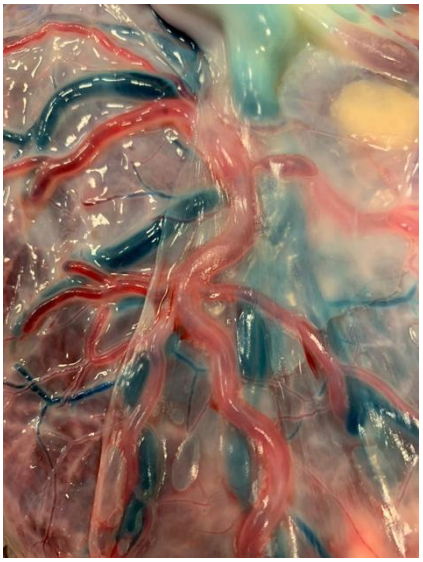
UNIVERSITÀ
DI PAVIA

Dipartimento di Scienze Clinico Chirurgiche, Diagnostiche e Pediatriche

Development of Dye-Perfused Human Placenta Model for Vascular Microsurgery Training: Preparation Protocol and Validation Testing in Hands-on Courses



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1. INTRODUCTION

Microsurgery is very challenging and requires specific bimanual abilities to perform dissection and anastomosis of vessels and nerves. The long and difficult learning curve in the acquisition of microsurgical skills, together with the risk of failure on live patient determined the need of training outside the operating room.[1] A regular exposure and practice in surgical procedures help surgeons to avoid errors.[2] Dissection and anastomosis techniques can be achieved by means of experience in surgical procedures, together with constant laboratory training. Implementation of reliable vascular models is pivotal for daily practice. Three main classes of microsurgery and microvascular training model are classically reported, namely, synthetic, ex vivo, and in vivo. Synthetic models make use of latex gloves, silicon tubes, endovascular protheses, and 3-dimensional printed samples, whereas in vivo models make use of live rats and pigs. In the context of the reported poor reliability of synthetic models and the ethical limitations of in vivo models, ex vivo models, like chicken wings, turkey neck, bovine and human placenta, and human and animal specimens, could achieve a good compromise.[2] In 1979, Goldstein first reported the use of fresh human placenta for microsurgical training.[3] Later, other authors highlighted the potential advantages of human placenta for laboratory use, especially because of its availability and low cost.[4] Nevertheless, despite many well-known advantages, research on the optimal laboratory preparation, storage technique and application in hands-on courses of human placenta are scant.

1.1 History of Microsurgery: An Inspirational Journey

Microsurgery has become an integral part of many highly specialist surgeries and even of the general one, whose evolution is closely linked to some specific elements of microsurgery. Many surgical techniques in the fields of neurosurgery, reconstructive surgery, cardio-surgery, and

otolaryngology require magnification instruments. In 1897 it was Jhon Benjamin Murphy who reported the first systematic experimentation on anastomoses.[5] Only in the early 1900s, thanks to the French surgeon Alexis Carrel, the world of medicine begin to recognize the importance of vascular surgery techniques; these techniques are still used in organ transplantation.[6] Carrel developed the principles of vascular anastomosis (tensionless anastomosis and continuous endothelial-endothelial contact) which, 100 years later, are still current. In the neurosurgical field, the introduction of the microscope has allowed the development of microsurgical techniques. In 1953, the first Universal Operative Microscope (OPMI 1) was marketed by the German manufacturer Carl Zeiss and was initially used by Otolaryngologists and Ophthalmologists; only later it slowly enters other surgical fields. In 1957 the neurosurgeon Theodore Kurze observed an otolaryngology operation in which the OPMI 1 was used, from that moment he began to use it for training the approach to cerebellopontine angle.[7] In the late 1950s, Julius H. Jacobson II at the University of Vermont, used the OPMI 1 to improve the "end-to- end" anastomosis technique on a canine carotid artery (diameter 3 mm).[8] The advent of the microscope made possible some surgical techniques, such as the anastomosis between the external and internal carotid artery, performed in 1951 by Charles Miller Fisher. In 1965 a Neurosurgeon from the University of Zurich moved to Burlington to study microsurgery techniques under Donaghy's supervision. In 1999 the Turkish neurosurgeon, M. Gazi Yasargil, was recognized as "Neurosurgery's Man of the Century 1950-1999" by the Congress of Neurological Surgeon.[9] Yasargil developed his microsurgical skills by practicing on the peripheral vessels of dogs using nylon threads 8-0. After completing 120 operations with this technique, he moved on to sutures on the brain vessels of the dogs, specifically the basilar artery. Yasargil continued to practice and perfect his technique thanks also to the advent of the bipolar forceps to coagulate and of the 9-0 sutures. In 1967, on October 30, after returning to Zurich, Yasargil performed the first bypass between the superficial temporal artery and the middle cerebral artery on an adult man and in 1968 a permanent Laboratory, for microsurgical

training, was establish in Zurich University Hospital. Yasargil was considered the founding father of microneurosurgery. The following decades were characterized by the preponderant development of microsurgery in various fields, for which microsurgical training programs were created in various countries including the United States and Japan. To date, it is unthinkable that a microsurgeon can use these techniques without having acquired the basic skills through specific educational programs. To date, microsurgical practice laboratories have become necessary for the acquisition of fundamental technical skills to apply on live patients. This has led some countries to request a certificate that testifies the achievement of a level of performance in microsurgery that allows surgeon to operate on patients. However, in many countries the development of surgical training laboratories is not yet advanced, which is why it is essential to spread the importance of microsurgical practice in laboratories even before in the operating room.

1.2 The Philosophy of Microsurgical Practice

Four essential principles rule the philosophy of microsurgical practice: "*(1) close replication of the microsurgery environment results in familiarity and ease with actual procedures: (2) repeated practice of the same techniques builds muscle memory and enhances mindfulness; (3) thoughtful preparation and rehearsal; and (4) training on increasingly more complex microsurgical tasks builds higher level skill and aids progress through the stages of competence*". [10]

These principles, reported in 2020 by Belykh and Nakaji, encompass the essence of how well-planned, and repeated microsurgical practice within a laboratory setting can improve the operative skill set of the microsurgeon. Such philosophy, described for microneurosurgeons, can be extended to all specialties dealing with microsurgery. Continuous training refines manual skills and enhance mindfulness, helping surgeons to overcome dexterity and

psychological limits. Thorough preparation and self-confidence are the cornerstone to push forward surgical indication and to treat complex cases in vascular disciplines.

2. MODELS FOR MICROSURGICAL TRAINING

The 1960s marked the era of microsurgery. At that time, it became evident that microsurgery training had to begin, first in the laboratory, since the acquisition of manual skills and familiarization with a new environment (microscope, microinstruments, microsutures), that required several hours of training. In the 70s and 80s, the worldwide expansion in this field involving various surgical specialties, was made possible through the organization of “micro” seminars with practical training.[11] The best way turned out to be 3-5 days (10-12 hours) training on synthetic and ex vivo models (knots, suturing of tubes, suturing of chicken vessels) and then on in vivo models such as rats and rabbits. The need for integration of different skills makes the live rat an indispensable part of any training program. Any academic program dealing with microsurgery needs recognition of the "3 R principle": (1) Reduce the number of animals used,[12] (2) Replace as many models as possible,[13] (3) Refine the experimental design.[14] Simulation is the basis of training in the field of microsurgery. Vascular and nerve microsurgical simulations need two elements: a binocular microscope to help young surgeons to master the visuospatial aspects of microsurgery and a training model that emulates the architecture and texture of living structures. Preferably, the complexity of the model should reflect the technical capabilities of the trainee. The more experienced the surgeon, the more complex and reality-like the model should be. The aim of a continuous training program is to reduce complication rate and surgical time in the operating room.[15] Microsurgical training models are typically classified into 3 groups: synthetic, ex vivo and in vivo.

2.1 Synthetic Models

The synthetic models allow the acquisition of basic surgical techniques through high repetition at low cost.[16] They are typically: latex gloves, Silicon or Polyvinyl alcohol (PVA) gelatin tubes. They can help to understand the principles of anastomosis and to learn how to set up a microscope and find the optimal focal distance. However, the elastic properties of tubes do not correspond to those of live tissues, which is their major limit. Silicon tubes are manufactured in different sizes and are available from industrial stores. Also training cards (microvascular practice card, Muranaka medical instruments Co., Ltd., Osaka, Japan) can be used. PVA gelatin tubes have better qualities than silicone for simulation of the properties of human arteries. Other simulators based on synthetic tubes are widely used, such as a microvascular simulator[17] or MD-PVC Rat Model (Braintree Scientific, Inc., Braintree, Massachusetts, USA).[18] Latex gloves can be also used for training. A thin leaf made from glove simulates a wall of microvessels that can be cut and sutured in different ways.

2.2 Ex Vivo Models

Ex vivo models such as chicken wings and legs, turkey wings and necks, bovine, porcine and human placenta and human cadavers fit into this scenario.[19] Chicken and turkey parts certainly constitute a more complex training model than synthetic ones. They are low cost, easy to find and allow anastomosis of small vessels. However, the vessels available are all with similar caliber, without great dimensional variability.[20] Turkey wings and necks have larger arteries than chicken ones. The turkey arteries are similar in diameter to the human artery of middle caliber.

The human cadavers constitute the model with the most likely surgical environment. This model allows to work in contact with the real tissues and allows to reach an excellent knowledge of the anatomy. However, fixed brain tissue and vessels have different mechanical properties

and all the extra-time spent on the dissection of fixed cadaveric heads is inappropriate for the regular practice. Such models are better only in case of whole surgical procedure simulation.
In the neurosurgical field, several cadaveric head models with pressurized vessels have been described for use in hands-on courses.[21,22] Lastly, cadaveric models are very expensive, difficult to find, and its use is prohibited in many countries.

2.3 In Vivo Models

In vivo models, such as live rat, are the gold-standard for the learning and training of young surgeons, they, nevertheless, require complex bureaucratic process to obtain the permits, along with the lack of both economical and logistical resources, ethical considerations and hygiene issues, make the use of this model difficult.[23,24] Training with laboratory animals is considered as a final preparatory step before performing microvascular anastomosis on a live patient.
Common sites of anastomosis are the femoral neurovascular bundle, exposing the femoral artery and vein in the iliac region. Dissection of neck vessels, such as jugular vein and carotid artery is generally used to perform end-to-end carotid artery anastomosis and end-to-lateral jugular vein to carotid artery anastomosis. Midline laparotomy provides the exposure of the aorta and vena cava. Aorto-cava dissection is one of the most complex dissection because of the fragility of the cava wall. Latero-lateral aorto-cava anastomosis is typically considered the final step of microsurgical training.
As general rule, basic manual and visuospatial skills must be acquired on low-fidelity simulator before the introduction to a high-fidelity one. In this way, the surgeon will benefit more from the in-vivo model without wasting complex one and consequent cost reduction.[25]

3. HUMAN PLACENTA TRAINING MODEL

3.1 Anatomy of The Human Placenta

Mammalian placentas consist of embryonic fetal envelopes of the chorion and allantoides. It is divided in a bigger fetal portion and a smaller maternal one. It is possible to distinguish 4 types of mammalian placenta: diffuse, cotyledonary, ringed, and discoid, based on the distribution of the villi on the fetal surface. The human placenta is part of the group of discoid types, in which the villi are distributed in a single circular plate.

The full-term human placenta is a circular discoidal organ with a diameter of about 18 cm, a central thickness of 2.5 cm, and an average weight of 500 g. However, when planning a morphometric analysis of the placenta, factors such as when and where the umbilical cord has been clamped are critical because loss of maternal and/or fetal blood clearly affects the dimensions of the placenta.[26] Describing the macroscopic anatomy, two surfaces are considered, the fetal and the maternal ones.

Fetal surface

The chorionic plate represents the fetal surface of the placenta, and this part is also covered by the smooth amnion. The amnion is composed of a single layered epithelium and of an avascular connective tissue, the amnionic mesenchyme. The amnionic mesenchyme is only weakly attached to the chorionic mesenchyme and can easily be removed from the delivered placenta. The umbilical cord is not always inserted centrally to the placenta, on the contrary, most of the time it is in a slightly eccentric position into the chorionic plate. The umbilical cord vessels continue with the chorionic vessels contained in the chorionic mesenchyme. The chorionic arteries, deriving from the two umbilical arteries, split up in a centrifugal pattern into their final branches, which supply the villous trees. The low blood pressure of the placental vessels does not require a thick vascular wall, and this justifies the absence of an internal elastic lamina and elastic fibers, unlike the brain arteries. The pressure is a maximum of 60-80 mmHg during the

final weeks of pregnancy.[2] The veins of the villous tree continue directly in the chorionic veins, which usually cross the chorionic arteries underneath. The chorionic veins give rise to the single umbilical vein (Figure 1).

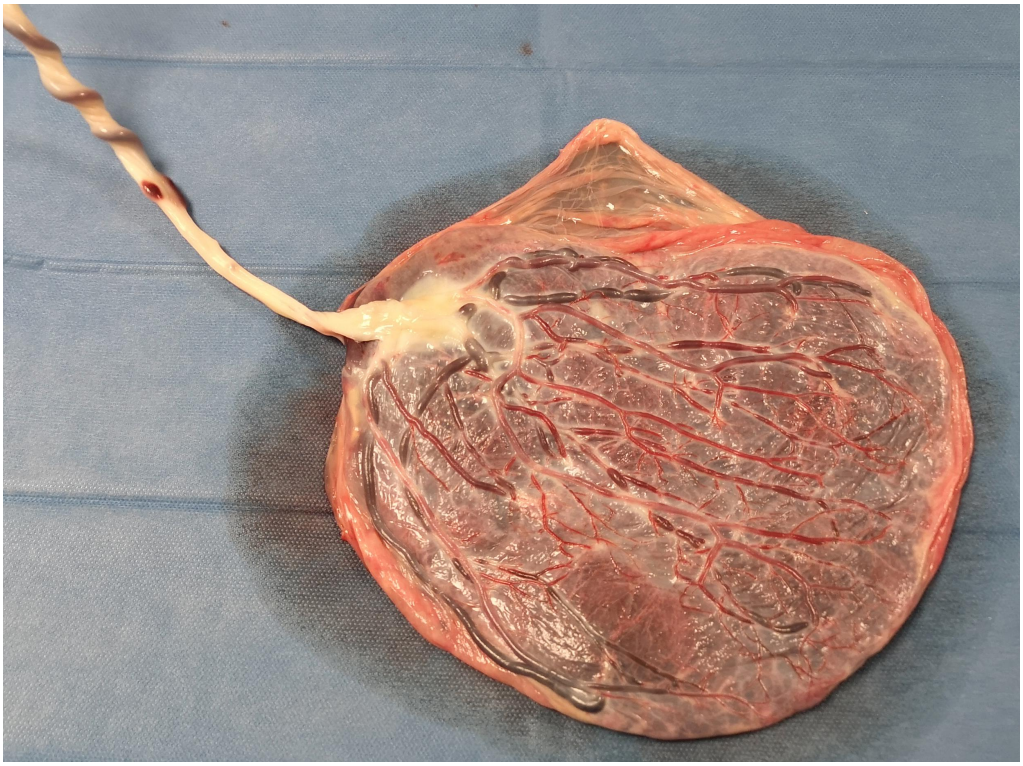


Figure 1. Fresh human placenta, fetal surface.

Maternal surface

The basal plate represents the maternal surface of the placenta. During delivery, from the separation of the placenta from the uterine wall, the basal plate emerges. This artificial surface is a colorful mixture of fetal extra villous trophoblasts and decidual stroma cells, natural killer cells, macrophages, other immune cells, and all kinds of maternal cells of the uterine decidua. A large amount of extracellular matrix, fibrinoid and blood clots are also contained by the basal plate. The latter is also divided into lobes: 10-40 slightly raised regions, divided by a system of flat grooves or deeper clefts. Inside the placenta, the septa (which correspond to the grooves) only trace the lobar borders as irregular pillars or short sails. On the maternal surface of the placenta, the lobes are visible, and they show a good correspondence with the position of the

villous trees arising from the chorionic plate into the intervillous space. From the chorionic plate of a full-term placenta, arise almost 60–70 villous trees (or fetal lobules).[27,28] Placentome has been defined as the presence of a single hairy tree occupying a single lobe.[29] The chorionic and basal plates merge at the level of the chorionic placental margin and form the smooth chorion the fetal membranes or the chorion leave. The chorion leave is composed of three layers: the amnion (with its epithelium and mesenchyme); the chorion (with a layer of mesenchyme and a layer of extra villous trophoblast); and the decidua capsularis[30] (Figure 2).

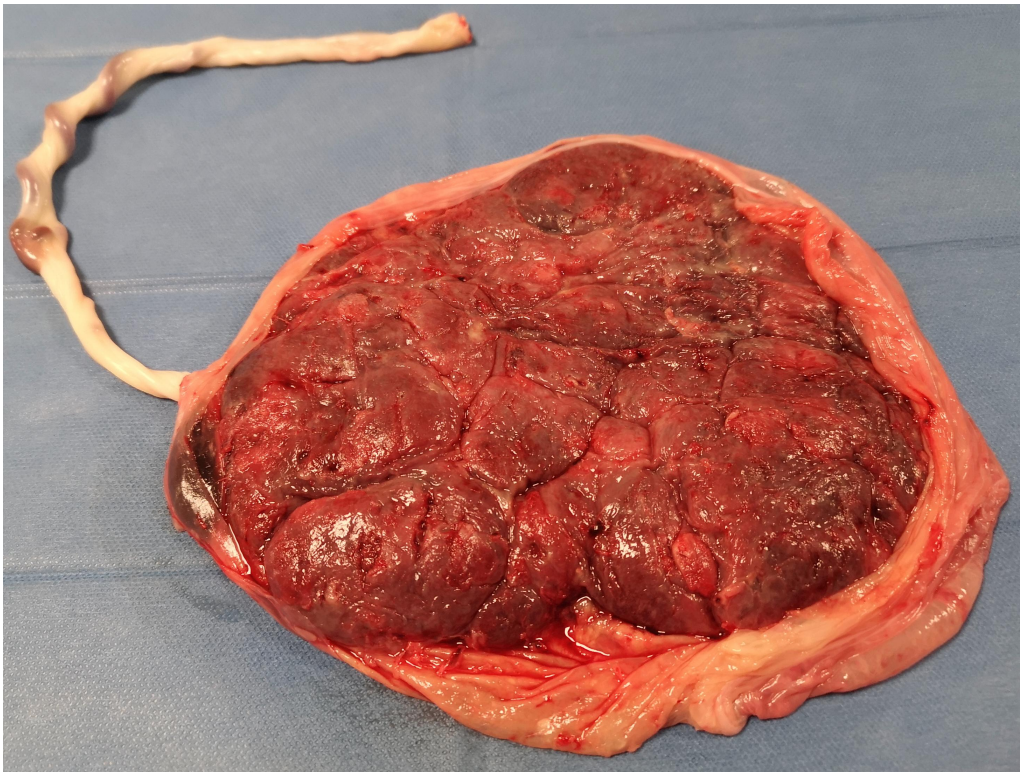


Figure 2. Fresh human placenta, maternal surface.

3.2 Application of Human Placenta in Lab Activities

In the group of ex-vivo training models, the placenta stands out for its characteristics: an inexpensive microsurgical training tool, easy to find and to prepare, with lesser ethical implications that concern other types of models. Moreover, the placenta anatomy is, itself, particularly suitable for microsurgical exercises.[2] These are the main aspects that justify the

rapid evolution, in importance and use, of the human placenta in lab activities. In 1979 Goldstein published the first report about the use of fresh human placenta for microsurgical training.[3,31] Later, in 1983 McGregor revised 10 human placentas to describe in detail their anatomy and to analyze the histological aspect of the vessels wall, characterized by a thick adventitial layer and a lack of elastic fibers of the tunica media, thus affecting technical aspect of dissection and anastomosis.[32] In 1992 Ayoubi highlighted the close similarity between length and diameter of the placental vessels in relation to those of the brain.[33] It was in 2008 that Romero F.R. analyzed twenty-five human placentas, and for the first time described step-by-step suture exercise on placenta vessels, in order to show its usefulness for training.[34] In the following years, the use of placenta as a microsurgical tool has become more and more widespread. Several studies were developed to create increasingly difficult exercises with the aim to make the model more realistic and like in vivo ones. A great contribution in the study and development of this specimen must be assigned to Magaldi Ribeiro de Oliveira M. His team, in 2014, isolated human placenta vessels to create aneurysm of different shapes, developing a useful tool for microsurgical exercises (sylvian fissure-like dissection and aneurism clipping), that allows to acquire and maintain the microsurgical skills needed for vascular neurosurgery.[31] The placenta was also used for simulating brain tumor removal, with the aim to provide a valid exercise for tumor resection.[35] The spread of the placenta model has required the evidence of consistency, face, content, and construct validities. So that, in 2016 Belykh at al., assess the usefulness of microvascular bypass simulation models on human and bovine placenta vessels, employing the Northwestern Objective Micro-anastomosis Assessment Tool (NOMAT) scale and a post-training survey.[2] In order to measure specific operative parameters of aneurysm clipping surgery and to objectively assess construct validity of this training model, the same group has exploited the Objective Structured Assessment of Aneurysm Clipping Skills (OSAACS).[36] In 2019 The Skill Assessment in Microsurgery for Brain Aneurysms (SAMBA) scale was created in order to be used as an interface between

learning and practicing, highlighting the importance of this microsurgical training model, in influencing the outcome in a real surgical environment.[37,38,39,40] Recently, the group of Gomar-Alba described in details how to use a model of infused human placenta to train residents in the vascular control and intraoperative vessels rupture management.[41]Last, the human placenta was used to simulate microsurgical thrombectomy in the scenario of an acute ischemic stroke.[42]

In table 1 are summarized all the mentioned reports (Table 1).

Table 1. Use of Placenta as Microsurgical Training Tool: Literature Summary

Authors	Year of Publication	Type of Placentas	N° of Placentas	Preparation Process	Type of Exercise	Aim of the Article
Goldstein M.	1979	human	n.a.	n.a.	n.a.	n.a.
McGregor JC et al.	1983	human	10	n.a.	vessel dissection	human placenta anatomy description and training model
Leconte D.	1988	n.a.	n.a.	n.a.	n.a.	n.a.
Ayoubi S. et al.	1992	human	25	n.a.	n.a.	use of placenta for microvascular training
Romero FR et al.	2008	human	25	n.a.	vessels anastomosis	training model description and exercises
Magaldi Ribeiro de Oliveira M et al.	2014	human	40	aneurysm model creation	sylvian-like dissection/aneurysms clipping	to describe model for aneurysms clipping
Magaldi Ribeiro de Oliveira M et al.	2016	human	16	tumor model preparation	microsurgical tumor removal/vessels anastomosis	to simulate tumor resection using placenta
Belykh E et al.	2016	human and bovine	30	placenta and bypass preparation	vessels anastomosis	to describe microvascular bypass simulator
Belykh E et al.	2017	human and bovine	n.a.	aneurysm model creation	sylvian-like dissection/aneurysms clipping	to validate human placenta aneurysm model
Magaldi Ribeiro de Oliveira M et al.	2018	human	30	aneurysm model creation	aneurysms clipping	to compare aneurysm clipping in cadaver and placenta models
Magaldi Ribeiro de Oliveira M et al.	2018	human	100	n.a.	to perform bypass	to evaluate IC-IC bypass apprenticeship
Magaldi Ribeiro de Oliveira M et al.	2019	human	2	n.a.	aneurysms clipping	to validate aneurysm simulator
Magaldi Ribeiro de Oliveira M et al.	2019	human	n.a.	n.a.	aneurysms clipping	to describe and validate a skills assessment instrument
Gomar-Alba et al.	2021	human	9	Placenta and 3D skull preparation	Management of vessels rupture	to describe a realistic training in vessels rupture scenario
Magaldi Ribeiro de Oliveira M et al.	2021	human	6	n.a.	Microsurgical thrombectomy	to describe a stroke simulator

n.a. = not amenable; IC= Intracranial

3.3 Anastomosis technique on human placenta vessels

Four different exercises were commonly reported to train dissection and anastomotic dexterity using human placenta vessels.

3.3.1 Allantoid and vessels dissection

The exercise starts with the identification and elevation of the allantoid membrane with forceps. Then, a linear incision of the allantoid membrane is performed with micro-scissors. The membrane is reflected to expose a selected segment of an artery or a vein. A sharp dissection of the infero-lateral side of the vessel wall from the underlying stroma is suggested with a progressive gentle elevation of the vessel segment. It's recommended to use the micro forceps on the adventitia of the vessel, to elevate and move it without damaging the vessel wall. The dissection of the vessel is completed through small and delicate cuts with micro scissors or cutting dissector. These steps are more difficult on veins, as they have a thinner wall than the arteries and have a more intimate fusion with the stroma (Figure 3).

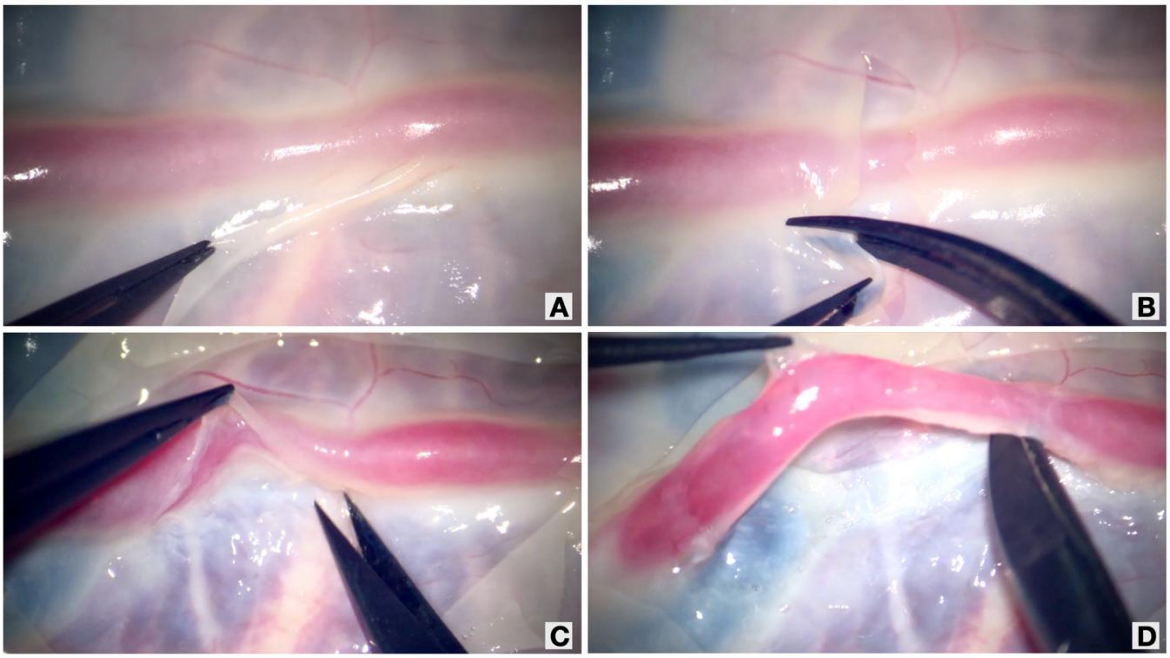


Figure 3. Allantoid and arterial dissection: (A) Identification and elevation of the allantoid membrane. (B) Sharp dissection of the allantoid and arterial exposure. (C-D) Arterial dissection from the underlying stroma.

3.3.2 End-to-end anastomosis

Under the microscope, the placenta is carefully examined to choose the best working zone. The allantoid is removed with the micro-scissors. A dissection of the chosen segment of the vessel is performed with micro-forceps, micro-scissors, and microsurgical knife. Dissection is performed until a segment of at least 2-2.5 cm is released from the stroma of the underlying placenta (as described in the previous sub-chapter 3.3.1 Allantoid and vessel dissection). A latex leaf is placed to highlight the dissected vessel from the underlying stroma and make it easier to view under the microscope. The micro-spacer is positioned to isolate 1.5 cm of the vessel. Release of the vessel from the adventitia and removal of the connective tissue with micro-scissors. A linear incision is done perpendicular to the vessel to obtain two separate ends. The lumen of the vessel is cleaned with saline solution. Reconstruction of the vessel with detached stitches. The dimension of the suture strictly depends on the dimension of the vessel. The first suture is performed in the upper border of the vessel (12 o'clock). The second suture is performed in the lower border of the vessel (6 o'clock). Then, the anterior wall is sutured with other three single stiches. The vessel is turned around with the microforceps to expose the posterior segment of the anastomosis. Other three or four stiches are performed (Figure 4). Each suture must be tied three times. The micro-spacer must be removed after the end of the anastomosis and the vascular patency must be tested opening the infusion set.[34]

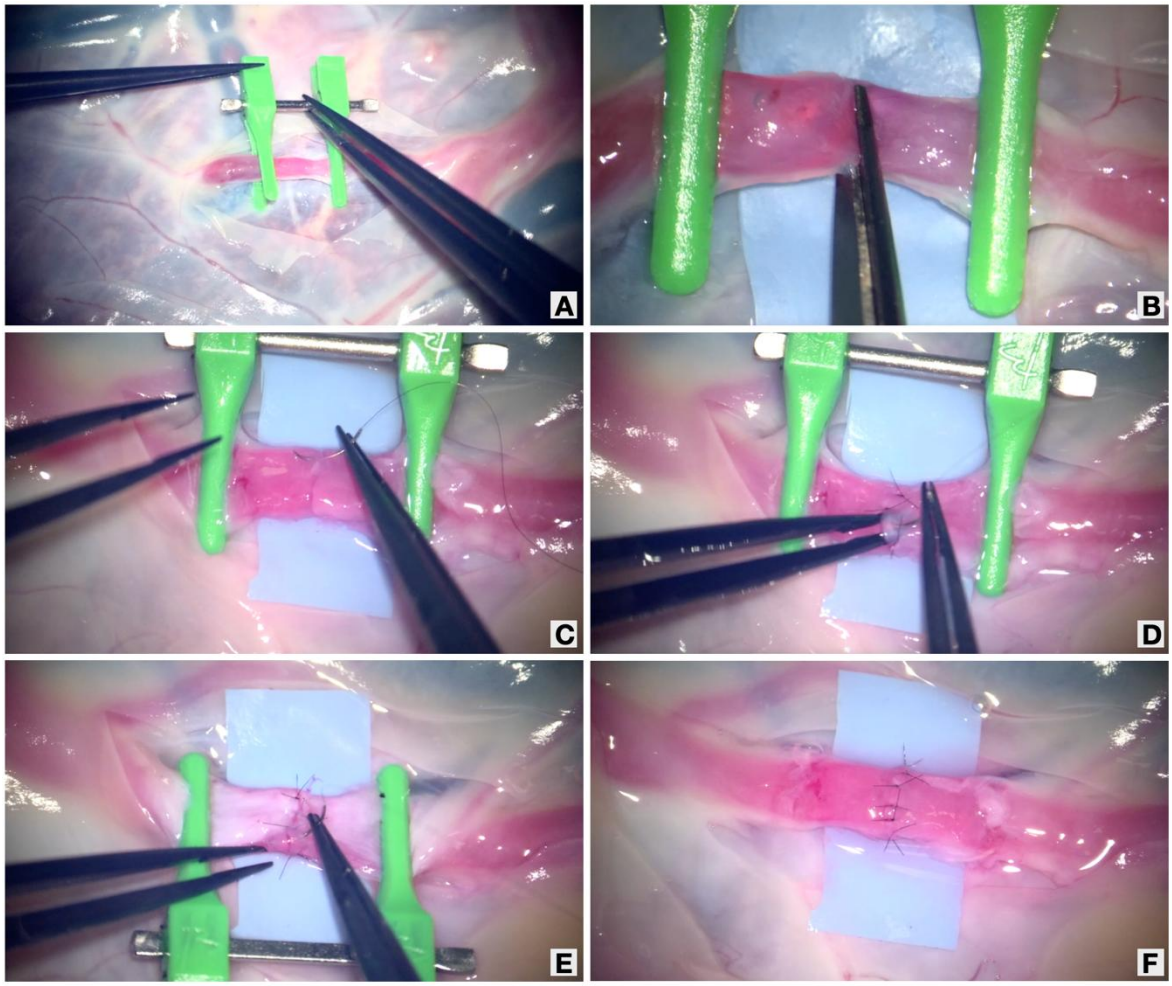


Figure 4. Arterial end-to-end anastomosis. (A) After dissection of an artery from the stroma the micro-spacer is positioned. (B) Linear cut of the vessel. (C) The first suture was done at 12 o'clock. (D) Suture of the anterior wall completed. (E) Suture of the back-wall. (F) Final appearance of the end-to-end anastomosis and its evaluation.

3.3.3 End-to-side anastomosis

A donor and a recipient vessel are chosen with a similar caliber and in close position. The allantoid is removed with the micro-scissors. A dissection of the chosen segment of the vessel is performed with micro-forceps, micro-scissors or microsurgical knife (as described in the sub-chapter 3.3.1 Allantoid and vessel dissection). Dissection is performed until a segment of at least 2-2.5 cm is released from the underlying stroma. A latex leaf is placed to highlight the dissected vessel from the underlying stroma and make it easier to view under the microscope. The donor vessel is cut at 45° with the aid of micro-scissors and micro-

forceps and is brought closer to the recipient vessel. The receiving vessel is prepared, and an elliptical hole is done on the vessel wall. The first two sutures are performed at the extremities of the elliptical hole. First, the suture is performed in the posterior wall and then the procedure in repeated on the anterior side (Figure 5). Each suture must be tied three times. The clips must be removed after the end of the anastomosis and the vascular patency must be tested opening the infusion set.[34]

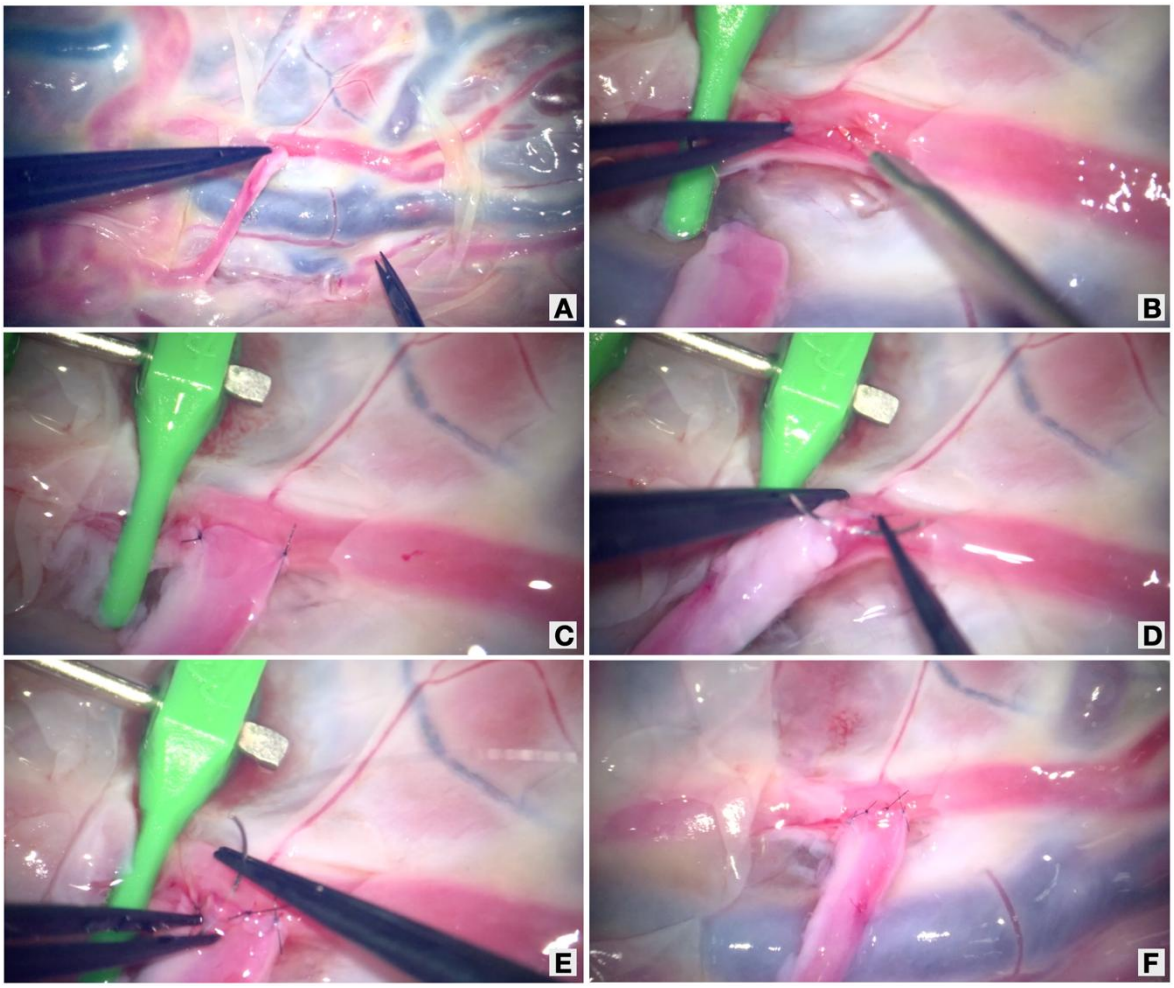


Figure 5. Arterial end-to-side anastomosis. (A) Dissection of "donor" artery and its transposition to the "recipient" artery. (B) Incision of the recipient vessel with micro-blade. (C) The first and the second suture were done at the extremities. (D) Suture of the back-wall. (E) Suture of the anterior wall. (F) Final appearance of the end-to-side anastomosis and its evaluation.

3.3.4 Side-to-side anastomosis

The exercise starts with the selection of two parallel vessels, close together and with the same diameter. An arterio-venous side-to-side anastomosis should be done. Proceed with the dissection of both vessels obtaining a similar length (at least 1.5 cm). Verify that the dissected vessels are free from the underlying stroma. The dissection technique is the same described in the sub-chapter 3.3.1 (Allantoid and vessel dissection). Place a leaf of latex under both vessels and proceed with the removal of the adventitia. Two temporary clips are placed to bring the vessels close together and occlude flow. A pair of separate micro-clips could be used to stabilize each vessel and avoid excess tension. An arteriotomy is performed parallel to the direction of the vessel**.** The lumen of the vessels is washed with saline and the margins to be sutured are identified**.** Start the suture by reconstructing the posterior wall of the vessel (usually a running suture is advised) (Figure 6). Single stiches must be tied three times. The clips must be removed after the end of the anastomosis and the vascular patency must be tested opening the infusion set.[34]

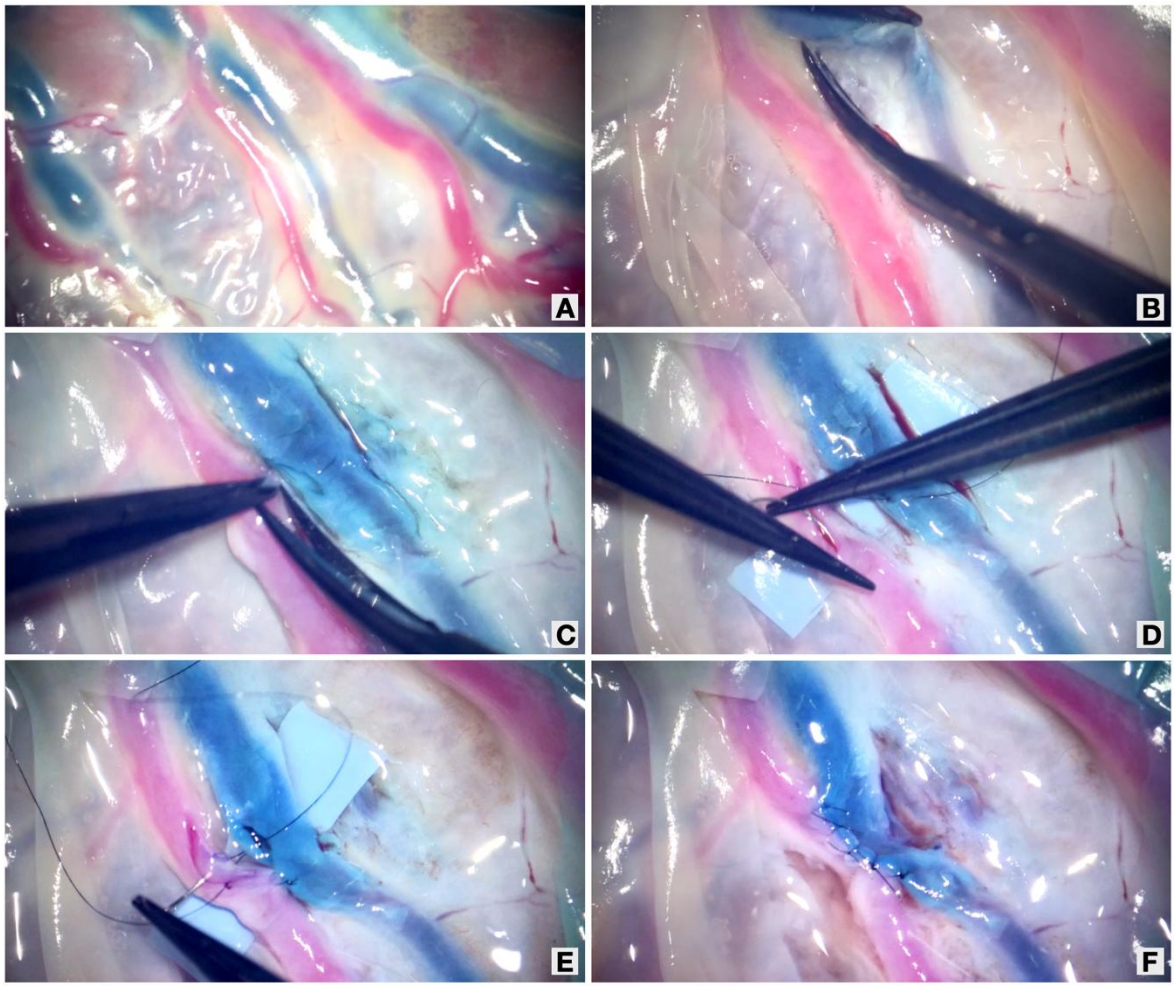


Figure 6. Arterio-venous side-to-side anastomosis. (A) Choice of two parallel vessels with similar diameter (B) Dissection of vessels from the stroma. Venous dissection is more difficult. (C) Cut of the vessel wall with micro-scissor. (D) The first suture is done at lower extremity. (E) Running suture of the back-wall. (F) Final appearance of the side-to-side anastomosis and its evaluation.

4. MATERIALS AND METHODS

This study was performed by the collaboration between the Department of Neurosurgery, the Department of Otolaryngology and the Department of Gynecologist and Obstetrics of the Fondazione I.R.C.C.S. Policlinico San Matteo of Pavia and mainly conducted in the Experimental Laboratory of Microsurgery "Botta 2" of the University of Pavia.

Informed consent regarding the use of placentas for surgical practice was obtained from "appropriate" obstetrics patients with negative blood test for common infections, who accept to donate their placentas before delivery. Only non-pathologic placentas were collected.

4.1 Protocol of human placenta preparation

After delivery, non-pathological placentas were stored in a non-sterile box. In case of night delivery, they were crammed under refrigeration at 3° Celsius (C) and prepared within 24 hours. A step-by-step process is proposed:

Step 1

Placentas with the umbilical cord were cleaned with warm water (almost 36° C) in a specific washbasin to remove superficial blood clots, with special attention to avoid damage to the allantoid membrane, preserving the anatomical integrity of the specimen.

Step 2

Placentas were positioned over a pad to become dry. Then, each placenta was weighted with a common weigher, and each diameter were calculated with a measuring tape.

Step 3

The umbilical cord was cut between 7 and 10 cm from the fetal surface of the placenta. The transversal section of the umbilic al cord was irrigated with saline solution and the umbilical vein, and the two arteries were identified inside the connective tissue.

Step 4

The extremity of the umbilical vein was irrigated with warm saline solution and then cannulated with a 14 or 16 catheter (Abbocath, Abbott, Chicago, IL) with the end catted at 45°. The choice of the caliber of the venous puncture catheter was proportional to the vessels' diameter. The first irrigation was done with saline solution to check the flow inside the vein, if the flow was correct a second irrigation with 1 vial of sodic heparin (5000 U.I./ml, 5 ml) in saline solution (1:4) was done. Then, another preliminary irrigation with warm saline solution was done at the extremities of umbilical arteries to facilitate their cannulation with a 14 or 16 catheter. If the flow was correct again, another second irrigation with 1 vial of sodic heparin (5000 U.I./ml, 5 ml) in saline solution (1:4) was done. This procedure is essential for the preliminary removal of blood clots inside the vascular system.

Step 5

With a 0.0 silk wire, the cannulation system was fixed to the umbilical cord to prevent system pull-out during infusion (Figure 7.1)

Step 6

Continuous infusion with a pressurized system at 70-90 mmHg was done, to obtain a perfect blood clots removal and avoid endangering of vessels wall. This step has a variable duration and the process finish when all vessels are perfectly transparent.

It is possible to perform this step manually, with repeated irrigation using saline solution in almost 20 mL syringe.

Step 7

Removal of excessive saline solution in vessels to avoid over-relaxing of vessels wall.

Step 8

Placentas were stored in sterile plastic bags identified by date of preparation, n. of vessels cannulated, an overall quality of the model (Figure 7.2).

Step 9

Storage in fridge at 3°C (until four days) or in common refrigerator at -18° C (until 40 days). Some placentas were also stored in industrial refrigerator at -80° C (until 40 days).

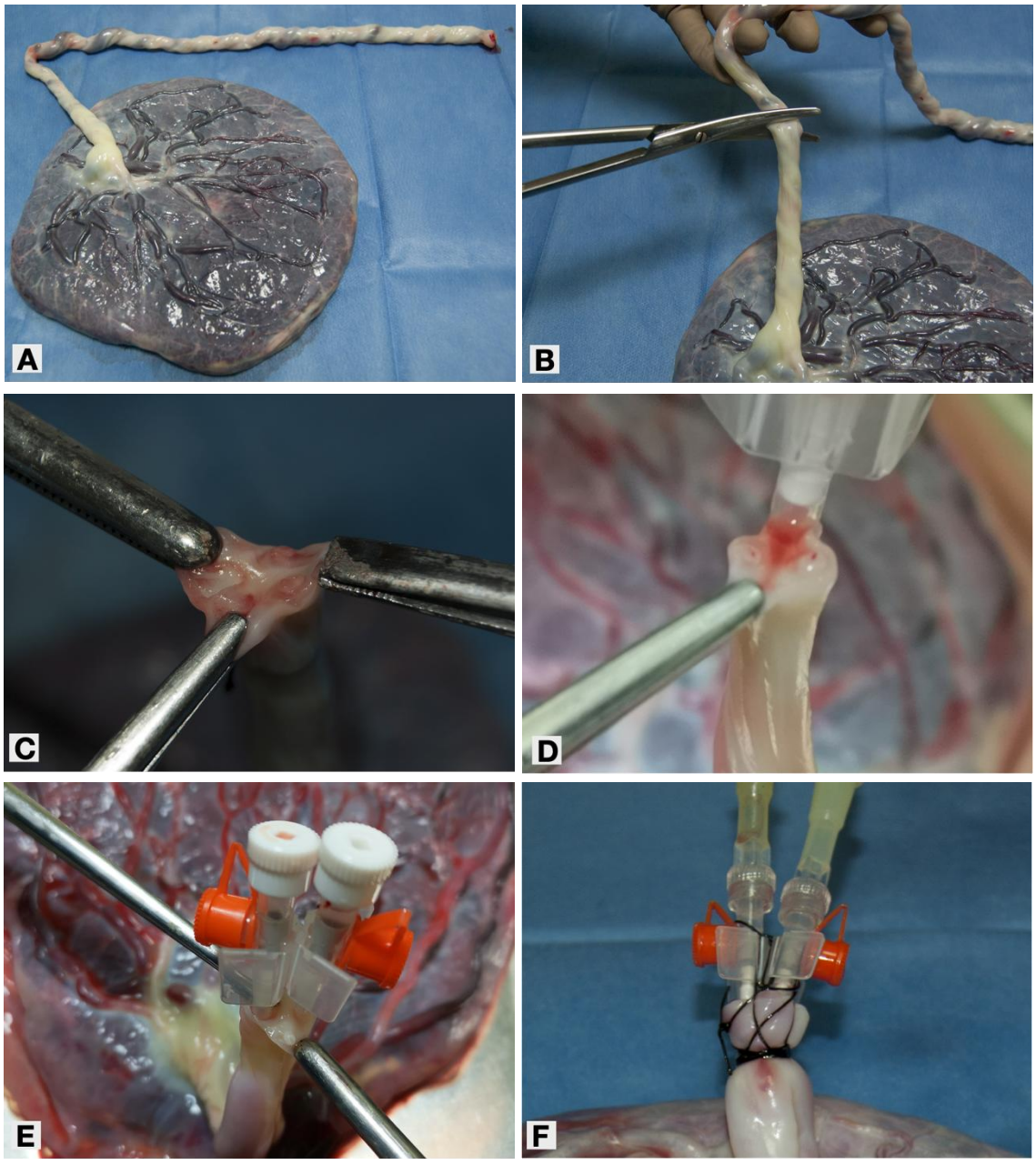


Figure 7.1 Stepwise description of the laboratory protocol for implementation of a color-perfused human placenta model. (A) Fresh human placenta with the entire umbilical cord. (B) Axial cut of the umbilical cord. (C) Identification of the vein and arteries. (D–F) Cannulation of the vein and arteries and locking of the system.

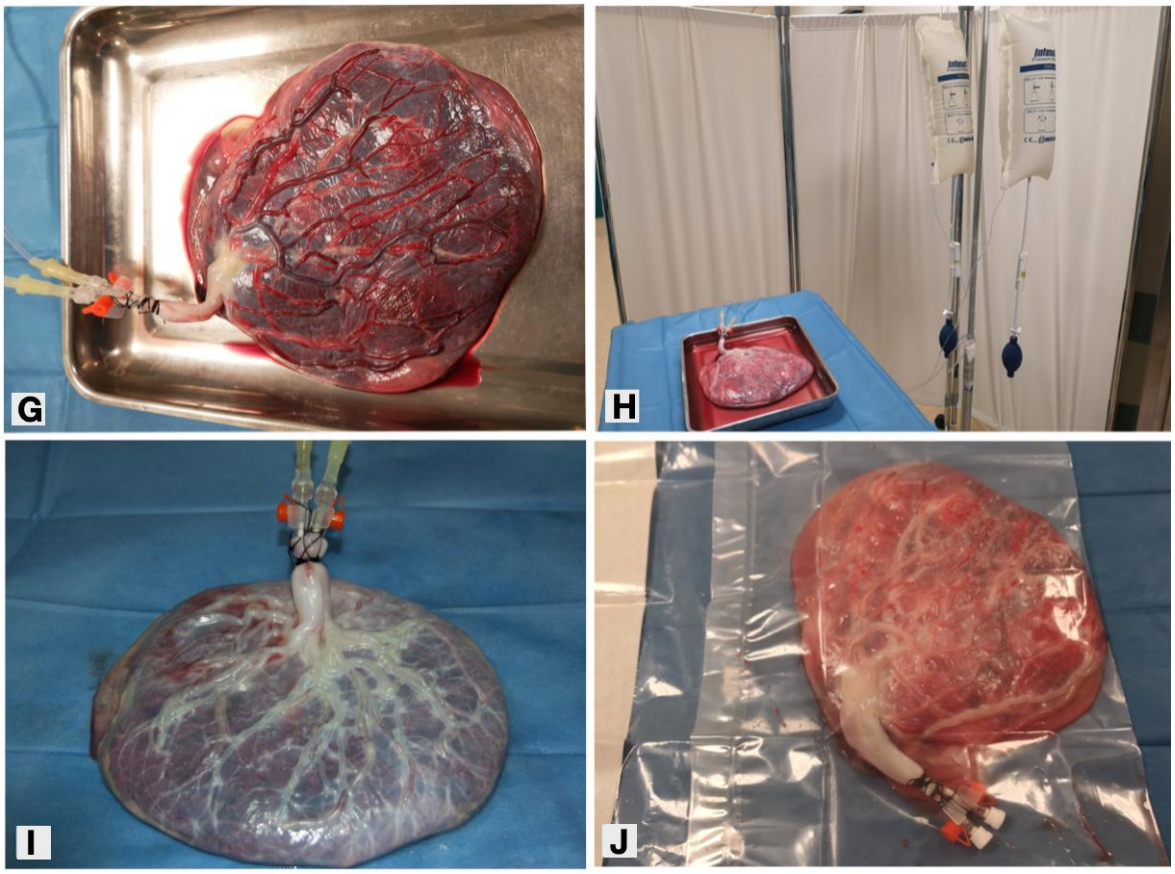


Figure 7.2 Stepwise description of the laboratory protocol for implementation of a color-perfused human placenta model. (G–I) Infusion of saline with the pressurized bag at 70–90 mmHg. (J) Storage bag.

4.2 Protocol of microvascular training model preparation

A step-by-step process is used also to describe the preparation of the dye perfused human placenta microvascular training model:

Step 1

Frozen placentas stored in refrigerator at -18° C were defrosted for 15 hours at 20 – 22 ° C. Rather, the ones preserved at -80° C, were defrosted for 24 hours.

Step 2

Each model was pulled out from the bags and cleaned with warm water.

Step 3

The anatomical integrity of each model was checked together with the position and patency of cannulas at the umbilical cord.

Step 4

The model was dried with pads.

Step 5

The model was placed on a container with a pad ready to use in the training workstation.

Step 6

At the training workstation the placenta was perfused with 2 different infusion sets, 1 for the arteries and 1 for the vein, which released 2 different dyes. Carmine/Cochineal (E120) red colorant (Bioindustria L.I.M., Fresonara, AL, Italy) (3 mL/vial) and methylthioninium chloride blue dye (100 mg/10 mL vial) (Bioindustria L.I.M.) were used for the arteries and the vein, respectively. Both dyes were diluted with saline (1:100) (Figure 8).

Step 7

When the perfusion is completed, and the vessels distension was considered appropriate it has been possible to stop the in-flow and start the training (Figure 9).

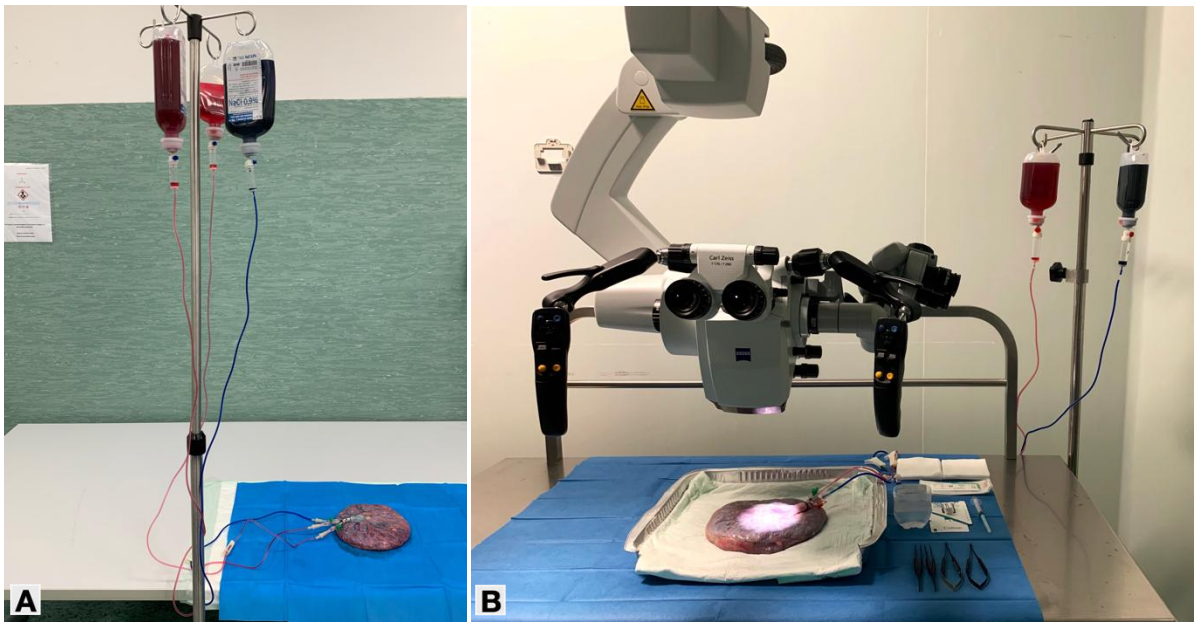


Figure 8. Placental perfusion. (A) 3 vessels perfusion. (B) 2 vessels perfusion at workstation.

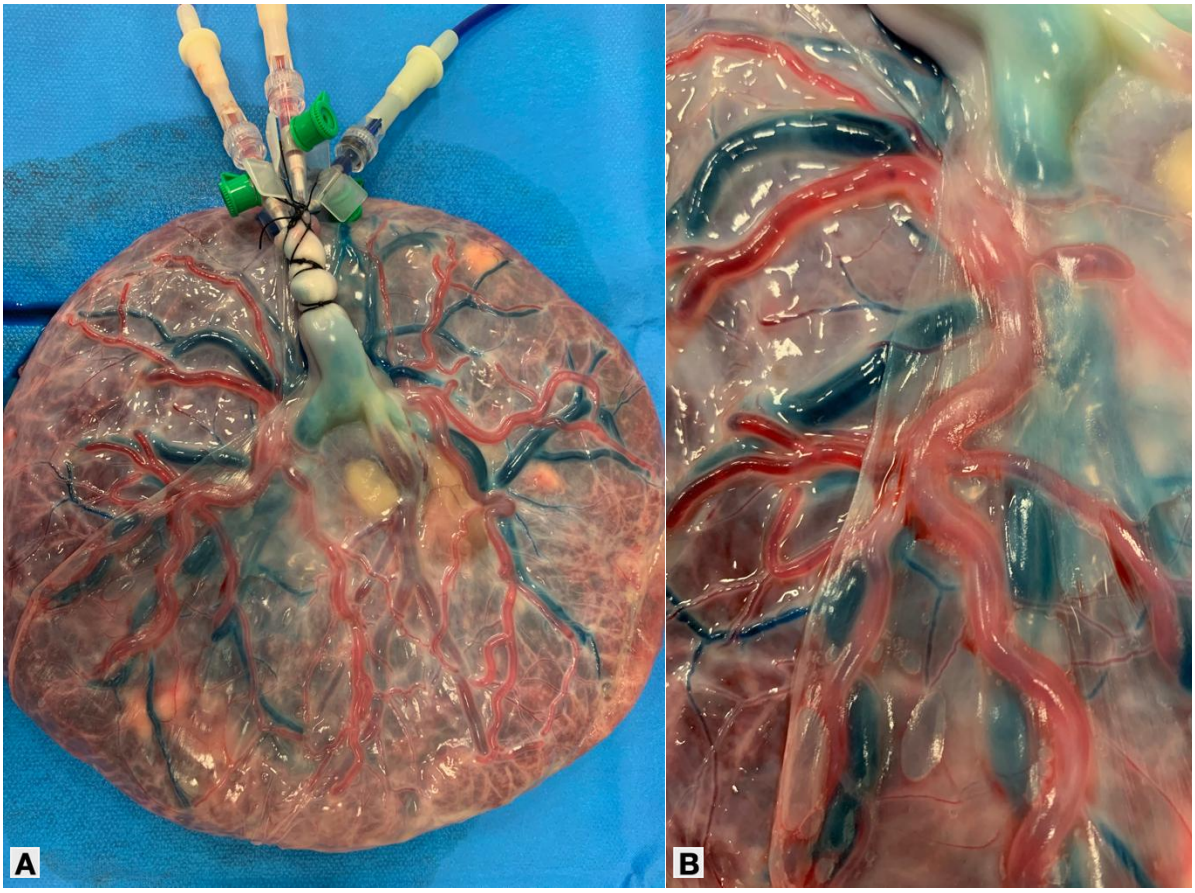


Figure 9. (A) Dye-perfused human placenta model. (B) Arterio-venous detail.

4.3 Set up of training workstation

The workstation was provided with a microscope (Zeiss OPMI pico™ – Oberkochen, Germany) and a drip stand for the infusion set. Each workstation was completed with standard microsurgical instruments: two forceps; straight scissor; needle holder, insulin needle, suture thread 8.0, 9.0 and 10.0; q-tips, vessel micro-approximator, vascular mini clips, saline solution, and small blue rubber leaf (Figure 10).

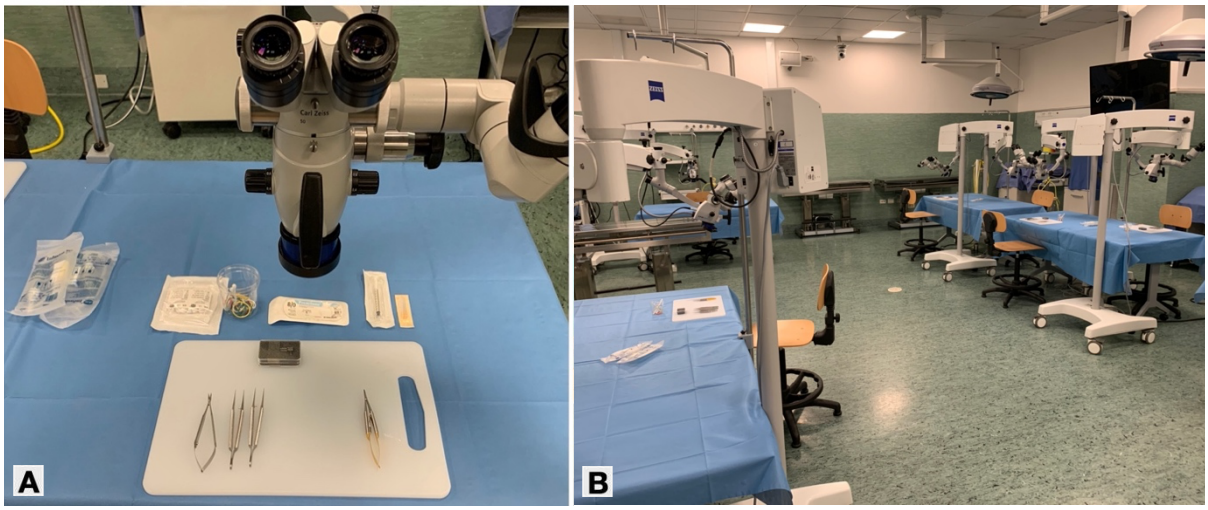


Figure 10. (A) Standard microsurgical training workstation. (B) Laboratory of Experimental Medicine "Botta 2" (Pavia, February 11, 2020).

4.4 Exercises

Four different exercises with a progressive degree of difficulty were commonly performed with the techniques described in the previous chapter (3.3 Anastomosis technique on human placenta vessels). During Courses, each trainee must complete the exercise correctly before performing the next one.

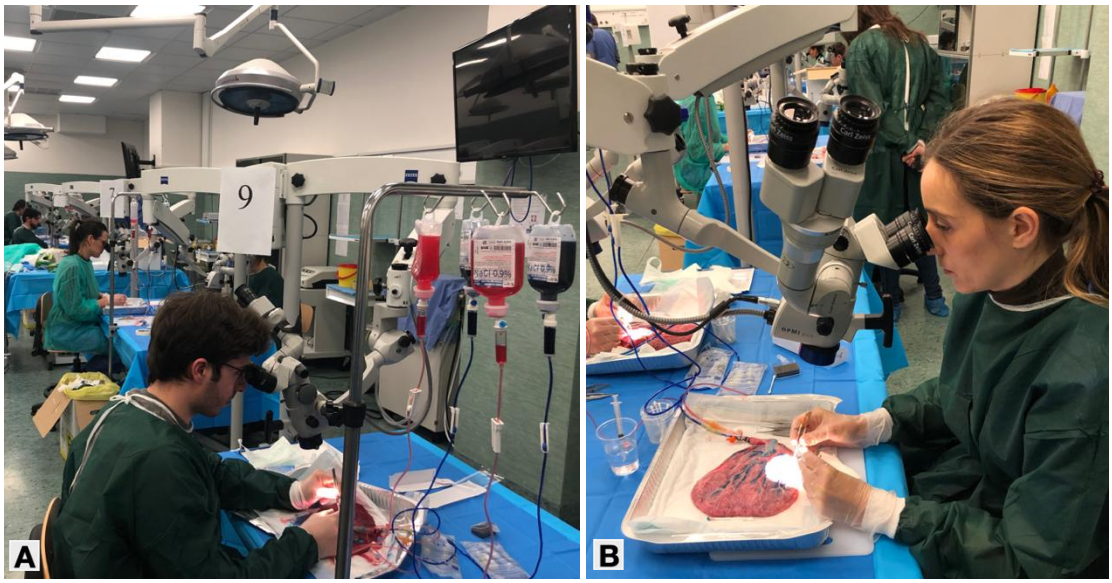


Figure 10. (A-B) Trainee during the 12th Edition of the Hands-on Course of Microsurgical Techniques. Pavia, February 2019.

4.5 Training model usefulness survey

The human placenta model was validated during 4 consecutive editions of a hands-on microsurgical training course held at the Experimental Laboratory of Microsurgery, University of Pavia, Italy, between July 2018 and February 2020. The course objective was to achieve a good level of skill and dexterity in performing microdissection of arterial and venous vessels and to successfully execute the 3 main types of microvascular anastomosis, namely, end-to-end anastomosis, end-to-side anastomosis, and side-to-side anastomosis. The course participants were young attendants (<3 years from the residency) and residents in neurosurgery, otolaryngology, and maxillofacial surgery; a total of 40 participants were enrolled in the courses. No vascular neurosurgeons were involved. Based on their experience and regardless of their specialization, all attendees were assigned to 1 of 3 groups: a low-experience (1st to 3rd years of residency), an intermediate-experience (4th and 5th years of residency), and an advanced group. A single prepared placenta was assigned to each workstation, and participants worked on the same model for 2 consecutive days. Participants were introduced to each exercise in a step-by-step fashion. At the end of each course, an on-line survey-based questionnaire, using the platform SurveyMonkey®, was administered to each participant. The survey specifically included 5 consecutive questions aimed to assess the validity and reliability of the color-perfused human placenta model for vascular microsurgical training. The survey questions about the reliability of the model and the relative possible answers are reported in Table 2. Differences in categorical responses between the 3 groups were analyzed using a c2 test using commercially available software (STATA 16, StataCorp LLC, College Station, TX).

Table 2. Survey Questions

Question n.	Question	Possible Answers
1	Do you think that the proposed training model faithfully reproduces a possible real microsurgical scenario?	"absolutely yes" "somewhat", "absolutely no"
2	According to your surgical experience, is the consistency of the placental vessels comparable to the one of the in-vivo tissues?	"very similar," "quite similar", "different"
3	Do you think that the dissection of human placenta vessels is similar to in-vivo vessels dissection?	
4	Do you think that practice on this type of surgical training model can improve the surgical technique and reduce errors on the patient?	"absolutely yes" "somewhat", "absolutely no"
5	Do you think you will reuse or propose to use of this microsurgical training model?	"yes", "no"

5. RESULTS

5.1 Human placenta preparation

Forty of 45 placentas were considered suitable for microsurgical model. Three frozen placentas were barred because of allantoid damage and two were excluded because of leakages at the proximal segment of the umbilical cord for arterial damage. Ten placentas were prepared after 24 h from delivery. The average weight of the selected 40 placentas was 480 gr and their mean diameter was 17,5 cm. They were all characterized by an approximately central position of the umbilical cords' attachment. Transverse section of the umbilical cords showed in all cases the presence of two arteries and one vein. In every placenta, the arteries could be easily distinguished from the veins because at the crossing point the former were superficial. Five placentas presented small and fragile umbilical cord arteries. In three of these cases arterial cannulation was done with a 20-gauge catheter, while the other one was solved cannulating more proximal to the attachment to the umbilical cord

to the placenta surface. However, the latter solution created the problem of a slight reflux of the dye used. In consideration of the vessel fragility of these 4 human placentas, they have been infused with a pressurized system at 40-60 mmHg instead of 70-90 mmHg. Almost one vein and one artery were cannulated in all placentas. Eighteen human placentas were stored in the fridge at 3 °C and were used within 4 days. The remaining placentas were freeze, 10 of these were used 30 days later. At the thawing time, in 24 placentas the allantoid membrane was perfectly preserved.

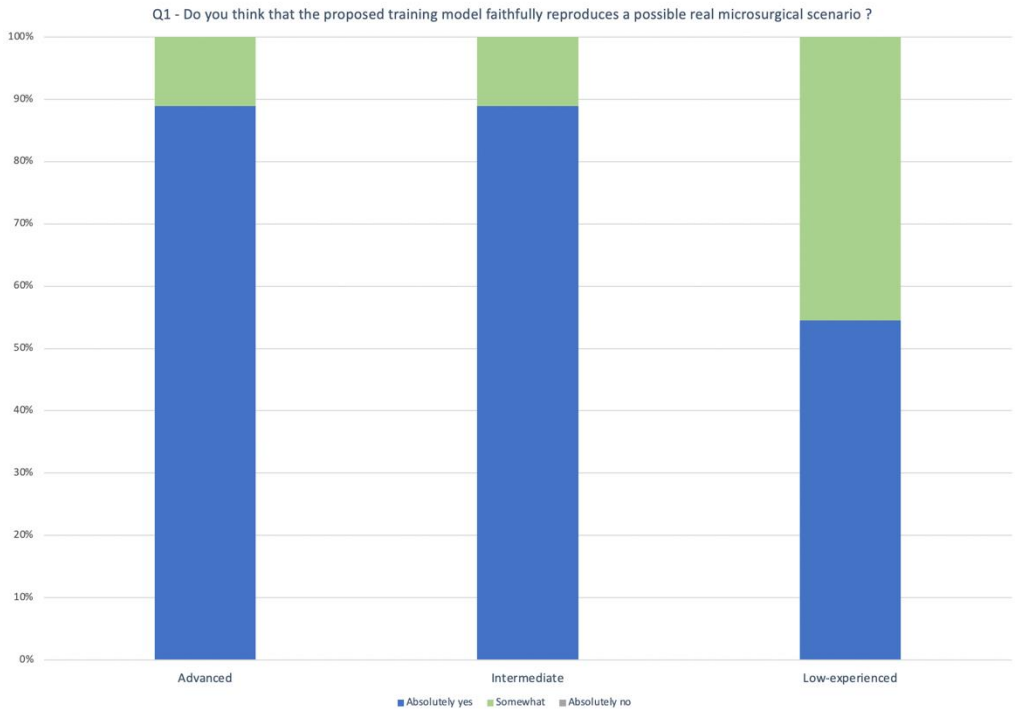
5.2 Groups demographics

The trainee cohort of the four courses consisted of 40 surgeons of different branches: specialists and residents of Neurosurgery, Otolaryngology and Maxillofacial surgeons. The mean age was 31,06 ranging from 27 to 43 years.

Twenty participants were neurosurgeons of which 16 residents (5 of 1st year, 3 of 2nd year, 5 of 3rd year and 3 of 4th year) and 4 specialists. Ten participants were Otolaryngology of which 3 residents (2 of 3rd year and 1 of 4th year) and 7 specialists. Ten participants were maxillofacial surgeons of which 6 residents (3 of 3rd year and 3 of 4th year) and 4 specialists. All attendees were further assigned in three groups based on their experience regardless of specialization. The group of low-experienced consists of fourteen residents (1st, 2nd, and 3rd year). The intermediate-experience group consists of eleven residents (4th and 5th year) whereas the advanced group comprises 15 specialists.

5.3 Survey

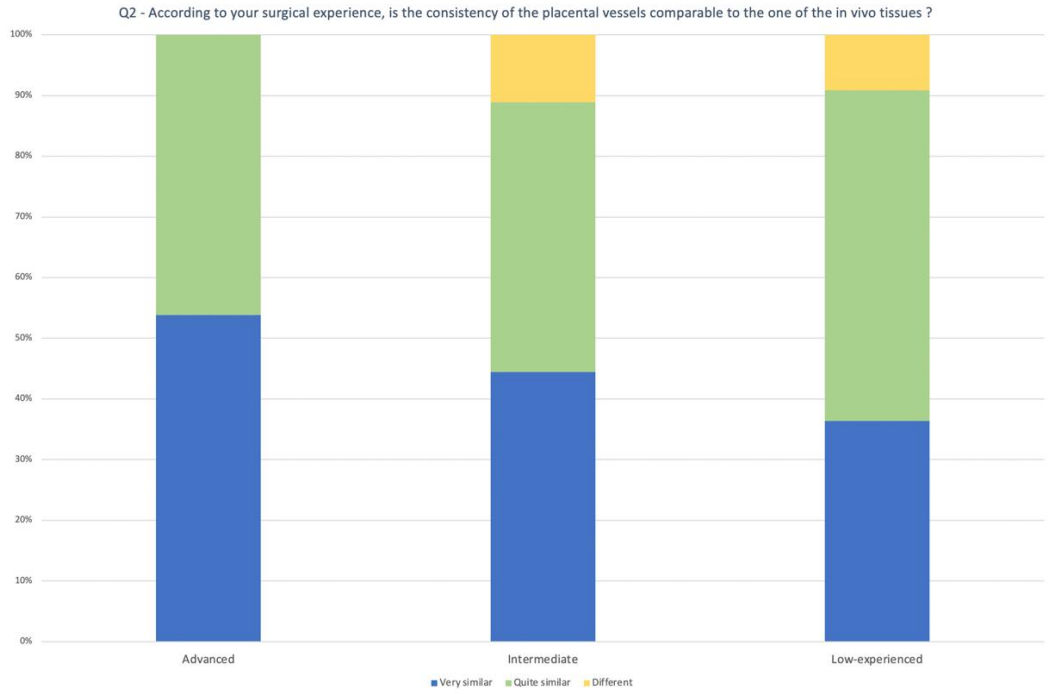
The aim of the survey was to assess the quality of the proposed model and to validate it for microsurgical training. Of the 40 participants, 33 completed the questionnaire of which 13 of the advanced group, 9 of the intermediate-experience and 11 of the low-experienced one. To question n°1, regarding the possibility to reproduce a real surgical scenario thanks to this model, 11 of 13 advanced (89%), 8 of 9 intermediate experience (89%) and 5 of 11 low-experienced (59%) answered "absolutely yes". The remaining 8 participants (24%) indicates that the model "somewhat" replicated real surgery. The difference between the three groups was not statistically significant (Pearson $\chi 2$ (2) = 4.0954, Pr = 0.129) (Graph Q1).



Graph Question 1

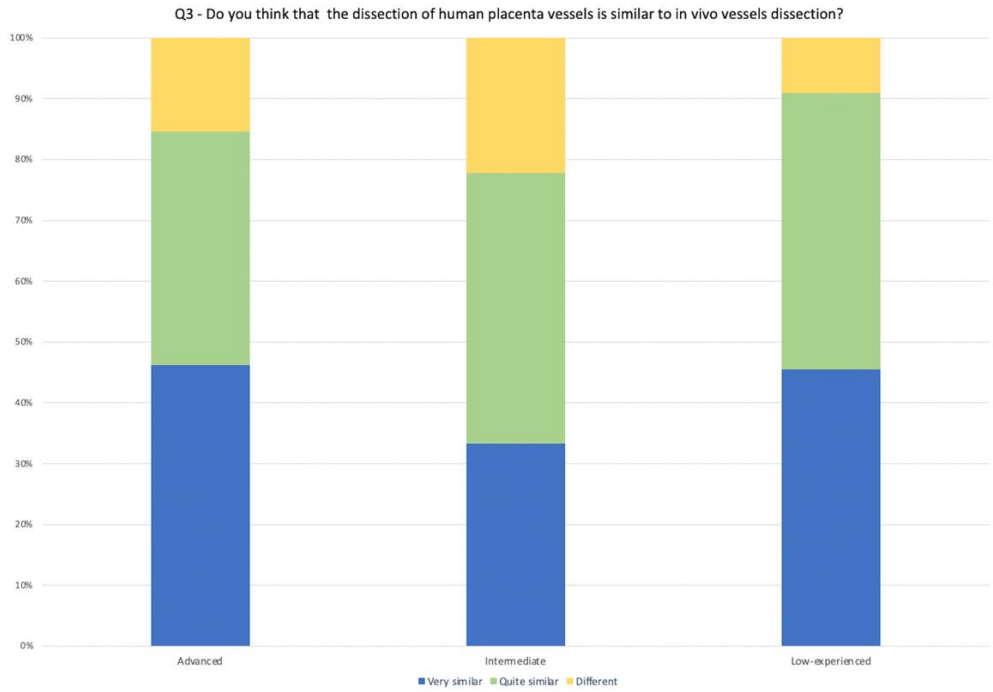
Regarding the comparison between the consistency of the placental model tissues with those in vivo (question n°2), 7 of 13 advanced (54%), 4 of 9 intermediate (44%) and 4 of 11 (36%) of low-experienced replied "very similar" while 16 participants (48,5%) answered "quite11 similar". One of the low-experienced and one of the intermediate groups (6%) replied

"different". The difference between the three groups was not statistically significant (Pearson $\chi 2$ (4) = 1.8650, Pr = 0.761) (Graph Q2).



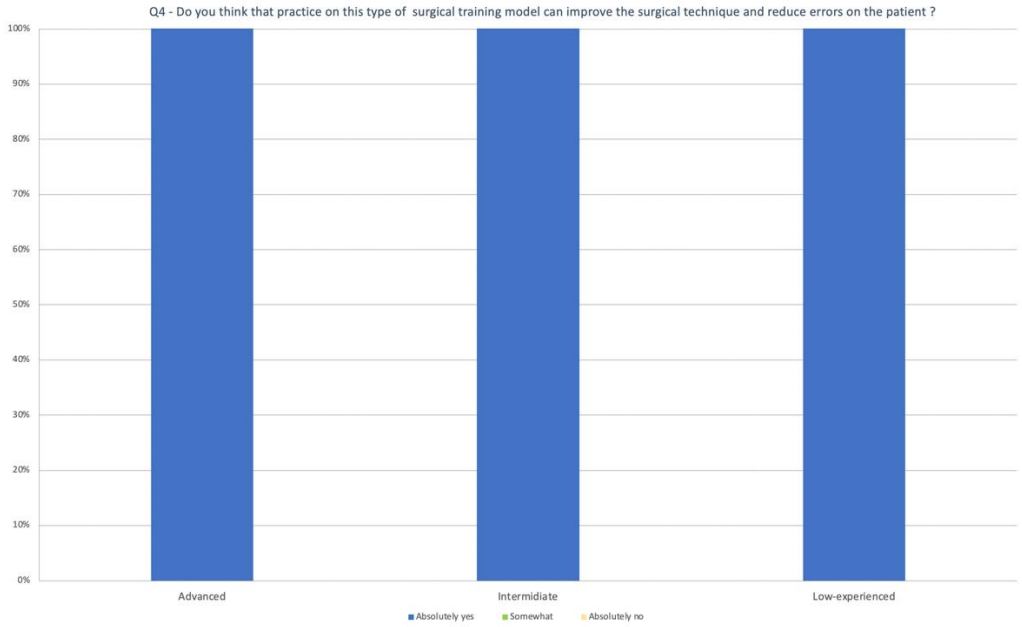
Graph Question 2

To question n°3, concerning the comparability between placental vessels and in vivo vessels dissection, 6 of 13 advanced (46%), 3 of 9 intermediate (33%) and 5 of 11 (45%) of low-experienced answered "very similar". The answer "quite similar" was selected by 14 attendees (42%) and five participants (15%) replied "different". The difference between the three groups was not statistically significant (Pearson $\chi 2$ (4) = 0.8864, Pr = 0.926) (Graph Q3)



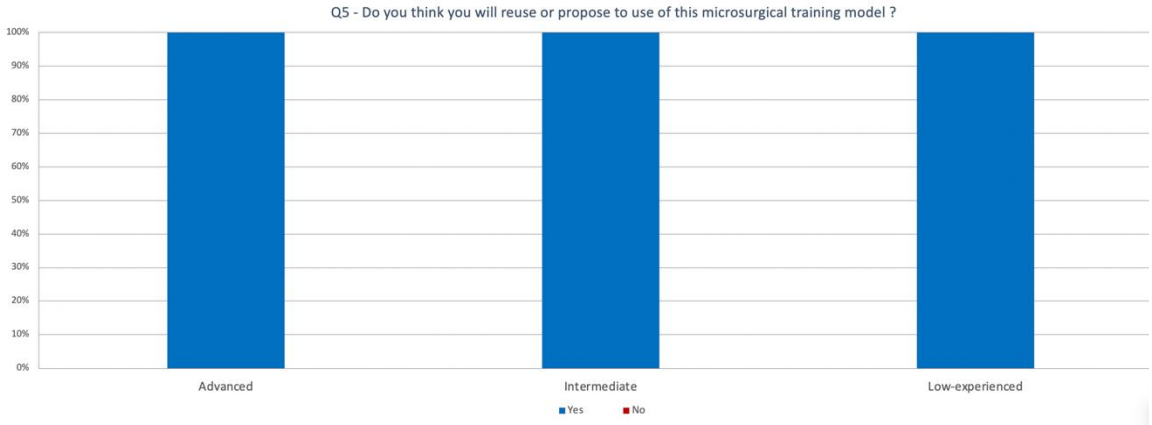
Graph Question 3

All participants (100%) answered “absolutely yes” to question n° 4 about the impact on the improvement of the surgical technique training on the proposed model (Graph Q4).



Graph Question 4

Lastly, all participants (100%) replied “yes” to question n° 5 regarding the possibility to reuse or propose to use this microsurgical training model (Graph Q5).



Graph Question 5

6. DISCUSSION

In 1979 Goldstein introduced the idea that the human placenta can be used as a microsurgical training model.[3] Thereafter, several authors have begun to highlight the properties of the novel proposed ex-vivo model: easy to find, low cost and free from ethical implications.[4] The growing interest in the development of microsurgical laboratory courses as a crucial part of educational programs and the continuous search for complementary models to animal ones, has allowed the diffusion of the human placenta in microvascular lab activities.[11] Starting from 1983 several authors mastered the study of the anatomy of the human placenta, analyzing the diameter and composition of its vessels, to validate the efficacy and the realism of the placental training.[32,33,2] In the following years, different studies were developed to ameliorate such ex vivo model, enhancing advantages and approximate it to in vivo models.[31,35,2] Detailed descriptions of microsurgical exercise on placental vessels are well

represented in the literature (Table 1). However, an accurate protocol of preparation, storage and dye-perfusion is not reported.

We developed a specific protocol, improved, and validated during four hands-on microsurgical training courses (From July 2018 to February 2020), set up with the collaboration between the Department of Neurosurgery, the Department of Otolaryngology, the Department of Gynecologist and Obstetrics of the Fondazione I.R.C.C.S. Policlinico San Matteo of Pavia and conducted in the Experimental Laboratory of Microsurgery "*Botta 2*" of the University of Pavia.

The protocol allowed us to reduce the placenta packaging time, achieving an average preparation time of 20 minutes. This was especially important during the courses in which it was necessary to prepare many placentas. Furthermore, the step-by-step preparation and storage protocol allowed to reduce staging errors, thus avoiding wasting human placentas. Very important is to emphasize the reproducibility of the model that can be taught and learned easily.

The experience gained during these years, has allowed us to verify that the placenta remain excellent for use even if it has been frozen. The placenta thawing must be taken place for almost 15 hours, to obtain the right consistency of the tissues. It is possible to use the same placenta for 4 days,[31] however, to ensure better quality, the participants of the courses practiced on same placenta for two days, trying to perform vessels dissection and all types of anastomoses (end-to-end, end-to-side, side-to-side). Since placental vessels are thinner than those of the human head and neck region, due to the absence of an intern elastic lamina,[2] some participants found it more difficult to execute anastomoses. We have observed that, a very important step, to correctly complete the exercises, is the choice of the "working zone". In 2018 M.M. de Olivera et al. compared the human placenta to a clock with the vein and the umbilical cord pointing to 12 h and 6 h respectively. Each artery was named with the letter A, followed by the letter L or R depending on left or right direction. The next symbol

was a number corresponding to the level of bifurcation (1 to 4), relative to the central vein. The letter V was assigned to the veins, followed by the number indicating the bifurcation, while the space between one bifurcation and the other was called the "working zone".[39]

The tortuosity and immersion of the vessels in the underlying stroma also play an unfavorable role in the execution of the dissection. The natural arborization and the different diameters of the placental vessels makes this model particularly suitable to perform all the proposed exercises with different grade of difficulty. Perfusion of the placental vessels played an important role in verifying the tightness of the vascular anastomoses performed by trainees. The use of red and blue dye simplifies the identification of arteries and veins respectively, however in case of unintentional damage of vessel's wall the spreading of the dye, especially the methylthionine can mess up the sample. So that, during the last course, we decided to use only the red dye for arteries. It must be stressed that the perfusion system doesn't allow the pulsatility of the vessel wall, and this is one of the gaps with in vivo model.

The large number and dimensional variability of the placental vessels grant to perform any type of anastomosis, having the opportunity to do many practices improving progressively the surgical technique. The placenta microsurgical training model falls within a multi-stage learning paradigm. All exercises performed on the placenta model are naturally propaedeutic for the execution of anastomosis in rats and others in-vivo models. The high cost and the ethical impact regarding these models reduce the chance of mistakes on them, therefore, to be considered the last step in the microsurgical training.

To estimate and validate the potentials of this model, we created a questionnaire. Face and content validity were outlined by the participants concerning the usefulness in practicing on this model to improve microsurgical skills. Face validity is a subjective measurement used to determine whether the model provides the reproduction of a work environment like that of a surgical scenario in head and neck region. Content validity is a subjective measurement that relates to whether the model can teach participants about microdissection and vessel

anastomosis. The results of the survey, even if not statistically significant, show how the proposed micro-surgical training model was appreciated by all participants, coming from different specialties (Otolaryngology, Maxillo-Facial surgery and Neurosurgery). For most of the specialists and residents, vessel anatomy of this model reproduces vessels anatomy of different body districts and they found that this model is certainly useful to improve microsurgical skills. Focusing on neurovascular surgery, the development of simulation model to import in neurosurgery training program is of paramount importance in the current era of decreased exposure to open vascular cases. Residents and young neurosurgeons must perform lots of lab activities on anastomosis training models to deal at the best with aneurysms, vascular arteriovenous malformations as well as all pathologies requiring a by-pass procedure.

Despite the listed advantages of this model, there are still limitations. First, the absence of real blood implies that the influence of coagulation cascade cannot simulate the formation of blood thrombus after the anastomoses. Furthermore, the absence of natural vessels pulsation does not allow to verify the tightness of the anastomoses in a realistic way. Last, the proposed model offers to work only in a superficial field, so it is necessary to combine it with other simulation tools (*e.g.* 3D printed skull, glass) or with another placenta to generate a more difficult surgical scenario in deeper and narrow spaces. Among the limitations of this research, it must certainly be emphasized the short duration of our experience, the few numbers of placenta models prepared, and the small cohort of survey participants do not allow a statistically significant analysis. Then, we need to applicate rating scales and an evaluation of the exercises performed that allow an objective examination of the teaching efficacy of this training model.[37,38]

The described experimental model, designed at the University of Pavia, had also national and international resonance. In December 2019, neurosurgical residents of the University of Zurich came at the Experimental Laboratory of Microsurgery “Botta 2” to study the model

and to train themselves. Moreover, despite the blocking of live courses caused by the SARS-Cov-2 pandemic, in October 2020, the dye-perfused human placenta was used during a hands-on course held at the Institute of Anatomy of the University of Turin, to perform vascular anastomosis with the Exoscope (Aesculap AEOS® Robotic Digital Microscope) (Figure 11). Lastly, in September 2021, the model was used both in microscopic and exoscopic workstations, during a microsurgical training course for young neurosurgeons organized by the University "La Sapienza" and hosted at the S. Andrea Hospital in Rome. (Figure 12).

Figure 11. Arterial end-to-end anastomosis in Exoscopic view.
Institute of Anatomy. Turin, October 7, 2020

Figure 11. Arterial dissection in Exoscopic view.
Sant'Andrea Hospital. Rome, September 23, 2021

7. CONCLUSION

The human placenta is technically excellent for microsurgical vascular laboratory training. Its low cost, availability, ease of preparation, ethical acceptance, and strong vascular resemblance to other anatomical structures make it a valuable model. The quantity and variability of the placental arteries and veins allows a prolonged and complete training. Due to the possibility of choosing exercises with different degrees of difficulty, this model perfectly matches the philosophy of microsurgical practice. It can be considered a real training ground for the microsurgeon, propaedeutics, and in some cases an alternative, for in vivo models.

Adhering to the proposed preparation and storage protocol allows the use of such model in hands-on courses. Dye-perfusion of placental vessels enhances the overall reliability of this

model, which has proven to be a worthwhile training tool by most attending trainee, regardless of their experience and their field of interest in microsurgical procedure.

Efforts to implement peristaltic perfusion and further validation studies, based on quantitative rating scales, are both necessary to increase the reliability of the dye-perfused placenta model.

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All pictures of this research are original and produced by the author Mattia Del Maestro.