral cutaneous nerve (LcFN) formed primarily by L4, although there are connections with L3-L5; femoral nerve (FN) formed primarily by L5 and

73 portions of L4-L6; obturator nerve (ON), that arises from L4-L5-L6, and
74 sciatic nerve (ScN) formed by L6-L7-S1-occasional contribution from S2
75 (Evans & de Lahunta 2013). Stifle sensory innervation is provided by
76 posterior articular nerve (PAN) which is a branch of tibial nerve, lateral
77 articular nerve (LAN) which arises from the peroneal nerve and the
78 medial articular nerve (MAN) which mainly originates from saphenous
79 nerve but contains also branches of the femoral and obturator nerves in
80 up to 50% of dogs (O'Connor & Woodbury 1982). It has been proposed
81 that, an unblocked ON in humans could account for some cases of failure
82 of pelvic limb block (Sakura et al. 2010) and improved surgical
83 analgesia is achieved when specific ON blockade is added to FN and ScN
84 (McNamee et al. 2002; Macalou et al. 2004; Sakura et al. 2010). The
85 anatomical evidence (O'Connor & Woodbury 1982) suggests that the ON
86 is also relevant for dogs undergoing stifle surgery.
87 One of the most common orthopaedic procedures concerning pelvic limb
88 is tibial plateau levelling osteotomy (TPLO), which involves a medial
89 approach to the stifle and proximal tibia. Graff and colleagues (2014)
90 evaluated FN and ON as the two main nerves components of surgery area
91 innervation during TPLO and, in order to obtain the coverage of both
92 nerves, they suggested to approach to the lumbar plexus at L7 level. The
93 US-guided approach for FN is still strongly debated and different
94 approaches with various landmarks have been developed until now.
95 Briefly, the techniques developed for FN can be summarized by the two
96 anatomical compartments involved: inguinal compartment approaches

(Campoy et al. 2010; Costa et al. 2009; Echeverry et al. 2010; Shilo et al. 2010) and iliopsoas muscle compartment approaches (Mahler 2012; Echeverry et al. 2012a; Graff et al. 2015; Monticelli et al. 2015). Until now only one of these approaches proposed involves the ON, too. The success of the FN US-guided block has been evaluated as not optimal (50.0-62.5%) when an inguinal compartment approach was used in fact it has not been identified in 4/8 (50%) of the pelvic limbs examined in vivo by Echeverry and colleagues in 2010. Us-guided localization of FN during an inguinal approach resulted difficult and the peripheral nerve stimulator was essential to prove the nerve location by observing a positive evoked muscular response (Echeverry et al. 2010).

In our knowledge there is still a lack about the US-guided FN and ON block performed within iliopsoas muscle and no clinical study has been performed until now, in order to evaluate the US technique proposed. The aim of the present study was to evaluate a clinical usefulness of a targeted acoustic window obtained in the IPM compartment which involved simultaneously FN and ON. In the present study FN-ON block was performed using two different concentrations of ropivacaine and the efficacy, safety and duration were evaluated intra and postoperatively in dogs undergoing TPLO surgery.

Materials and methods

The study was conducted in compliance with the European Welfare Act and with the approval of the local Ethical Committee. Prior written informed owner's consent allowing the collection and use of data for a research purpose was obtained. The study was divided in two phases. The second phase was designed considering the results of the first phase.

Phase-1 anatomical study. This phase was performed in order to define the approach to the nerves. Six mongrel dogs, weighting between 10-40 kg, euthanized for reasons unrelated to this project have been used for this purpose. The cadavers were immediately frozen after euthanasia, except for one, and before the procedure were thawed at ambient temperature. Two of the six cadavers (four pelvic limbs) were used for the gross anatomy study and dissections were performed in order to expose femoral and obturator nerves from vertebral rise until their exit form the psoas compartment. With the dog laying in lateral recumbency and pelvic limb in a neutral position, the dissection was performed removing the superficial fascia and muscle until the lumbar plexus with FN and ON have been exposed. On the basis of this anatomical observation landmarks and the way to approach to FN and ON were registered. Other three cadavers (six pelvic limbs) were used to research an US window called as "target" which allowed to detect simultaneously both nerves within psoas compartment. Afterwards FN and ON US-guided approach was designed. With the dog in lateral recumbency, a high frequency 12 MHz, linear array transducer (Venue 40; GE Medical

144 Systems, China) was placed perpendicularly to the spine and under the
145 cranial margin of ileus wing in the area between L6-L7 and tangent to
146 the iliopsoas muscle (IPM). Furthermore with a movement starting from
147 cranial to caudal, the belly of IPM was inspected and identified as
148 described in a previous study (Cannon & Puchalski 2008) as a
149 hypoechoic structure with an internal pattern of scattering echoes.
150 Afterward, a simultaneously US image of both FN and ON was
151 researched in the IPM belly, the target nerves were reached using an in-
152 plane technique and a volume of 0.1 mL kg^{-1} of new methylene blue
153 solution was injected around the nerves producing a "halo" of liquid
154 (commonly referred as the donut sign, Marhofer et al. 2005).
155 Subsequently the iliopsoas area was dissected in order to confirm the
156 involvement of both nerves and the accuracy of the injection was
157 evaluated by determining the dyeing of the nerve, which was evaluated
158 effective with staining ≥ 2 cm (Raymond 1992). In order to compare the
159 US images with the anatomical structures, a fresh cadaver weighted 10
160 kg has been frozen at -40° C for 1 week immediately after the injection
161 of new methylene blue around the nerves with the same procedure
162 described above. Subsequently using a highspeed bandsaw at the
163 thickness of 2 cm transverse cryosections of the area between L4 and
164 sacrum were made.
165
166 **Phase-2 clinical study.** Twenty dogs that underwent elective unilateral
167 TPLO were enrolled for this phase. Before starting the procedure,

physical examination, haematological and serum biochemical analysis, were carried out. Only dogs ranked as American Society of Anaesthesiologist's classification system (ASA) physical status 1 and 2 were included in the study. For the animals that received NSAIDs in the period prior to the surgery, treatment was discontinued 24 hours before surgery. Food was withheld from dogs for 12/18 hours prior to surgery but free access to water was guaranteed until two hours before the premedication. Written informed consent was obtained from the owners before the participation of any dog in the study. Exclusion criteria consisted of ASA physical status ≥ 3, skin infections, intractable behaviour, neurological or neuromuscular disease and owner's refusal.

All the dogs were premedicated intramuscularly (IM) with acepromazine (0.01 mg kg^{-1}) (Prequillan; Fatro, Italy) and methadone (0.1 mg kg^{-1}) (Semfortan; Dechra, Italy) and after 20 minutes a catheter (20 gauge; Delta-Ven 1; Delta Med, Italy) was aseptically placed in a cephalic vein, and fluid therapy with lactate ringer solution at 5-10 ml kg^{-1} h^{-1} was started. Afterward a bolus of fentanyl 5 μg kg^{-1} (Fentanest; Actavis, Italy) was administered intravenously (IV) and anaesthesia was induced with propofol (Proposure; Merial, Italy) IV titrated to effect. Induction was considered complete when orotracheal intubation was feasible. All dogs were connected to a rebreathing circuit and anaesthesia was maintained with isoflurane (IsofluraneVet; Merial, Italy) with a fraction of inspired oxygen (FiO_2) between 60%-70%. A catheter (22 or 20

gauge) was placed in the dorsal pedal artery to monitor blood pressure by measurements of invasive arterial pressure.

In order to provide adequate intraoperative and postoperative analgesia an US-guided PNB for FN-ON and ScN was performed in all patients. The ScN block was achieved with the mid-femoral approach (Campoy et al. 2010) and the FN-ON block was executed using the technique defined in the phase-1 of the present study.

Dogs were randomly assigned via a computer-generated random numbers list (https://www.random.org/sequences) to one of the two treatment groups. In the R3 group, nerve blocks were performed with ropivacaine 0.3% (Naropina; AstraZeneca, Italy), while in the R5 group was used ropivacaine 0.5%. The two groups received a volume of 0.1 mL kg^{-1} for FN-ON block and for ScN block. During anaesthesia heart rate (HR), respiratory rate (f_R), systolic, mean and diastolic invasive arterial blood pressure ($SAP_{inv,}$ MAP_{inv}, DAP_{inv}) end-tidal carbon dioxide pressure ($P_E'CO_2$) side stream system, end-tidal isoflurane concentration ($F_E'Iso$) pitot based, peripheral oxygen saturation (SpO_2) were monitored continuously and recorded every five minutes and during specific surgical time points (Table 1) until the end of the procedure. A multiparametrical monitor ($S5^{TM}$ Compact Anaesthesia Monitor; Datex Ohmeda, Italy) was used to measure the parameters described above. For the SAP_{inv}, DAP_{inv} and MAP_{inv} a pre-calibrated transducer (Transpac® IV Disposable Pressure Transducer; ICU Medical Europe, Italy), placed at the right atrium level and zeroed to the atmospheric pressure, was

used. All parameters, recorded five minutes before the start of the surgery, were registered as T0 values. The same surgeon performed all the surgeries using the technique proposed by Slocum and colleagues (1998). During the surgery the anaesthetic plane was maintained decreasing the F_E'Iso of 0.1 % – 0.2 % each five minutes in order to achieve the minimal concentration necessary to prevent movements and maintain the patient on a light general anaesthesia plane; in case of movements an IV bolus of propofol at the dose of 1 mg kg^{-1} was administered and the F_E'Iso increased of 0.1%-0.2%. Increase of more than 20% of HR, f_R and MAP_{inv} was considered as nociception and rescue analgesia with a bolus of 1 µg kg^{-1} of fentanyl IV was administered. If more than two boluses were required to re-establish the cardiorespiratory parameters, a variable rate infusion of fentanyl (2.5-10 µg kg^{-1} hr^{-1}) was started. In case of hypovolaemia, a bolus of 2 mL kg^{-1} of colloids (Gelplex; Fresenius Kabi, Italy) was administered IV. In case of hypotension, dopamine (Dopamina Hospira; Hospira, Italy), at the range dose of 2-5 µg kg^{-1} hr^{-1} IV, was administered. In case of bradycardia, atropine (Atropina Solfato; ATI, Italy) 0.02 mg kg^{-1} IV was administered. Intermitted positive pressure ventilation was performed in case of $P_E'CO_2$ was more than 45 mmHg.

At the end of surgery and after extubation, carprofen at the dose of 2 mg kg^{-1} (Rimadyl; Pfizer, Italy) was administered IV. Post-operative pain assessment started when dogs were aware of their surrounding, using the Glasgow composite pain scale short form (Reid et al. 2007), each hour

until the first injection of rescue analgesia. Rescue analgesia was provided by methadone 0.2 mg kg^{-1} IV when the patients reached a score of 5/20. Intraoperative monitoring and pain assessment were performed by a clinician trained in the use of the pain scales, blinded about the belonging group of the dogs. Time elapsed from PNB and first rescue analgesia was recorded.

Statistical analysis

Data were analysed for normal distribution with the D'Agostino & Pearson test with a statistical software (Prism 6-2; GraphPad Prism Inc., USA). Parametrical data were expressed as mean and standard deviation while no parametrical data with median and range. For the parametrical data a one way ANOVA test for repeated measures with a Bonferroni post hoc test, was used to assess differences inside each group over the time. Differences between groups were investigated before with two way ANOVA and in case of significance a student T-test between all time points of the two groups was performed. For non-parametrical data (pain score and n. of fentanyl boluses) a Kruskal-Wallis test was done to evaluate differences over the time inside each group, whereas a Mann-Whitney test was used to compare the two groups. Values of $p<0.05$ were considered significant.

Results

263

264 **Phase-1 anatomical study**. The gross anatomical study was performed in
265 two cadavers weighted 20 and 28 kg respectively. Dissection involved
266 both sides at the level of lumbar and proximal lateral abdominal region.
267 After removing of superficial epaxial muscles, thoracolumbar fascia and
268 quadratus lumbar muscle, iliopsoas minor an iliopsoas major were
269 exposed. From the ventral branches of $L4^{th}$ $L5^{th}$ as described in a
270 previous study (Echeverry et al. 2012a; Portela et al. 2013) the nerves
271 ran caudally into the psoas compartment. FN rose at the level of $L5^{th}$ and
272 ran caudally within the IPM while ON emerged from the $L4^{th}$ and ran in
273 to caudomedial portion of IPM. At the level of the $L7^{th}$-$S1^{th}$ some other
274 branches reached ON. After that ON moved away from the FN and
275 entered into the pelvis canal while FN escaped from IPM and became,
276 with the superficial branch, saphenous nerve, while with the deeper
277 branch continued as FN (Fig. 1).
278 In the other 3 cadavers a US image of FN and ON was obtained
279 positioning the dog in lateral recumbency with the interested limb
280 uppermost. Drawing two imaginary lines, the first tangent to the iliac
281 crest and the second tangent to tuber coxae (Fig. 2A), an acute angle of
282 about 30° was created. Using a linear US-probe positioned inside of the
283 ventral angle obtained by the two straight lines mentioned before the
284 visualization of the IPM was obtained by rotating the probe around its
285 short axis of 30-50° (Fig. 2B). With a slightly medial pressure movement
286 of the probe US-imagine of the FN was achieved as a rounded

hypoechoic hole in the middle of IPM. From the US-imagine of FN the US probe was tilted caudally in order to detect the target image where both FN and ON were simultaneously identified as two adjacent hypoechoic structures (Fig. 3). In all cadavers US-guided target image was correctly identified (6/6). In 6/6 cases target US image was detected between the body of $L6^{th}$ vertebra and the transvers process of $L7^{th}$ vertebra. After the visualisation of the nerves a needle was introduced close to the transducer from dorsal to ventral direction using an in-plane technique. In all of the cases it was possible to follow the needle and its advancement (Fig. 3). After an injection of 0.1 ml kg^{-1} of new methylene blue, the distribution around the nerves was observed in real time by ultrasonography. Anatomical dissection showed that the entire circumferences of the FN and ON were stained for a length greater than 2 cm in 6/6 cases. No evidence of dye spread was seen on the epidural or paravertebral space and no traces of dye were detected in the abdominal cavity. The transverse cryosections of the area between $L4^{th}$ and sacrum confirmed the position and path of the nerves within IPM detected with the gross anatomical and ultrasound study; caudally to the target point the obturator nerve appears no more close to the FN, but it is visible between the FN and the ScN (Fig. 4A-B). The spreading of the new methylene blue throughout the IPM was from $L5^{th}$ up over $L7^{th}$ with a dyeing of femoral nerve (Fig. 5A) and its branches until it exited from the IPM (Fig. 5B)

311 **Phase-2. Clinical study.** All dogs completed the study without any
312 major complications. No adverse events were observed during the
313 recovery and no signs of neurological disorders as a result of a nerve
314 injury were detected.
315 Population data resulted homogenous in terms of age and weight. The
316 mean and standard deviation (mean ± SD) of age and weight in the R3
317 group was 44 ± 31 months and 32.1 ± 12 kg and in the R5 group 53 ± 36
318 months and 30 ± 7.5 kg, respectively.
319 The propofol dose used for induction was not statistical different
320 between the two groups: R3 group 2.143 ± 0.508 mg kg^{-1} and 1.833 ±
321 0.477 mg kg^{-1} for R5 group. Regarding F_E'Iso values, no statistical
322 differences were detected between and inside both groups (Fig. 6 C);
323 with a F_E'Iso mean values lower than minimum alveolar concentration of
324 isoflurane (ISO_{MAC}) reported in literature for dog (Steffey et al. 2015).
325 The time elapsed between the execution of PNB and the beginning of the
326 surgery did not show significant difference between the 2 groups: R3
327 group 67.00 ± 3.26 minutes and R5 56.67 ± 7.40 minutes. No statistical
328 difference was detected between two groups regarding surgery time (T2-
329 T12): R3 group 152.8 ± 12.39 minutes and R5 group 171.0 ± 10.61
330 minutes. No statistical difference was detected in elapsed time between
331 PNB execution and the end of the surgery: R3 group 213.9 ± 10.92
332 minutes and 232.0 ± 11.31 minutes for the R5 group.

The parameters collected during the surgery time (HR, f_R, SAP_{inv}, MAP_{inv}, DAP_{inv}, F_E'Iso, P_E'CO_2) did not show any statistical differences between groups.

Statistical differences were detected for of each group regarding the following parameters: The MAP_{inv} values were significant higher compared to T_0 at time T_3, T_4 for the R3 group and at time T_{10}, T_{11}, T_{12} for the R5 group (Fig. 6 B). The SAP_{inv} values were significant higher in comparison to T_0 at T_4, T_8 for the R3 group and at T_7 and T_{12} for R5 group (Fig. 6 C). The DAP_{inv} values were significant higher compared to T_0 at the time T_3 for the R3 group and at T_5, T_6, T_7, T_{10}, T_{11} for R5 group (Fig. 6 D).

During the surgery 7/10 dogs for both groups required fentanyl as rescue analgesia; median and range dose were achieved and recorded as following: R3 group median dose of 2 μg kg^{-1} (0-3) while for R5 group median dose of 1.5 μg kg^{-1} (0-3). In particular for R3 group, 2/7 dogs required one bolus of 1 μg kg^{-1} to return to the base values, while 4/7 dogs received two boluses of 1 μg kg^{-1} for the same propose. In R3 group 1/7 dogs fentanyl a continuous infusion was carried out at the rate of 2 μg kg^{-1} hr^{-1} to return and maintain baseline values. In R5 group 2/7 dogs received one bolus of 1 μg kg^{-1} and 2/7 dogs two bolus of 1 μg kg^{-1}; in this group 3/7 dogs required fentanyl as continuous infusion at the rate dose ranging from 1 to 3.5 μg kg^{-1} hr^{-1}.

Incidence of intraoperative hypotension, administration of vasopressor and anticholinergic drugs was registered and reported in (Table 2).

Concerning pain evaluation during the postoperative period for the R5 group no significant differences were detected between the first hour after the end of surgery and the subsequent time points, conversely in R3 group a significant increase of the pain score was detected between the first (T_1) and the fifth (T_5) after the end of surgery (Fig. 8). Time elapsed from PNB performing and the first rescue analgesia administration for each group did not show any significant difference. It was 550.9 ± 58.52 minutes after the end of the surgery for R3 group, while for the R5 group first rescue analgesia was necessary at 664.2 ± 151.4 minutes from the end of the surgery (p=0.06).

The pain scores obtained showed a statistical difference between the two groups at T_3, T_4, T_5 (p=0.019) with lower values in the R5 group compared to R3 group.

Discussion

This study demonstrated the clinical usefulness of an US-guided approach to the two mains component of the lumbar plexus, FN-ON, performed in the IPM using 0.1 mL kg^{-1} of ropivacaine. The FN-ON block, combined with a US-guided ScN block, in dogs, undergoing general anaesthesia during TPLO, provided sufficient analgesia, in order to minimise the nociceptive stimulus during the surgery and post-operatively too. Two different concentrations of ropivacaine 0.3% and

0.5% were studied resulting in a whole duration of the PNB respectively of about 9 and 11 hours and for R3 and R5 groups, respectively.

Based on the anatomical results gained from the phase-1 of the present study, an individual variability was detected regarding the localization of the target US image, but, according to previous studies, (Graff et al. 2015; Monticelli et al. 2015) FN and ON were successfully imaged in all cadavers as hypoechoic bundle surrounded by a thin hyperechoic layer as previously described (Echeverry et al. 2012b). Not important differences were detected between the US appearance of the nerves in living and in dead animals although a slight difference was registered for the walls of the nerves, that appeared more hyperechoic in dead animals compared to the living ones. In the present study the US image of FN was located in the dorsal portion of the IPM at the level of $L5^{th}$ while it was in a central portion of the IPM, between the L6 vertebral body and of L7 transvers process and progressively moved towards a more ventral and lateral position in cranio-caudal direction until it escaped from the IPM becoming FN and saphenous nerve. Concerning the US image of ON it was properly located only between the $L6^{th's}$ vertebral body and the $L7^{th's}$ transvers process that is partially in accord with Graff and colleagues 2015, in fact at this level the ON moved away from the FN, visible as a second hypoechoic hole.

The phase-1 of the present study demonstrated that FN-ON within IPM can be adequately covered using 0.1 mL kg^{-1} of new methylene blue solution whit an in-plane technique when US is used an the “target”

image is achieved. In literature doses used for the same propose ranged between 0.1 and 0.4 mL kg^{-1} (Campoy et al. 2010; Echeverry et al. 2012b) and the recommended dose resulted 0.2 mL kg^{-1}. The lower dose used in the present study suggests that the target US image and the approach were enough precise to obtain a complete coverage of the nerves. No evidence of intra-abdominal or epidural contamination based on the anatomical dissection and cryosections was detected which is in contrast with a preview study, Monticelli and colleagues 2015, where intra abdominal spread of the dye was detected in 2/4 cadavers. One explanation could be the different US-guided technique used. In fact Monticelli and colleagues used an out-of plain approach which did not allowed to follow the needle during the progression through the IPM. Another explanation could be the greater volume of dye 0.4 mL kg^{-1} used by Monticelli instead of 0.1 mL kg^{-1} used in the present study. In the present study a peculiar US image was searched, where FN and ON could be detected simultaneously. In order to achieve this "target point" the IPM was scanned from $L5^{th}$ to $L7^{th}$ and the results gained from the *phase-1* showed that the target US could be achieved at different levels of the lumbar region for each cadaver (between vertebral body of $L6^{th}$ to vertebral body of $L7^{th}$) due to individual anatomical variability. This is the reason why the authors are not able to indicate a fix landmark. In order to overcome this drawback, following the nerves throughout the IPM belly, resulted mandatory. Regarding the amount of staining obtained, it involved all the nerves and in 6/6 cases for a length higher

than 4 cm. Furthermore at the same level of the target US in 6/8 cases also ScN nerve was gained near to the shadow of the $L7^{th}$ body (Fig 5). This evidence was confirmed during the phase-2 of the present study where in 15/20 cases ScN was also detected contextually to FN-ON using the same US window, suggesting that one single approach could be applied, in order to obtain the block of the three nerves. Future studies are necessary to investigate these results and if the block of ScN at this level (closer to its roots) could be more suitable for surgery involving the proximal part of the pelvic limb.

During the phase-2 of the present study in all patients, 20/20 dogs, the target images were achieved and the PNBs performed resulted in adequate block for the dogs undergoing TPLO surgery. Anaesthesia plane was maintained using a low concentration of volatile anaesthetic agent and this is in accordance with other studies (Portela et al. 2013; Vettorato et al. 2013).

The amount of fentanyl administered as rescue during the surgery was higher for R3 in comparison to R5 group, but no statistical differences were detected. For group R3 most of the rescues were administered at time T_7, which corresponded to the tibial periosteal incision. Regarding R5 group most of the rescues were administered at time T_{10} corresponding to osteosynthesis plate placement showing a possible lack in ScN cover, more than FN-ON, for both groups. However it is not possible to exclude the possibility that fentanyl could have been administered after sympathetic response was caused by insufficient

hypnosis and not for a nociceptive response, this option might be supported by the low post-operative pain score registered in both groups. Considering that overall in 3/20 dogs intraoperative PNB was not considered completely successful because of the fentanyl infusion at 2 μg kg^{-1} hr^{-1} for R3 group and 1-3.5 μg kg^{-1} hr^{-1} for the R5, the success rate of the PNB was 85% during a light anaesthesia plane which was maintained with 1.00 % of Fe'Iso. As reported in literature (Barrington et al. 2012), US-guided loco-regional anaesthesia rate success is also operator's skill level dependant, suggesting that an improvement with training is possible. In fact the iliopsoas muscle compartment block is considered an advanced level technique, mainly because of the depth of the needle placement and it is recommended to practice after appropriate training (Campoy & Mahler 2013).

Furthermore regarding the volume of the LA used in the present study (0.1 mL kg^{-1}) was the same used in a previous study by Portela and colleagues in 2013 but was lower that the volume suggested by Echeverry in 2012 (0.2 mL kg^{-1}) for the same propose. Considering that the aim of the present study was to evaluate the efficacy and the usefulness of a PNB of FN and ON, the anaesthesia plane used was scheduled to permit to evaluate the block. In the R3 group there is the possibility that the lower concentration, with the low volume used, produced a less solid block which can explain the difference detected regarding the blood pressure.

Ultrasound guided peripheral nerve block of FN-ON beside of ultrasound

guided ScN provided a sufficient analgesia capable to minimise response to surgical stimulation in the region innervated by these nerves, in dog during TPLO surgery. The present FN-ON block was able to postpone the first rescue analgesia administration at least to 5.6 hours after the end of the surgery for the R3 group while after 7.2 hours for the R5 group, which was considered a quite good result. Postoperative pain score trend showed a difference between the 2 groups: in fact while in the R5 group the scores were low with slightly increase over the time in group R3 the trend showed a cut off 5 hours after the end of the surgery with a severe increase of the pain score.

Ultrasound approach to FN-ON in the psoas compartment was previously investigated by other authors (Echeverry et al. 2012a; Graff et al. 2015; Monticelli et al. 2015) but all this studies did not evaluate the clinical efficacy of the block, for this reason it is no possible to make any comparison with the present study. The FN-ON block performed within the iliopsoas muscle could be considered an advanced level technique but, on the other hand, compared to the FN block, performed at the level of the inguinal region, it permits to avoid the risk of puncturing the femoral artery or vein (Mahler & Adogwa 2008) because no big vessels are around or close to the nerves. The approach proposed in the present study was performed using an in-plane technique which has the opportunity to follow the needle progression inside the muscle planes.

The safety of the present PNB was confirmed by the absence of contrast spread in the abdomen and in the epidural space, during the anatomy

trial and the absence of clinical signs of epidural involvement during clinical phase, however, we cannot rule out the potential spreading of LA. Another advantage that emerged during this study was the possibility to perform the two block in one-side recumbency; the need not to change the position from lateral to dorsal in this study permitted not to use supports to maintain the animals in dorsal recumbency making the block viable by one operator alone without aids and consequently in a faster procedure.

In conclusion the potential clinical advantage gained by performing the FN and ON block in the IPM, achieving the nerves before their departing from this muscle in different branches, resulted in effective perioperative analgesia capable to minimise the response to surgical stimulation during TPLO surgery in dogs and to putt off the first rescue analgesia requirement at almost 5 hours after the end of the surgery.

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618 **Table 1** Surgery time points registered during the procedure.

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Time	Procedure
T0	five minutes before preparation of the operative field
T1	during the preparation of the surgical field
T2	incision skin and subcutaneous tissue
T3	incision joint capsule
T4	distraction of the knee joint
T5	meniscectomy
T6	suture joint capsule
T7	periosteal incision
T8	positioning external fixator
T9	osteotomy
T10	positioning plate osteosynthesis
T11	suture muscle planes
T12	suturing skin and subcutaneous tissue

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634 **Table 2** Number of animals that received a rescue treatment for
635 bradycardia (atropine 0.02 mg kg^{-1} IV), vasodilation (dopamine 2-5 µg
636 kg^{-1} min^{-1} IV) or hypovolaemia (colloids 2 mL kg^{-1} in bolus IV).
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Group	Atropine	Dopamine	Colloids
R5 Group	2/10	2/10	0/10
R3 Group	2/10	1/10	2/10

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Figure 1 Anatomical dissection of the iliopsoas muscle (IPM) between the 6th (L6) and the 7th lumbar (L7) vertebra. In order to put in evidence the femoral nerve (FN), a slight traction of it was necessary. At this level the FN sends some branches to the lumbar emergence of the sciatic nerve (ScN) and to the iliac wing (IW), the obturator nerve (ON) emerges at this level too.

Figure 2 Positioning of the probe for the individuation of the ultrasound window for the iliopsoas compartment. Two lines were drawn (A), the first (1) tangent to iliac crest (IC) and the second (2) tangent to tuber coxae (TC). The probe was positioned inside of the ventral angle obtained by the two lines (ventrally to the ilium wing, IW) and the visualization of the IPM was obtained by rotating the probe around its short axis of 30-50° (B).

Figure 3 Ultrasound image of the femoral nerve (FN) and the obturator nerve (ON) at the target level. The left side is the cranial (CR) side and the right side is the caudal one (CD). The nerves are visible as hypoechoic holes: the FN is the most cranial near to the needle, the ON is recognizable as a hypoechoic structure caudal and lower to the FN. Caudally to the FN it is possible to recognize another structure that

represents the iliac branches (IB) of the FN. The sciatic nerve (ScN) is merely visible outside the belly of the iliopsoas muscle (IPM).

Figure 4 Cryosection and ultrasound images of the iliopsoas muscle (IMP) caudally to the target point. The femoral nerve (FN) is visible in the left side of the picture, the obturator nerve (ON) is between the FN and the sciatic nerve (ScN).

Figure 5 Cryostections done at 7th lumbar (L7) (A) and 1st sacral vetrebrae (S1) (B) level. It is possible to see the dyeing of the iliopsoas muscle (IPM) and of the femoral nerve (FN) inside it. In the non coloured portion the obturator nerve (ON) and the sciatic nerve (ScN) are visible. Laterally to the vertebral body is apparent the ileus wing (IW).

Figure 6 Mean values and standard deviation of HR (A), MAP_{inv} (B), SAP_{inv} (C), DAP_{inv} (D), in the 2 groups, R3 group (ropivacaine 0.3%) R5 group (ropivacaine 0.5%) over the time-points registered during the surgery. Significant difference ($p<0.05$) in comparison to T0 *for R3 group # for R5 group.

Figure 7 Mean values and standard deviation of f_R (A), $P_E'CO_2$ (B), F_E'Iso(C) in the 2 groups, R3 group (ropivacaine 0.3%) R5 group (ropivacaine 0.5%) over the time-points registered during the surgery.

Figure 8 Median values and ranges of GPS scores in the 2 groups, R3 group (ropivacaine 0.3%) R5 group (ropivacaine 0.5%), starting from T1 that is 1 hour after the end of the surgery.

Table 1 Surgery time points registered during the procedure.

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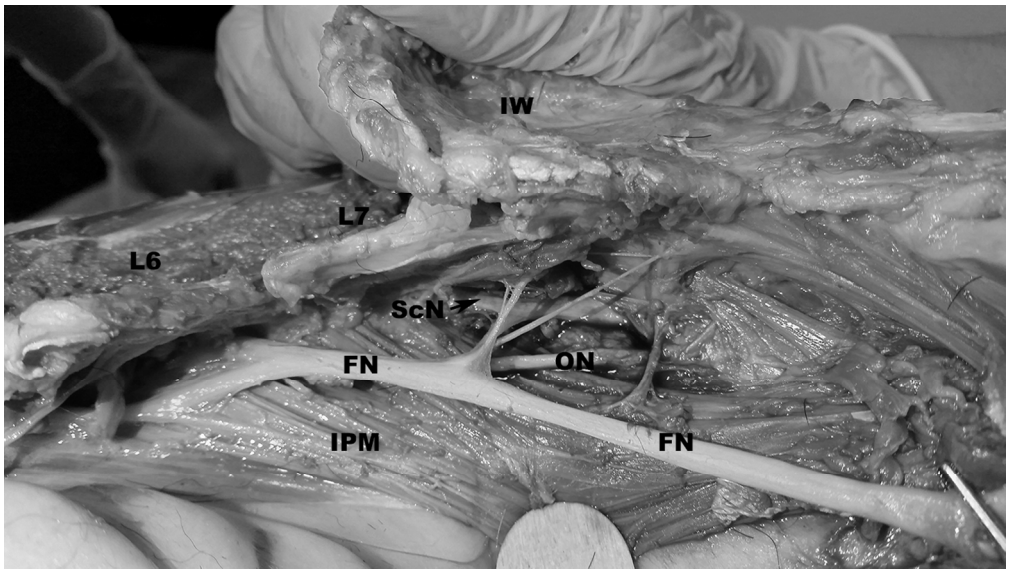
Table 2 Number of animals that received a rescue treatment for bradycardia (atropine 0.02 mg kg^{-1} IV), vasodilation (dopamine 2-5 µg kg^{-1} min^{-1} IV) or hypovolaemia (colloids 2 ml kg^{-1} in bolus IV).

Group	Atropine	Dopamine	Colloids
R5 Group	2/10	2/10	0/10
R3 Group	2/10	1/10	2/10



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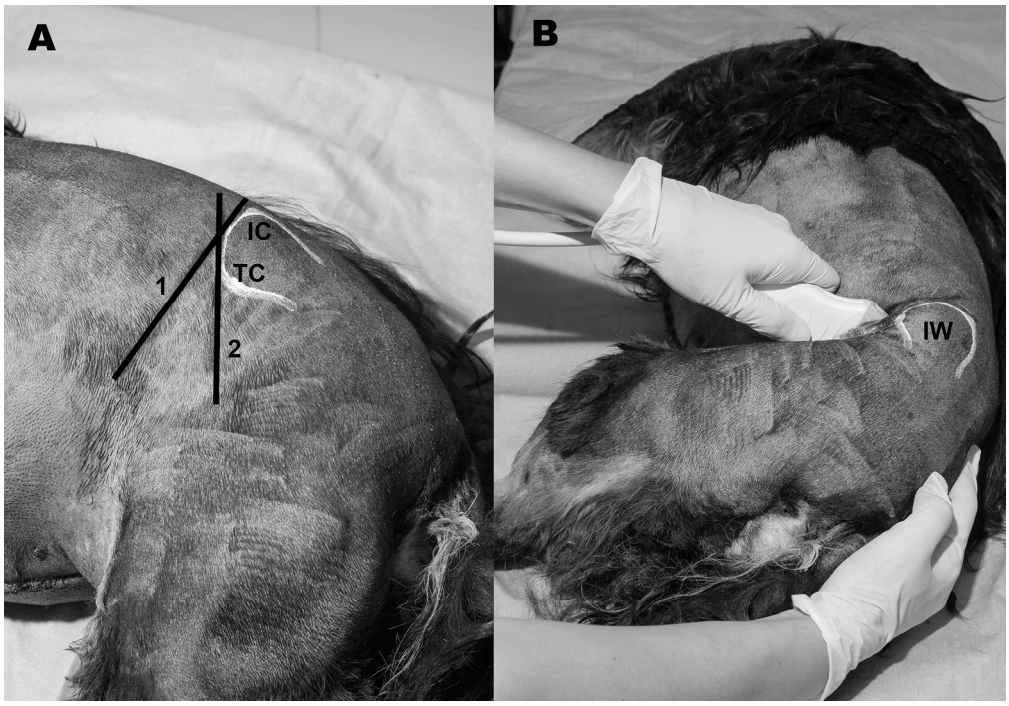

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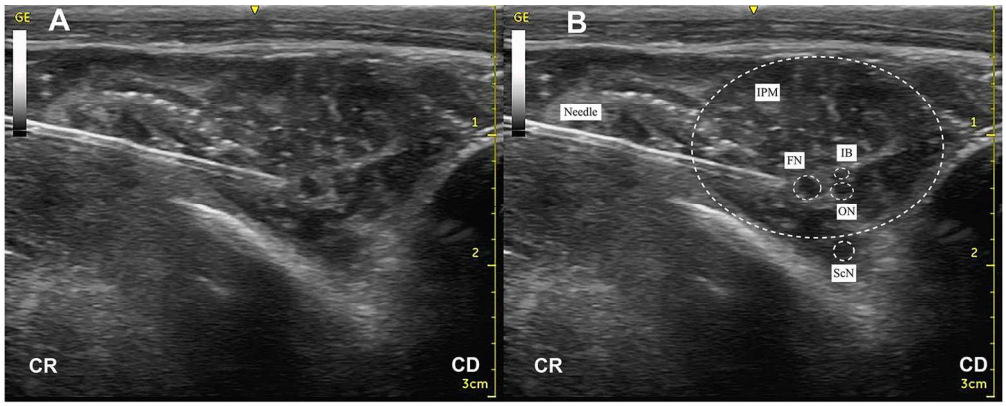

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150x59mm (300 x 300 DPI)

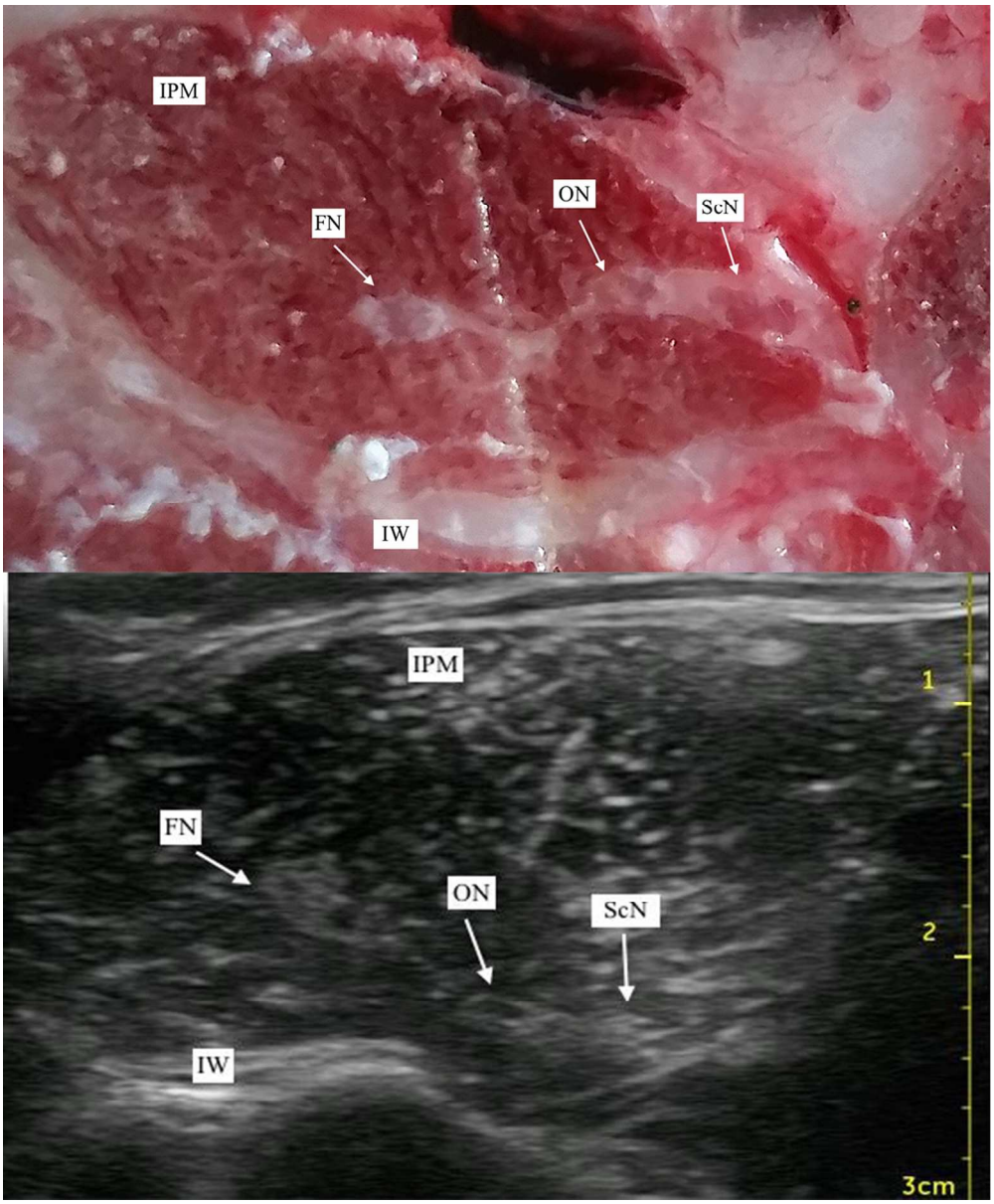
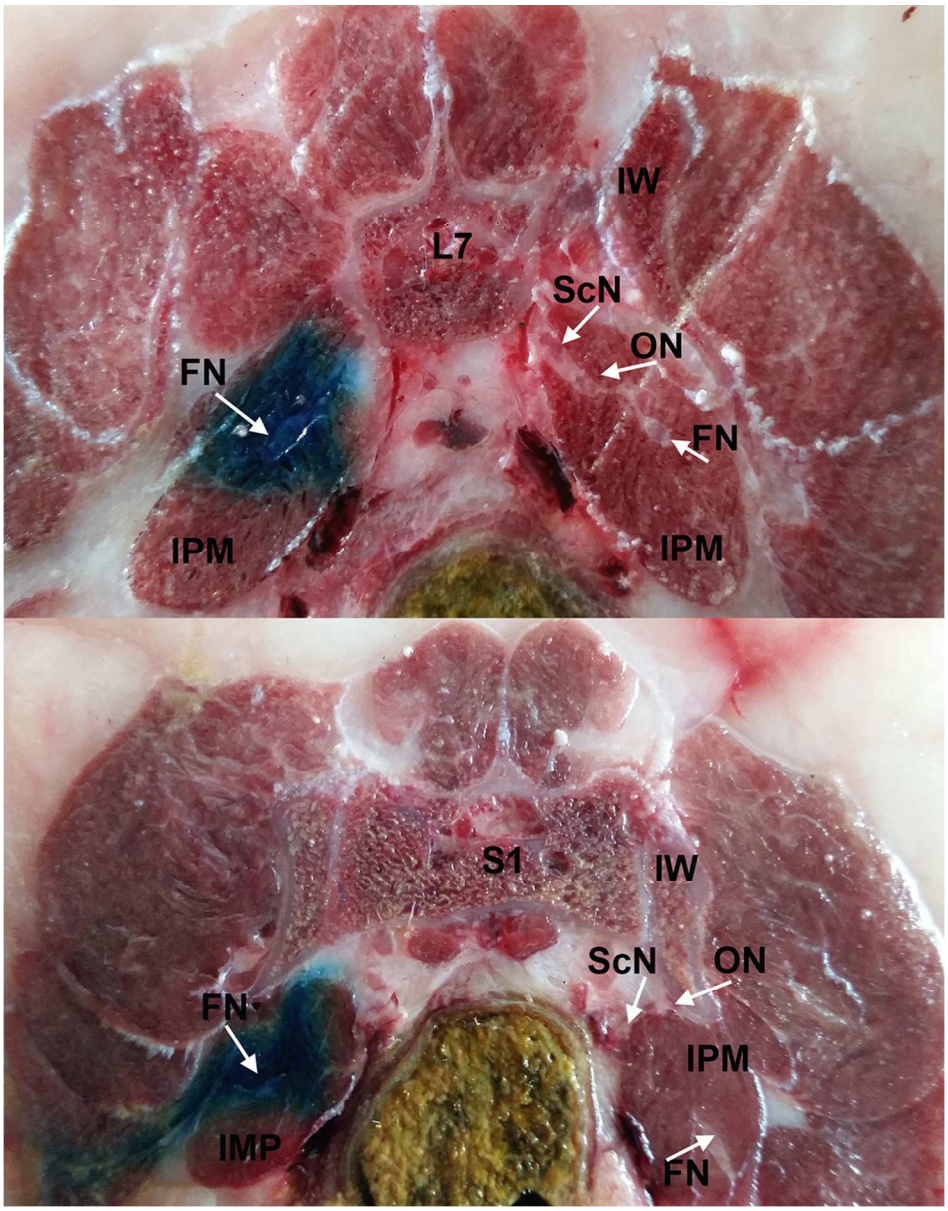


Figure 4 Cryosection and ultrasound images of the iliopsoas muscle (IMP) caudally to the target point. The femoral nerve (FN) is visible in the left side of the picture, the obturator nerve (ON) is between the FN and the sciatic nerve (ScN).
99x120mm (300 x 300 DPI)



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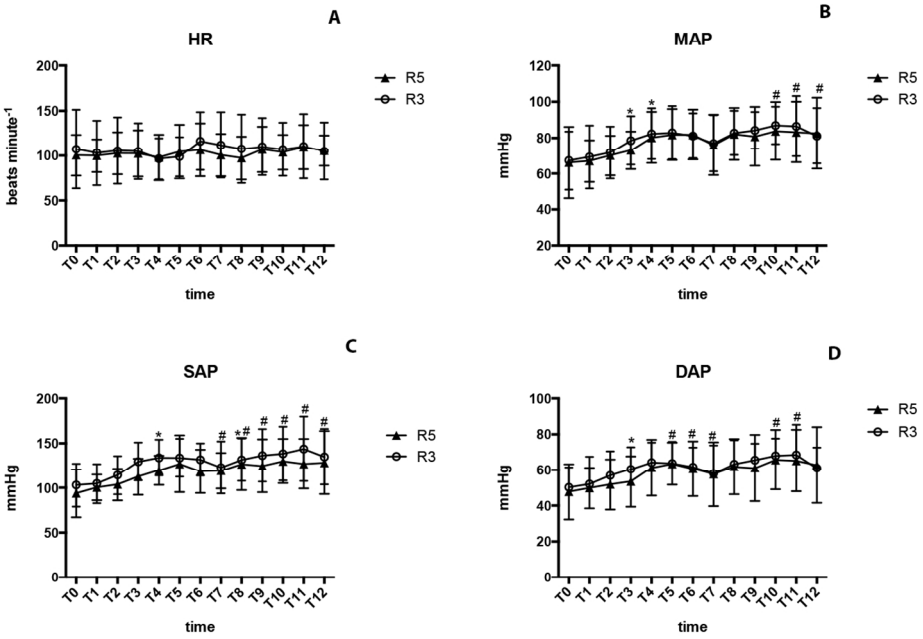
94x119mm (300 x 300 DPI)



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119x89mm (300 x 300 DPI)

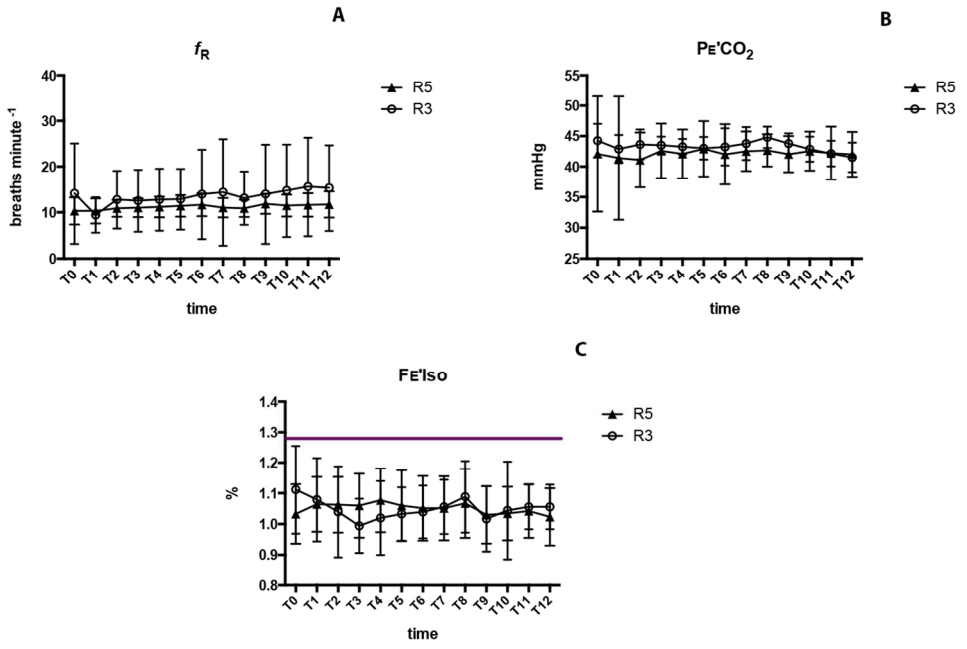

Figure 7 Mean values and standard deviation of fR (A), PE'CO2 (B), FE'Iso(C) in the 2 groups, R3 group (ropivacaine 0.3%) R5 group (ropivacaine 0.5%) over the time-points registered during the surgery.

119x80mm (300 x 300 DPI)

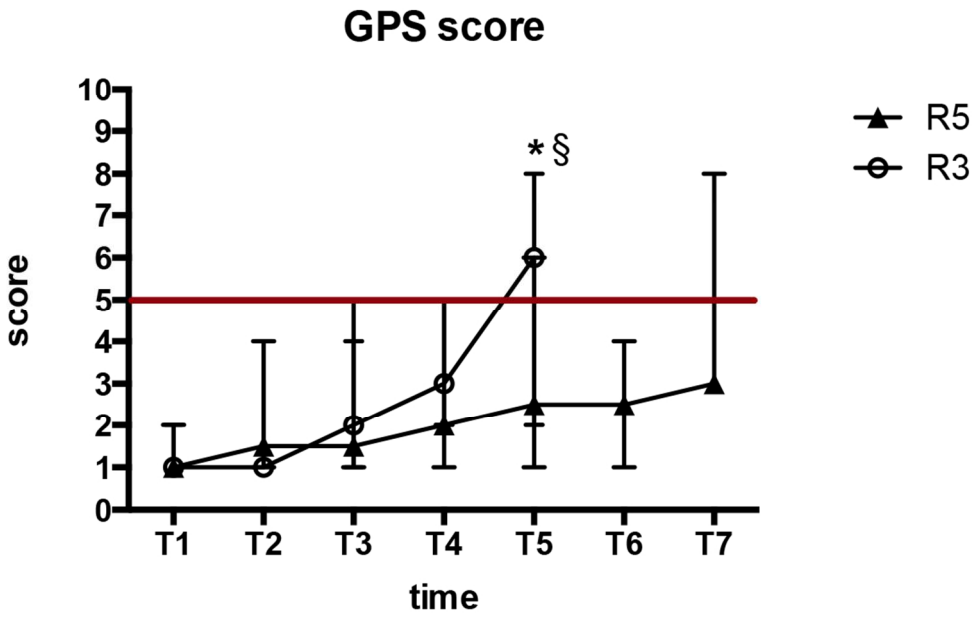

Figure 8 Median values and ranges of GPS scores in the 2 groups, R3 group (ropivacaine 0.3%) R5 group (ropivacaine 0.5%), starting from T1 that is 1 hour after the end of the surgery.
99x64mm (300 x 300 DPI)