Peter Girman · Jan Kriz
Peter Balaz *Editors*

Rat Experimental Transplantation Surgery

A Practical Guide

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Preface

Transplantation medicine is a rapidly developing field comprising progress not only in surgical techniques but also in research of new immunosuppressants, technologies, biological agents and tissue engineering. Taking into consideration the abovementioned fact, many researchers interested in experimental transplantation are not trained in surgery, even though they very often perform surgical experiments. Moreover, it is becoming more frequent for medical graduates preparing at transplantation surgery to start their career with an experimental work. For all those, we try to write a practical guide, describing surgical procedures of single organ transplantation amended with tips and tricks written by experienced authors. Rats were used as an experimental animal model, which are well accepted for transplantation research for many reasons (well-described genetic conditions, physiological and pathological behaviour). The procedures are written in "step-by-step" fashion, with detailed photos and diagrams. All contributors are skilled senior researchers, with long-term practical experience in experimental and clinical transplantation. We hope that this book will be helpful not only for young colleagues to avoid mistakes and so improve their results in experimental transplantation but also for skilled researchers to expand their view in this interesting scientific field.

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Chapter 1
Laboratory Rats: Anatomical and Physiological Notes

Ivan Misek

Abstract The laboratory rat was domesticated and bred from initially wild-ranging brown rats. While it is not clear when and where this process occurred, it is obvious that this animal was used as an object for experiment as early as in Egypt, although the first documented reports come from Greece, about 250 BC, when Aristotle conducted autopsies in dogs. A sharp increase in experimental work with animals occurred after World War II as a result of sprouting military industry and big capital projects in pharmaceutical industry and biomedical research. In the 1930s, there were three trends in the management of experimental animals. Firstly, there was a tendency seeking to produce experimental models at the level of tissue and cell cultures. Secondly, introduction of new species was underway. The last trend was the development of inbred (blood relatives) and outbred (unrelated by blood) strains, whilst working to improve the overall health condition of the animals. The chapter describes briefly history of rat as an experimental animal and more detailed anatomy of single systems.

Keywords Rat • Anatomy • Cardiovascular system • Gastrintestinal system • History

1.1 Introduction

The laboratory rat was domesticated and bred from initially wild-ranging brown rats. While it is not clear when and where this process occurred, it is obvious that this animal was used as an object for experimenting as early as in Egypt, although the first documented reports come from Greece, about 250 BC, when Aristotle conducted autopsies in dogs. A sharp increase in experimental work with animals occurred after World War II as a result of sprouting military industry and big capital projects in pharmaceutical industry and biomedical research. In the 1930s, there were three trends in the management of experimental animals. Firstly, there was a

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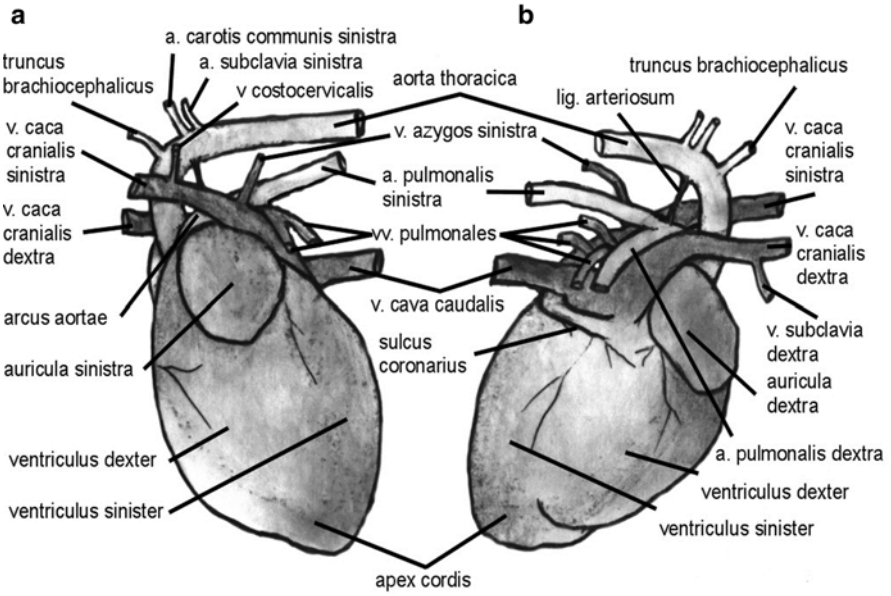

Fig. 1.1 Heart from left site (**a**) and right site (**b**). *a* artery, *lig* ligament, *v* vein, *vv* veins

tendency seeking to produce experimental models at the level of tissue and cell cultures. Secondly, introduction of new species was underway. The last trend was the development of inbred (blood relatives) and outbred (unrelated by blood) strains, whilst working to improve the overall health condition of the animals. In 1982, the brown rat was one the most exploited species amongst laboratory animals (39 % compared to 54 % in mice) [2] (Figs. 1.1, 1.2, 1.3, and 1.4).

Sometimes incorrectly referred to as black rats or albino black rat in the Czech language, laboratory rats are usually an albino form of the brown rat *(Rattus norvegicus varietas alba*). The laboratory rat was domesticated and bred from initially wild-ranging brown rats (*Rattus norvegicus norvegicus*). Brown rats came into Europe from East Asia in the same way as did the black rat, i.e. with the assistance of humans. For wild-ranging brown rats, sewers, places under the ground, as well as commercial premises and human dwellings form the habitat. Unlike the black rat, brown rats become faster adapted to laboratory conditions. In this country, brown rats occur throughout the territory. Easily confused with black rats, with this species however ranging locally only in north-western Bohemia, particularly in the region of Polabská nížina (the Elbe Basin), brown rat's most famous groups are three strains from which most of the other strains evolved by crossing: the Wistar rat (Philadelphia; W), Sprague Dawley (Madison, Wisconsin), and Long-Evans (LE), the last mentioned differing with its black-coloured head, neck, and the dorsal part of the body, the "hood" (often incorrectly called "capuchins"). Strains, however, may feature essential differences between each other [6].

Brown rats are omnivores, hunting even other smaller animals. Their body length being about 21–27 cm, the bare squamous tail measuring 17–23 cm, brown rats are utilised as experimental animals in labs in several forms, of which pure white with

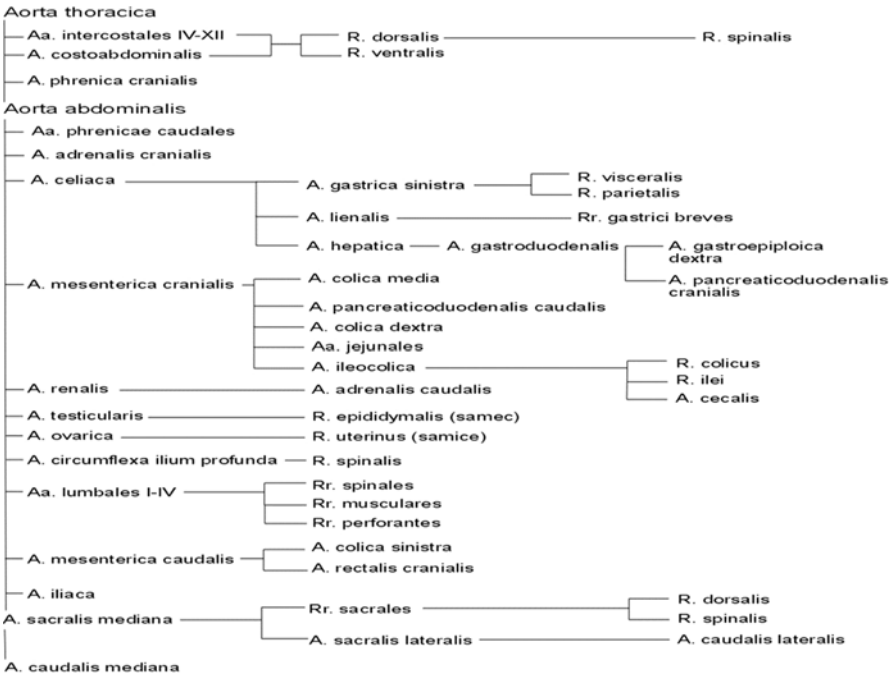

Fig. 1.2 Table of branches of thoracic and abdominal aorta. *A* artery, *Aa* arteries, *R* branch, *Rr* branches

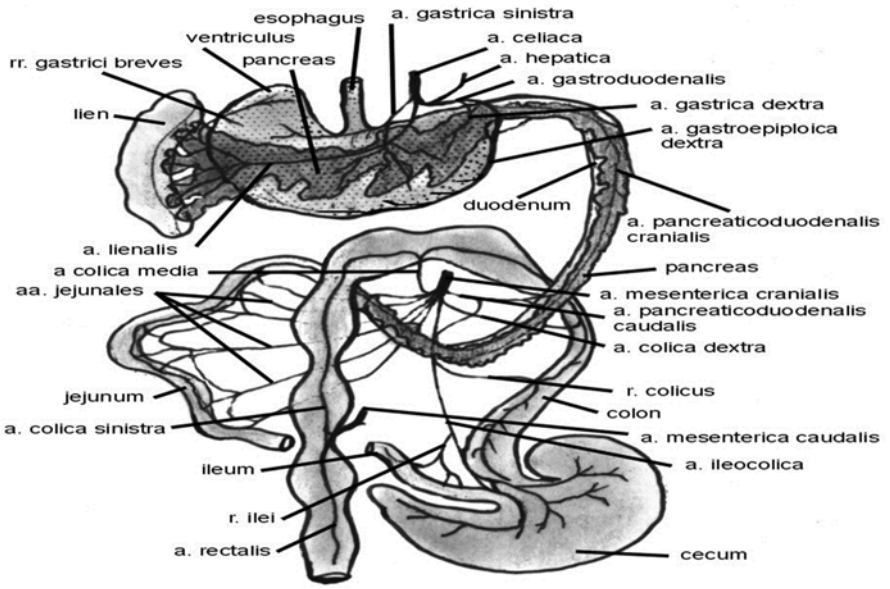

Fig. 1.3 Scheme of gastrointestinal tract from dorsal site. *a* artery, *aa* arteries, *r* branch

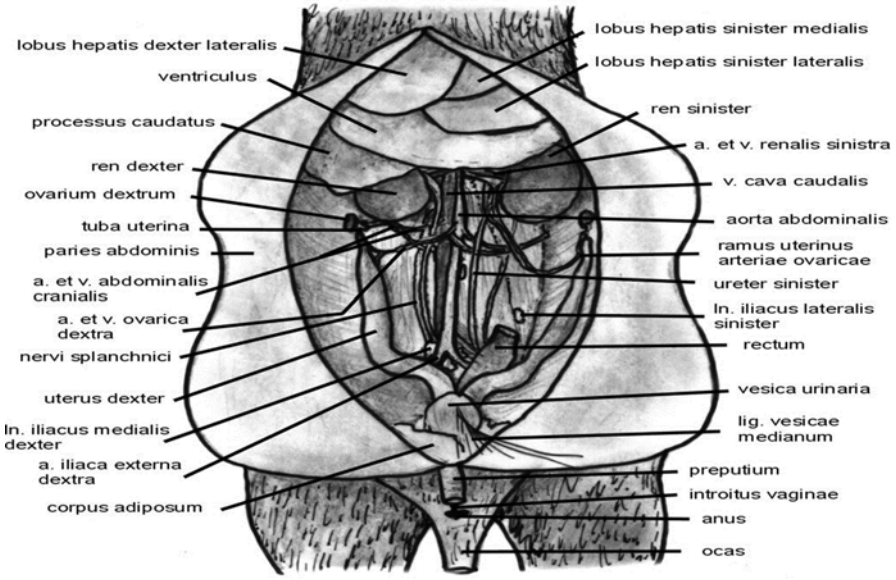

Fig. 1.4 Arteries and veins from dorsal site of abdominal cavity. *a* arteries, *lig* ligament, *ln* lymph node, *v* vein

red eyes is the most common, followed by the black or yellowish-white version with black eyes. The hallmark of laboratory rats is their tameness and low biological variability. Unlike mice, brown rats are more resistant to infectious diseases. Under good welfare, their lifespan can be about 3 years. The weight varies according to diet, adult animals being able to reach 400 g or more. They reach sexual maturity in 3 months.

1.2 External Description and Determining the Age of Laboratory Rats in the Postnatal Period

Laboratory rat's body is elongated with a relatively small rostrum-shaped head. The body is covered with long stiff hairs except the nose, tail and load-bearing pads on the limbs. In albino strains, eyes possess an iris of pink colour, with eyelids clearly developed. The body is elongated in the movement, while bow-bent in the rest position. The tail is long (approximately 85 % of the body length), covered with horny scales under which there is a cover of sparse and indistinctive short hairs. Under the root of the tail there is anus. In males, there is scrotum at the tail root, which contains loosely placed testicles that may be present even intra-abdominally. Cranially before the scrotum, there is raised *ostium preputiale*, where one can palpate fine *preputium* and a penis in it. In females, there is a vaginal entry with a faint *vulva* anterior to the anus. Close below its ventral commissure, there is evident preputial

sac with a distinct *ostium preputiale*, where one can pull a *clitoris.* On the basis of *clitoris*, there is an opening of *urethra* into the preputial sac. The terminal of the urinary tract in females is thus separated and even more clearly isolated from the genital organs than in laboratory mice. At the base of the trunk in lactating females, there are very well noticeable six pairs of milk nipples ventrally in the thoracic, abdominal and groin regions. The thoracic limbs are shorter and weaker than the pelvic limbs. They are used, among others, for digging, holding food and wiping the hair on the body. The thoracic and pelvic limbs have five fingers/toes. Both palmar and plantar section of the autopodium of the limbs are bare.

At birth, the newborn is bare. Palpebral fissures and external auditory canal are closed with an epithelial plug. Teeth are not yet erupted. Hair growth begins on day 2 to day 3. On day 4, the external auditory canal opens. On day 8 to day 10, incisors erupt. Eye slits open between day 10 and day 17. On day 12 to day 14, the young animal starts to eat solid food. On day 18, both upper and lower M1 erupt, followed by M2 on day 21. This is the time of weaning. M3 erupt on day 35. The initial coat is replaced in females on day 36 to day 38, whilst in males this occurs on day 36 to day 40. Around day 40, the process of descent of the testes is complete. Between day 35 and day 45, females begin their first ovulation. On day 42, the juvenile is fully independent. Sexual maturity for both sexes is reported to arrive within day 35 to day 90 of postnatal development. On day 40 to day 70, the epithelial plug of the vagina disappears in females. Laboratory rats reach the adult size on month 4 to month 6 [3, 6, 10].

1.3 Blood System and Anatomy of the Heart

Cor – located rather to the left of the median plane ventrally at the level of rib 3 to 6/7. The tip can be achieved in interchondral space 6. The size is about 2 cm per 1 cm. The lateral projection of the heart reaches cranially to the caudal margin of *costa III*, ventrally to the sternum and dorsal-caudally to the caudal periphery of *costa V* and its cartilage. In its dorsoventral projection, the heart extends cranially to the plane between the caudal margin of Th4 and the middle part of the second sternebra. Caudally, its extension is defined by the plane lying between the caudal margin of Th8 and the caudal end of the fourth sternebra. *Apex cordis* is directing slightly to the left. The brown rat heart weight varies from 0.9 g (for body weight of 200–300 g) as much as 1 g (body weight up to 450 g) in males, whilst in females the range is from 0.67 g (body weight 170–200 g) to 0.8 g (body weight of up to 280 g). Out of the total weight of the body, the heart makes around 0.289 % [3]. The heart structure is generally the same as in other placental mammals, with just a few minor differences to be noted. Right *v. cranialis cava* enters *sinus venosus* of the right atrium craniodorsally (*v. cava cranialis* is doubled in the brown rat), *v. cava* caudally, left *v. cranialis cava* from the left and two cardiac veins from the right, ventrally. *Sinus venosus* is separated from the atrium by two valves formed by the muscle of the *sinus* wall. Ventricles stretch almost equally to the tip of the heart.

Papillary muscles of the left ventricle are represented by two strong strips of muscle that extend, from the cranial and caudal views, from the lateral wall of the ventricle. Right ventricle papillary muscles are usually made only as a conical ridge. In the walls of both ventricles there are numerous *vv. cordis minimae*, the occurrence being greater in the right ventricle. In the right ventricle, they are usually found in the interventricular septum, while in the left ventricle they are enclosed in the base of the papillary muscles. Cardiac ganglia occur in the region surrounding the terminal of the aorta and *truncus pulmonalis*, and then around the opening of venae cavae and in the wall of the atrium. The atrioventricular node is located in the interatrial septum near the right atrioventricular valve. The accessory atrioventricular node is normally present near the beginning of the aorta. The horseshoe-shaped sinoatrial node is located at the entrance of *v. cava cranialis*. A part of the fibrous skeleton of the heart is adjacent to the atrioventricular node; bypassing the right atrioventricular orifice, it runs between the opening and the aorta to form a complete fibrous ring around the left atrioventricular orifice. Subsequently, the atrial muscular system plunges into the fibrous base. The fibrous ring near the aorta contains cartilage, which may partly become calcified at month 6, this being furthered as the animal is getting older. Blood supply to the heart is one of a double system, meaning that in addition to the coronary arteries, parts of the heart are supplied by so-called accessory arteries. The fundamental arteries are *a. interventricularis, a. coronaria dextra, a. coronaria sinistra* and *a. cardiomediastinalis. A. cardiomediastinalis* is an accessory cardiac artery that comes, to the right, from *a. thoracica interna* (49 % of cases), from *a. subclavia* (31 % of cases), *a. carotis communis* (3 % of cases), or, as a separate cardiac branch, from *a, thoracica interna* (17 % of cases). On the left side, this artery comes from *a. thoracica interna* (89 % of cases), from *a. subclavia* (10 % of cases) or *a. intercostalis suprema* (1 % of cases) [1].

The aorta begins with the ascending aorta *(aorta ascendens)* which exits cranially to the heart. While still inside the pericardium, it produces coronary arteries that run in the ventrolateral direction. The aorta is then penetrating pericardium at the level of Th4, forming the aortic arch *(arcus aortae)* pointing left with a peak at approximately Th2. The aorta then runs caudally as the descending aorta (*aorta descendens*). Approximately 10 mm from the terminal of the aorta from the aortic arch, *a. anonyma* is exiting to branch, after about 4 mm of its course, into right *a. subclavia* and right *a. carotis communis*. Shortly after the peak of the aortic arch, left *a. carotis communis* is produced and a branch of left *a. subclavia* is formed immediately afterwards. The branching of each of the arteries in the laboratory rat is evident from the enclosed tables [3].

Due to frequent experimental interventions in the abdominal cavity, a more detailed description is provided of the *aorta abdominalis* branching. After *aorta thoracica* passing through the diaphragm in *hiatus aorticus*, the abdominal part of the aorta *(aorta abdominalis)* runs between the median arms of the diaphragm pillars, to some extent, near the cranial end of lumbar vertebra 2, with one or multiple *aa. phrenicae* caudales being the first arteries that extend from *aorta abdominalis*, these being usually asymmetric. Along with these or separately, *a. adrenalis cranialis* exits immediately caudally to the abdominal aorta that on the right side may exit

a. renalis dextra [8]. Unpaired *a. celiaca* exits at the level of lumbar vertebra 3 (at the cranial pole of the right kidney) to split, after 10 mm, into *a. splenica,* left *a. gastrica* and *a. hepatica. A. splenica* runs into the splenic hilum, where it splits, prior its entry into the splenic parenchyma, to form 5–8 branches. *A. gastrica sinistra* runs caudally to the stomach, approaching its small curvature to the right of the oesophagus. Here, it is branching into fully anastomosing visceral and parietal branches that form ramifications on the corresponding surface of the stomach. *A. hepatica* runs cranially between *v. portae* and the right side of the papillary tip of the liver (processus papillaris hepatis) into *porta hepatis*. Before moving from the small curvature of the stomach, it produces *a. gastroduodenalis,* which then splits to form *a. gastroepiploica dextra* and *a. pancreaticoduodenalis cranialis*. The aforementioned trunk runs caudally through the pylorus to the large curvature of the stomach, where its course forms considerable meanders. The branch for the pylorus is called, by Mickwitz [8], *a. gastrica dextra. A. pancreaticoduodenalis cranialis* runs caudally in the mesoduodenum, where it is producing numerous branches for the pancreas to eventually anastomose with *a. pancreaticoduodenalis caudalis*. One of the branches stemming from the final branching of the hepatic artery is supplying the caudal portion of the oesophagus through the ascending and descending arterioles (called *a. hepatoesophagica* by [7]). Another branch exiting *aorta abdominalis* is *a. mesenterica cranialis*, this taking place about 3–5 mm caudally behind *a. celiaca.* The first branch of this is (often doubled) *a. colica media* that supplies colon transversum and the front segment of colon descendens. It is often anastomosing with *a. colica sinistra* that exits *a. mesenterica caudalis*. Another artery to be produced by *a. mesenterica cranialis* is *a. pancreaticoduodenalis caudalis*. Shortly after its exiting, it splits into its right branch and left branch. While the left branch is producing branches for the pancreas, splitting near the duodenum to form its cranial part and caudal part, the right branch supplies the ascending duodenum. Both of the branches anastomose with one another as well as the nearby intestinal arteries. Next, *a. colic dextra*, runs to *colon ascendens*, producing 16–20 loosely branched *aa. jejunales* that supply the jejunum and the proximal portion of the ileum. The primary continuation of *a. mesenterica cranialis*, *a. ileocolica* that produces a branch of the colon for colon ascendens and the ileal branch for the ileum to approximate, as *a. cecalis*, the caecum near ostium ileocecale. Here, there is this vessel's bifurcation and branching along the small curvature of the caecum. In accordance with the location of the kidney, there is another artery exiting aorta abdominalis immediately after *a. mesenterica cranialis – a renalis dextra. A. renalis sinistra* exits the abdominal aorta more caudally about 5 mm further. *A. renalis dextra* is running dorsally around *v. cava caudalis* to direct towards the renal hilum, where it splits into several branches. Before branching, it produces *a. adrenalis cranialis* (a. adrenalis dextra) [5]. *A. renalis sinistra* turns slightly caudally to the hilum of the left kidney, before which it is producing *a. adrenalis caudalis* (a. adrenalis sinistra) [5]. Approximately at the level of the caudal pole of the right kidney, paired *a. testicularis* or *a. ovarica* exits the abdominal aorta. Within its course, *a. testicularis* is producing *rami epididymales*. A. ovarica runs on each side caudolaterally, producing *r. uterinus.* About 10–15 mm caudally behind the terminal of a. renalis sinistra, there are *a. circumflex*

ilium profunda sinistra and *a. circumflex ilium profunda dextra* exiting aorta abdominalis under the right angle to the left and more caudally to the right, respectively. These arteries run dorsally to the urethers to head further to the lateral margin of m. quadratus lumborum, into which they enter. They further supply ventral lumbar muscles and the muscles of the abdominal wall and continue as far as the sacrum level to supply the skin. During its course through the abdominal cavity, aorta abdominalis is producing, dorsally, 5 unpaired lumbar arteries – *aa. lumbales*, each of these splitting to form its left branch and right branch shortly after exiting. The branches supply paraxial and lumbar muscles *(rami musculares),* the skin *(rami perforantes)*, and, through foramina intervertebralia, the spinal cord *(rami spinales).* Just before the caudal bifurcation of the abdominal aorta, before the exit of *a. sacralis mediana*, aorta abdominalis is producing penultimate unpaired branch – *a. mesenterica caudalis* that may also be exiting directly in the bifurcation. This artery is directed caudally to split, after about 10 mm, into a cranially directed *a. colica sinistra* supplying colon descendens, and *a. rectalis* running caudally to the anus. At the level of the caudal end of the last lumbar vertebra, the abdominal aorta splits into (paired) *a. iliaca* and unpaired *a. sacralis mediana*.

1.4 Lymphatic System

Lymphonodi – lymph vessels too are not fundamentally different from what is generally known in mammals. It should be noted, however, that the published data sometimes quite differ on when the clustering of the lymphatic tissue should be referred to as lymphonodus (ln.) or lymphocentre (lc.). Lymphocentre usually refers to a group of lymphatic nodes with a stable location in certain areas of the body, receiving afferent lymph vessels from the same region in all species. For the laboratory rat, the following lymphocentres can be detected:

Lc. mandibulare consists of two to three lymphonodes at the craniolateral end of the submandibular salivary gland. Occasionally, 2–3 minor lymphonodes may follow caudally. The drainage area of the aforementioned lymphocentre is tongue, the mucous membrane of the oral cavity, and the rostral region of the head. Efferent lymph vessels drain the lymph into lymphocentrum cervicale superficiale, or profundum.

Lc. cervicale superficiale is represented by one to two lymph nodes on or caudally to the bifurcation of the surface jugular vein and located on the ventral muscles of the neck. In some cases, a small node may be formed near the ear base and inserted in the parotid gland. This lymphocentre drains the ear, the parotid gland, the skin of the surrounding region, the occipital region, and the neck

Lc. cervicale profundum consists of the cranial and caudal node located laterally to the trachea, with its initial and intermediate portion based near *m. sternothyroideus.*

The drainage region is the palate of the oral cavity, chewing region, tongue, pharynx, larynx, thyroid gland, the initial portion of oesophagus, lc. mandibulare and lc. cervicale superficiale and the beginning of the trachea.

Lc. axillare incorporates its own, i.e. axillary, nodes, as well as several associated nodes. Two/four *lnn. axillares proprii* are arranged in a straight axillary line from the large axillary vessels to the ventral margin of *m. latissimus dorsi* and are covered by *m. cutaneus trunci*.

Lc. thoracicum dorsale is formed of multiple small unstable nodes *lnn. thoracici aortici* that are nested in a narrow strip of brown adipose tissue along both sides of the aorta, extending ventrally to the bodies of the thoracic vertebrae.

Lc. mediastinale is confined to the region of praecardial mediastinum, formed by a group of small lymph nodes located ventrally in the thoracic sinus and another 2–4 rather large dorsal lymph nodes connecting to the thymus, cranially to the aortic arch and laterally to the venae cavae.

Lc. lumbale is composed of several small *lnn. lumbales aortici* located along the abdominal aorta, and of paired *ln. renalis, located cranially from renal veins near the hilum of the kidney. The drainage area is the lateral abdominal wall, abdominal cavity, lc. iliosacrale,* kidney, testes/ovaries and the main part of the uterus.

Lc. celiacum is located cranially to the beginning of the abdominal artery. Composed of several lnn. gastrioduodenopancreatici and lnn. cisternales located caudal and lateral to cisterna chyli, its drainage region being the stomach, the end portion of the oesophagus, duodenum, pancreas, spleen, ometum, truncus intestinalis, peritoneum, and the dorsal part of the diaphragm.

Lc. mesenterium craniale incorporates *lnn. jejunale, cecales et colici.* It can be formed by over 8 lymph nodes nested in the intestinal mesenterium. Two to three lymph nodes are placed near the wall of the caecum close to the entry of the ileum. Two more nodes are nested into the transverse mesocolon, generally varying in size. The drainage area comprises the distal duodenum, jejunum, ileum, caecum, plus there is ascending colon and transverse colon.

Lc. mesenterium caudale usually contains two lymph nodes. It drains the descent colon and the rectum.

Lc. iliosacrale consists of a group of nodes located in the abdominal aorta bifurcation region.

Lc. ischiadicum is located lateral to *incisura ischiadica* above the sciatic nerve, draining the tail, the sacral region, and the sex organs and glands.

Lc. popliteum is a single lymph node associated with the lateral head of *m. gastrocnemius.*

Lc. inguinofemorale is composed of a series of one/three nodes nested into the rather bulky pad of fat. Located dorsolaterally to *v. epigastrica superficialis*, it drains the skin of the thigh, genital region, lateral abdominal wall, and the base of the tail.

1.5 Lymph Vessels

Usually, there are two efferent lymph vessels leading from *lc. mandibulare*; after passing through *lc. cervicale superficiale*, they run into *lc. cervicale profundum caudale,* which also receives a variety of efferent lymph vessels from *lc. cervicale profundum craniale*. Along the course of the neck, the body and the limbs, additional lymph tracts are formed, such as *tr. brachialis, tr. femoralis, tr. lumbalis, tr. intestinalis, cisterna chyli, tr, paravertebralis, tr. parasternalis* and, eventually, *ductus thoracicus,* into which the major amount of lymph from the body is flowing.

1.6 Blood Lymph Nodes

Located in the vicinity of lien and kidney vessels and nested along the surface of the thymus, there are greyish-red blood nodes with the average of 1–3 mm. They contain a thin connective tissue and a massive lymphoreticular tissue.

1.7 Thymus

Initially formed from the third pharyngeal slit, it reaches its maximum size on day 40–60, approximately. In terms of morphology, it has two lobes and a triangular apex which cranially extends to the larynx. Its curved base is attached, both cranially and caudally, to the pericardium. While most of the thymus is located in the praecardial mediastinum, its minor – cervical – portion is placed ventrally to the trachea. In young individuals, it is located on the ventral side of the *trachea* in the thoracic cavity. Its right lobe and left lobe extend from *apertura thoracis cranialis* almost to the heart. It undergoes gradual involution with age.

1.8 Lien

Lien – is an elongated, flat organ, 3–5 cm long, of stable width of about 1 cm, brownish-red in colour. It is located on the left side along the large curvature of the stomach and the left kidney. It is pointing caudoventrally. *Hilum* is found along the whole length of the spleen.

1.9 Anatomy of the Liver

Most of the front surface of the liver is in close contact with the diaphragm, richly structured/split to the liver lobes. As regards these, there is *lobus sinister lateralis, lobus sinister medialis, lobus dexter medialis*, minor *lobus dexter lateralis*, very

small *lobus quadratus*, and *lobus* caudatus. *Processus papillaris* is doubled and elongated. It extends, with a lobe, as far as the visceral surface of the stomach via the small curvature of the same. *Processus caudatus* touches the right kidney *(impressio renalis)*, connecting with it via the ligament *lig. hepatorenale.* The brown rat does not possess gall bladder [3, 4]. The individual bile terminals join to form *ductus choledochus*, which is placed ventrally to and to the right of vena portae. It is crossing dorsally the beginning of the duodenum. It is about 1.2–4.5 cm long and 0.1 cm wide. Penetrating *pancreas*, it opens, about 0.7–3.5 cm distal to *pylorus*, atop a conical, 0.1 cm high, duodenal papilla, into *duodenum.* It receives throughout its course several (2–8) terminals from the pancreas.

1.10 Anatomy of the Kidney and Urinary Tract

Kidney – dark brown in colour, elongate and bean-shaped, it is about 1.5 cm long with a smooth surface. When cut, it shows apparent joint *crista renalis. Sinus renalis* is deep, filled with a spatious renal pelvis. Kidneys lie ventrally along the lumbar spine, the range being L_1-L_2 for the right kidney and L_2-L_3 for the left kidney. *Urether* – a course similar to that of laboratory mice. *Vesica urinaria* – has a bulbous shape. The body and vertex are located in the abdominal cavity, while the neck is placed in the pelvis. The bladder has a great expansion capacity. *Urethra* – urethra masculina has *pars pelvina*, where there are accessory sex glands connecting, and *pars spongiosa*, which passes through *penis* to open into *ostium urethrae externum*. Urethra feminina is running within the pelvis along the ventral wall of *vagina*, opening into the preputial sac at the root of *clitoris.* Urine flows through *ostium preputiale* [9].

1.11 Anatomy of the Pancreas

Pancreas – made of small lobes, which are scattered in *mesoduodenum*, extending to *omentum maius* towards the spleen. It consists of two main lobes and the body. While the right lobe is accompanying *duodenum descendens* and is about 5 cm long, the left lobe points to the stomach and the spleen in the greater omentum and measures about 3 cm. Fifteen to forty excretion terminals join to form two, but sometimes also five to eight main terminals opening into *duodenum*. Weight of *pancreas* varies in adults between 0.5 and 1 g.

1.12 Anatomy of the Intestine

Intestinum tenue – small intestine splits in brown rats into three segments. *Duodenum* is short and points behind the right kidney along the right abdominal wall to turn ventrocranially below the spine and convert into *jejunum.* In

mesoduodenum, there is *lobus dexter pancreatis. Jejunum* is in brown rats the longest segment of the small intestine, measuring, along with *ileum*, 70–90 cm. It consists of numerous loops located mainly to the right between the liver, stomach and caecum. At the site of *ostium ileale*, there is a lymphatic tissue concentrated and forming *sacculus rotundus*. Macroscopic differentiation of individual sections of the small intestine relates to the knowledge of topography in the abdominal cavity. *Jejunum* generally makes the brightest, orange-brownish portion of the small intestine. *Ileum* has, in its wall, evident subserosally prominent districts of lymphoreticular tissue *(*Peyer's plaques).

Intestinum crassum – the large intestine is divided into basic parts as commonly seen in other mammals. *Caecum* is 6-9 cm long. Scimitar-shaped, it takes up a considerable portion of the left half of the abdominal cavity immediately behind the stomach. *Corpus ceci* is located ventrally and may, in males, be in contact with an intra-abdominally placed testicle. *Apex ceci* is projecting to form *processus vermiformis*. In its wall, there is plenty of lymphatic tissue. *Curvatura ceci major* faces ventrally. Generally, the caecum wall concentrates much of lymphatic tissue. There is *colon ascendens* leaving *basis ceci* at *curvatura ceci minor*. Somewhat wider than *jejunum*, it forms several loops, whereas pointing to the right towards the liver, where turning, at right angle, into *colon transversum* in the *flexura coli dextra* region cranially to *a. mesenterica cranialis. Colon transversum* runs from the right to the left, turning caudally in *flexura coli sinistra*. Afterwards, it continues caudally in the median plane on a relatively long suspension and then to the left as *colon descendens*. Throughout its course, the intestine already contains formed faeces. Lumen of *colon descendens* is slightly extended, narrowing toward the rectum. *Rectum* is narrow, placed in the pelvis, turning caudally into *canalis analis*, which opens under the tail root. There are no anal glands developed. Macroscopic differentiation of individual sections of the large intestine needs to be derived from the topographical conditions in the abdominal cavity and generally applicable rules for intestinal tract sectioning.

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Chapter 2
Basic Equipment for Microsurgical Experiment

Peter Balaz

Abstract Unlike of human organ transplantation, the most of the experimental procedures in small animal are performed under magnification and using specific micro-instruments and microsurgical suture material. In this chapter, we briefly describe the crucial features of the surgical microscope and its use, microsurgical instruments and suture materials which are usable in experimental organ rat transplantations.

Keywords Microscope • Magnification • Instruments • Sterilization • Suture material

2.1 Surgical Microscope

The same kind of surgical microscopes can be used for experimental and clinical microsurgery. There are several basic levels of microscopes, ranging from basic models with simple equipment to microscopes combined with an external monitor, a recording camera and/or an accessory ocular for a second viewer of the surgical field. Although the price can limit the purchase of a microscope, it must at least be binocular with a focal distance of at least 20 cm and the magnification should range between 4 and 16 times.

As a general rule, the lowest magnification allowing the secure handling with instruments and a clear orientation for the surgeon within the field should be used. The range and the depth of surgical field in focus diminish with increasing magnification. Therefore, using a high magnification makes clearly visible only the object in focus (e.g. vascular anastomoses) and many surrounding structures become fuzzy.

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The distance between the optics of microscope and the object (the anatomic structure) is called a working distance which has to provide space enough for comfortable manipulation with instruments and adequate light source.

In short, low magnification and clear lenses are recommended for most of surgical procedures.

2.2 Surgical Equipment and Instruments

In general, each animal needs eyes treated with eye ointment (w) and the surgical field has to be cut from hair and optimally shawed with detergent and razor (y).

The surgical pad is one of the most important pieces of equipment for surgery. Based on our experience, an optimal pad should provide supportive heating of the animal and fixing of surgical retractors and all under the sterile conditions. A stainless steel desk 15 × 30 cm with internal window 10 × 15 cm can serve as an example (p, r). An adequate size makes easy the rotation of the pad together with animal, which allows a change of view angle of the surgical field.

Experimental microsurgical instruments are identical to the instruments used in human microsurgery. It is strongly recommended that each surgeon to have their own separate set of fine instruments. Every instrument should be identifiable with colored tape (autoclavable) to identify the owner. In a small microsurgery department, it is possible to have common set of big instrument for all members. In this section the only basic set of instruments is described (Fig. 2.1). Special instruments needed for a specific surgical procedure are described in each particular transplantation model.

Medical scissors (a) and surgical tweezers (b) can serve for laparotomy preparation, a needle holder for abdomen closing (c) as well as a pair of small tissue forceps (d). For the tissue dissection/preparation, a pair of 11–12 cm long microsurgical tweezers with straight and angled tip (e, f, g) can be used. For vascular clamping, vascular clamps (m, n) some of them are placed on the vessel using special tweezers (h) can be used. For preparation, dissection, cutting of the tissue and suturing, use various types of the micro scissors with length with sharp or blunt tips (i, j).

Precise suturing is possible with atraumatic stitches inserted using a microneedle holder (k, l) in combination with straight micro forceps. Sterile cotton Q-tips are an invaluable tool for the gentle manipulation of tissue (o). Sterile gauze squares are used for draping of the surgical field and for moisture cover of abdominal organs. Special triangles can be used for blood removal in case of bleeding (q). Some sterile beakers are useful for warming saline (x; 37° C) before being used for moistening of tissues. The flushing of vessels can be performed using fine metal cannula made from an injection needle by brushing of the tip.

Retractors can be used to hold back the edges of an incision to allow adequate visibility within the surgical filed (s). Massive bleeding should be prevented using a

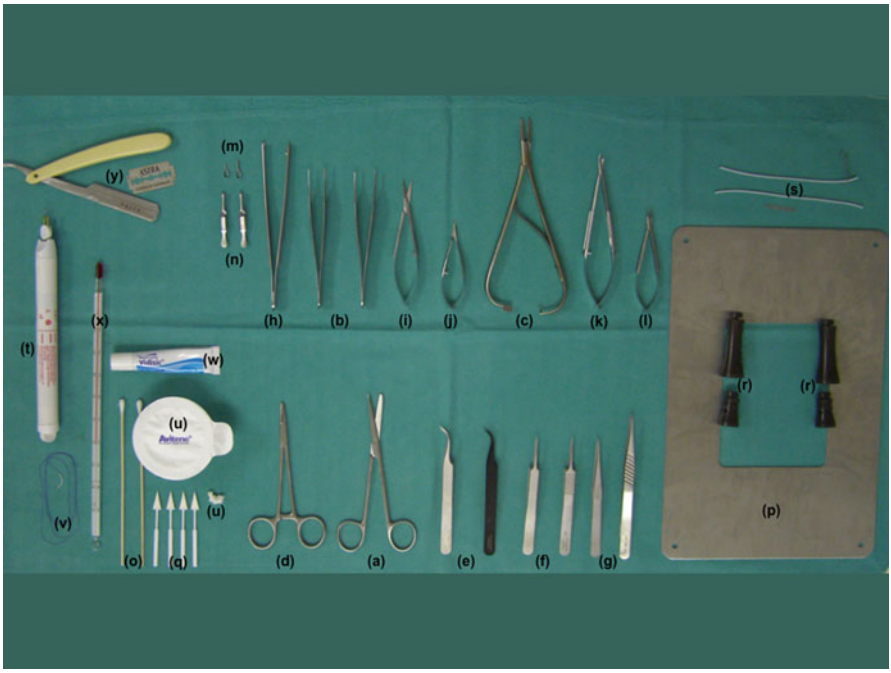

Fig. 2.1 Basic set of instruments

fibrin or collagen foam (u) or bipolar electro-cauter or much cheaper disposable thermal cauter (t).

Micro-surgical instruments are expensive and some of them are exceedingly delicate. Despite this, instruments have to be sterile at the beginning of experimental surgery. The gentle techniques of instruments sterilization are necessary as simple autoclaving is not adequate. Micro scissors should never been used for cutting of paper or skin. Instruments can be washed in mild soap and water to remove any blood after each procedure. Ideally, cleaning is best done with an ultrasonic cleaner. However, use caution because some very delicate microsurgical instruments can be damaged with ultrasonic cleaner. Special lubricants should be used to maintain the instrument condition and to prevent rust.

2.3 Suture Material

Similarly as surgical instruments, suture material is the same used in human surgery. Sutures are either absorbable or remain indefinitely in place. The adequate indication of suture tape is necessary.

Absorbable sutures, called “catgut”, are made from sheep intestines or synthetic material. VICRYL™ (v) is a synthetic absorbable sterile surgical suture composed of a copolymer made from 90 % glycolide and 10 % L-lactide. This material is usually used internally, for instance for vessels ligation, bowel anastomosis; however, it should not be used for vascular anastomosis or skin closure.

Non-absorbable sutures are usually used for skin closure and for vascular anastomosis. PROLENE™ is polypropylene (clear or pigmented), sterile surgical suture composed of an isotactic crystalline stereoisomer of polypropylene, a synthetic linear polyolefin. Generally, for experimental vascular connections, use monofilament suture with thickness 8/0 to 10/0. The optimal length of filament is 13 cm with round tip needle 3/8 and “chord length” 5 mm.

Chapter 3
Immunosuppresive Drugs Commonly Used in Transplantation Models

Peter Girman

Abstract The chapter describes the four most commonly used immunosuppressive drugs in transplantation rat models: polyclonal antibody, tacrolimus, mycophenolic acid and mTOR inhibitors. The aim of the chapter is to provide available information about the administration and doses of single drugs with appropriate trough levels or AUC curves that will enable the reader to better estimated therapy in his or her experiments. Furthermore, we outline available data about rejection times of different organ transplantations in various strain combinations.

Keywords Immunosuppression • Rat model • Kinetics • Dose

3.1 Monoclonal and Polyclonal Antibodies in Rat Organ Transplantation

In human transplant medicine, two antibodies are available for organ recipients: basiliximab (monoclonal antibody against receptor for interleukin-2) and ATG (polyclonal anti thymocyte or anti T-lymphocyte globulin). Only limited data are available about the effects of basiliximab in rat models. Therefore, this section will be focused on experiments done with ATG.

3.1.1 Anti Thymocyte Globulin

Currently, there are three commercially available preparations of ATG (ATG Fresenius – rabbit, Thymoglobuline Genzyme – rabbit, AtGam Roche – horse). Rabbit preparations are preferred to equine ATG due to better tolerance and a lower incidence of side effects. Generally, polyclonal antibodies are prepared by immunizing rabbit models with cell suspension from human thymic tissue. Then,

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polyclonal globulins are obtained from harvested rabbit serum and samples from several thousands of rabbits are pooled to obtain batch-to-batch consistency.

3.1.2 Mechanism of Action

The primary mechanism of immunosuppressive action is T-cell depletion. Depleting effects are mostly mediated through the complement based cell lysis though other mechanisms, including antibody mediated toxicity or activation of apoptosis, can be involved. Treatment with ATG decreases T-cells not only in peripheral blood but also in secondary lymphoid tissue, except for the thymus. Modulating effects are demonstrated with down-regulation of expression of CD2, 3, 4, 5 and 6 on the T-cell surface. Decreased expression of integrins and intercellular adhesion molecules results in decreased adhesion of leukocytes during the reperfusion period. Moreover, ATG binds to the number of receptors on the surface of B-lymphocytes and activates apoptotic pathway in these lymphocyte cell subsets [1, 2].

3.1.3 ATG in Prevention of Rejection

The effect of ATG on lymphocyte cell count was investigated by Olausson, et all in heterotopic heart transplantation in PVG to DA strain combination. Rabbit ATG was administered in two doses, 0.1 and 0.01 ml. Untreated recipients of the heart demonstrated decline in CD5, CD4 and CD8 cell counts. The number of cells return to their pre-transplant level in the first week post-surgery. Both doses of ATG effectively decreased numbers of CD4, 8 and 5 cells, while the count of B-cells remained unchanged. Animals treated with lower dose of ATG rejected their grafts and demonstrated higher counts of t-cell subsets in comparison to those recipients with functioning grafts. In another experiments, treatment with 25 mg/kg of ATG on day-3 and 5 mg/kg on days -2,-1 and 0 resulted in indefinite survival of PVG and LEW heart allografts transplanted in the DA recipient [2, 3]. The same regimen did not prolonged function of lung grafts in the same strain combinations [3]. In the PVG to DA strain, the combination 0.02 ml of ATG was ineffective in prolongation of heart allografts, while doses of 0.1 and 0.2 ml significantly increased survival of the graft to the median of 100 days. Friedmann et all demonstrated that 4 mg/kg of rabbit ATG prolonged the survival of allogeneic islets to 11 days in Wistar/Furth to LEW combination and to more than 100 days in Fischer to LEW model [4].

ATG has been widely used in human organ transplantation as a part of induction protocols and an anti-rejection treatment. The efficacy of the treatment may depend on the presence of antibody specificities. Popow et al. comprehensively described composition of commercially available ATG preparations and found out differences in the amount of specific antibodies. For instance, in ATG-Fresenius they did not detect antibodies against CD1a, CD27, CD68, CD80 and CD209, while in

Thymoglobulin they did not detect antibodies against CD 102, CD298, CD58 and CD68 [5]. In another experiment, Penack et al. demonstrated significantly toxigenic effects of ATG – Fresenius and Thymoglubuln Genzyme on NK cells in comparison to Lymphoglobulin [6]. At present, it seems that these differences among preparations have little or no impact in clinical settings. In any case, with increasing knowledge about tolerance mechanism and immune network, these differences may play a significant role in prolonging the graft survival.

3.2 Tacrolimus

3.2.1 Mechanism of Action

Tacrolimus binds to its intracellular receptor FKBP (FK binding protein). Once bound, it forms an inhibitory complex that blocks the enzymatic activity of calcineurin. Calcineurin inhibition results in complete blockage of the translocation of cytoplasmatic NF-ATc, which consequently leads to T-cell inactivation and inhibition of cytokine gene transcription.

3.2.2 Pharmacokinetics of Tacrolimus Rat Model

Tacrolimus is poorly absorbed drug with almost 50 % first- past elimination after absorption. The mean 24 h AUC were 16.2, 76.9 and 450.2 (ngxhr/ml) after oral administration of tacrolimus in solid dispersion formulation as demonstrated in experiment with fed Sprague- Dawley rats. For all dosing regimens, maximal concentrations were achieved 30 min after administration. The mean maximal blood concentrations of tacrolimus were 8.8 ± 4.9, 11.6 ± 5.3 and 40 ± 19.4 ng/ml after oral administration of 1, 3.2 and 10 mg/kg, respectively. Tacrolimus is stable in gastric juice and intestinal secretions. Studies of the absorption site were done using a close loop model at five different sites and show that tacrolimus is predominantly absorbed in the jejunum and duodenum. Significantly lower absorption was observed in the ileum and colon. Only a minimal amount of tacrolimus gets into the blood through the stomach. Almost 50 % of tacrolimus is eliminated in the liver [7].

Bioavailability after intraintestinal and intraportal administration of tacrolimus 1 mg/kg in Wistar rats were 26.2 % and 39.8 %, respectively. Hepatic extraction at a dose of 1 mg/kg was about 60 %. At the same dose, 34 % of tacrolimus was metabolized in the small intestine. Experiments with the everted sacs method showed that 23 % of tacrolimus disappear after 1 h incubation with the inhibitor of CPY3A, providing evidence that intestinal enzymes moderately participate in the metabolism of tacrolimus [8]. Jejunal or ileal segmental small bowel transplantation decreases bioavailability of tacrolimus by 40 % of 5 mg/kg in comparison to control non-transplanted group (LEW RTA[1]) [9].

Bioavailability of tacrolimus can be improved by rectal application. In experiment with Sprague-Dawley rats, 2 mg/kg of tacrolimus administered rectally in suppository form resulted in significant increase of 24 h AUC when compared to per oral administration of the same dose (707 ± 28.6 and 103 ± 6.7 ng.h/ml, respectively). Similarly, blood trough levels of tacrolimus were higher after rectal administration in comparison to oral administration (25.8 ± 4.8 and 1.6 ± 0.8 ng/ml, respectively). The tacrolimus was applied under general anesthesia [10].

In experiments with LEW rats, it has been established that the pharmacokinetics of tacrolimus was influenced by the circadian rhythm. Tacrolimus administered at 10 h p.m. resulted in a higher maximal concentration in comparison to a morning dose (46.4 ± 12.6 and 15.9 ± 4.1 ng/ml, respectively). Consequently, 24 h AUC was 2.9 times higher in rats treated with tacrolimus at 22 h p.m. [11].

Oral administration of tacrolimus in 4 mg/kg dose to Lewis rat concomitantly with MMF 20 mg/kg did not change availability of mycophenolic acid. The mean AUC 2–24 of MPA in controls and Tacro/MMF group were 79 ± 10 84 ± 26 mg/L, respectively [12].

3.2.3 Prevention of Allograft Rejection

Tacrolimus effectively prolongs graft survival from doses of 0.2 mg/kg given intramusculary [13–24]. Peroral doses are generally 10 times higher, which results from intensive metabolism of tacrolimus in the liver and small intestine. For practical use, intramuscular administration is the simplest approach. Intravenous, inhaled and peroral administration are more complicated, requiring general anesthesia (intravenous and inhaled) or a special technique (peroral – gastric feeding tube). In our experiments with islet transplantation in LEW to BN strain models, we administered tacrolimus in daily regimen at dose of 0.05 mg/kg intramusculary, which resulted in long term survival of the islet graft and trough levels of tacrolimus in the range of 5–10 ng/ml. Rejection times in various strain combination and tacrolimus doses are described in following tables.

Donor strain (heart)	Recipient strain	Tacrolimus dose (mg/kg)	The mean graft survival (days)	Administration of the drug	References
Brown-Norway $RT1^n$	Lewis $RT1^l$	1	10–15	Oral	Deuse et al. [13]
Brown-Norway $RT1^n$	Lewis $RT1^l$	2	15–20	Oral	Deuse et al. [13]
Brown-Norway $RT1^n$	Lewis $RT1^l$	8	20–25	Oral	Deuse et al. [13]
Brown-Norway $RT1^n$	Lewis $RT1^l$	0.3	7.8	Oral	Kinugasa et al. [14]
Brown-Norway $RT1^n$	Lewis $RT1^l$	0.6	9.8	Oral	Kinugasa et al. [14]

Donor strain (heart)	Recipient strain	Tacrolimus dose (mg/kg)	The mean graft survival (days)	Administration of the drug	References
Lewis RT1^{l}	Lewis RT1^{l}	0.7	17	Oral	Kinugasa et al. [14]
ACI	Lewis RT1^{l}	0.032	16	Intramuscular	Fang [15]
F344 (RTA^{lvl})	Lewis RT1^{l}	0.025	11	Intramuscular	Li et al. [16]
F344(RTA^{lvl})	Lewis RT1^{l}	0.05	13	Intramuscular	Li et al. [16]
F344(RTA^{lvl})	Lewis RT1^{l}	0.1	52	Intramuscular	Li et al. [16]
Lewis RT1^{l}	Lewis RT1^{l}	0.2	40	Intramuscular	Jeske [17]
DA RT1^{avl}	PVG RT1^{c}	2.4	13	Oral	Qi et al. [18]
DA RT1^{avl}	PVG RT1^{c}	4.8	18	Oral	Qi et al. [18]

Donor strain (islet)	Recipient strain	Tacrolimus mg/kg dose	The mean graft survival (days)	Administration of the drug	References
ACI RT1^{a}	Lewis RT1^{l}	5	30	Intramuscular	Rastellini [19]
ACI RT1^{a}	Lewis RT1^{l}	10	30	Intramuscular	Rastellini [19]
Wistar	ACI RT1^{a}	1	71	Intramuscular	
WKA RT1^{u} (renal subcapsular grafts)	Lewis RT1^{l}	0.32	13	Intramuscular	Yasunami [20]
WKA RT1^{u} (renal subcapsular grafts)	Lewis RT1^{l}	1	20	Intramuscular	Yasunami [20]
WKA RT1^{u} (intrahepatic grafts)	Lewis RT1^{l}	0.1	7	Intramuscular	Yasunami [20]
WKA RT1^{u} (Intrahepatic grafts)	Lewis RT1^{l}	0.32	42	Intramuscular	Yasunami [20]
WKA RT1^{u} (intrahepatic grafts)	Lewis RT1^{l}	1	45	Intramuscular	Yasunami [20]

Donor strain (lung)	Recipient strain	Tacrolimus dose	The mean graft survival (days)	Administration of the drug	References
Brown-Norway RT1^{n}	Lewis RT1^{l}	3 mg/kg	8.7	Intramuscular	Misao [21]

Donor strain (pancreas)	Recipient strain	Tacrolimus dose	The mean graft survival (days)	Administration of the drug	References
DA RT1^{avl}	Lewis RT1^{l}	1 mg/kg	16	Intramuscular	Sakuma [22]

Donor strain (kidney)	Recipient strain	Tacrolimus dose	The mean graft survival (days)	Administration of the drug	References
Brown-Norway RT1^{n}	Lewis RT1^{l}	0.32 mg/kg	10	Oral	Jiang et al. [23]
Brown-Norway RT1^{n}	Lewis RT1^{l}	1 mg/kg	23	Oral	Jiang et al. [23]
Brown-Norway RT1^{n}	Lewis RT1^{l}	3.2 mg/kg	More than 100	Oral	Jiang et al. [23]
WKAH	Lewis RT1^{l}	5 mg/kg	68 (tacro administered 4,5,6 poTx days)	Oral	Hayakawa et al. [24]

3.3 Mycophenolate Mofetil/Sodium

3.3.1 Mechanism of Action

Mycophenolic acid is a fermentation product of Penicillium species that effectively blocks purine synthesis through allosteric inhibition of inosine monophosphate dehydrogenase. Purine nucleotides are synthetized by two pathways. The primary one is a de novo synthesis of inosinemonophosphate (IMP) from PPRP. The IMP is then used for synthesis of adenosine and guanosine by inosinemonophosphate dehydrogenase and adenosine deaminase, respectively. The second pathway for synthesis of purine nucleotides is their recyclation from guanosine and adenosine mediated by hypoxanthine guanine phosphoribosyltransferase and adenosine deaminase. Both pathways used PRPP.

The amount of PRPP in T and B lymphocytes is significantly increased after antigenic stimulation of the cells and is proven to be an important step before cell proliferation. Adding mycophenolic acid into the mixed lymphocyte reaction has effectively inhibited cell proliferation. Analyzing the cells by flow cytometry proved that lymphocyte passed through the G1 phase and stopped their mitosis in the S-phase. Another mechanism of action included depletion of GTP that may result in inhibition of G-protein based transduction signals. In immunized rats, the GTP pool significantly decreased after 4 days treatment with 20 mg/kg of MPA. MPA was shown to have a unique dual activity, both immunosuppressive and antimicrobial. Rats infected with Pneumocystis carinii did not develop pneumonia if they were treated with MPA [25].

3.3.2 Pharmacokinetics and Dynamics

Pharmacodynamics effects of MMF were tested on Lewis recipients of BN hearts after an 8- day treatment at oral doses of 5, 10 and 20 mg/kg. The AUC values after 8 days of treatment were 8.2 ± 1.5, 24 ± 2.6 and 41.2 ± 5.1 for above mentioned doses, respectively. AUC values significantly correlated with inhibition of lymphocyte proliferation and rejection grading in heart allografts. In a dose dependent manner, MPA effectively inhibited CD25, CD134, 71, CD45 positive cells, with the strongest effect at 20 mg/kg. The rejection changes were significantly worse in lower doses of MPA treatment [26]. Therapeutic window of MPA was very narrow ranging from 10 to 30 mg/kg.

3.3.3 Tolerability and Safety

Side effects and tolerability of mycophenolic acid were tested in experiments with Lewis recipients of either BN kidneys and heart or DA aorta. A dose of 40 mg/kg was not tolerated and most of the animals were terminated due to adverse events. Mycophenolate mofetil or sodium were tolerated at doses from 10 to 30 mg/kg with only minor to moderate side effects observed. The most frequent events included a decrease in red and white blood cells counts, thymic atrophy and villous atrophy in the jejunum [27, 28].

3.3.4 Prevention of Allograft Rejection

Mycophenolic mofetil/sodium was shown to dose-dependently inhibit intima thickening of aortal allograft in DA to Lewis strain combination. However, the prevention of rejection changes in aorta grafts was incomplete, perhaps due to the lower effect of MPA on intimal myocytes. 5 mg/kg of MPS was shown to be the minimal dose for effective kidney survival in BN to the Lewis model. 10 mg/kg of MPS prolonged kidney graft survival in the same model indefinitely. The same dose was not effective in kidney allotransplantation of DA to the Lewis model resulting in rejection of the grafts. MPS administered at 20 mg/kg was not tolerated in Lewis recipients of DA kidney grafts. In DA to Lewis model of heart transplantation, a dose of 10 mg/kg of MPS prolonged the time of rejection for several days, while a dose 20 mg/kg seemed to be effective with survival more than 14 days in all animals. All Lewis recipients of BN hearts showed rejection changes after treatment with 20 mg/kg of MMF. The same dose did not prevent rejection in DA to Lewis heart transplantation. 40 mg/kg were not tolerated resulting in termination of almost half of the recipients due to serious adverse events [27]. Similar results in DA to Lewis heart transplantation were ascertained by Matsumuto et al. [29]. Experimental

studies with left lung transplantations showed that MMF at a dose 30 mg/kg blocks acute lung rejection in Fischer 344 to Wistar Kyoto strain combination, but only in grafts with no or minimal rejection changes. Administration of MMF in a later phase of rejection was not successful [30]. In the liver allotransplantation of PVG (Piebald Viral Glaxo) to Lewis strain combination, 5 days treatment with 40 mg/kg of MMF, if given subcutaneously, prolonged graft survival to more than 100 days [31]. Monotherapy with 20 mg/kg of MMF has resulted in median islet survival of 12 days when a strain combination Wistar to Lewis was used. Islet survival was similar to recipient treated with cyclosporine at a dose of 5 g/kg [32].

3.4 MTOR Inhibitors

Sirolimus and everolimus are two representative drugs that belong to the mTOR inhibitor group. Sirolimus is a macrocyclic lactone drug obtained from Streptomyces hygroscopicus. Everolimus is derived from sirolimus by chemical modification and has the same mechanism of action on cellular and molecular levels.

3.4.1 Mechanism of Action

Both drugs bind to intracellular binding protein called FKBP (FK binding protein) which is the substrate for tacrolimus as well. Molecular complex sirolimus/FKBP blocks pathway different from that blocked by tacrolimus/FKBP. Sirolimus/FKBP inhibits the so-called mTOR molecule (mammalian target of rapamycin). MTOR phosphorylates S6 kinase which became activated and further phosphorylates S40 subunit of ribosome. Activation of this pathway results in increased translation of mRNA transcripts. MTOR inhibitor effectively diminished these events. Another mechanism of action includes inhibition of CD28 mediated costimulating pathway through decreased translocation of c-REL protein to nucleus and consequent inhibition of lymphokine production. Furthermore, mTOR inhibitors blocks cdk2/cyclin E and cdk4/cyclin D complexes in G1 phase of cell cycle. Sirolimus acts synergistically with both cyclosporine and tacrolimus. The synergistic effects of cyclosporine and tacrolimus are not surprising as both drugs bind to a different molecule and acts at different pathways. On the other hand, tacrolimus binds to the same kind of intracellular protein as sirolimus; in spite of that, both drugs acts syngergically. The explanation seems to be in the excess amount of FKBP12 present in cells which is sufficient for both drugs. Accordingly, at therapeutic levels, the drug did not compete for the substrate. Competition can occur only in 50–1000 molar excess of tacrolimus [33].

3.4.2 Pharmacokinetics and Dynamics

Trough levels of sirolimus, if administered in therapeutical doses, range between 5 and 30 μg/l. Sirolimus is metabolized by P45O 3A enzyme group in rat liver and intestinal cells. Metabolite products usually have less than 10 % activity of the drug. Everolimus, a drug derived from sirolimus, seems to have similar pharmacokinetic properties. In a rat model, both drugs had been proved to have similar AUC values after oral dosing of 1.5, 5 and 15 mg/kg/day (435, 1468, 7076 and 228, 1104, 4071 ng.h/mL for everolimus and rapamycin, respectively). The slightly higher levels of AUC were reported for everolimus, perhaps due to increased bioavailability. Experiments with Lewis rats showed that whole blood levels correlated with graft survival. Oral therapy with sirolimus at doses 0.3, 0.8, 2 and 6 mg/kg resulted in whole blood levels of 0.47 ± 0.04, 1.55 ± 0.16, 7.13 ± 1.2 and 12.5 ± 1.4 ng/ml in Lewis rats, respectively [34].

3.4.3 Prevention of Allograft Rejection

14 days intravenous continuous therapy of sirolimus at doses 0.01 and 0.02 mg/kg was ineffective in prolonging cardiac survival of BUF (Buffalo) hearts transplanted into WF (Wistar Furth recipients). Sirolimus at 0.04 mg/kg increased survival of heart allograft in the previous model to 14 days. In the same strain, combination sirolimus doses 0.01, 0.02 and 0.04 effectively increased survival of kidney grafts from 11 days in untreated control animals to 21, 42 and 61 days [35]. In the same strain combination, sirolimus administered orally at doses 0.3, 0.8, 2 and 6 mg/kg prolong cardiac allograft survival to 13 ± 1, 15.5 ± 0.8, 32 ± 5 and 30 ± 4 days, respectively [34]. Continuous administration of sirolimus by osmotic pump at doses 0.08, 0.32 and 0.8 mg/kg extended heterotopic cardiac survival to 354, 55 and 74 days in BUFF to WF strain combination, respectively [36]. Sirolimus given orally at doses 0.5, 1.2 and 4 mg/kg provided dose dependent prolongation of cardiac allograft function to 12, 18, 52 and 90 days in the same strain combination [37]. Survival of pancreas allograft in above mentioned strain combination was prolonged from 9 days to 14.5 ± 1.4 and 15.3 ± 2.6 after 30 days oral therapy with sirolimus at doses 0.2 and 0.4 mg/kg. The same dosing regimen in the same strain combination prolonged kidney graft survival from 7.7 ± 0.8 in untreated controls to 10 ± 2.2 and 14.4 ± 7.5 days, respectively [38]. In the lung transplantation rat model (BN to LEW), oral therapy with 2.5 mg/kg of everolimus failed to prevent graft failure [39].

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Chapter 4
Basics in Rat MHC Genomics

Peter Girman

Abstract The chapter is focused on basic information about genomic organization of MHC system of laboratory rat. Nomenclature is briefly explained and at the end of the chapter impact of MHC incompatibility on organ survival is mentioned.

Keywords Rat • MHC system • Antigen

Basic knowledge about rat MHC genomics is important for the selection of the right donor and recipient strain combination in organ or tissue transplant experiments. The rat MHC system is called the RT1 system. Genes of RTA system are localized on chromosome 20 and they are grouped into the 4 regions. The first region, called RT1A, is localized in the centromeric part and includes 1–3 loci of the Class Ia genes. The second region is RTA B/D and includes locuses of Class II genes. Class III region includes non-MHC genes. The fourth region is called RT1-C/E/M and comprises class Ib genes. The organization of RT1 complex is described in Fig. 4.1 [1].

Using serological and histogenetic typing, several haplotypes (combination of alleles in RT1-A and RT1-B/D regions) were defined. The haplotype is marked with upper case letters (a, b, c, d, f, g, h, k, l, m, n, q, s, u or e, i, j, o, or p). Each haplotype is defined with combination of alleles in standard RT1-A regions (a, b, c, d, f, g, h, k, l, m, n, q, s, u) and standard RT1-B/D (a, b, c, d, f, h, k, l, m, n, u). The Nomenclature is explained in Fig. 4.2.

RT1-A regions contains class Ia genes, usually 1–3 genes depending on the haplotype. For example, haplotypes RT1 a,k,u contains only one class Ia gene in the RT1-A region, while haplotype c carries 2 genes and haplotype n contains 3 genes in the RT1-a region. Class Ia genes encode the highly polymorphic part of MHC I molecule that are responsible for presentation of antigens to αβ part of T-cell receptor.

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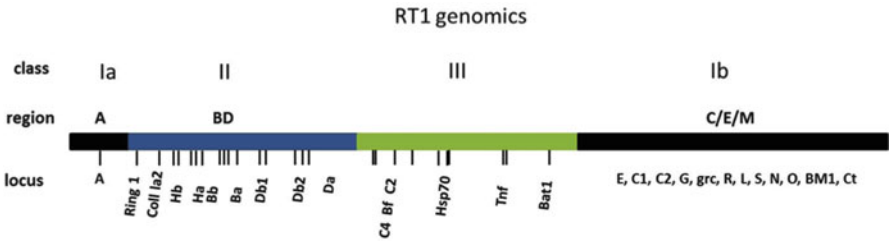

Fig. 4.1 RT1 genomics

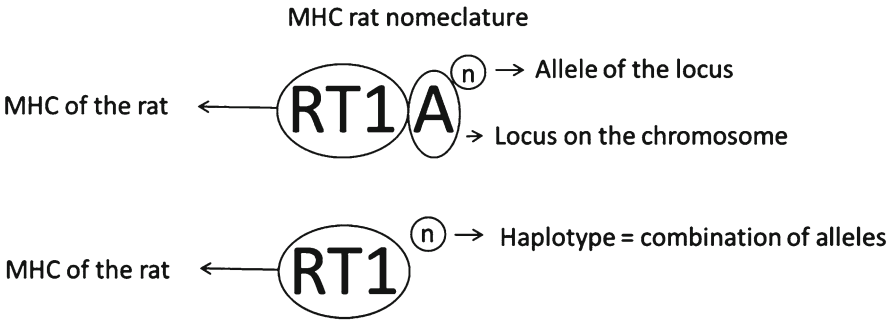

Fig. 4.2 Nomenclature of RT1

RT1-C/E/M region contains several class Ib genes depending on the haplotype. Most of them are responsible for coding of non-polymorphic or monomorphic peptides of MHC antigens.

MHC class II molecules are coded by class II genes in the RT1B/D region. RT1-H genes are orthologous to HLA-DQ genes, RT1-B genes are orthologous to HLA-DP genes and RT1-D genes are orthologous to HLA-DR genes [2].

Class III region includes the heat shock protein gene family, the tumor necrosis factor cytokine family and genes of the complement system. Class III genes present in human HLA class III system are the same as those present in rat class III region.

4.1 Impact of RT1 Haplotypes on Rejection

Allografts fully mismatched in RT1 antigens are usually rejected in an acute manner. However, in some RT1 mismatched rat strains (particularly liver) can be accepted spontaneously for longer time. For example, liver from the DA rat strain can survive spontaneously in PVG rats. Similarly, kidneys from F344 rats transplanted into LEW recipients survive for longer time and this model is often used for studying chronic rejection changes. F344 differs from LEW in the RT1C region [1].

Table 4.1 Selected haplotypes in rat strains

RT1 haplotype	Strain
A	AVN
av1	DA (dark Agouti), ACI
B	BUF (Buffalo)
C	PVG (piebald viral glaxo)
L	LEW (Lewis)
lv1	F344 (fischer)
N	BN (Brown-Norway)
U	WF (Wistar Furth), BB (BioBreeding rats), WAG (Wistar albino Glaxo)

Histoincompatibility in the RT1C region usually results in long-term acceptance of most of the organ allografts with the exception of skin grafts and pancreas grafts. When strains are different selectively in the RT1A region, transplanted organs are clearly rejected in very short time. Class II molecules are strong histoincompatible antigens and organs transplanted between RT1B/D incompatible RT1A compatible strains survive for short time due to acute rejection. Furthermore, the rejecting response depends on the presentation of donor alloantigens by recipients RT1 molecules. Generally, RT1c strains are considered as low responders with lower production of antibodies and RT1u are considered as high responders to RT1a strains [3].

Selected haplotypes are shown in Table 4.1. More information about registered rat strains and their genomic can be found at http://rgd.mcw.edu.

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Chapter 5
Anesthesia in Experimental Microsurgery

Ladislav Hess and Hynek Riha

Abstract An appropriate anesthesia is essential for successful experimental animal research; it should be administered to animals undergoing surgical procedures or procedures that cause more than light or transistory pain or distress. This chapter comprehensively discusses the preparation for the anesthesia, types of anesthetic drugs and their proper selection as well as options for postoperative analgesia which is an integral part of the most of experimental interventions in animals.

Keywords Atropine • Benzodiazepines • Neuroleptics • Alpha2-agonists • Opioids • Ketamine • Euthanasia • Isoflurane • Sevoflurane • Vaporizer • Minimal alveolar concentration • Induction chamber • Endotracheal intubation • Mechanical ventilation • Respiratory rate • Cardiovascular monitoring

5.1 Injectable Anesthesia (by Ladislav Hess)

An appropriate anesthesia is essential for successfully mastering experimental animal research and should be administered to animals undergoing surgical procedures or procedures that cause more than momentary or slight pain or distress. This chapter comprehensively discusses the preparation of the anesthesia, proper selection and type of anesthesia as well as post-operative analgesia, which is an integral part of each experimental intervention on animals.

Section "Injectable Anesthesia" was written by Ladislav Hess and section "Inhalational Anesthesia in Rats" was written by Hynek Riha.

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5.1.1 Preparation of the General Anesthesia

Due to the high metabolic activity of small rodents, pre-anesthetic fasting should not exceed 2 h. Several hours of food deprivation is not desirable because it leads to disturbances in balance, metabolic acidosis and hypoglycemia. During prolonged fasting, important intestinal flora dies, which may result in the resorption of endotoxin. Because rats cannot vomit, a long fasting before anesthesia induction is not necessary. Water should never be restricted during the pre-anesthetic period. For easier handling with the organs in the abdominal cavity, solid food can be replaced by liquid food approximately 8–12 h before surgery.

A complete physical examination of the animal should be performed prior to surgical procedure for a smooth duration of general anesthesia. Pay attention particularly to a nutritional status, quality of fur (thinning, dirty) and behavior (movements of the limbs and trunk, rigid walking and a flattened abdomen as a sign of pain). Furthermore, examine natural orifices (discharge from the nose, increased salivation and impurities around the anus and genitals) and condition of the eyes ("spectacle eyes" – a red secretion of the Harder's glands accumulate in the inner corner of the eyes in animals that are not sufficiently clean). It is also important to observe respiration because just the occurrence of non-manifesting subclinical pulmonary diseases can lead to severe respiratory failure in general anesthesia with subsequent death of the animal.

Animal handling should be calm and gentle; otherwise, stress hormones are intensely released and can cause tachyarrhythmias with subsequent cardiac arrest during the general anesthesia.

5.1.2 Anticholinergic Premedication

Atropine or glycopyrrolate can be applied as pre-anesthetic medications to prevent bradycardia and to suppress bronchoconstriction. Rat livers produce atropine esterase, which causes resistance to the atropine effect and therefore repeated injections may be required. The recommended dosage of atropine is 0.05–0.2 mg/kg i.m.

5.1.3 Drugs Used in General Anesthesia

For general anesthesia of laboratory rats mostly parenteral anesthetics are used.

5.1.3.1 Sedatives

For sedation, benzodiazepines such as diazepam, flunitrazepam and midazolam are mainly used, which in clinical dosage only slighty affect circulation and respiration. The first two named benzodiazepines are insoluble in water, are transformed to

active metabolites and their effect is long lasting. Midazolam is a water-soluble benzodiazepine with a short half-life (1.5–2.5 h). The effects can be reversed by the use of a specific benzodiazepine antagonist, flumazenil.

The second group of sedatives represents neuroleptics, foremost derivatives of butyrophenone. Dehydrobenzperidole is no longer manufactured and therefore azaperone is used instead. Neuroleptics induce psychomotoric sedation. Moreover, these drugs have a slightly negative inotropic effect, they cause vasodilation, a mild decrease of blood pressure and marginally stimulate respiration.

The sedatives also include the group of alpha2-agonists (xylazine and derivatives of detomidine or medetomidine), which have sedative, hypnotic and analgesic effects. Blood pressure is initially increased (peripheral vasoconstrictor effect), then decreased (central vasodilator effect). Alpha2-agonists decrease heart rate, act as diuretics and affect respiration only minimally. The effect can be antagonized by atipamezole which causes the release of catecholamines, especially norepinephrine.

5.1.3.2 Analgesics

Analgesics are receptor specific drugs. Significant analgesia is a result of the interaction with μ1 opioid receptor while binding to the μ2 opioid receptors, which cause suppression of respiration. Spinal analgesia is caused by interaction with opiatic receptors. Blood pressure is not significantly affected by analgesics and heart rate is decreased due to the vagomimetic effect.

Fentanyl and its derivatives are the most commonly used opioid analgesics in laboratory animal care (mainly fentanyl and sufentanil). Fentanyl is a hundred times stronger analgesic than morphine and sufentanil even a thousand times. To antagonize their effects, a specific opioid antagonist naloxone can be given.

Ketamine is a dissociative anesthetic. Depending on a dose, the effect of ketamine is sedative, hypnotic, analgesic and cataleptic. Blood pressure, heart rate and muscle tonus are increased. Respiration is not considerably affected at the clinical dosage. The analgesic effect is related to somatic rather than to visceral pain. Ketamine is often combined with benzodiazepines or alpha2-agonists. The pharmacological effects of both drugs are complementary. The specific antagonist of ketamine is not known because it affects a number of neurotransmitter systems.

Not only pre-operative, but also immediate post-operative analgesic administration is important for adequate pain relief. For post-surgical analgesia, non-steroidal anti-inflammatory drugs (NSAID) are frequently used which act by inhibiting cyclooxygenase activity. Blockade of prostaglandin production is essential for their analgesic, antiflogistic and antipyretic effects. The most commonly used analgesics of this type include carprofen, ibuprofen or flunixin.

Metamizole is a derivative of pyrazolone and thus belongs to non-opioid analgesics. It acts both peripherally and centrally.

5.1.4 Injectable Anesthesia

Injectable anesthetics are administered most often intramuscularly and intraperitoneally or, only rarely, intravenously. Subcutaneous administration of anesthetics is not recommended because the induction of anesthesia is prolonged and variable in onset. However, this method can be used for premedication or for administration of specific antagonists to prolong their effects. Intramuscular injections are applied into the musculus semitendinosus and the semimembranaceus of the hind leg. A larger volume of solution is administrated intraperitoneally; however, there could be a problem during abdominal surgery when you cannot add an anesthetic that way. For intravenous administration into the tail vein, proper restraint or sedation is required [1, 2]. For parenteral anesthetics is important to calculate an accurate dosage based on the actual body weight of the animal to avoid either over- or underdosing. Longer periods of anesthesia can be accomplished by repeated injections.

Prior anesthesia induction is advisable for sedation of animals with the following drugs (Table 5.1). The most commonly used anesthetic drug in laboratory rats is ketamine combined with alpha2-agonist xylazine or more specific medetomidine (Table 5.2 A, B). In addition, a combination of ketamine with benzodiazepines, mostly flunitrazepam, in a dosage of ketamine 25–40 mg/kg i.m., and flunitrazepam 1.0 mg/kg i.m. can be used. Benzodiazepine components can be antagonized by flumazenil at a dose 0.2 mg/kg s.c. A combination of tiletamine/zolazepam [3] (Table 5.2 C, D) is suitable for short-term surgical procedures lasting for 15–20 min.

The opioid anesthetics fentanyl or sufentanil can be combined with neuroleptic azaperone (Table 5.3). Mantell [4] used fully antagonizable general anesthesia in a combination fentanyl + medetomidine + midazolam. The reversion was performed by a combination of atipamezol, flumazenil and naloxone (Table 5.4). Perantoni used partiall agonist of the opioid receptor buprenorfin instead of naloxon to antagonize the above mentioned anesthetic combination. The use of buprenorfin instead of naloxon results in prolongation of analgesic effect even in the postoperative period.

5.1.5 Post-operative Analgesia

Analgesics should be given in the post-operative period to enhance the recovery of animals and a more rapid return to normal behavior (food and water intake, movement). Adequate pain relief should also be provided from ethical reasons and based on the animal protection act.

Table 5.1 Recommended dosage of sedatives

Sedation
Midazolam 2.5 mg/kg i.m. or i.v.
Diazepam 2.5 mg/kg i.m. or i.v.
Xylazine 5.0 mg/kg i.m.
Medetomidine 0.135 mg/kg i.m.
Azaperon 1.0 mg/kg i.m.

Table 5.2 Anesthesia with alfa2-agonists

A	**B**
Ketamine 100 mg/kg i.m. + Xylazine 5 mg/kg i.m.	Ketamine 60–75 mg/kg i.m. or s.c. + Medetomidine 0.25–0.5 mg/kg i.m. or s.c.
Surgical anesthesia stage lasts 20–30 min. For prolongation, one third of the starting dose can be added. Sleep after anesthesia is lengthy. Diuresis is increased. Danger of hypothermia. The effect can be partially antagonized by atipamezole	The length of surgical anesthesia stage fluctuates from 30 to 90 min. Danger of hypothermia. Polyuria. The partial reversion by atipamezole is possible no earlier than 45 min after anesthesia induction
C	**D**
Tiletamine/zolazepam 20 mg/kg i.p. or i.m. + Xylazine 10 mg/kg i.p. or i.m.	Tiletamine/zolazepam 20–40 mg/kg i.m.
Surgical anesthesia stage lasts 30–45 min. Recovery may be prolonged. The effect of zolazepam and xylazine can be partially antagonized by specific antagonists	It is used less often because recovery is prolonged

Table 5.3 Anesthesia with opioid components

A	**B**
Fentanyl 0.1–0.15 mg/kg i.m. + Azaperon 1.0 mg/kg i.m.	Sufentanil 0.01–0.02 mg/kg i.m. + Azaperon 1.0–2.0 mg/kg i.m.
After 10–15 min, a stage of surgical analgesia for 30–45 min is reached. The effect of the opioid component can be reversed by naloxone at a dose 0.1 mg/kg	After 10–15 min, the stage of surgical analgesia for 60–120 min is reached. Naloxone (0.1 mg/kg) can be administrated to antagonize the effect of opioid component
C	
Hypnorm (fentanyl 0.08 mg/kg – fluanizon 0.25 mg/kg) + Medetomidine 0.25 mg/kg	
Occasionally Atropin 0.05 mg/kg	
Therapeutic range is good. Animals can tolerate double of the recommended dose. Anesthesia can be prolonged by half of the initial dose of hypnorm/medetomidine or by combination of fentanyl/medetomidine at a dose as for anesthesia induction or by combination droperidol 1.0–2.0 mg/kg with medetomidine 0.15–0.2 mg/kg. Specific antagonists can reverse the effects of fentanyl and medetomidine	

Though we are able to provide effective analgesia during surgery, the use of analgesics in the postoperative period of small rodents has not yet became a general rule The post-operative analgesion has still a minor tradition in small rodents. Although general anesthesia produces loss of consciousness, painful stimuli can still be transmitted and processed by the spinal cord and brain and also hypersensitivity can still develop. Administration of analgesics before surgery can prevent these events and also the duration and intensity of post-operative pain is reduced. This approach is known as preemptive analgesia and combines analgesics with different mechanisms of action, such as ketamine and alpha2-agonist. Peri-operative analgesion in laboratory rats use strong analgesics (opioids), mostly centrally acting antipyretic (e.g. pyrazolones) and more peripherally acting weak non-steroidal antiflogistics. Post-operative analgesia is listed in the following table (Table 5.5).

Table 5.4 Fully antagonizable anesthesia

Anesthesia	Antagonization
Fentanyl 0.005 mg/kg i.m. + Midazolam 2.0 mg/kg i.m. + Medetomidine 0.15 mg/kg i.m.	Naloxon 0.12 mg/kg s.c. + Flumazenil 0.2 mg/kg s.c. + Atipamezol 0.75 mg/kg s.c.
Duration of the anesthesia is 45–60 min. One third of the initial dose can be administered i.m. or i.p. for anesthesia prolongation	Analgesia maintaining in the post-operative period can be achieved by replacing naloxone with buprenorphine at a dose 0.05 mg/kg s.c.

Table 5.5 Post-operative analgesia

	Dose	Duration of action
Opioids		
Petidin	10–20 mg/kg s.c. or i.m.	2–3 h
Morphine	2.5 mg/kg s.c.	4 h
Butorphanol	2.0 mg/kg s.c.	4 h
Buprenorphine	0.01–0.05 mg/kg s.c. or i.v.	8–12 h
	0.1–0.25 mg/kg per os	8–12 h
Antipyretics		
Metamizol	1–2 drops per os	4–6 h
Paracetamol	200 mg/kg per os	4 h
Aspirin	100 mg/kg per os	4 h
NSAIDs		
Carprofen	4 mg/kg i.v., i.m. or s.c.	12 h
	2 mg/kg per os	12 h
Ibuprofen	30 mg/kg per os	12 h
Flunixin	2.5 mg/kg s.c	12 h

5.1.6 Euthanasia

Euthanasia of healthy or diseased animals must be carried out fully in accordance with ethical guidelines. Unconsciousness must be induced rapidly and death must occur with minimal fear and pain. Therefore, euthanasia should be performed by an experienced person. Also, the presence of other animals during killing is not recommended because the smell of blood affects plasma levels of catecholamines; norepinephrine and dopamine are in particular increased.

Physical methods or pharmacological agents (inhalant or injectable anesthetics) are used for euthanasia. The choice of appropriate euthanasia method is important, although both methods can impact on post-mortem findings. Decapitation with surgical scissors or by guillotine are very quick and effective methods, however, the tissue in the neck area is seriously devastated. Cervical dislocation is the least devastating technique. With inhalation techniques, an overdose of diethylether or another inhaled anesthetic can be used. If an animal is killed at the end of the experiment and is still in general anesthesia, the combination product T61 can be

administrated intraperitoneally. From injectable anesthetic, thiopental overdose can be used. The use of parenteral anesthetics results in stasis of blood in the organs, which may complicate post-mortem examination.

5.2 Inhalational Anesthesia in Rats (by Hynek Riha)

5.2.1 Introduction

General anesthesia is required for the successful performance of many animal experiments [6] with the main advantage being immobilization of the animal (i.e., reversible reduction or complete elimination of sensory and motor responses of the animal). Both major types of general anesthetics, i.e., inhalational and injectable types (administered most frequently intraperitoneally but also subcutaneously or intramuscularly), are used in rats [7]. One of the main advantages of inhalational agents in contrast to injectable anesthetics is the possibility for precise anesthetic control. Injectable drugs have a slow onset of action, their absorption after intraperitoneal or intramuscular application may significantly vary and relatively large doses are required due to the high metabolic rate in rats, which results in variable duration and depth of anesthesia with prolonged recovery times.

The most important anatomical, physiological and pathophysiological aspects of general anesthesia in rats [7] are:

- the small diameter of superficial veins with restricted opportunity for the application of intravenous anesthetics;
- the high ratio of body surface area to body weight, which predisposes the animal to rapid heat loss and subsequent hypothermia (moreover, most anesthetics depress thermoregulation to some degree and other insults, e.g., shaving, cold fluids and environment, can also promote hypothermia);
- the small larynx with relatively difficult access for endotracheal intubation;
- the small body size, which predisposes the animal to respiratory compromise due to excessive traction or pressure from the surgeon's hand or putting surgical instruments on the rat's chest;
- the occurrence of subclinical lung disease with the risk of development of respiratory distress or failure during or after general anesthesia.

5.2.2 Inhalational Anesthetics

Inhalational (volatile) anesthetics are small molecules of liquids that evaporate very easily. In chemical composition, they represent halogenated hydrocarbons: alkanes (halothane) and ethers (isoflurane, enflurane, sevoflurane and desflurane). Some of the noble gases also have anesthetic properties (e.g., xenon). Currently, the most frequently used inhalational anesthetics in the experimental field are isoflurane and sevoflurane.

Inhalational anesthetics reach the alveoli by ventilation, which is either spontaneous or artificially (mechanically) achieved by a ventilator. In the alveoli, anesthetics diffuse into the circulating blood and finally reach the site of their primary effect, i.e., the central nervous system. General anesthesia with inhalational agents in the rat can be divided into three specific phases: induction of anesthesia, its maintenance and a recovery phase.

The "dose" of inhalational anesthetics in an inhaled gas mixture is expressed either as an absolute value in percent volume (vol %) or as a relative value in multiples of minimal alveolar concentration (MAC). MAC is defined as the concentration of inhalational anesthetic in the alveolar gas that prevents purposeful movement in response to a standardized noxious stimulus (e.g., skin incision) in 50 % of subjects and it reflects the anesthetic potency of the particular agent. MAC does not vary significantly in different species (interspecies variation is approximately 10–20 %) and is not significantly influenced by the sex of the animal or the duration of anesthesia. However, it is significantly influenced by age (younger animals require higher concentrations of anesthetics to reach the same depth of anesthesia), the addition of nitrous oxide (administration of N_2O in an inhaled gas mixture significantly decreases MAC) and by other medications (analgesics decreases MAC). The MAC of isoflurane and sevoflurane in adult rats was determined to be 1.3 % and 2.3 %, respectively; in 9-week-old rats, the MAC of sevoflurane is 2.7 %. Reported concentrations used for inhalational anesthesia in the rat vary among various centers and in different types and durations of conducted procedures and they also depend on concomitant medication. Commonly used concentrations are 2–3 MAC for the induction of inhalational anesthesia and 1–2 MAC for the maintenance of anesthesia.

5.2.3 Practical Conduction of Inhalational Anesthesia

Inhalational anesthetics are administered via a vaporizer that is specifically calibrated for a particular anesthetic agent; in the vaporizer, the saturated vapor of the inhalational anesthetic is diluted by fresh gas flow (precisely controlled by flowmeters) to achieve an accurate target volume concentration (%) that is set on the vaporizer. The inhaled gas mixture that is used for anesthesia is typically composed of air and oxygen; the commonly used inspiratory fraction of oxygen (FiO_2) is in the range of 21–40 % and depends on the type and duration of the experimental procedure or examination. Intraperitoneal administration of injectable sedatives (e.g., opioids [fentanyl], benzodiazepines [midazolam], xylazine, and medetomidine) or anesthetics in lower doses (e.g., ketamine) before the induction of inhalational anesthesia for major procedures (i.e., premedication) facilitates handling of the rat, reduces stress associated with the induction and decreases agent concentrations that are required for anesthesia.

The induction of inhalational anesthesia can be conveniently performed in an anesthetic induction chamber (either commercially available or custom-made) [8];

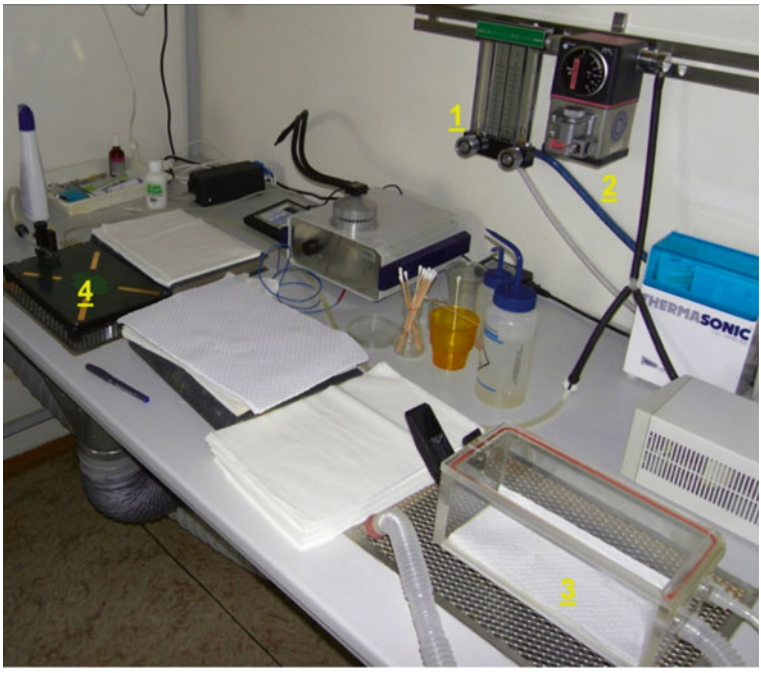

Fig. 5.1 *1*. Flowmeters regulating fresh gas flow, *2*. Vaporizer, *3*. Anesthetic induction chamber, *4*. heated pad with gold-plated electrodes (ECG) and nose cone for delivery of inhalational anesthetic

depending on the anesthetic concentration that is set on the vaporizer and fresh gas flow into the chamber, the anesthesia is induced after a few minutes (Fig. 5.1). Afterwards, the rat is removed from the chamber and very brief procedures can be carried out. Otherwise, the maintenance of anesthesia is secured by a face-mask, sealed nose cone or nasal catheter (put in the back of the pharynx) with spontaneous ventilation and continued administration of the inhalational agent.

Longer surgical procedures obviously require mechanical ventilation. As the first, endotracheal intubation or tracheotomy with subsequent cannula insertion is performed after intraperitoneal or intramuscular administration of an adequate dose of injectable sedatives or anesthetics. Intubation should be used for survival experiments to avoid the risk of tracheal occlusion from edema after the removal of tracheostomy cannula (inner diameter of the rat's trachea is 2–3 mm). Intubation of the rat is typically performed in semivertical position with the animal anchored to the inclined metal plate by upper incissors (Fig. 5.2).

The difficulty of intubation is caused by small oral cavity, narrow and highly mobile larynx, and difficult visualization of the trachea [7]. After opening of the oral cavity, the tongue is pulled out and displaced; the larynx is exposed by small metal blade/utensil or custom-made instrument. Percutaneous transillumination using strong light source focused on the ventral area of the neck is a very useful tool

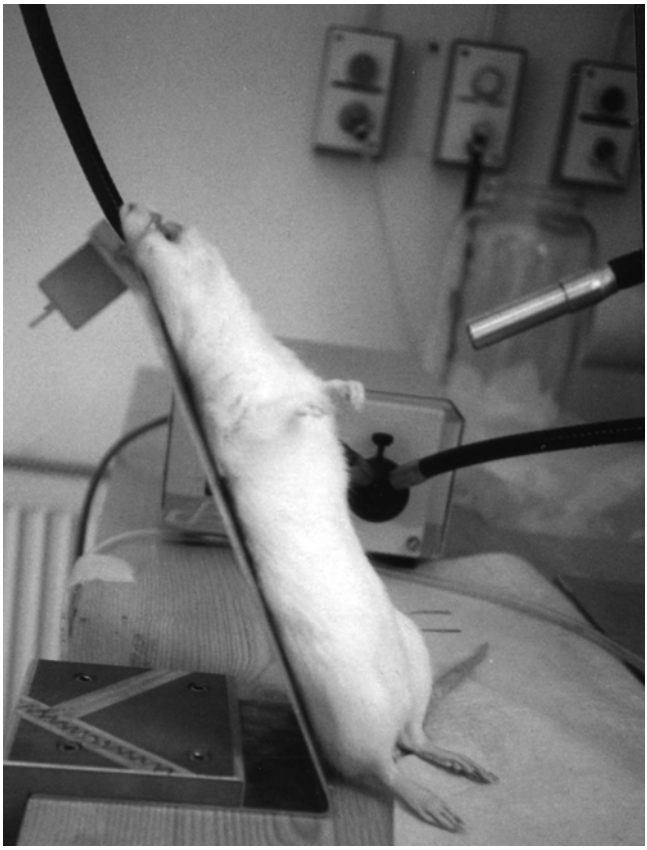

Fig. 5.2 Intubation of the rat is typically performed in semivertical position with the animal anchored to the inclined metal plate by upper incissors

to aid with visualizing the vocal cords and the entrance to the trachea. In most cases, a 12–16G intravenous cannula is appropriate for endotracheal intubation of the rat. The stylet should be used to increase the stiffness of the cannula (e.g., over-the-needle cannula); the stylet (i.e., the needle) has to be shortened to prevent tissue injury. Subsequently, an inhalational agent from a vaporizer is delivered by mechanical ventilation; rodent ventilators are used to achieve a very small tidal volume (1.2 mL/100 g of body weight) and high respiratory rate (65–100 breaths/min) with the ratio of inspiration to expiration of 1:1.5–1:2 [7].

The emergence from inhalational anesthesia, which is characterized by reacquisition of righting reflex, coordination and consciousness, is relatively fast (5–30 min) depending on the anesthesia duration. The risk of unnecessary hypothermia continues until the rat regains normal motor activity.

5.2.4 Monitoring and Analgesia

Monitoring during general anesthesia is largely dependent on the type and duration of the surgical procedure. Temperature monitoring is crucial and, with available thermometers, is easily ensured, e.g., measuring rectal temperature.

Respiratory monitors use sensors to monitor the respiratory rate; these sensors can become inaccurate in smaller rats (<200 g). Consequently, it is necessary to use a pulse oximeter to monitor respiratory function. The probe (clip) is typically put on the hind feet and, in some cases, on the tail (tail-wrap clip); with significant vasoconstriction (e.g., after ketamine administration or severe fluid loss), the probes are prone to signal loss. During mechanical ventilation, capnography can be used.

Cardiovascular monitoring includes electrocardiography, blood pressure measurements (non-invasive or invasive, typically via the carotid artery) and pulse oximetry. Pulse oximeters display heart rate, which is derived from the peripheral pulse waveform. Due to the elevated heart rate in rats (300–400 beats/min), oximeter devices must be selected based on their suitability for use with this species.

Analgesia represents an important part of perioperative care in the rat; it improves recovery and reduces pain after surgery [9]. Frequently used drugs are opioids (e.g., buprenorphine 0.05–0.1 mg/kg s.c. q 8–12 h) and non-steroidal anti-inflammatory drugs (e.g., ketoprofen 2.5–5.0 mg/kg s.c. q 24 h). In extensive surgical procedures, it is advisable to use preemptive analgesia, i.e., to administer analgesics before surgery. Because opioids cause dose-dependent respiratory depression, this effect should be taken into account during subsequent general anesthesia with spontaneous ventilation to avoid hypoventilation. At the end of major procedures, surgical site infiltration with a long-acting local anesthetic (e.g., bupivacaine, which has an onset of action of 15 min and lasts 4–6 h) can greatly reduce postoperative pain. Afterwards, subcutaneously administered opioids and non-steroidal anti-inflammatory drugs (alone or in mutual combination) are typically used.

5.2.5 Caveats of Inhalational Anesthesia

1. Inhalational anesthetics have a profound impact not only on the central nervous system but also on other organs. Major side effects can be observed in the cardiovascular [10] and respiratory systems. These adverse effects are directly proportional to the anesthetic concentration and, to a lesser extent, the duration of anesthesia. Both isoflurane and sevoflurane depress myocardial contractility and reduce systemic vascular resistance and the resulting hypotension is accompanied by an increase in heart rate, which is due to baroreflex activity, surgical stimulation and fluid loss.
2. Respiratory depression (hypoventilation) induced by isoflurane and sevoflurane can cause hypercarbia, hypoxia and respiratory acidosis; severe hypoxia is associated with the risk of cardiac failure and arrest. In contrast, respiratory depression induced by injectable anesthetics is typically more profound. In spontaneously

ventilating rats developing severe hypoventilation, one can help to inflate the lungs by gentle blowing using a syringe barrel placed over the nose and mouth. In particular surgical models, especially in extensive or lengthy procedures, it is useful to verify the effects of inhalational anesthesia on arterial blood gases (blood samples taken from the ventral tail artery by a 22G intravenous needle); this verification can lead to modification of the anesthesia parameters (e.g., lowering the concentration of the volatile agent or increasing FiO_2).

3. The use of inhalational anesthetics necessitates the need for their scavenging (passive or active) to prevent pollution of the work environment and exposure of animal-care personnel. In the case of mechanical ventilation, the use of a scavenging hose is a simple solution. In anesthesia with spontaneous ventilation using a face-mask or sealed nose cone, scavenging of exhaled gases could be more difficult but an extract tube for waste gases placed near the nose can be useful.

5.2.6 Tips and Tricks

1. It is not necessary to withhold food and water before general anesthesia unless the surgical procedure would involve the stomach; feeding is reestablished as soon as possible after the procedure. Inappetence after major surgical procedures can be caused by postoperative pain.
2. The heat loss during general anesthesia can be rapid and serious. Maintaining normothermia with a heated pad and lamp, which is sometimes supplied with insulating material covering the rat, is strongly advisable. During recovery from anesthesia, suitable comfortable warm bedding is important.
3. A precisely defined anesthesia protocol, i.e., standardized delivery of inhalational anesthesia (including agent concentration and duration) in all the rats during the experiment, is crucial. Otherwise, unpredictable confounding effects could be introduced into the experiment.
4. The whole setup and procedure of inhalational anesthesia should be thoroughly tested before running the experiment to avoid unwanted side effects (e.g., hypothermia, severe respiratory depression or death).
5. The lowest possible concentration of inhalation agent should be used. In most procedures, it is rarely necessary to use >3 MAC for the induction and >2 MAC for the maintenance of anesthesia.

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Chapter 6
Basic Techniques for Microsurgery Experiment

Peter Balaz and Jan Kriz

Abstract Exactly performed vascular anastomosis between graft and recipient is important step in all organ transplantation, and its basis of success of the each transplantation procedure. The chapter provides technical "know how" of surgical training for the surgeons in the learning curve time and in details described all types of the common vascular anastomosis with its advantages and disadvantages. Tips and tricks in each part of the chapter facilitate training period not only for the trainee surgeons, but could be important for also for skilled experimental surgeons. The second part of the chapter provides the simple manual how to collect blood samples from anesthetized and/or conscious rat.

Keywords Blood sampling • Microsurgery • Catheter • Surgical training • Knotting • Vessel preparation • EtE anastomosis • EtS anastomosis • Cuff technique • Patency test

6.1 Vascular Anastomosis

Experimental microsurgical techniques using intraoperative microscopy at present, enables a spectrum of organ transplantations to be performed in rats and as such has considerably expanded experimental surgery and given a new impetuses in clinical surgery [1]. However, for the inexperienced microsurgeon, most organ transplantations are a very demanding procedure, mainly because of the difficult vascular connection for which a long learning curve is usually needed. Proper education and training not only benefit trainees, who can acquire special competence, but also the research institutions, since established models and methods can be continued by

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qualified surgeons [2]. Therefore, the fundamental principles of microsurgery play an important role in successful experimental research, especially as a training model for surgeons. Vascular anastomosis is a fundamental technique that enables reperfusion of a transplanted organ and its absolutely precise performance is basis of experimental success.

This chapter provides technical "know how" of surgical training for the beginner in experimental microsurgery and in detail describes different techniques of vascular connection. The techniques of biliary anastomosis, ureteral anastomosis and reconstruction of the intestinal tract are separately described in relevant chapters dedicated for organ transplantation in the special parts of this book.

6.1.1 Surgical Training "Ex Vivo"

For an inexperienced surgeon, it is important to be familiar with the surgical microscope and handling with instruments to be able to make clear vascular anastomoses. Where the saving of life of an experimental animal remains paramount, we strongly recommend before experiment to improve surgical skills on an "ex vivo" model. This exercise enhances the future experimental procedures and prepares surgeons for another surgical dimension – microscopic surgery. Ex vivo training allow surgeons to obtain spatial orientation under magnification so that the hand movements are precise and viewing under magnification helps to perfect the interplay with hands. Firstly, the surgeon must be familiarly with surgical microscope with siting and adjusting. Before starting the work, remove every object from the way of the legs and make enough room for the knees. Hand tremors must be absolutely eliminated and therefore the both forearms should rest firmly on the surgical table. The microscope must be adjusted at 0 diopter, after the focus is adjusted to the fine position. Set the interpupillary distance. Choose the lowest magnification and focus on the spot that you previously marked by using the coarse focus and after choose the highest magnification and adjust the fine focus. Finally, switch to the lowest magnification without modifying the focus, and set the eyepieces to the lowest possible diopter. Adjust the diopters separately for each eye by rotating the lens of the eyepiece clockwise. It is important is to adjust the light source on the minimum intensity where the object is clear. Training exercises of the hand and tools position are also very important. Instruments should be held like a pen using three fingers (Fig. 6.1) and the wrists and under sides of the forearms should rest on the table.

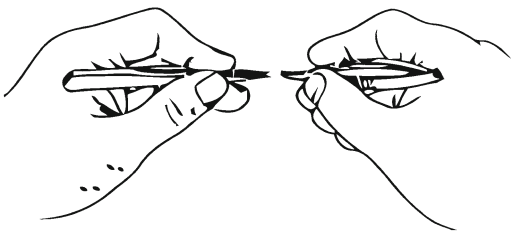

Fig. 6.1 Holding of the instruments (Source: http://www.szote.u-szeged.hu/expsur/hefop/angol/microsurgery/microsurgery_advanced_invivo.htm#TOP)

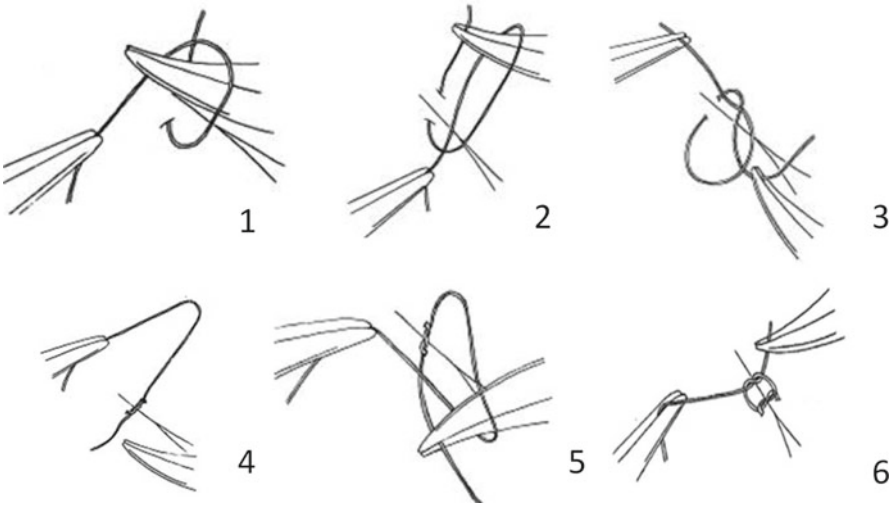

Fig. 6.2 The knotting technique (Source: http://web.med.u-szeged.hu/expsur/hefop/angol/microsurgery/microsurgery_exercises.htm#endtoend)

There exist some techniques for knotting, but in all types of knotting in microsurgery is performed with using instruments (microsurgical needle holder and tweezers). The first knot is mostly a double and another is simple. Tightening of the suture must be reasonable, because over tightening can lead to disruption of the vessel wall and damage of the intimal layer, which may result in the initiation of coagulation and thrombosis. On the other hand, too loose tightened of sutures may expose thrombogenic subintimal layers of the vessel wall and patency of the anastomosis is also seriously threatened. A schema of knotting techniques are described in Fig. 6.2.

For the training of previously described practice, it is the ideal to use an inexpensive model which can be easily prepared from a surgical glove stretched over a Petri dish where the target area is painted with a square (Fig. 6.3). Initially, put the instruments into the field of microscope view in a predetermined place. This exercise teaches the surgeon tool orientation and gentle movements of the instruments in vertical and horizontal lines. After perfectly mastering the safe orientation in the field of the microscope, then proceed to practice stitching. As the most effective training, it is best to make the sutures on the perforated glove in previously described model. This model allows surgeons to practice incision, excision and their suture technique under the magnification.

6.1.2 Vascular Anastomoses

Organ transplantation depends on restoration of blood flow to the new organ and, for this reason, the precise vascular connection between graft and recipient vascular system is the "cornerstone" success of the procedure. In contrast with human organ

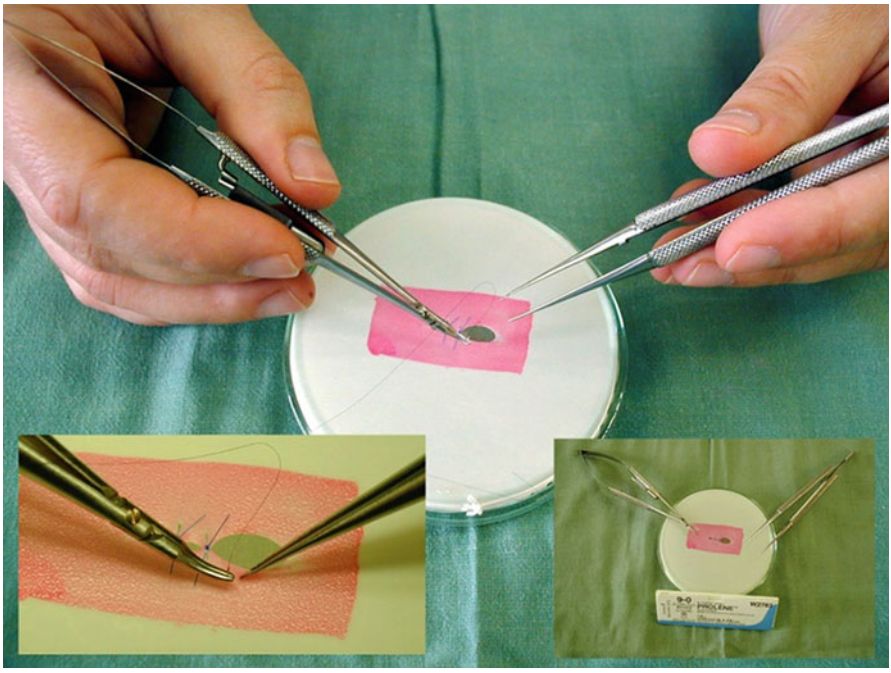

Fig. 6.3 Surgical training (From Balaz et al. [13])

transplantation, experimental rat transplantation is performed with microsurgical technique similarly as in human reconstructive microsurgery. Therefore, the main rules with vessels handling are similar to the human microsurgical technique. Microsurgical anastomoses are performed using meticulous technique and with the following principles. The recipient vessels and the match between the size of the recipient and pedicle vessels must be carefully assessed. Preparation of vessels must be to a healthy untraumatized part; blood clots from the edge of the vessels must be removed and irrigated with heparinized saline. Vessel side branches are examined and ligated to prevent hematoma. Either end-to-end or end-to-side anastomoses may be performed, depending on the recipient vessel and the match between the size of the vessels. Avoidance of vessel tension, kinking, and twisting is important. Vascular anastomoses are finally examined with patency test and the control of bleeding must be precise. Small bleeding should be stopped with pressure applied using a cotton pad or adding sutures. Keep in mind that small bleeding can be fatal in experimental rat transplantation.

6.1.2.1 Preparation of the Vessel

For the better visualization and to avoid contamination of the anastomotic area, it is recommend to put a strip of silicon glove behind the dissected vessel. A vascular clamp is gently placed as first on proximal part of artery (distally on the vein) and

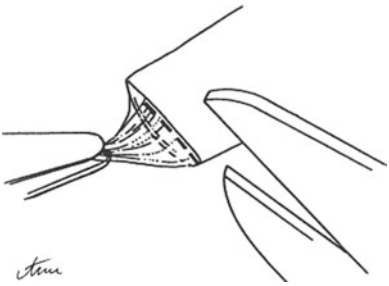

Fig. 6.4 Adventitial stripping using scissors (Source: http://www.szote.u-szeged.hu/expsur/hefop/angol/microsurgery/microsurgery_advanced_invivo.htm#TOP)

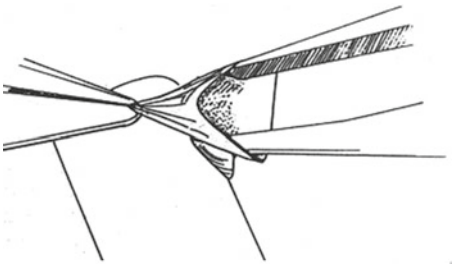

Fig. 6.5 Dilatation of the vessel (Source: http://www.szote.u-szeged.hu/expsur/hefop/angol/microsurgery/microsurgery_advanced_invivo.htm#TOP)

after distally (proximally on the vein). The space between the clamps and the place of the future anastomosis must be enough large so that there is about a 1 mm distance between the vessel ends. The cutting of the vessels must be completely through with one cut using scissors perpendicularly to the vessel axis. Both lumen are irrigated in with a solution containing heparin (1 ml of solution contains 5000 UI of heparin) and the adventitia should be removed in a few millimeter distance from the edges (Fig. 6.4). If the distal end of the vessel is not used for the anastomosis, it must be carefully ligated.

It is recommend to perform a gently dilatation the lumen of the dissected vessel with tweezers to avoiding a spasm and enlargement the diameter to a maximum of 1.5 times the natural diameter (Fig. 6.5). During surgery, it is important to rinse the wounds with isotonic solution and wash the vessel lumen with heparin solution for better visualization and vasospasmus prevention. Flushing solutions should be at room temperature as a cold solution for possible induction of vasospasm is not suitable.

6.1.3 End to End Anastomosis

6.1.3.1 Continuous Suture

While the interrupted suture technique has been generally preferred for microsurgical end-to-end and end-to-side anastomosis, studies which preferred continuous suture technique have been also reported [3–5]. Additionally, this technique is faster

than an interrupted suture: there are no knots to tie, no thread ends to cut, and therefore there is no need to change the instruments in the hands. It is important to use a two short thread (3–5 cm) in order to reduce vessel trauma which occurs when the suture passage through the vessel wall. In additional, in texts, the upper pole is defined as "12 o'clock" and the lower pole as "6 o'clock".

1. the first suture is placed at the center of the posterior wall at the position opposite the surgeon (12 o'clock) (Fig. 6.6, A2)
2. the second suture is placed on the anterior wall (6 o'clock) (Fig. 6.6, A1)
3. the one end of each suture is temporarily fixed in gently tension to maintain the vessel lumen in a flattened and expanded condition
4. the anastomosis begins from 12 o'clock from the posterior wall of the vessel towards 6 o'clock (to avoid the accidental stitching of the dorsal wall the plastic cannula with appropriate diameter can be placed into the lumen during the suture) (Fig. 6.6, B)
5. the first thread of the suture is knotted with one of both threads at 6 o'clock (Fig. 6.6, C3)
6. finally, the continuous suture is finished with sutures of the anterior wall from 6 o'clock toward to the 12 o'clock, where the thread is knotted with one of the threads at 12 o'clock (Fig. 6.6, C4)

Apart from the previously described technique, the suture of the posterior wall with rotation of the vessel can be facilitated so that the stitch placed on the 6 o'clock is turn up to the 12 o'clock position and the stitch placed at 12 o'clock is turned down at the 6 o'clock position. With this maneuver, twist the vessel and place the posterior wall to the anterior position which will facilitate the suture. After finishing the suture of the posterior wall, the vessel is turned back to the initial position and the anterior wall is performed.

Usually after the first two initial stitches at both ends, a further 4–5 stitches are necessary and must to include the muscle and also the intima layer of the vessel wall into the each stitch. The technique of the continuous suture is similar for the vein and artery, however, the artery anastomosis is easier than the vein because the fine venous wall and the lumen collapse when the suture is performed.

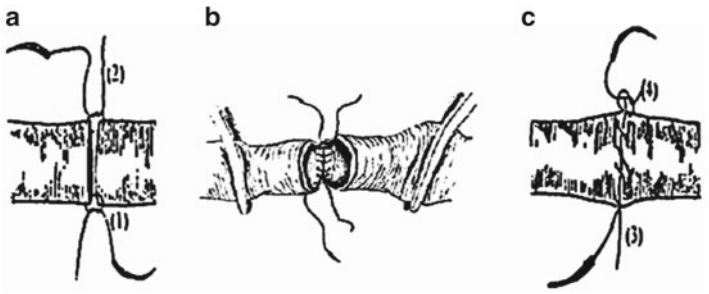

Fig. 6.6 Technique of end-to-end anastomosis using continuous suture (Source of pictures **a**, **c** – Chen et al. [5], Source of picture **b** – Ball and Feliciano [14])

6.1.3.2 Interrupted Suture

The classical end-to-end interrupted suture is performed after vessels clamping with the placing of three main sutures which divide the circumference of the vessels into three equal 120° segments. Additional sutures are then placed between the main sutures on the anterior vessel wall. The clamps are then rotated 180° to allow suturing of the posterior wall [6]. In the next paragraph, we described a simplified technique developed by Yu et al. [7] which allow the safe end-to-end anastomosis without vessels rotating which can cause a endothelial damage, sometimes resulting from even slighter manipulations [8].

Course of the technique described by Yu et al. [7]:

1. the posterior and anterior sutures are placed (Fig. 6.7, A1, A2)
2. the vessel is rotated 90° by traction of the two stay sutures, allowing for half of the vessel to be presented anteriorly (Fig. 6.7, B)
3. the third, fourth and fifth sutures are placed (Fig. 6.7, C)
4. after traction on the two stay sutures, the vessel is first rotated back 90° to its original position (Fig. 6.7, D)
5. in a further 90° rotation, the opposite unsutured half of the vessel is presented anteriorly (Fig. 6.7, E)

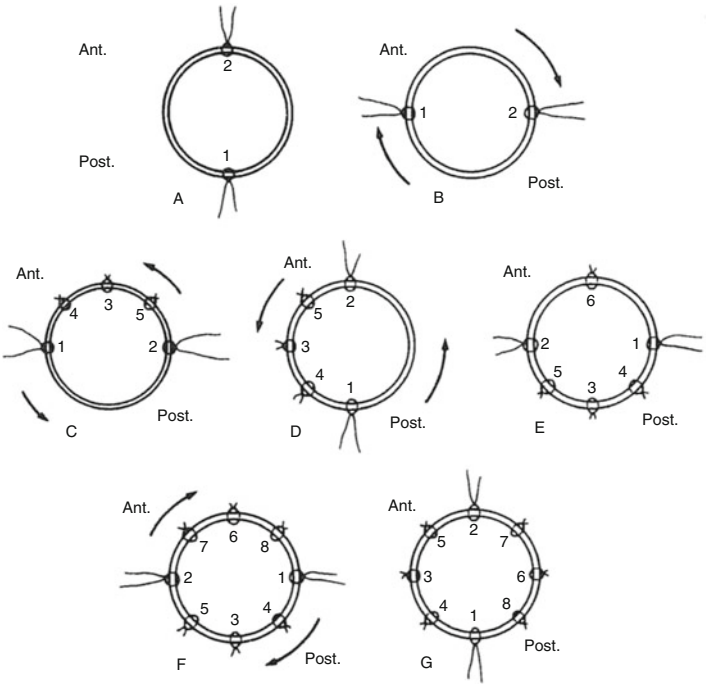

Fig. 6.7 Cross-sectional drawings of the end-to-end interrupted anastomosis technique (Source: Yu et al. [7])

6. complete the suture (Fig. 6.7, F)
7. reposition of the vessel to the neutral position (Fig. 6.7, G)

6.1.4 End to Side Anastomosis

Although end-to-side anastomosis is technically more challenging than the end-to-end anastomosis, its importance is a good patency rate and safety. This type of anastomosis represents a solution for the using with discrepancies in the vessel diameters and, additionally, it does not interrupt the distal blood flow. The main difference is in the performance of arteriotomy, which can be demanding with the small caliber of the vessels. For this purpose, facilitate the arteriotomy with placing a stitch into the area of the future excised vessel part, lift it up and cut out an ellipse-shaped piece with a single move with a curved scissor (Fig. 6.8). This excised area can further be enlarged when necessary. In very small caliber vessels, a simple longitudinal incision can be also performed. After flushing the lumen, free adventitia is removed. The length of the arteriotomy is recommended at one and a half times more than the diameter of the vessel that will provide the terminal end of the anastomosis.

Similarly, end-to-end anastomosis can be performed with in a continuous or interrupted fashion. From the literature, there exist a few experimental works comparing the efficiency of both techniques. Little and Salerno [9] suggested that a continuous suture method is faster, more hemostatic with equivalent patency rate compared to an interrupted suture method. On the other hand, Hamilton and O'Brien found that the continuous suture was less favorable because of entrapment and breakage of the suture [10]. In comparative study designed by Peerless et al., it was found that the continuous suture has an equivalent growth and patency figures and is much faster than the interrupted suture method [4]. Based in our long term experimental experience with rat organ transplantation, we recommended for the majority of transplantations an end-to-side anastomosis with continuous suture technique. The next paragraphs will describe both techniques step by step.

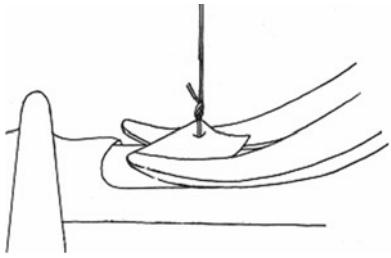

Fig. 6.8 Performing of the arteriotomy in small caliber artery (Source: http://www.szote.u-szeged.hu/expsur/hefop/angol/microsurgery/microsurgery_advanced_invivo.htm#TOP)

6.1.4.1 Continuous Suture

Place the first corner knot at the heel of the anastomosis and tie the knot leaving the short end longer so that later it will be used for tying with another knot (Fig. 6.9 A/1)

1. Take a second thread and suture the apex of anastomosis at 180° angle from the first corner knot (Fig. 6.9 A/2)
2. Use the thread from the "heel stitch" and make a continuous suture of the posterior wall (Fig. 6.9 B).
3. Tie the knot with the shorter end of the "apex stich" after gently tightening of the suture (Fig. 6.9 B/1).
4. Use the longer end of the "apex stitch" and continue with anterior wall (Fig. 6.9, C)
5. After gently tightening of the suture, tie a final knot with shorter end of the "heel stitch" (Fig. 6.9, D)

6.1.4.2 Interrupted Suture

1. place the corner knot by stitching into the graft vessel from the inside towards the outside
2. use the second needle of the same thread and place a stitch from inside towards the outside in recipient vessel
3. approximate both vessels and make the first knot (Fig. 6.10/1)
4. place the second corner stitch at 180° angle from the first one in the same fashion (Fig. 6.10/2) and knot it.
5. place other stitches of the posterior wall with a back-hand technique. The stitches should be placed starting from to corner toward the center of the anastomosis (Fig. 6.10/7,8,9,10).
6. after placing the all stitches, make knots consecutively on the posterior wall ("insert suture first, tie later" principle).

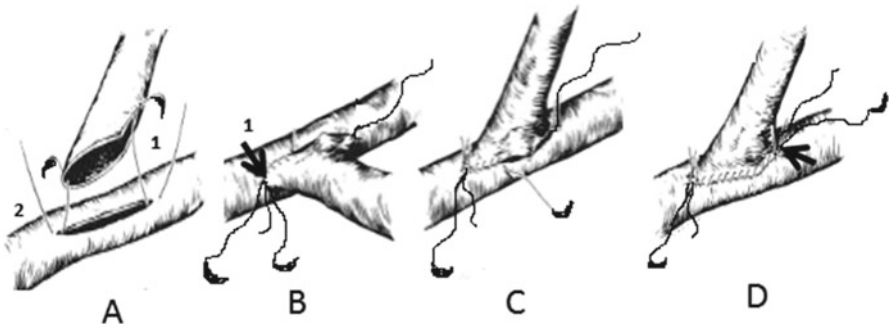

Fig. 6.9 Technique of continuous suture (Source: Peerless et al. [4] – modified by Balaz P. (Eds))

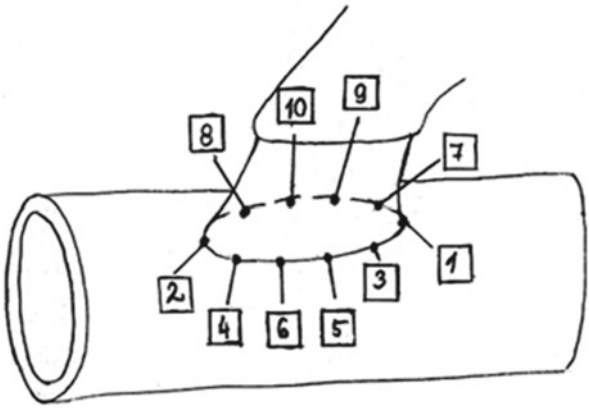

Fig. 6.10 Order of the stitches of the interrupted suture (Source: http://www.szote.u-szeged.hu/expsur/hefop/angol/microsurgery/microsurgery_advanced_invivo.htm#TOP)

7. turn over the graft and expose the anterior wall.
8. inspect the interior of the anastomosis and check to make sure that the sutures were placed correctly.
9. suture the anterior wall of the anastomosis in the same manner as the posterior wall with placing the stitches (Fig. 6.10/3,4,5,6).

6.1.5 Vascular Anastomosis with Using "Cuff Technique"

Even if the traditional suture of vascular construction uses thread with needle which can traumatize vascular tissue and can cause frequent technical complications, it is still the "gold standard" for graft revascularization in all organ transplantation. However, the proper performance of vascular anastomosis requires time-consuming learning curve and costly education of the trainees. A number of techniques can be tried to simplify microvascular anastomosis; however, they accomplish procedural drawbacks, poor efficiency, and scanty clinical significance [11]. The Cuff technique is a simple method of microvascular anastomosis where at the vascular connection polyethylene cuffs are inserted to the donor and recipient vessel and are thus connected together. The Cuff technique is suitable as alternative method to standard vascular anastomosis for many transplantation models in rats in end-to-end vascular anastomosis. This technique is usually used for large rat vessels e.g. portal vein, vena cava, aorta, pulmonary artery/vein, but can be used in a modified fashion for biliary anastomosis after liver transplantation. The main advantage of this technique is that it is an inexpensive, easy and fast even by an unexperienced experimental surgeon. The disadvantage is the cuff persistence inside anastomosis, possible foreign body reaction and the risk of thrombosis. Even if histopathological studies confirmed only a mild and acceptable foreign-body reaction of the graft and

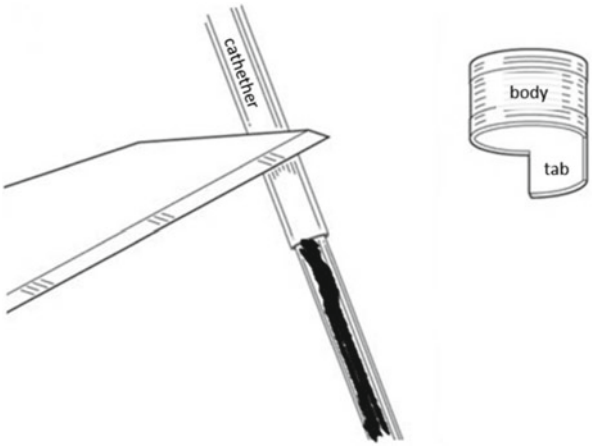

Fig. 6.11 Creation of the cuff (Source: Alexander et al. [15])

polyethylene cuff on the vessel anastomotic site [12], we recommend this technique in models for organ transplantation without planned long term survival of animals e.g. research of ischemic-reperfusion injury.

The cuff is usually constructed from a polyethylene or Teflon angiocatheter in various sizes (16–20G) depending on the diameter of the cuffed vessels. After insertion of angio guide wire or other metal rod into catheter, create with the scalpel blade a 1.5 mm-long tab (hanger) and cut the 1.5 mm-long body of the cuff (Fig. 6.11). The tab is used to hold the cuff with a clamp during the implantation.

The basic principles of the cuff technique involve pulling the donor vessel though the circular cuff which is held in place by (a) the extension handle of the cuff. The vessel is folded over the cuff (b) and secured to the cuff with a 10–0 nylon tie (c). The cuffed donor vessel is then inserted into the recipient vessel and secured with another 10–0 nylon tie (d) (Fig. 6.12).

6.1.6 Patency Test of Vascular Connection

The test of the anastomosis patency is very important procedure which must be performed at the end of each vascular anastomosis. Unfortunately, any signs of stenosis or other problems with anastomosis must be carefully recorded and usually the animal is excluded from the experimental group due to possibility of the negative impact of technical error. Here is a list of the main patency tests which help investigator to assess the quality of the anastomosis:

"Observation test", is performed through the operating microscope without additional instrumental maneuvers. The sign of patent arterial anastomosis is present when the dynamic changes in diameter (transversal pulsation) of the vessel from

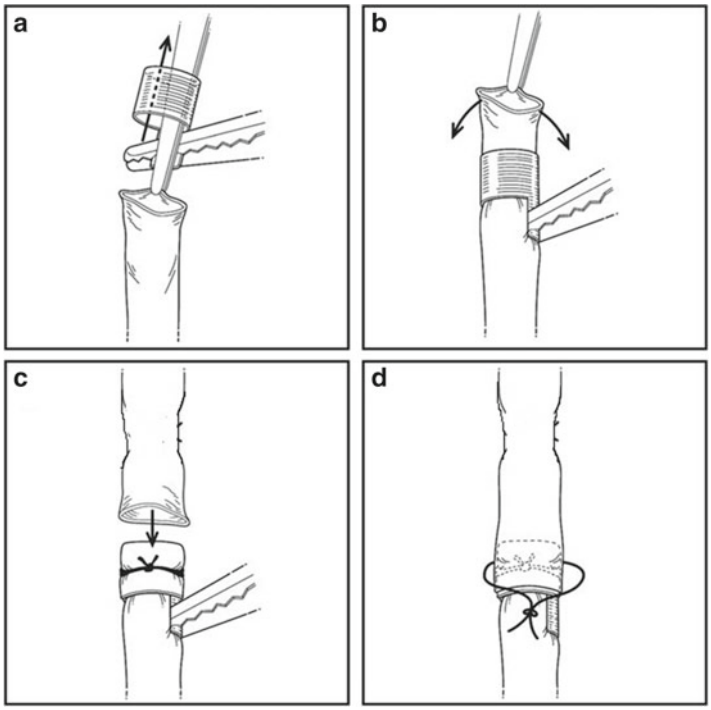

Fig. 6.12 Technique of cuff implantation (Source: Alexander et al. [15])

the anastomosis and to the caudal segment of the vessel are observed. On the other hand, longitudinal pulsations usually reveal obstruction of the anastomosis. In the vein anastomosis, after removing the clamps, the vein is fully dilatated and the diameter is the same distally and proximally to the anastomosis.

"Uplift test", is performed by lifting the vessel caudally to the anastomosis with forceps until it collapses. It will pulsate, when lowered, if the anastomosis is correctly done (Fig. 6.13).

The "empty-and-refill test" is performed by clamping the artery with a forceps immediately caudal to the anastomosis, and then closing the artery again with a second forceps, immediately near the first one. Slide the second forceps over the artery in the direction of the blood flow, keeping it closed and release the first forceps. An almost instant filling of the emptied segment of the artery proves patency of the anastomosis. Any delay is a sign that the anastomosis is partially blocked (involvement of the adventitia in the lumen or to a through -stitch). If there is no filling and the vessel remains collapsed, the anastomosis has completely failed (Fig. 6.14).

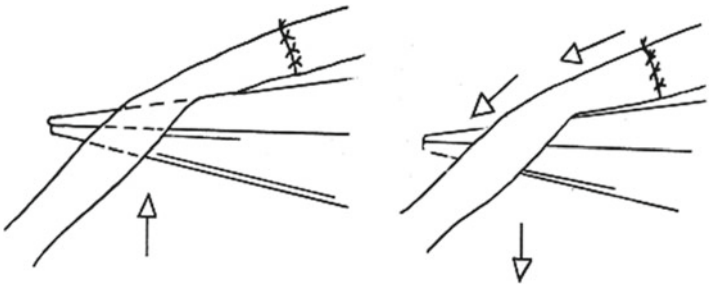

Fig. 6.13 The uplift test (Source: http://www.szote.u-szeged.hu/expsur/hefop/angol/microsurgery/microsurgery_advanced_invivo.htm#TOP)

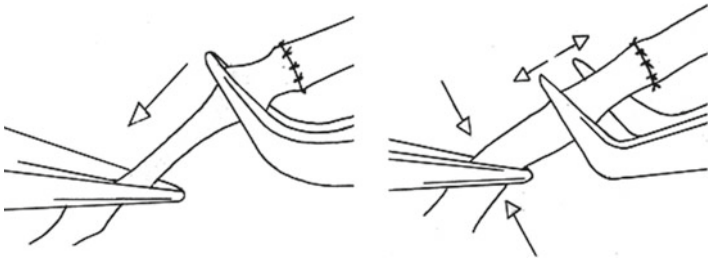

Fig. 6.14 The "empty and refill test" (Source: http://www.szote.u-szeged.hu/expsur/hefop/angol/microsurgery/microsurgery_advanced_invivo.htm#TOP)

6.2 Blood Sampling

Blood sampling is one of the most frequently used technique for examination of experimental animals. There are several approaches to collect blood from rat and the surgeon should choose the proper one for each purpose.

1. For frequent collection of very small blood volumes (i.e. measuring of blood glucose levels with portable glucometer) can be punctured lateral tail vein.
2. For acute (minutes – hours) monitoring of single i.v. injected (lateral tail vein) substance blood levels the puncture of saphenous vein can be optimal
3. For single collection of larger volume (up to 500 ml) the periorbital venous plexus bleeding could be used
4. For total blood collection can be used decapitation or direct heart puncture
5. For "long term" (tens of minutes) or repeated i.v. injections and/or blood sampling the catheterization of lateral tail vein can be used

6.2.1 Simple Puncture of Lateral Tail Vein

Tail vein sampling is quick and easy to perform by trained personel. It can be used repeatedly for collection of small volume of blood (several drops), without any specific equipment and procedures (of course a basic environment is requested – quiet room with adequate light conditions, room temperature 21 °C is sufficient). The crucial is the knowledge about anatomy of tail blood vessels (Fig. 6.15). Typically the single puncture of lateral tail vein using 30G needle is sufficient for taking 1–2 drops of blood for glucometers. If more blood is needed (3–5 drops) then the bigger size of needle is recommended (25G).

1. prepare the needle (25–30G) – unwrap it from paper and also from the plastic cover
2. prepare the gauze square for cleaning of the tail from blood
3. prepare the glucometer or glass capillary for sampling
4. take the animal off the cage and look at the tail – if it is injured the sampling from tail vein is not recommended
5. hold the tail within the palm of the left hand. Between the first and second finger hold the cranial part of the tail at the sides – it can cause the temporary occlusion of veins. Between the fourth and fifth fingers hold the distal part of the tail (Fig. 6.16)
6. then puncture the lateral vein and wait a few seconds when the drop of blood appears
7. collect the blood to the glucometer or the glass capillary

Whether larger sample of blood is required, then the prewarming of tail or whole animal is necessary as well as the restraining of animal by the assistant or plastic restrainer.

6.2.2 Puncture of Saphenous Vein

Exceptionally this technique can be performed by one experienced person, but typically this method requires two people – the first one for animal restraining and the second one for blood collection.

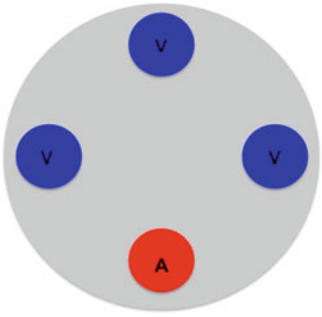

Fig. 6.15 The schematic tail anatomy – V in *blue* circles indicate veins and A in *red* circle indicates tail artery

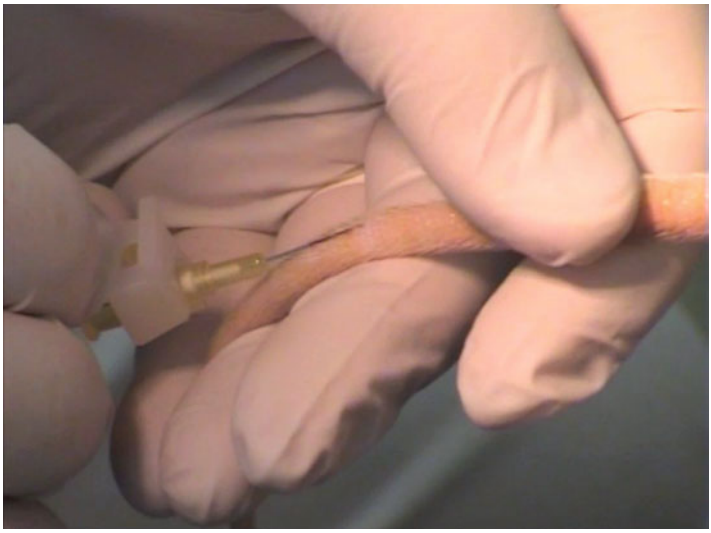

Fig. 6.16 The grip of the tail for blood collection

1. restrain the animal and let the hind leg free for further manipulation
2. shave or pluck the hairs on the inner thigh of the rat to make the vein visible – the adequate wetting easier hair shaving
3. when the area is shawed, spread a small amount of vaseline using a Q-tip in order to visualise the vein and to support the blood to constitute drops
4. using a 25–30G needle puncture the vein and collect the drops of blood
5. the bloodflow can be encouraged by pumping with the leg
6. when the blood is collected, the bleeding should be stopped by gently pressure on the puncture using a sterile Q-tip. As the bleeding from saphenous vein is plentiful, the adequate attention should be paid to stopping of it.

6.2.3 Blood Collection from Periorbital Venous Plexus

This rarely used technique can serve for a one-time collection of relatively large volume of blood when the recovery is requested. It can be performed only in generally anesthetized animal by staff experienced with this technique and the adequate attention should be paid to post procedure monitoring of animal due to frequent (1–2 %) potential side effects.

1. induce a general anesthesia
2. restrain the animal and gently constrict the neck skin between two fingers and pull it dorsally
3. the eyes bulges and the conjunctiva becomes visible

4. insert the glass capillary laterally or dorsally eye and graze the conjunctiva and venous plexus
5. due to capillary action the blood is coming into the capillary. The blood can be contaminated by tissue fluid.
6. the bleeding can be stopped by gentle pressure on closed eyelid for 20–30 s

Do not perform a sequential sampling due to increased risk of adverse effects of the technique.

6.2.4 Decapitation or Direct Heart Puncture

These techniques of "terminal" blood collection are useful whether the large volume of blood is requested (8–10 ml per animal). The decapitation is not recommended and is ethically controversial – the very experienced staff (with specific certificate in some countries) and specific equipment (guillotine or adequately sized sharp scissors) is required. The staff should train the decapitation on dead animals.

6.2.4.1 Decapitation

1. restrain the animal and let the head and neck free for the next manipulation
2. put the animal into the guillotine OR attach the large scissors and quickly cut the neck
3. collect the blood flowing from the trunck

6.2.4.2 Heart Puncture

The heart puncture can be performed only under the general anesthesia. It allows obtaining of relatively large volume of arterial blood sample of good quality.

1. induce a general anesthesia
2. restrain the animal on the back side
3. puncture the left ventricle percutaneously or through the diaphragm, whether the abdomen is opened, or the thoracotomy can be performed
4. collect slowly (prevention of the heart collapsing) the blood until the animal is dead

6.2.5 Tail Vein Catheterization

These technique is suitable for a sequential i.v. injection or/and blood sampling during tens of minutes until the animal can be permanently monitored by staff – rats typically try to bite the catheter.

1. induce a soft anesthesia (half dose of injection anesthesia necessary for surgery or 3 % isoflurane)
2. cover eyes with gel and pull the tongue
3. animal should be placed on the edge of the table in the prone position so that the tail hung loosely over the edge
4. grease tail by anesthetic ointment (Mesocain gel) for 3 min
5. put the tail into the warm water (45–48 °C) for 1–2 min
6. then dry the tail quickly and gently
7. hold it within the palm of the left hand. Between the first and second finger hold the cranial part of the tail, between the fourth and fifth fingers hold the distal part of the tail Fig. 6.16
8. Between the first and the second fingers compress the vein
9. 24GA catheter take out of the box, remove the cap and the cover of catheter. Using the right hand insert the catheter into the vein just 3–4 mm. Then pull the needle a little bit in order to prevent the perforation of the vein Fig. 6.17. Flexible catheter without the needle cannot easily perforate the vein wall
10. The blood fills spontaneously the cone of the catheter. Then the saline (0.5–1.0 ml) should be injected to verify the catheter patency and localization
11. Put the lid on and fix the catheter using surgical tape
12. The catheter is ready for repeated injections or blood collections

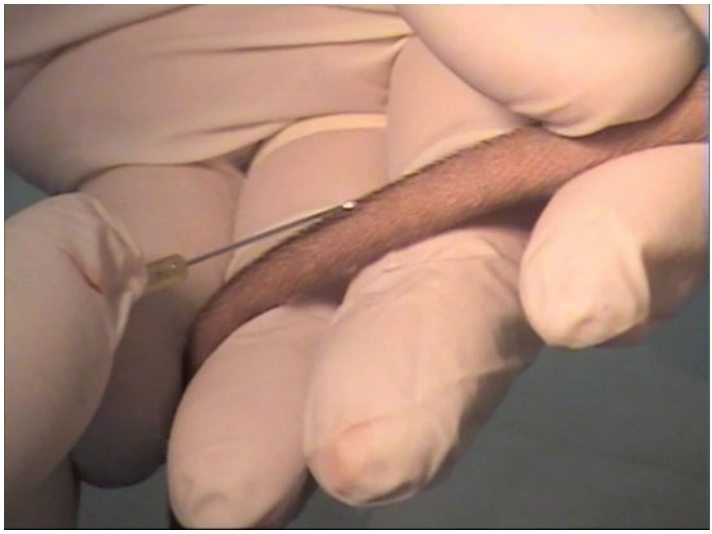

Fig. 6.17 The partially inserted catheter – the needle should be a little bit pulled out in order to prevent the perforation of the vein. The blood filling spontaneously the catheter is visible. The needle should not be completely removed until the catheter is completely inserted

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Chapter 7
Recovery After Surgery

Eva Fabryova and Jan Kriz

Abstract Postoperative care should be considered an important and integral part of the experimental study, which can dramatically impact the outcome of the surgery. Any surgical procedure is not complete until the animal is fully conscious and self-sufficient in accessing water and food. Especially in case of multiple surgeries performed sequentially, it is useful to have the assistant providing surveillance over the animal during the early post-op period. Most operating rules prohibit the return of animal to the animal facility until it is fully recovered. Postoperative management includes cage for recovery, maintenance of body temperature, fluid and nutritional support, pain management and eye protection.

Keywords Recovery • Pain management • Surveillance • Post-operative monitoring • Temperature maintenance

Postoperative care should be considered an important and integral part of the experimental study. Any surgical procedure is not complete until the animal is fully conscious and self-sufficient in accessing water and food. Especially in case of multiple surgeries performed sequentially, it is useful to have the assistant providing surveillance over the animal during the early post-op period. Most operating rules prohibit the return of animal to the animal facility until it is fully recovered.

7.1 Cage for Recovery

1. The cage should be smooth, safe and empty – without bedding and treats
2. Uncoordinated movements of the animal during recovery can cause injury of the corneas

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3. Uncontrolled (spontaneous) swallowing can cause the injury of the tongue or induce some aspiration of small pieces of bedding

7.2 Maintenance of Body Temperature

Rodents are small animals, which have a limited capacity of thermoregulation. Commonly used anesthetics significantly impair thermoregulation during total anesthesia [1] and even more in combination with a large abdominal surgery. Therefore, hypothermia can be easily induced during the surgical procedure without any adequate thermal support. Hypothermia is a serious complication leading frequently to a critical condition or even the death of experimental animal. The reasons are:

1. Decreased degradation of anesthetic drugs ⇒ prolongation of its action and significantly delayed recovery from anesthesia [2]
2. Increased blood loss [3]
3. Impaired wound healing [4, 5]
4. Heart arrest.

Supportive heating can be considered a difference between the successful surgery and the failure. Hypothermia can be prevented:

1. Preoperative: by increasing of pre-anesthetic peripheral body temperature, body condition [6]
2. Intraoperative: by minimizing the duration of open (chest, abdominal) cavity/procedures, length of the incision, exposure of viscera [7], by using of warm (37 °C) fluids for lavage
3. Intraoperative/postoperative: warming to 37 °C
    - passive surface warming (blanket covering the animal/tail, and between the animal and the surface operating table, cage)
    - active: heating pad, heating lamp, temperature supporting cage
4. Take care about checking of electric heating pads – there were reported cases of animals burned by malfunctioning pads. Do not place the animal directly on the surface of heating pad – interlay some towel or paper cloth.
5. Better are pads warmed by water – attention has to be paid to the risk of heating pad biting by recovering animal.
6. Heating lamps represents another alternative. The distance between the lamp and the animal should be optimized using a hand of the personnel. Put the hand to target area and test the temperature for 1 min. If it is OK for you, it is certainly safe for the animal.

7.3 Fluid Support

1. Water has to be freely accessible for animals almost until the induction of anesthesia even if the surgeon requested a fasting period for the procedure.
2. All animals undergoing anesthesia and/or surgery benefit from refilling of warm balanced solutions. It helps to maintain normothermia, corrects blood loss and also accelerates recovery following injectable anesthetics.
3. Permanent moistening of tissues in surgery field is necessary and useful. It prevents sticking of tissue to the instruments. The commonly used volume is 10–12 ml to an adult rat, either subcutaneously (1–2 ml) or intraperitoneally (as required during surgery and 1–2 ml after the closure of abdominal wall) [8].
4. In diabetic animals, fluids refilling is in particular even more important due to polyuria caused by hyperglycemia.
5. During the recovery period, water should be placed on the bottom of the cage to be easily accessible for the animal. Several animal food pellets should be moistened and placed in the small dish at the bottom of the cage.

7.4 Postoperative Pain Management

Effective postoperative analgesia is essential for performing a surgery with a high degree of success. Untreated pain causes stress and impairs wound healing and immune function [9, 10]. Some level of pain can enforce the animal to reduce movements, but the ethical concept and rules of animal care law are clear – pain must be efficiently treated.

Pain is eminently subjective symptom and thus its assessment in rodents is pretty difficult, but there are some symptoms:

1. Pay attention to common and nonspecific signs indicating pain.
2. The animal welfare regulations says “that unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.”
3. Rodents require at least 7 h of analgesia after abdominal surgery [11], varying with incision length, visceral manipulation, etc.

The specific analgesic drugs and its combinations are described in detail in Chap. 5.

7.5 Antidotes, Reversing Anesthetic Agents

The recovery period can be limited with drugs reversing anesthesia. The specific drugs are described in Chap. 5.

7.6 Eye Lubricant Use

More than 20 min of anesthesia requires an application of eye lubricant/ointment to avoid corneal desiccation which can induce lesions and pain.

7.7 Nutritional Support

Early after surgery, animals are limited in movements and cannot easily reach the food pellets located in elevated feeders. Pellets should be moistened and located at the bottom of the cage.

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Chapter 8
Kidney Transplantation

Ondrej Viklicky, Eva Girmanova, and Peter Balaz

Abstract Kidney transplantation represents the best option of renal replacement therapy with a 93 % first year graft survival rate and graft half-life reaching 10 years at most centers. Nevertheless, chronic rejection mediated by antibodies represents by far the most important cause of kidney grafts failure in the long-term follow-up while acute rejection affects 10–20 % of kidney allografts early after transplantation. The chapter briefly describes rejection models and models of renal failure and the procedures of kidney graft procurement and transplantation. Models used in kidney transplantation are divided into the models studying rejection (chronic, acute, antibody mediated) and models studying specific process of kidney graft damage (ischemia reperfusion injury, membranous changes, kidney failure, antibody mediated changes, toxic effects of single drugs). Kidney graft procurement and kidney graft transplant procedure are described step by step in detail with photos at important steps.

Keywords Kidney transplantation • Rat model • Rejection • Ischemia-reperfusion

8.1 Rat Models in Kidney Transplantation

Kidney transplantation represents the best option of renal replacement therapy with a 93 % first year graft survival rate and graft half-life reaching 10 years at most centers. Nevertheless, chronic rejection mediated by antibodies represents by far the

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most important cause of kidney grafts failure in the long-term follow-up while acute rejection affects 10–20 % of kidney allografts early after transplantation.

There are many kidney transplantation models used for studying various post-transplant pathologies and for testing the effects of novel immunosuppressants. Previously, chronic rejection was suggested to be associated with no alloantigen driven mechanisms and therefore several models of progressive nephropathies were used such as 5/6 nephrectomy, ischemia/reperfusion model and ratmodel of chronic rejection F334/LEW. However, observations based on the results of several rodent models were never replicated in human settings. There are several reasons for this. The F334/LEW model of chronic rejection resembles more human focal and segmental glomerulosclerosis than chronic rejection as defined in the last Banff classification. Moreover, the only similarity with human kidney transplantation is the histological presence of interstitial fibrosis and tubular atrophy. These are non-specific findings that occur in most renal pathologies, including non-transplant ones. Finding the correct dosage of several drugs in rodent models seems to be very difficult since the therapeutic window strictly differs between rodents and humans. As transplantation models, especially in rat and mouse, are dependent on the technical skills of surgeons, several groups have used other non-transplant models that mimic functional and morphological changes observed after kidney transplantation in the later period. Among those, the remnant kidney model or ischemia/reperfusion model have been used so far in experimental nephrology.

Thus, the only acute rejection models seem to be viable tools to test new drugs to prevent acute rejection or delayed graft function. For those models, allograft, syngraft and isograft orthotropic transplantation have been used.

8.1.1 Acute Rejection

For acute rejection experiments, adult male Lewis and Brown Norway (BN) rats weighing 210–250 g have been used. Lewis rats usually act as recipients and BN rats as donors. In another model, Dark Agouti (DA) rats are used as donors and Lewis rats as recipients. Acute rejection develops promptly and destroys the kidney within a week in the absence of immunosuppression because both BN and DA rat strains differ from LEW rats in the MHC system. On the other hand, if Fisher F334 strain is used as donor, kidney grafts survive roughly 180 days without immunosuppression because they differ from Lewis rat strain only in minor histocompatibility antigens. Since alloantibodies were detected in recipients, these models represent probably mixed rejection models, both T-cell and antibody mediated rejection. Bilateral nephrectomy just before transplantation is necessary to exclude the effect of remaining native kidney on global renal function.

8.1.2 Chronic Rejection

For studying of pathogenetic mechanisms occurring in chronic rejection, the Fischer to Lewis model of kidney transplantation was developed. These two strains have the same genetic information in RT1A region and differ in genes present in RT1C region. The Fischer kidney grafts transplanted into the Lewis recipient are rejected in a chronic manner. Histological and immunofluorescence examination has revealed three phases of processes in kidney allograft. Four weeks after transplantation glomerular basal membrane became thickening, IgG and IgM rise in the peripheral blood and simultaneously can be found as part of the deposits of basal membrane. Cellular infiltration is mild. At 8–12 weeks, lymphocyte infiltration increases and with the progression of changes at the basal membrane proteinuria develops. The last phase is characterized with increased expression of TGF beta, scarring and fibrosis. When animals are treated with short course of cyclosporine, the chronic process slows down and can be studied for at least 12 months. Allografts retransplanted back to F344 at 8 weeks show the histological changes. Reduction of glomeruli by 50 % results in hypertension and glomerular hyperfiltration. In some experiments, alloantibodies were detected and thus it has been speculated that this model may also mimic chronic antibody mediated rejection.

8.1.3 Non-transplant Models

Kidney transplantation in rodents is a demanding technique and until recently the proper model of chronic rejection did not exist. Therefore, instead of the previously frequently used F334/LEW model, several non-transplant models of progressive nephropathy that mimic chronic changes have been used instead. It must be noted that such models are highly artificial and may serve to study non-alloantigen driven processes only.

8.1.4 Remnant Kidney Model

Subtotal nephrectomy, the so-called 5/6 nephrectomy, represents the main way to establish a reliable model of chronic renal failure (CRF). This chronic renal failure model is achieved by partial ablation of renal parenchyma. Two of the most common surgical techniques are used: resection or infarction. The approach with infarction is typically associated with more severe proteinuria and hypertension than the one using excision and this probably due to induced up-regulation of the renin-angiotensin system in the peri-infarct zone [1].

The resulting stage of CRF injury is strain, gender and procedure dependent. Usually Wistar or Sprague-Dawleys rats are used. Three stages of injury can be

observed dependent on time after operation. In 2–4 weeks hypertrophy occurs, followed by minimal changes up to 10 weeks and by glomerular sclerosis and tubular injury after 10 weeks [2].

8.1.5 Resection Model

In resection (ablation) model 50 % of one kidney is removed 1–2 weeks after or before contralateral nephrectomy.

Protocol A 2–3 cm skin incision is made caudal to the rib cage on left side. After further muscle incision, the kidney is gently dissected from the overlying fat and adrenal gland. A single silk thread is placed around the renal pedicle. The upper and lower kidney poles are excised after lifting the thread (in order to temporarily occlude the vascular flow during cutting). A meticulous resection is made under eye control to ensure that the remnant kidney represents 25 % of the intact kidney. With the remnant kidney wrapped in homeostatic gauze, it is returned to the abdominal cavity. The muscle wall is then sutured. The average time from the first cut is about 5 min. One week after the first surgical procedure, the right uninephrectomy is performed.

8.1.6 Ligation Model

In the ligation (infarction) model, branches of the renal artery in the rat are ligated after contralateral uninephrectomy.

Protocol Kidney is exposed and decapsulated in a similar manner as above. Instead of surgical renal tissue, the left renal arterial branches are isolated with blunt forceps and ligated using sterile silk suture. Functional nephrectomy is thus achieved by renal tissue infarction. The average time from the first cut is about 10 min. One week after the first surgical procedure, the right uninephrectomy is performed [3].

8.1.7 Renal Ischemia/Reperfusion Injury Model

Renal ischemia and reperfusion (I/R) injury is also one of the common complications in clinical surgeries such as renal transplantation [4]. The I/R injury triggers an immune response and leads to both local and systemic inflammations. It disturbs renal function and immune system homeostasis.

Protocol Using a midline incision, the left kidney renal artery and vein are isolated, cooled and occluded for 45 min with clips. The right kidney is removed at the

time of surgery. Several rat strains are used, most frequently Hannover Sprague Dawley rats. If hypertensive rats are used, the progression of nephropathy is accelerated [5].

8.1.8 Other Nephrological Models

The Heymann nephritis ratmodel is a model of progressive glomerular injury. This setting allows us to study molecular mechanisms and the kinetics of immune deposit formation and glomerular capillary wall injury. It simulates one of the leading causes of nephritic syndrome in white adults, membranous nephropathy (MN). The disease is characterized by an accumulation of immune deposits on the glomerular basement membrane. In rats, the target antigen of antibodies is megalin, a multiligand receptor expressed at the podocyte cell surface [6, 7].

8.1.9 Active Heymann Nephritis

Heymann first described this model in 1959 [8]. Kidney extract of the rats own kidney with Freund's adjuvant is injected to the rat to induce nephritic syndrome. Nowadays this model is rarely used as it takes a longer period to induce disease and is much more variable [7].

8.1.10 Passive Heymann Nephritis

Insoluble subfraction of brush borders (BB) termed fraction 1A (F×1A) is isolated and combined with Freund's adjuvant. F×1A is administrated into the heterologous animal (rabbit, sheep, goat) and anti-rat BB are isolated. Rats then receive an injection of rabbit (sheep, goat) anti-rat BB antibodies [9].

Various strains such as Sprague-Dawley rats, Wistar rats, Munich Wistar rats, Lewis rats can be used. Generally, male rats are used. Antibody is administered intravenously through the tail or intraperitoneally [9].

8.1.11 Streptozotocin Induced Nephropathy

Streptozotocin (STZ) is toxic to insulin producing pancreatic beta cells. Its administration is widely used to induce experimental diabetes in animals [10].

Administration of single dose of STZ (40–80 mg/kg i.p. or i.v.) causes hyperglycemia within 72 h. The first signs of diabetic nephropathy (increased proteinuria,

serum creatinine, deposition of extracellular matrix, thickening of basal membrane) can be detected in rats between 4 and 12 weeks after the administration of single dose of STZ [10, 11].

The severity of diabetic hyperglycemia and heaviness of diabetic complications such as nephropathy are strongly dependent on the doses of STZ given [12].

In order to create a model that leads to the development of diabetic nephropathy in a shorter period, a combined model of STZ application (35 mg/kg i.p., once) and resection of half of one kidney and total removal of the other one can be used [11].

8.2 Kidney Graft Procurement

8.2.1 Microsurgical Equipment and Material

Scissors, forceps, 10-0 silk sutures, two micro-scissors (straight, curved), three micro-forceps (two straight, one curved), thermal cauterize unit, cotton swabs, gauze, heparin solution, heating pad, needles, syringes (5 ml).

Protocol

1. Open abdominal cavity with midline incision
2. Remove retroperitoneal adipose tissue surrounding renal artery and vein
3. Ligate and divide suprarenal vein (Fig. 8.1)
4. Release both renal vessel from retroperitoneal tissue (Fig. 8.2)

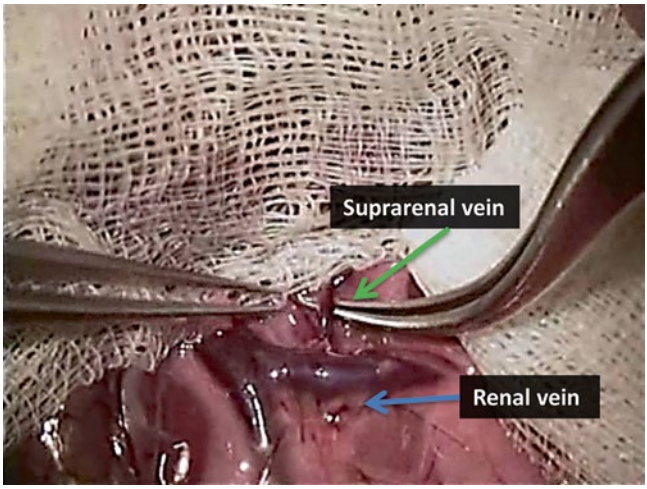

Fig. 8.1 Dissection of suprarenal vein

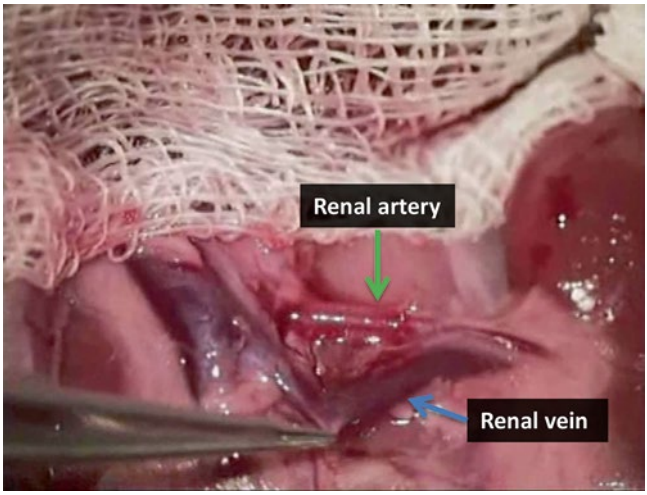

Fig. 8.2 Dissection of renal artery and vein

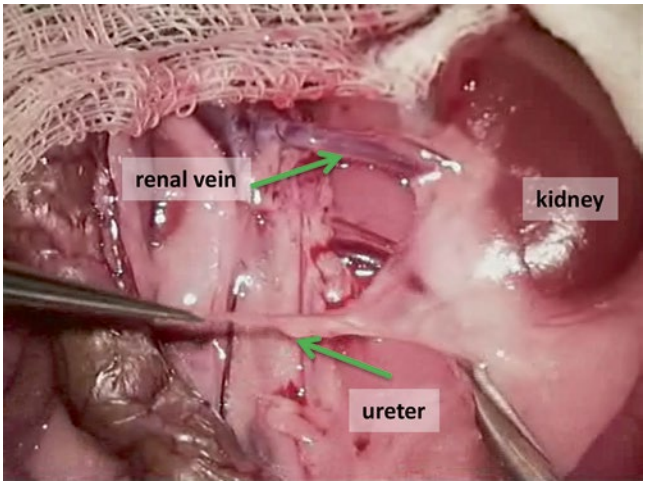

Fig. 8.3 Dissection of ureter

5. Release the ureter from retroperitoneal tissue and cut them at the distal end (Fig. 8.3)
6. Release the kidney from the surrounding tissue (Fig. 8.4)
7. Inject heparin solution (100 IU/1 ml) to the inferior caval vein
8. Put the clamp on the aorta above the stump of the renal artery

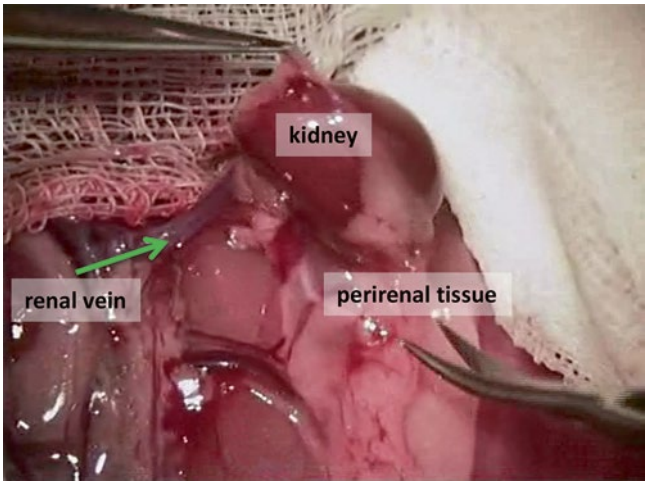

Fig. 8.4 Mobilization of the kidney from surrounding tissues

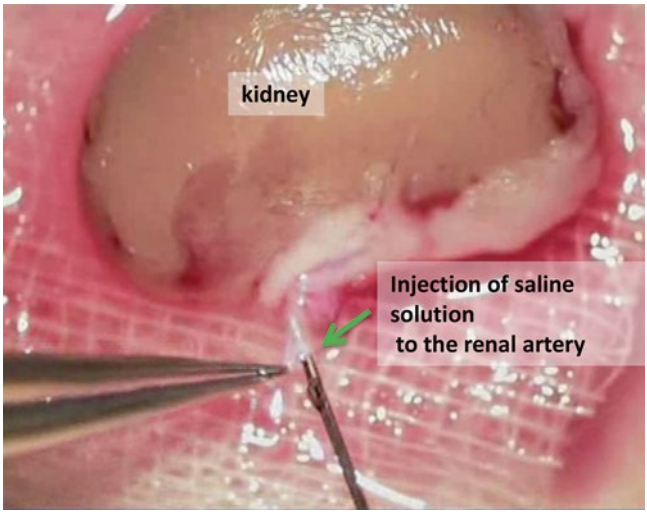

Fig. 8.5 Perfusion of the kidney

9. Cut the renal vessels as close as possible to the aorta and the caudal caval vein
10. At the back table, remove the redundant tissue from distal part of the ureter and renal vessels so that the ends are prepared for anastomosis and perfuse the kidney with saline solution through renal artery (Fig. 8.5)

8.3 Kidney Graft Transplantation

8.3.1 Equipment and Materials

Scissors, forceps, four vascular clamps, 9-0 nylon sutures, 7-0 and 4-0 silk suture, two micro-scissors (straight, curved), three micro-forceps (two straight, one curved), micro-needle holder, thermal cauterize unit, cotton swabs, gauze, heating pad, needles, syringes (5 ml, 10 ml).

Protocol

1. Open abdominal cavity with a midline incision
2. Cauterize capillary bleeding from the incision edge
3. Perform the recipient left nephrectomy so that the renal vessels and ureter are ligated and dissected as close as possible to the kidney hilus.
4. Flush the renal vessels stumps with cold heparin saline solution (100 UI/1 ml saline)
5. Start the arterial anastomosis with two fixating stitches (10/0) at the distance of 40 % in diameter from each other on the recipient renal artery (Fig. 8.6)
6. Put four stitches equidistantly on the anterior site of the arterial anastomosis (Fig. 8.7)
7. Rotate the artery by 180° to finish the posterior site of the anastomosis
8. Put the five stitches equidistantly on the posterior site of the anastomosis (Fig. 8.8)
9. Put two fixating stitches on the both edges of the recipient renal vein (Fig. 8.9)
10. Start the venous anastomosis of the posterior vein wall by performing a continuous suture. The suture starts with the upper polus thread toward to the distal polus (Fig. 8.10).
11. Likewise, perform the anastomosis on the anterior vein wall (Fig. 8.11)
12. Tighten the suture and remove the fixating stitches
13. Release the clamps and restore the blood flow in the graft (first from the vein after from the artery) (Fig. 8.12).
14. Stop the bleeding by pressing warm sterile gauze
15. Remove the adipose tissue from the recipient and donor ureters (Fig. 8.13)
16. Perform two opposite stitches on the ureter-ureteral anastomosis
17. Put two another stitches equidistantly between them and complete the anastomosis (Fig. 8.14).
18. The abdominal cavity is flushed with warm saline and closed in two layers

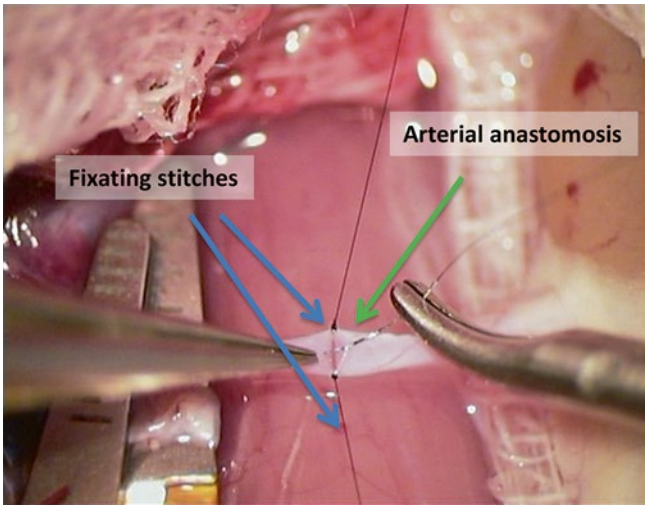

Fig. 8.6 Fixating polar stiches in arterial anastomosis

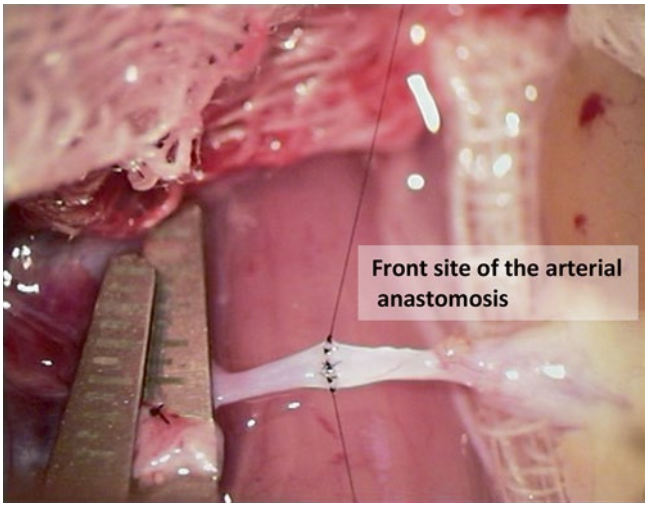

Fig. 8.7 Anterior wall of arterial anastomoses

8.3.2 Tips and Tricks

- For better visualization in surgical field, laparotomy can be accompanied with bilateral transversal incision of the abdominal wall
- Use left kidney for the procurement
- leave a small part of adipose tissue in the upper pole of the kidney

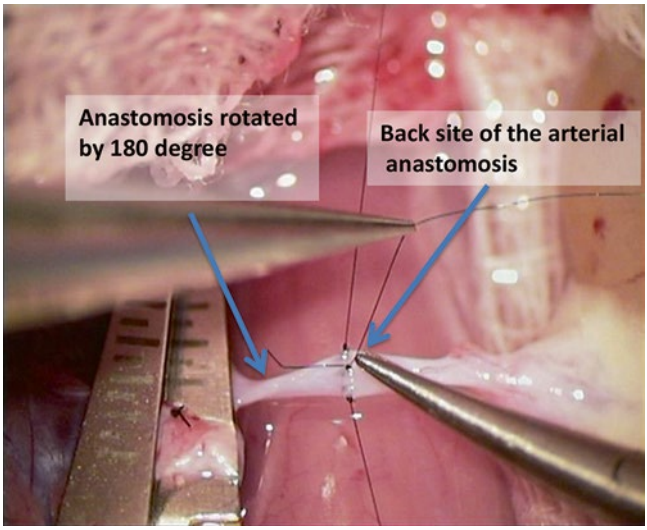

Fig. 8.8 Posterior wall of arterial anastomoses

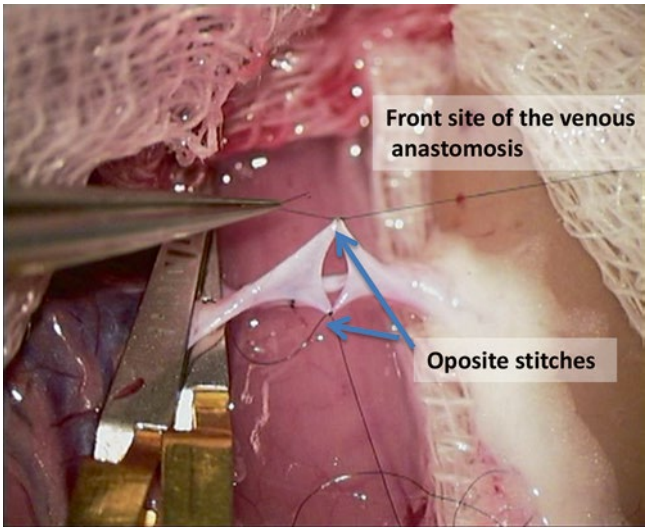

Fig. 8.9 Fixating polar stiches in venous anastomoses

- after perfusion of the graft, fulfill the container with preservation solution, to avoid desiccation of kidney surface
- put a strip of silicon glove below the dissected vessel when performing vascular anastomosis
- After completion of vascular and ureteral anastomosis fix the kidney to the retroperitoneum with fixating stitch between adipose tissue in the upper pole of the graft and retroperitoneum space of the recipient.

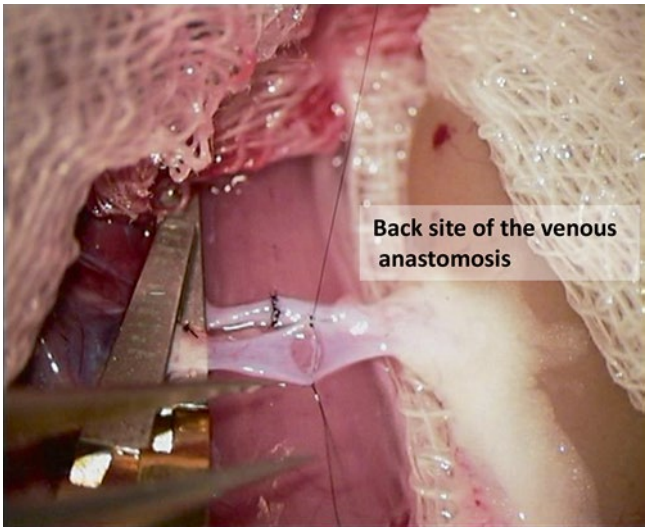

Fig. 8.10 Posterior wall of venous anastomosis

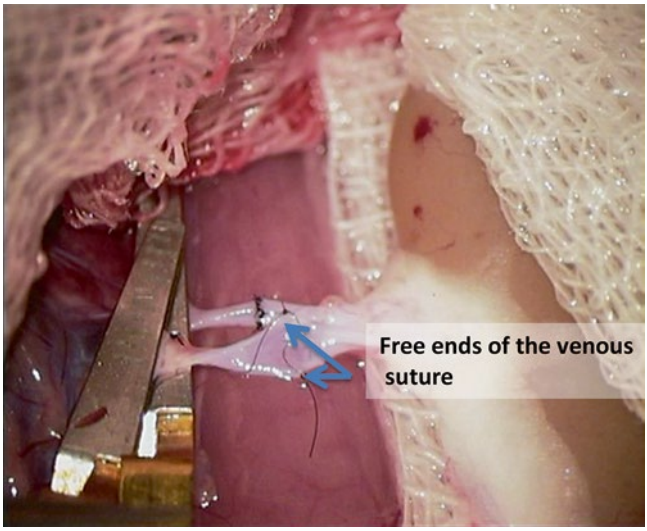

Fig. 8.11 Anterior wall of venous anastomoses

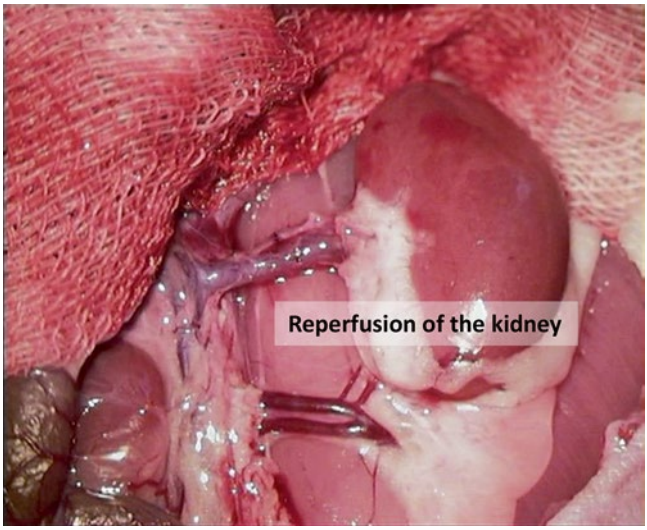

Fig. 8.12 Kidney graft after reperfusion

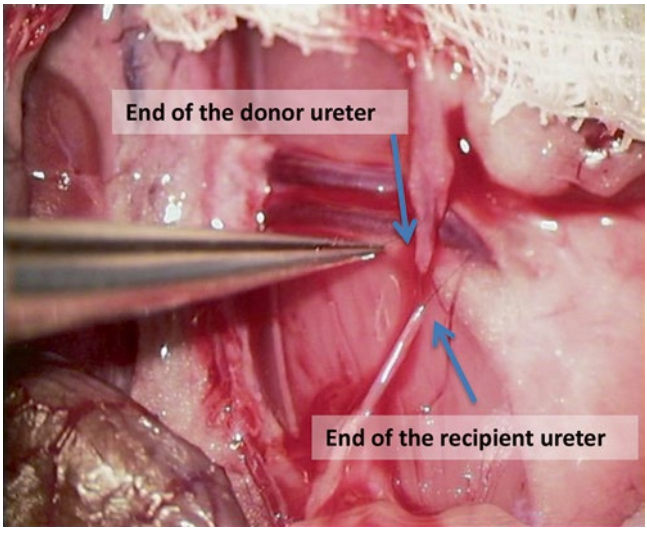

Fig. 8.13 Removing of the adipose tissue from the recipient and donor ureters

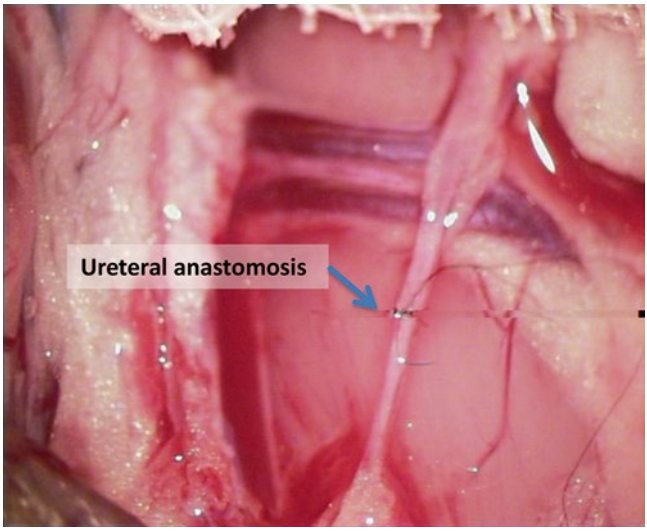

Fig. 8.14 Ureteral anastomosis

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Chapter 9
Heterotopic Abdominal Heart Transplantation in Rats

Martin Pokorny, Iveta Mrazova, and Jiri Maly

Abstract Heterotopic heart transplantation in rats has been accepted and widely used as a suitable model for transplant immunology investigation, for new cardioplegic solutions testing and also for heart mechanical unloading research. The heterotopic heart transplantation consists in microsurgical implantation of the donor heart into the host organism while the host heart is preserved untouched. Various spots have been routinely used for the heterotopic heart implantation (abdominal, cervical and femoral). In general, all the approaches mentioned above have the advantage of easy graft functionality assessment by palpation. The heterotopic abdominal transplantation model has become the most widely used one over the years. Gradually, many modifications have been adopted to make the microsurgical procedure faster and easier in order to improve the survival rate.

Keywords Rat • Heterotopic • Abdominal • Transplantation • Heart

9.1 Introduction

Heterotopic hearttransplantation in rats has been accepted and widely used as a suitable model for transplant immunology investigation, for new cardioplegic solutions testing and also for heart mechanical unloading research. The heterotopic heart transplantation consists of a microsurgical implantation of the donor heart into the host organism while the host heart is preserved untouched. Various spots have been routinely used for the heterotopic heart implantation (abdominal [1, 2], cervical [3] and femoral [4]). In general, all the approaches mentioned above have the advantage of easy graft functionality assessment by palpation. The heterotopic abdominal

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transplantation model has become the most widely used one over the years. Gradually, many modifications have been adopted to make the microsurgical procedure faster and easier in order to improve the survival rate.

9.1.1 History and Possible Applications

Heterotopic hearttransplantation was introduced for the first time in dogs [5] in 1933; in 1964, it was performed in rats [1]. In 1969, a modified model was presented where the donor's heart thoracic aorta was anastomosed end-to-side to the host's abdominal aorta and the pulmonary artery was anastomosed in the same manner to the recipient's inferior vena cava [2]. Over the years, this model has been accepted as a classical one. Originally, the model of heterotopic heart transplantation was used in research focusing on transplant immunology. With the aim of finding new immunosuppression agents and advances, heterotopic heart transplantations have been routinely used either as allografts [6] (outbred rat strains) or as xenografts (mouse [7] and hamster [8], respectively, as a heart donors). Simultaneously with the advancement of cardio surgery, the heterotopic heart transplantation model has been used for cardioplegic development [9, 10].

Later on, heterotopichearttransplantation was confirmed as an ideal, reproducible animal model for tracking beneficial or maladaptive changes that follow the mechanical unloading of the heart. The mechanical unloading of the heart, whose occurrence in clinical praxis is closely linked to the usage of ventricular assist device, became an intensively studied phenomenon. The main aim of these studies is to reveal the regulatory pathways that govern the reverse remodeling of the heart and subsequently to find the way to cardiac recovery [11, 12]. Nowadays, two fundamental operative techniques are routinely used in this field. In the first one, the model of complete volume unloading involves an aorto-aortic anastomosis and a pulmonary artery inferior vena cava anastomosis, where the left ventricle is completely volume unloaded, receiving only thebesian venous return. In the second technique, the model of partial volume unloading involves only one aorto-aortic anastomosis with conservation of two upper lobes of the right lung, where, in addition to thebesian circulation, the pulmonary blood flow (dependent only on coronary circulation) returning via the pulmonary veins also enters the left ventricle (see Figs. 9.1 and 9.2). The inbred rat strains (Lewis etc.) are mainly used in heart unloading experimental protocols in order to avoid rejection issues [13]. The heterotopic transplantation of the failing rat heart is also performed in order to achieve a deeper understanding of regulatory pathways during heart failure [14].

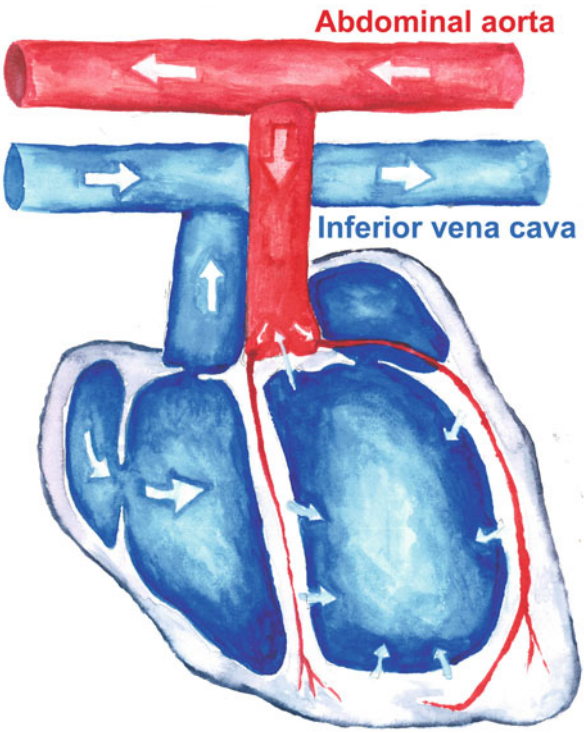

Fig. 9.1 Heterotopic heart transplantation - comlete volume unloading

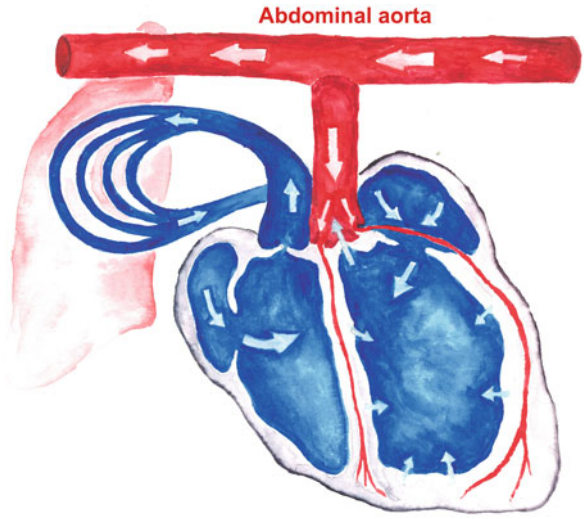

Fig. 9.2 Heterotopic heart transplantation - partial volume unloading

9.2 Preconditioning of the Animal Prior to Surgery

It is recommended to allow the animals to become familiar with the facility equipment and staff for at least 2 weeks after arrival from the supplier. This can provide the animals the necessary time to get accustomed to the environment and reduce stress. An added benefit is that the researcher can become familiar with individual animals, can identify those that are not in a perfect health condition and prevent them from inclusion into the experimental group. Ultimately, the animals used in chronic studies must be housed for at least 72 h prior to any procedures being performed.

Each animal must be clearly identifiable during the all phases of the study – they have to be explicitly marked before the first manipulation. Rats are unable to vomit, thus it is not absolutely required to let them fast before the surgery. But, in order to reduce the volume of intestinal content and therefore make the surgical manipulations within abdominal cavity easier, overnight fasting is recommended. This fasting does not mean starvation. Thus, in order to prevent ketosis and hypoglycemia, food pellets should be replaced by glucose solution and water freely accessible for the animal.

Both approaches for total anesthesia induction and maintenance can be utilized according to the routine of the researcher. Inhalation anesthesia used for heart donors is performed with a mixture (3–5 %) of isoflurane in the oxygen throughout the duration of the surgery. Recipient anesthesia can be based on thiopental (50 mg/kg) diluted in 1 ml warm saline and administered intraperitoneally.

During the anesthesia, temperature maintenance is crucial – the animal should be placed on safe heating pad, the room temperature should not be under 22 °C and the saline used for visceral moisturizing must be warmed to 37 °C.

9.2.1 DO's Just Before the Surgery

1. Check the donor visually, weight it and calculate the dose of anesthetic drugs.
2. Inject the anesthesia and let the animal sleep in the dark.
3. Donor anesthesia consists of isoflurane at 5 % for induction and 1.5 % for maintenance administered via the nose cone.
4. Shave an area large enough to include a complete incision line and some surrounding skin allowing efficient disinfection.
5. Treat the skin using detergent ⇒ alcohol ⇒ disinfectant.
6. Cover donor eyes with a proper unguent and put it onto the heating pad with the backside down.
7. Drape the animal using the sterile cloth to provide a sterile field around the incision
8. Intraoperative pain relief consist of a single bolus dose of buprenorfine (10 μg/kg) given via the intramuscular route

9.3 Heterotopic Abdominal Heart Transplantation

Heart transplantation in this manner leads to complete mechanical unloading of a donor heart. The heterotopic abdominal heart transplantation consists of anastomosis of the donor aorta to the syngeneic recipient aorta and the donor pulmonary artery to the recipient inferior vena cava. Blood flows from the recipient aorta into the transplanted aortic root. Due to the aortic valve competency, blood perfuses the coronaries without entering the left ventricle. Coronary flow is returned into the right atrium via the coronary sinus and then ejected by the right ventricle through the pulmonary artery. Only thebesian circulation allows a minute amount of blood to enter the left ventricle (Fig. 9.1).

9.3.1 Recipient Preparation

9.3.1.1 Equipment + Material

Microsurgical Instruments Scissors, forceps, 7-0 silk suture, two micro-scissors (straight, curved), three micro-forceps (two straight, one curved), thermal cauterize unit, cotton swabs, gauze, warm saline solution, heating pad, needles, syringes (5 ml, 10 ml).

9.3.1.2 Procedure

1. Open abdominal cavity with a midline incision.
2. Cauterize capillary bleeding from the incision edge and insert abdominal retractors.
3. Carefully retract the bowels to the animal left side and wrap them in wet gauze (Fig. 9.3).
4. Dissect the retroperitoneal fascia using wet cotton swabs and expose the infrarenal portion of the great vessels for a length of 4 cm. Later, using two forceps (one straight, one curved), carefully dissect around great vessels and underneath them. It is necessary to ligate all (even minor) branches with 7-0 silk (Fig. 9.4).
5. Using a fine curved forceps, divide the remaining tissues surrounding the great vessels and prepare spots for applying vascular microclamps on the inferior vena cava (Fig. 9.5a) and the abdominal aorta (Fig. 9.5b).
6. Return the bowels into the abdomen and wet them with warm saline during donor harvesting operation.

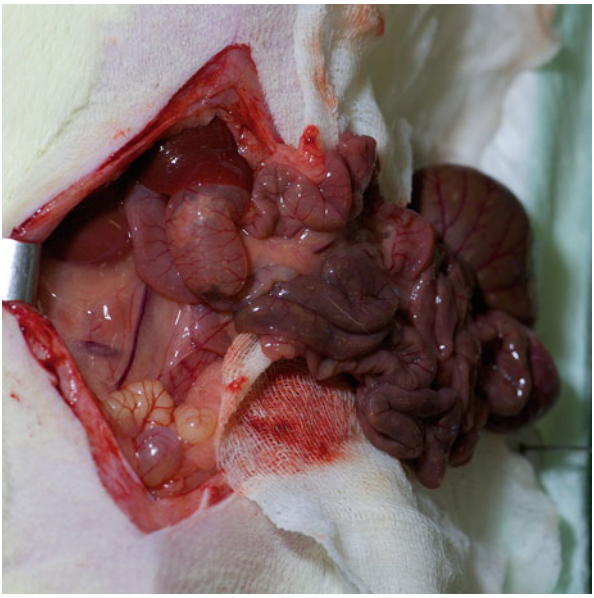

Fig. 9.3 Recipient - abdominal cavity opening

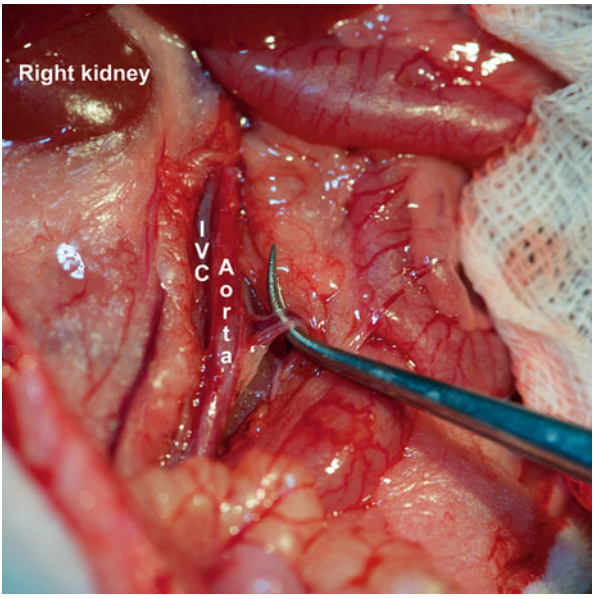

Fig. 9.4 Recipient - aortic side branches ligation

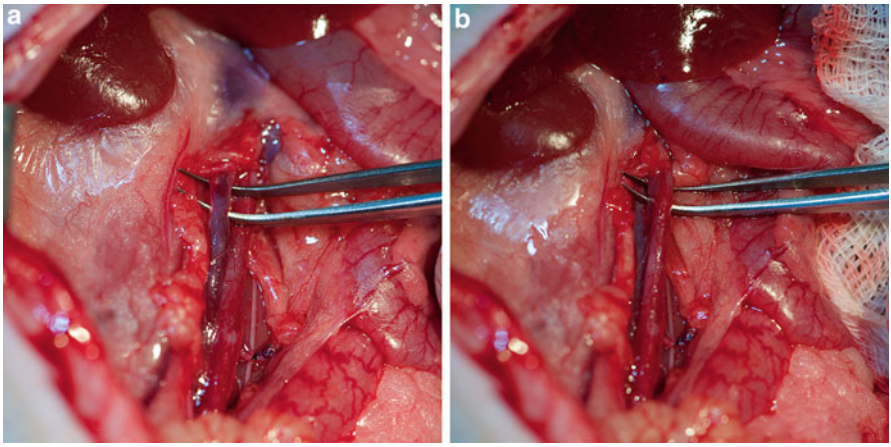

Fig. 9.5 Recipient - preparation of the inferior vena cava (**a**) and aorta (**b**)

9.3.1.3 Tips and Tricks

1. Wash the serosa with warm saline (37 °C) so that the tissue will not be sticky for surgical instruments.
2. Prepare the recipient as described above to minimize the cold ischemia time of the graft.
3. Carefully expose and ligate all branches in the desired area to prevent bleeding after vessel opening.

9.3.2 Heart Graft Procurement

9.3.2.1 Equipment + Material

Microsurgical Instruments Scissors, forceps, fine mosquito hemostat, 7-0 and 5-0 silk suture, two micro-scissors (straight, curved), three micro-forceps (two straight, one curved), cotton swabs, gauze, cold saline solution and ice, needles, syringes (5 ml, 10 ml), heparin (5000 I.U.) diluted in 5 ml of cold saline solution, cold cardioplegic solution (St. Thomas).

9.3.2.2 Procedure

1. Open abdominal cavity with a midline incision and expose the great vessels as before.
2. Use a 5 ml syringe and, via a needle, inspire approximately 5 ml of blood from the abdominal aorta. Immediately close the puncture site with hemostat.

3. Administer heparin (5000 I.U.) diluted in cold saline into the inferior vena cava via a 30-g needle (Fig. 9.6) and close the puncture site with hemostat as well.
4. Perform the midline sternotomy; expose the aortic arch by dissecting the thymus. Using fine forceps, clean the innominate artery (Fig. 9.7) and place a long silk suture around, which can be used to ease the innominate artery catheterization with a blunt 30-g cannula and also seal it using tourniquet or ligation.

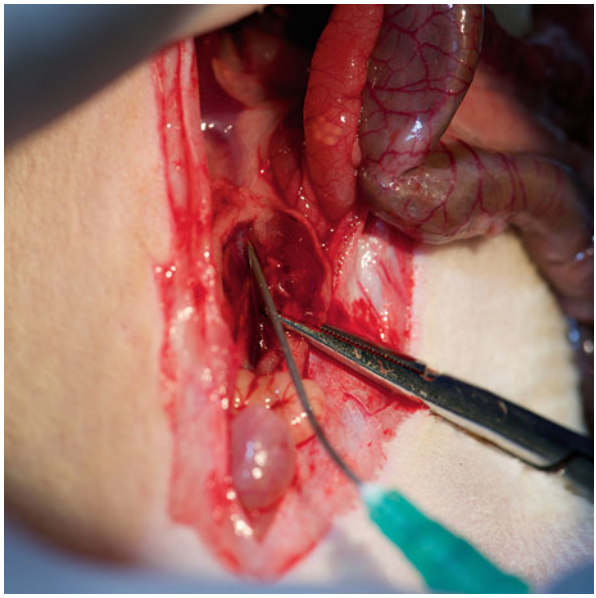

Fig. 9.6 Donor - heparin administration via the abdominal inferior vena cava

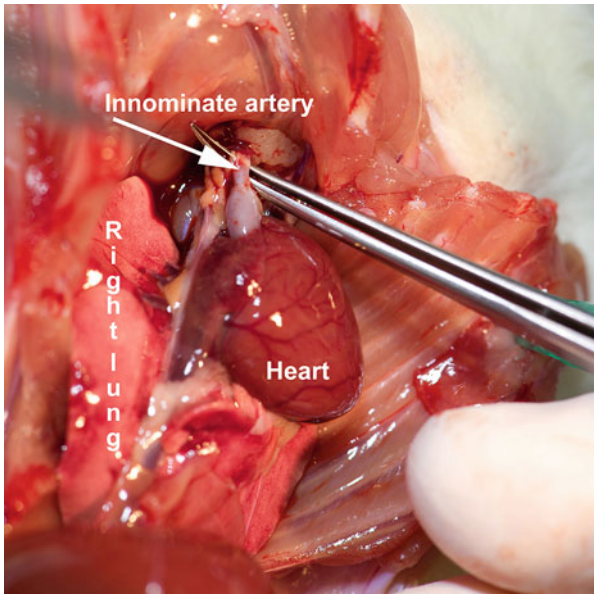

Fig. 9.7 Donor - situation after sternotomy

5. Using fine straight scissors, open the innominate artery, insert the blunt cannula heading to the aortic root and tighten the tourniquet (Fig. 9.8).
6. Clamp the rest of aortic arch with hemostat and arrest the heart by administering 50 ml cold cardioplegic solution very slowly via the aortic cannula and control the coronary vessels washout with cardioplegic solution. Meanwhile, cut superior and inferior vena cava to prevent heart extensive filling and distention. As soon as possible, apply ice on the heart (Fig. 9.9). After heart arrest, take the cannula out from the brachiocephalic trunk.

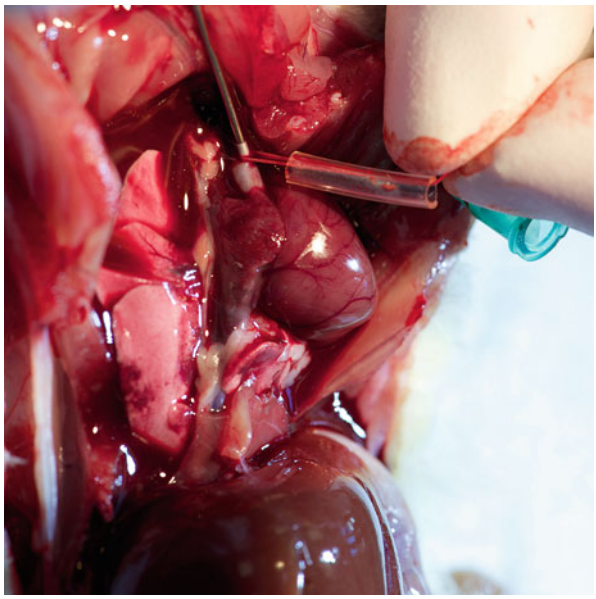

Fig. 9.8 Donor - cannula insertion into the ascending aorta

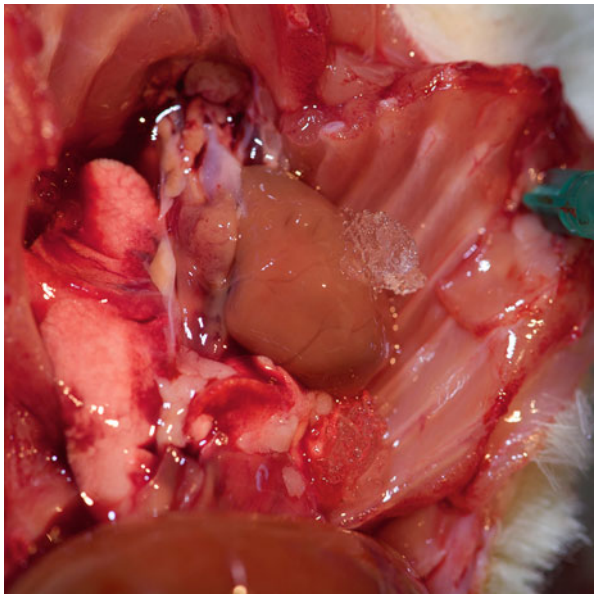

Fig. 9.9 Apply ice on the heart

7. Ligate with a silk 5-0 suture right and left lung close to the hilus and cut both lungs away (Fig. 9.10).
8. Perform blunt dissection, ligation (7-0 silk) and cut of the inferior vena cava (Fig. 9.11), left superior vena cava (Fig. 9.12) and right superior vena cava (Fig. 9.13).

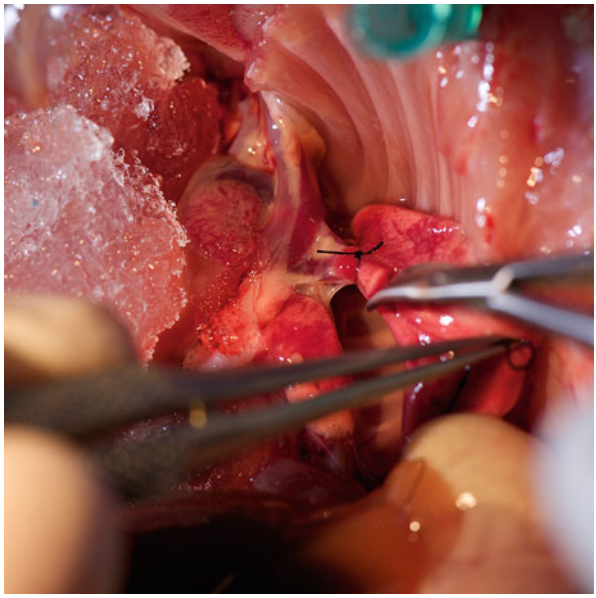

Fig. 9.10 Ligate with a silk 5-0 suture right and left lung close to the hilus

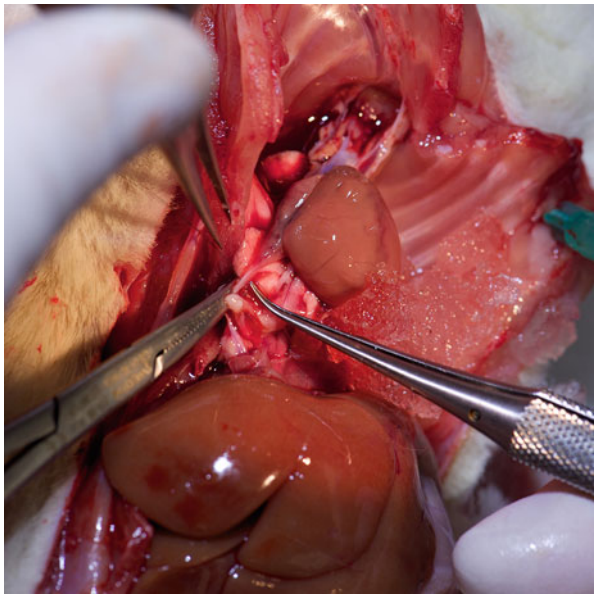

Fig. 9.11 Cut of the inferior vena cava

9. Clean the aorta and pulmonary artery. The aorta should be transected, just before the brachiocephalic trunk (Fig. 9.14), and the pulmonary artery, just below its bifurcation (Fig. 9.15), in one cut (straight microsurgical scissors).
10. Keep the heart in ice-cold cardioplegic solution until reimplantation.

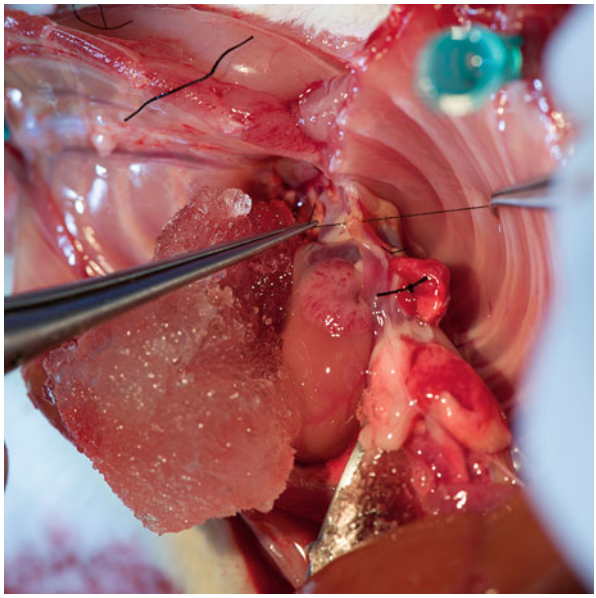

Fig. 9.12 Cut the left superior vena cava

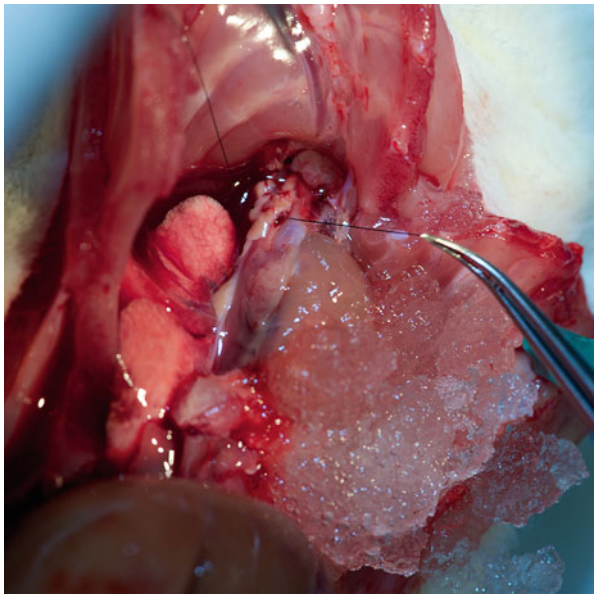

Fig. 9.13 Cut the right superior vena cava

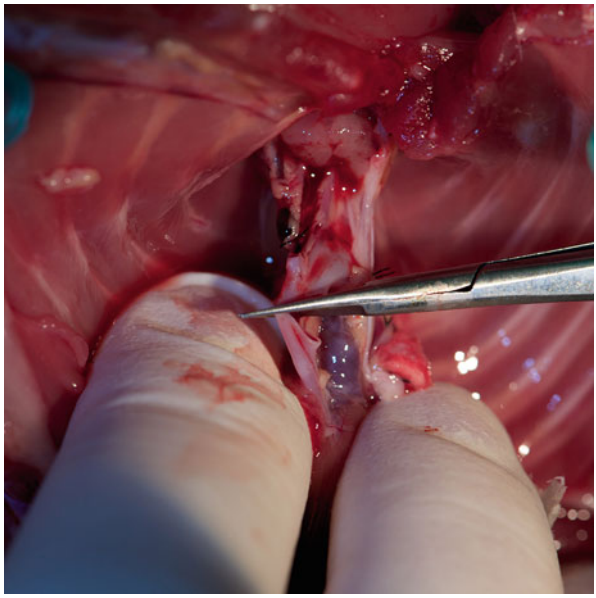

Fig. 9.14 The aorta should be transected, just before the brachiocephalic trunk

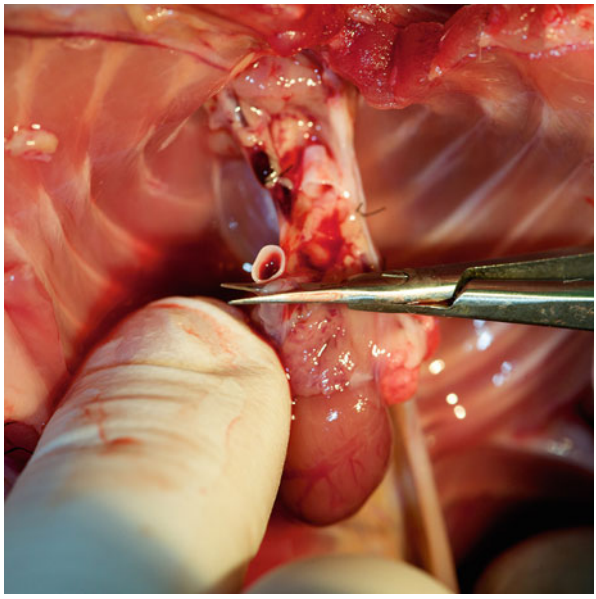

Fig. 9.15 Transect the pulmonary artery just below its bifurcation

9.3.2.3 Tips and Tricks

1. Be careful about any air in the needle or syringe during the administration of heparin or cardioplegia.
2. Insert the cardioplegic cannula very carefully to keep the aortic valve untouched.
3. Administer the cardioplegic solution very slowly to prevent distention of the heart and aortic valve damage.
4. The cut of the aorta and the pulmonary artery must be done at once in a proper angle to obtain optimal site for anastomosis.
5. It is very helpful to clean the aorta and the pulmonary artery of fat and fibrous tissue.

9.3.3 Heart Transplantation

9.3.3.1 Equipment + Material

Microsurgical Instruments Scissors, forceps, four vascular clamps, 9-0 nylon sutures with a 3 mm round-bodied needle, two micro-scissors (straight, curved), three micro-forceps (two straight, one curved), micro-needle holder, thermal cauterize unit, cotton swabs, gauze, saline solution, heating pad, needles, syringes (5 ml, 10 ml), heparin solution, ice.

9.3.3.2 Procedure

1. Carefully retract the bowels to the recipient left side and wrap them in wet gauze.
2. Apply vascular microclamps to the inferior vena cava (Fig. 9.16) and the abdominal aorta (Fig. 9.17) in prepared spots, leaving as much space between clamps as possible.
3. Using 30-g needle, make a small hole in the center of clamped aortic segment (Fig. 9.18), then using straight microscissors, perform a midline aortotomy approximately 4 mm long (to match with donor aorta circumference) and immediately wash the lumen with heparin saline solution (Fig. 9.19).
4. Similar procedure has to be done with the inferior vena cava; however, the vein incision should be slightly longer (6 mm) to prevent obstruction (Fig. 9.20).
5. Place the donor heart on the right side of the abdomen and correctly oriented so that the graft aorta leads to the abdominal aorta and the pulmonary artery to the inferior vena cava and wrap the graft into gauze soaked in ice cold saline and you can also add some ice if needed (Fig. 9.21).
6. The suture is performed with 9-0 nylon stitches with 3 mm round-bodied needle. Start with two anchor stitches placed at the heel and at the toe (Fig. 9.22a, b).

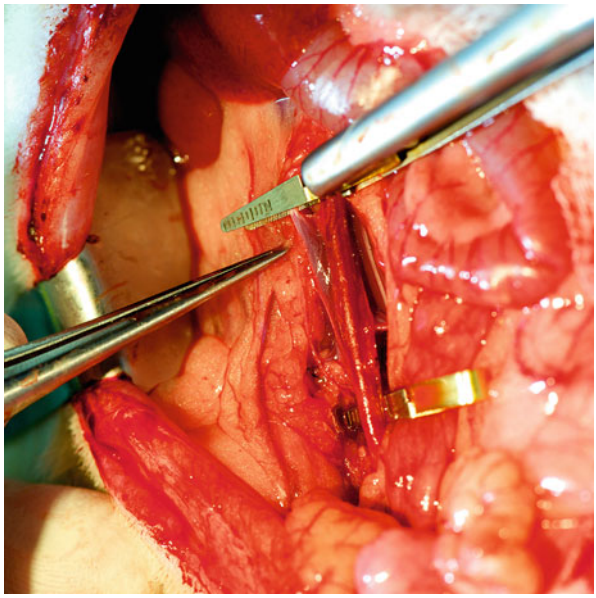

Fig. 9.16 Recipient - setting microclamps on the inferior vena cava

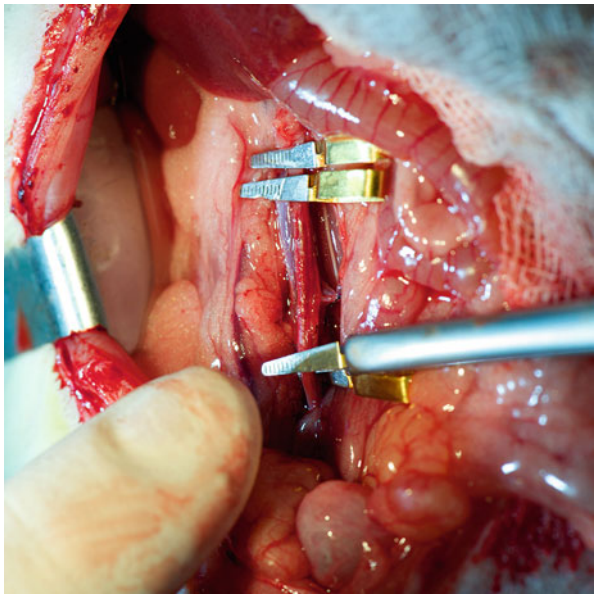

Fig. 9.17 Recipient - setting microclamps on the aorta

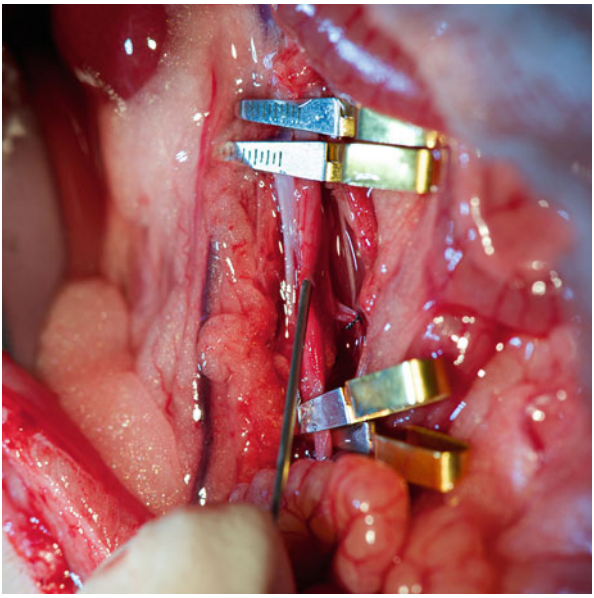

Fig. 9.18 Recipient - opening of the aorta

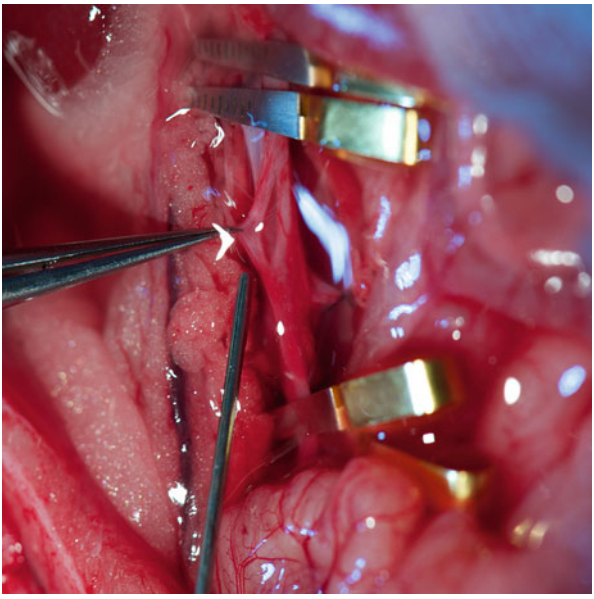

Fig. 9.19 Recipient - washing the opened aorta with heprin saline solution

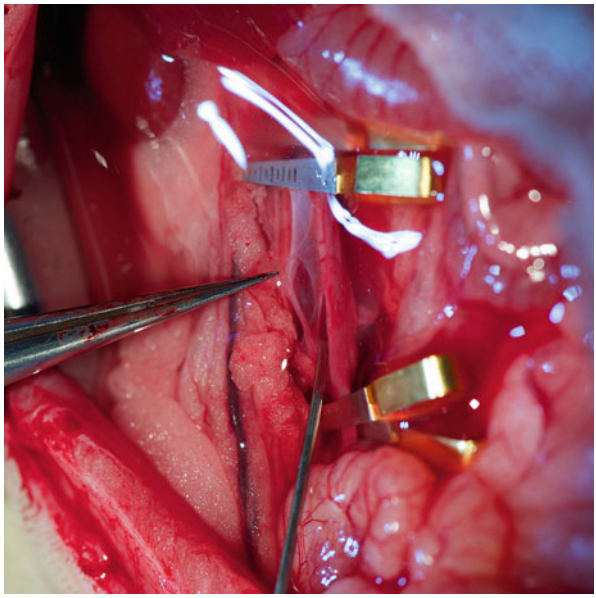

Fig. 9.20 Recipient - opening of the inferior vena cava

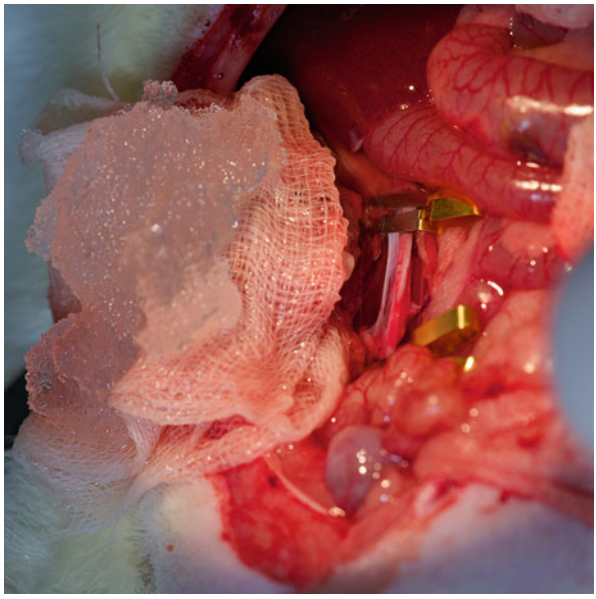

Fig. 9.21 Recipient - graft orientation

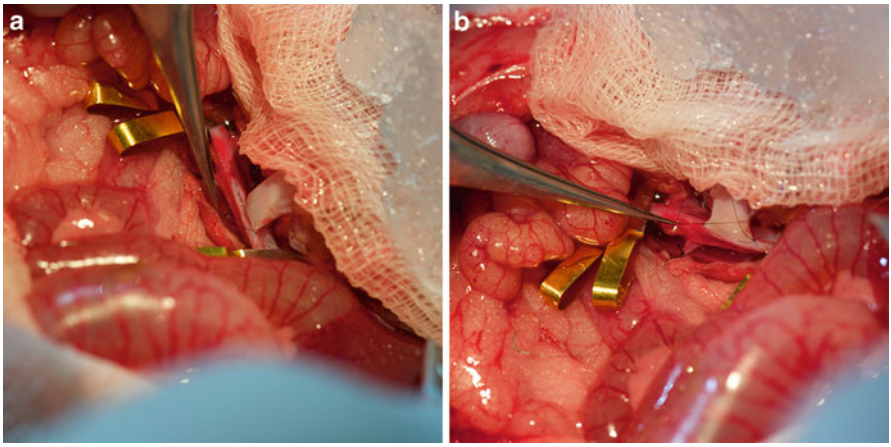

Fig. 9.22 Aortic anastomosis with anchor stitches

7. Make a continuous suture from one to the opposite anchor stitch with very fine steps. After reaching the opposite anchor stitch, it is advisable to tie the continuous suture to prevent purse-stringing the anastomosis (Fig. 9.23).
8. After finishing one side of the anastomosis, flip the heart to the opposite side of the abdomen and complete the other half of the anastomosis (Fig. 9.24).
9. Proceed with the anastomosis of the pulmonary artery and inferior vena cava in a similar fashion as the aortic anastomosis, but without flipping the heart. It is necessary to begin with the posterior wall (Fig. 9.25a) and then finish the anterior part of the anastomosis (Fig. 9.25b).
10. Remove the cold gauze and ice, examine both anastomoses for any gaps, which can be repaired with a single stitch. First remove the distal clamps and a look for any severe bleeding. Later, remove both proximal clamps and then hold the graft aorta closed with fine forceps and let the potential air exit via needle holes (Fig. 9.26).
11. Support the hemostasis by placing dry gauze around anastomoses.
12. Put gauze soaked in warm saline over the heart and the transplanted heart should start beating within 5 s.
13. Remove all instruments and gauze from the abdominal cavity and once again examine for potential bleeding (Fig. 9.27).
14. In the case of good graft function with no bleeding observed, replace the bowels carefully in its anatomical position, wash them with 3 ml of warm saline and close the abdominal cavity in anatomical layers (3-0 silk).

9.3.3.3 Tips and Tricks

1. Do not over-clean recipient vessels, a little fascia can support the suture.
2. Maintain both clamped segments of both vessels distended with blood to avoid cutting the back wall during opening.

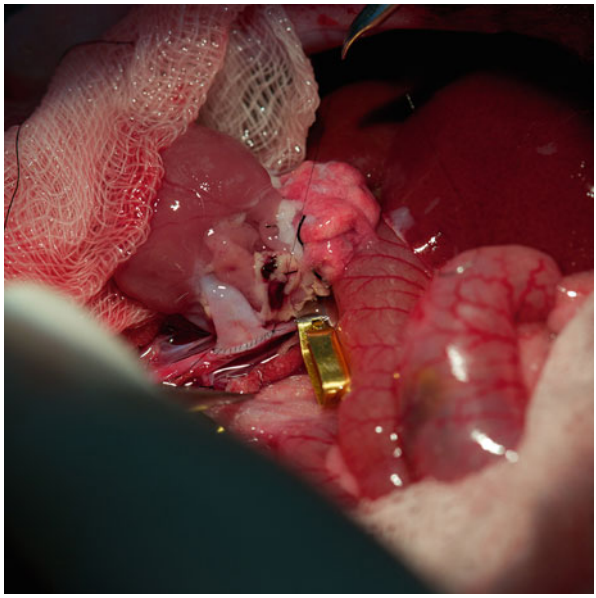

Fig. 9.23 First side of the end-to-side aortic anastomosis finished

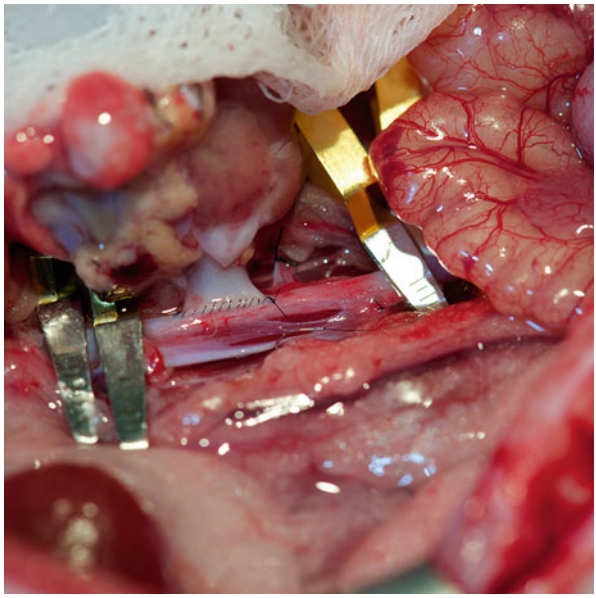

Fig. 9.24 Finished end-to-side aortic anastomosis

3. There should be no bleeding after opening clamped vessel segments; if there is, ensure that the clamps are applied properly and there are no patent branches remaining.
4. Good and precise orientation of the graft into the abdominal cavity is essential for successful anastomosis performance without the risk of kinking.
5. It is useful to support the anastomosis with surrounding retroperitoneum.

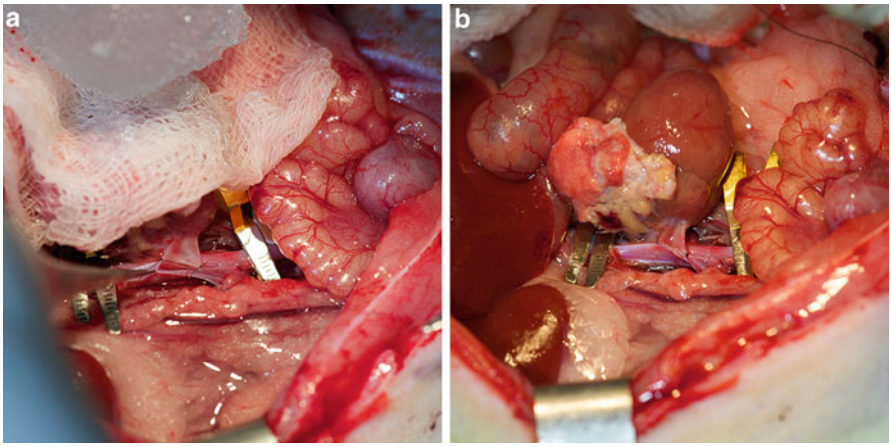

Fig. 9.25 Anastomosis of the pulmonary artery and inferior vena cava

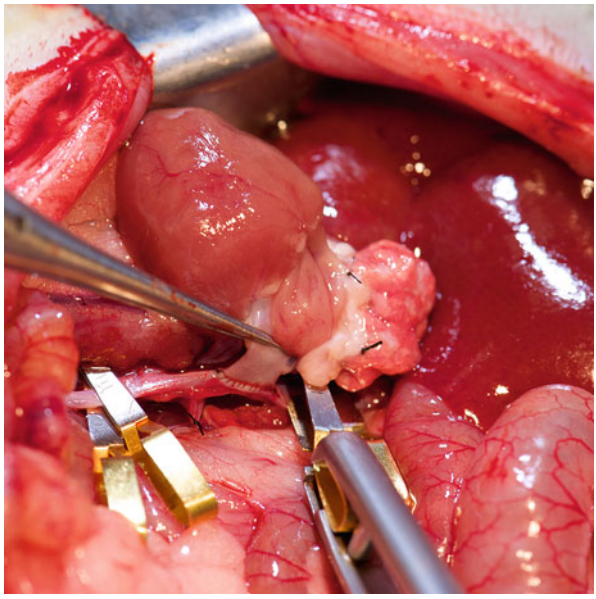

Fig. 9.26 Microsurgical clamps removal

6. During the operation, it is advisable to cover the graft with fresh ice in order to minimize ischemic injury.
7. Observe the graft color and function after unclamping. Ideally, the heart should be pink with no discolored area.
8. All walls should contract well without any ventricular distension. In the case of unsatisfactory graft function, a short gentle cardiac massage can be used to prevent ventricular distension and ischemia.
9. In the case of significant blood loss, up to 2 ml of warm saline can be administered via the femoral artery to maintain blood pressure.

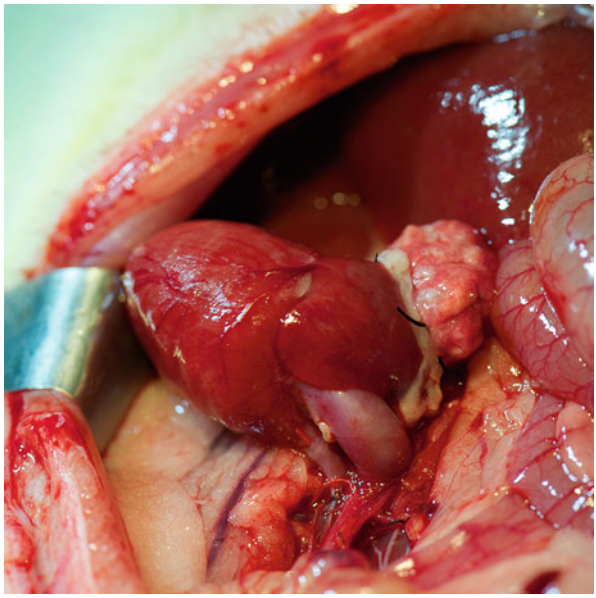

Fig. 9.27 Transplanted heart before the abdominal cavity closure

9.4 Heterotopic Abdominal Heart and Lung Transplantation (Partial Unloading)

Heart transplantation in this manner leads to partial mechanical unloading of a donor heart. Heterotopic abdominal heart transplantation consists of anastomosis of the donor aorta to the syngeneic recipient aorta only. Two upper right lobes remain connected to the graft. Blood perfuses the coronaries and the coronary flow is returned into the right atrium via the coronary sinus. Blood later flows via the vascular conduit, provided by connected part of the lung, to the left ventricle. The left ventricle is partially loaded with the small volume of coronary circulation. This kind of operation does not require operation on the very fragile pulmonary artery and vena cava. With this modification, you can achieve shorter graft cold ischemic time and there is no risk of the pulmonary artery anastomosis obstruction (Fig. 9.2).

9.4.1 Recipient Preparation

Recipient preparation is the same as described above with the only difference in the inferior vena cava preparation. There is no anastomosis on the inferior vena cava, so there is no need of cleaning and ligating any branches on the inferior vena cava.

9.4.2 Heart and Lung Grafts Procurement

9.4.2.1 Equipment + Material

Microsurgical Instruments Scissors, forceps, fine mosquito hemostat, 7-0 and 5-0 silk suture, two micro-scissors (straight, curved), three micro-forceps (two straight, one curved), cotton swabs, gauze, cold saline solution and ice, needles, syringes (5 ml, 10 ml), heparin (5000 I.U.) diluted in 5 ml of cold saline solution, cold cardioplegic solution (St. Thomas).

9.4.2.2 Procedure

Steps 1–6 are identical with the heart harvesting procedure which is described above.

7. Ligate with a silk 5-0 suture and inferior and pos-caval lobe (white arrows) of the right lung, so two upper lobes remain untouched (black arrows) (Fig. 9.28). Later ligate and cut left lung close to the hilus.
8. Perform blunt dissection, ligation (7-0 silk) and cut of the inferior vena cava (Fig. 9.11), left superior vena cava (Fig. 9.12) and right superior vena cava (Fig. 9.13).
9. Clean the aorta and transect just before the brachiocephalic trunk (Fig. 9.14) in one cut (straight microsurgical scissors).
10. Keep the heart in ice-cold cardioplegic solution until reimplantation (Fig. 9.29).

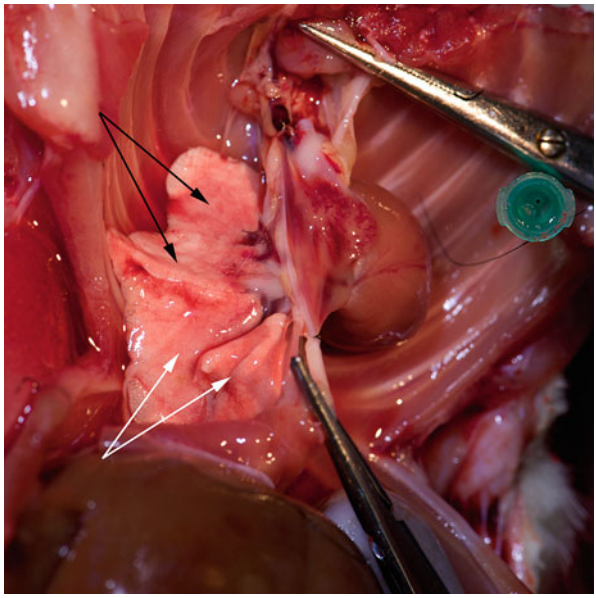

Fig. 9.28 Donor - right lung ligation wit 2 upper lobes preservation

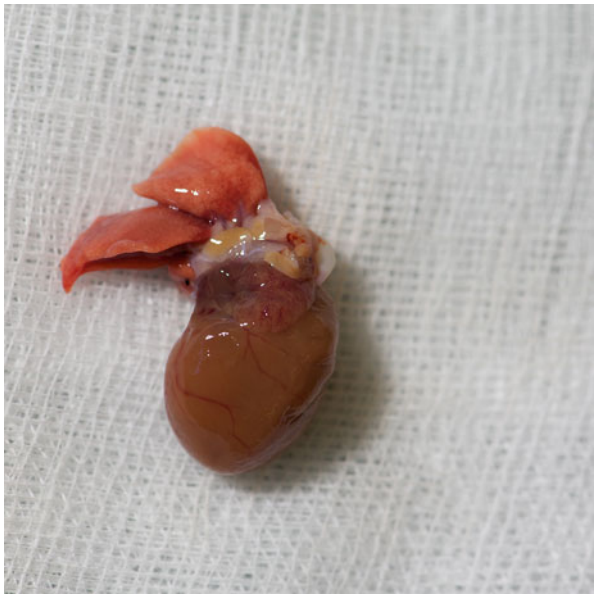

Fig. 9.29 Prepared graft for heart and lung transplantation

9.4.2.3 Tips and Tricks

1. Avoid touching the removed lungs with any sharp instrument to prevent later bleeding.
2. Be careful about any air in the needle or syringe during the administration of heparin or cardioplegia.
3. Insert the cardioplegic cannula very carefully to keep the aortic valve untouched.
4. Administer the cardioplegic solution very slowly to prevent distention of the heart and aortic valve damage.
5. The cut of the aorta must be done at once in a proper angle to obtain optimal site for anastomosis.
6. It is very helpful to clean the aorta of fat and fibrous tissue.

9.4.3 Heart and Lung Transplantation

9.4.3.1 Equipment + Material

Microsurgical Instruments Scissors, forceps, four vascular clamps, 9-0 nylon sutures with a 3 mm round-bodied needle, two micro-scissors (straight, curved), three micro-forceps (two straight, one curved), micro-needle holder, thermal cauterize unit, cotton swabs, gauze, saline solution, heating pad, needles, syringes (5 ml, 10 ml), heparin solution, ice.

9.4.3.2 Procedure

1. Carefully retract the bowels to the recipient left side and wrap them in wet gauze.
2. Apply vascular microclamps to the abdominal aorta (Fig. 9.30) in prepared spots, leaving as much space between clamps as possible.
3. Using 30-g needle, make a small hole in the center of clamped aortic segment (Fig. 9.18), then using straight microscissors, perform a midline incision of aorta approximately 4 mm long (to match with donor aorta circumference) and immediately wash the lumen with heparin saline solution (Fig. 9.31).
4. Place the donor heart on the right side of the abdomen and correctly oriented it so that the graft aorta leads to the abdominal aorta and wrap the graft into gauze soaked in ice cold saline and you can also add some ice if needed (Fig. 9.32).
5. The suture is performed with 9-0 nylon suture with 3 mm round-bodied needle. Start with two anchor stitches placed at the heel and at the toe (Fig. 9.22a, b).
6. After finishing one side of the anastomosis, flip the heart to the opposite side of the abdomen and complete the other half of the anastomosis (Fig. 9.24).
7. Remove the cold gauze and ice, examine both anastomoses for any gaps, which can be repaired with a single stitch. First, remove the distal clamps and a look for any severe bleeding. Later, remove both proximal clamps and meanwhile, holding the graft aorta closed with fine forceps, let the potential air exit via needle holes (Fig. 9.26).
8. Support the hemostasis by placing dry gauze around anastomoses.
9. Put gauze soaked in warm saline over the heart and the transplanted heart should start beating within 5 s.

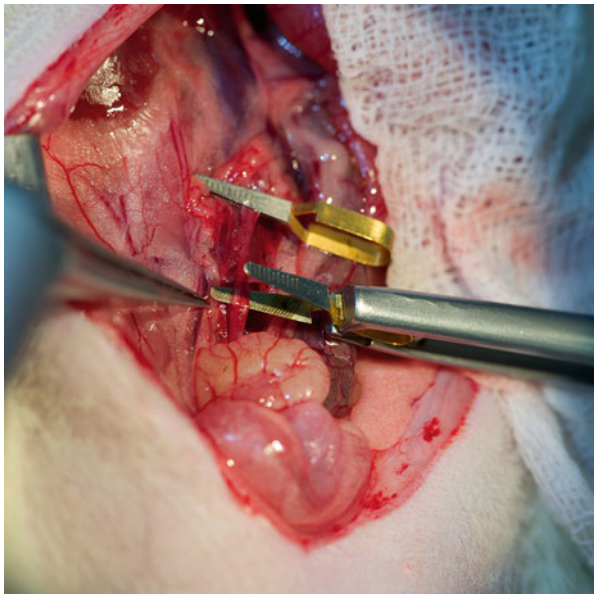

Fig. 9.30 Recipient - clamping of the abdominal aorta

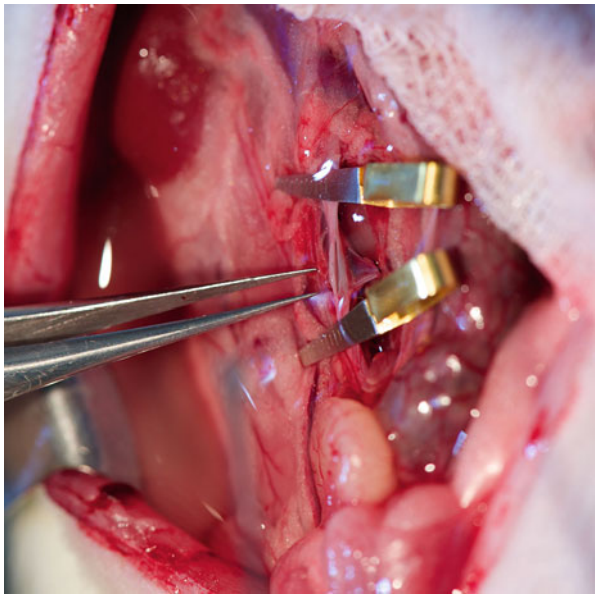

Fig. 9.31 Recipiet - opening of the abdominal aorta

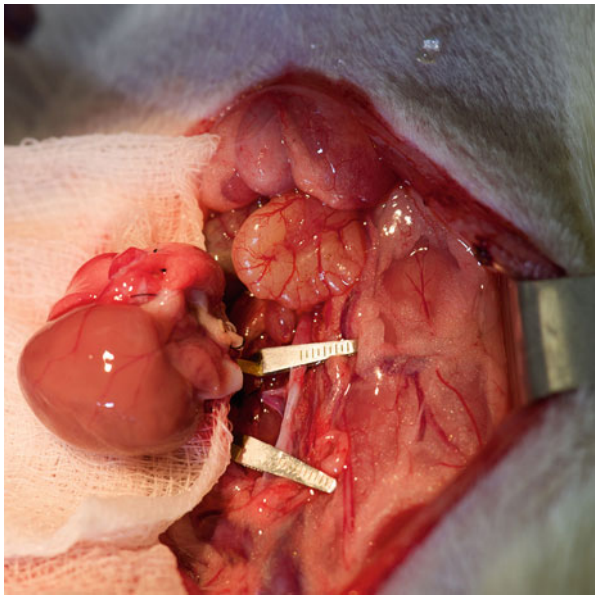

Fig. 9.32 Recipient - graft orientation

10. Remove all instruments and gauze from the abdominal cavity and once again examine for potential bleeding (Fig. 9.33).
11. In the case of good graft function with no bleeding observed, replace the bowels carefully in its anatomical position, wash them with 3 ml of warm saline and close the abdominal cavity in anatomical layers (3-0 silk).

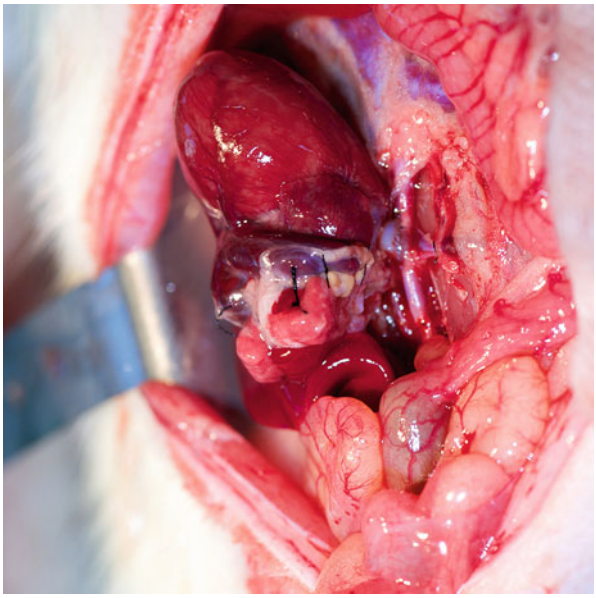

Fig. 9.33 Transplanted heart and lungs before the abdominal cavity closure

9.4.3.3 Tips and Tricks

1. Avoid any lung injury.
2. Do not over-trim recipients vessels; a little fascia can support the suture.
3. There should be no bleeding after opening clamped aorta; if so, ensure that clamps are applied properly and there are no patent branches remaining.
4. It is useful to support the anastomosis with surrounding peritoneum.
5. During operation, it is advisable to cover the graft with fresh ice in order to minimize ischemic injury.
6. Observe the graft color and function after unclamping. Ideally the heart should be pink with no discolored area.
7. All walls should contract well without any ventricular distension. In the case of unsatisfactory graft function, a short gentle cardiac massage can be used to prevent ventricular distension and ischemia.
8. In case of significant blood loss, up to 2 ml of warm saline can be administered via the femoral artery to maintain blood pressure.

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Chapter 10
Orthotopic Experimental Lung Transplantation

Andreas Habertheuer, Dominik Wiedemann, Gunther Laufer, Alfred Kocher, and Martin Andreas

Abstract Various techniques of orthotopic single lung transplantation in rats have been reported; however, their widespread use has been limited owing to the complexity of the procedure and the high mortality, respectively. We present a simplified and reproducible microsurgical lung transplantation model in rats with a high survival rate, rendering surgical assistance unnecessary.

Overall recovery rate from anesthesia and 1 day survival including the early period of establishment was 77.0 % (n = 97), however survival could be increased to and maintained at 100 % in later series. The overall long-term survival (28 days) of animals successfully recovered from anesthesia was 75.0 %. However, we found the long-term survival to be dependent on different animal suppliers and series with a variation between 61.5 % and 100 %. The most common postoperative transplantation related cause of death was pleural effusion. The lungs showed excellent perfusion and ventilation immediately upon transplantation. Blood gas samples drawn from the left pulmonary vein and histologic sections revealed excellent graft function. The donor operation lasted 21.6 ± 6.8 min, donor left lung dissection required 21.9 ± 4.8 min, and implantation required 100.9 ± 19.8 min with a tendency of improvement over the course of time.

This innovative method of orthotopic left single lung transplantation can be safely performed by one experienced surgeon and is associated with excellent results and a high degree of reproducibility. The model presented is a valuable tool for research in ischemia reperfusion injury, acute lung edema, vascular inflammation and chronic rejection.

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10.1 Experimental Models of Acute Lung Injury

Acute lung injury (ALI) and its most severe manifestation, acute respiratory distress syndrome (ARDS), is a clinical syndrome defined by acute hypoxemic respiratory failure and bilateral pulmonary infiltrates consistent with edema but with normal cardiac filling pressures. Histopathologically, ALI and ARDS are characterized by neutrophilic alveolitis, injury of the alveolar epithelium and endothelium (capillary-alveolar interface) with increased permeability, hyaline membrane formation and microvascular thrombi resulting in respiratory failure.

The acute physiological changes at the onset of ALI/ARDS in humans include severe hypoxemia that is usually responsive to positive end-expiratory pressure (PEEP) and a decrease in total thoracic compliance caused by reduced lung compliance. A marked increase in extravascular lung water is caused by an increase in endothelial and epithelial permeability, associated with impairment in the ability of the alveolar epithelium to actively clear alveolar fluid [1–3]. These changes cause severe ventilation/perfusion mismatching and an increase in shunt fraction.

Animal models provide a bridge between patient wards and the laboratory bench. Most animal models of ALI are based on clinical disorders that are associated with ALI/ARDS in humans. These include sepsis, multiple transfusions, multiple trauma, aspiration of gastric contents, and ischemia reperfusioninjury [4].

The goal of this chapter is to review models of lung injury that primarily target the vascular endothelium or the alveolar epithelium and to summarize their strengths and weaknesses.

10.1.1 Models Targeting the Vascular Endothelium

10.1.1.1 Oleic Acid

Oleic acid is the most common free fatty acid in mammals, representing 60 % of the free fatty acid pool [5]. Oleic acid also represents 50 % of the total fatty acids present in pulmonary emboli in patients with long bone trauma [6]. The oleic acid model was developed as an attempt to reproduce ALI due to lipid embolism.

Mechanism of Action Oleic acid is directly toxic to endothelial cells at concentrations of 5x104 M in vitro [7]. The damage to the endothelium appears to be due to necrosis rather than apoptosis [8]. Endothelial injury is followed by epithelial injury, with swelling and necrosis of type I alveolar cells, but no evidence of apoptosis [8].

Application and Dosage Within 1 min of intravenous oleic acid administration (0.04 ml/kg), electron microscopy reveals severe vacuolation of endothelial cells, and passage of an intravascular marker, Evans blue dye, into the subendothelial space. Oleic acid is insoluble in water and must be dissolved in ethanol or emulsified in blood before administration. Within 30 min, oleic acid becomes detectable in the air spaces. Oleic acid is not detected intracellularly and remains in the extracellular space and the air spaces [7].

10.1.1.2 Endotoxin

Lipopolysaccharide (LPS) is a glycolipid present in the outer membrane of gram-negative bacteria that is composed of a polar lipid head group (lipid A) and a chain of repeating disaccharides [9]. LPS is an important mediator of sepsis and acute lung injury in response to gram-negative bacteria, and systemic administration of LPS was one of the earliest approaches used to model the consequences of bacterial sepsis.

Mechanism of Action The capillary endothelium is the initial site of injury. The cellular injury induced by LPS appears to be related to apoptosis, in contrast to the oleic acid model, in which the injury is due to a direct toxic effect resulting in necrosis. Most of the biological effects of LPS are produced by lipid A, a sub-complex of LPS. LPS activates the CD14/TLR4 receptor structure on monocytes, macrophages, and other cells, triggering the production of inflammatory mediators, such as TNFα and IL-1 [10, 11] triggering ALI/ARDS.

Application and Dosage The intratracheal instillation of LPS via a tracheal intubation tube at doses ranging from 1 to 4 ng/kg rapidly triggers apoptosis of endothelial cells.

10.1.2 Models Targeting the Alveolar Epithelium

10.1.2.1 Acid Aspiration

The development of acute respiratory failure following aspiration of gastric contents was first described in 1946 by Mendelson and constitutes an important risk factor for the development of ARDS [4, 12] One of the main characteristics of gastric contents is a low pH and high osmolarity, apart from food particles, bacterial cell wall products, and cytokines such as IL-1β. All of these factors may contribute to the pathogenesis of aspiration-induced lung injury [13].

Mechanism of Action Low pH acid aspiration is a neutrophil-dependent form of lung injury that is characterized by injury of the airway and alveolar epithelium, including type I alveolar epithelial cells, followed by a repair process that involves proliferation of alveolar type II cells [14, 15].

Application and Dosage Introducing HCl directly into the trachea or bronchi while an animal is mechanically ventilated causes acid injury. Most studies use 0.1 N HCl, although higher concentrations (up to 0.5 N) have been used [16]. Because this results in a pH that is generally lower than that of gastric juice, an alternative method is to use 0.3 % NaCl titrated with HCl to a pH of 1.2–1.5. This results in a solution with pH and osmolarity approaching the gastric juice of some patients.

10.1.2.2 Surfactant Depletion by Saline Lavage

The saline lavage model was developed by Lachmann et al. in 1979 [17] based on the observation that ARDS is associated with depletion of surfactant which is produced by type II alveolar epithelial cells. In this model, warmed isotonic saline solution is introduced into the lungs and then removed by aspiration. The lavage is repeated until a target degree of hypoxemia is reached.

Mechanism of Action Repeated lavage with saline reduces the surfactant lipid concentration in alveolar lining fluids, altering alveolar surface tension. Pulmonary surfactant is a complex mixture of phospholipids and proteins that has several important functions. Surfactant decreases surface tension and prevents collapse of the alveolar spaces at low lung volumes. Depletion of surfactant facilitates alveolar collapse and increases the likelihood of mechanical injury to the alveolar walls during repeated cycles of opening/closure during mechanical ventilation. By decreasing lung compliance, more negative pressures are required to inflate the lungs additionally triggering pulmonary edema.

Application and Dosage 4.0 to 8.0 ml of 0.9 % saline solution is introduced via an orotracheal intubation tube, depending on the degree expected of lung injury [18]. Repeated saline lavage (0.9 % NaCl) leads to almost immediate hypoxemia and widening of the A-a O2 difference causing a pathophysiological cascade similar to that of ARDS. The saline lavage by itself has little consequence in terms of permeability changes or inflammation [19].

10.2 Animal Preparation

10.2.1 General Aspects

In terms of animal weight and size, it is recommended to use slightly larger and bigger recipients. Rats weighing between 250 and 300 g should be used as donors and rats weighing between 300 und 350 g should be used as recipient animals, respectively. Almost every recipient animal will develop a mild pleural effusion within the first postoperative days. The presented setting is associated with the most favorable animal survival, giving a mild pleural effusion place to expand without compressing other major thoracic organs [20].

Orthotopic ratlung transplantation is a valuable tool in transplant research. The most widespread experimental design to induce chronic rejection is the use of major histocompatibility complex incompatible rats. Common rat strains are Fischer 344 rats and Wistar Kyoto (WKY) rats which serve as donors and recipients, respectively.

10.2.1.1 Anesthesia

Animals are anaesthetized using 100 mg/kg Ketamine (Ketasol®) i.p. and 4 mg/kg Xylazine (Rompun®) i.p. All recipient animals should be additionally pretreated with 1 mg/kg Flunixin (Finadyne®) s.c. and 0.2 mg/kg Fentanyl (Fentamed®) s.c. to obtain best possible analgesia. It is recommended to administer 0.2 mg/kg Atipamezol (Antisedan®) i.m. and 0.1 mg/kg Nalxon (Narcanti®-Vet) i.m. following skin closure to guarantee early recovery from general anesthesia and increase animal survival.

10.2.2 Intubation and Ventilation Management

Animals are intubated using a 14G intravenous catheter and mechanically ventilated with small animal ventilator (Biegler Medizin Elektronik, Fig. 10.1). The correct ventilation management is illustrated in Table 10.1. Transillumination of the neck affords direct vision of the glottis and facilitates quick intubation. Figure 10.2 shows transillumination of the neck with subsequent intubation). All transplantations are performed using a binocular surgery microscope (we used the standard

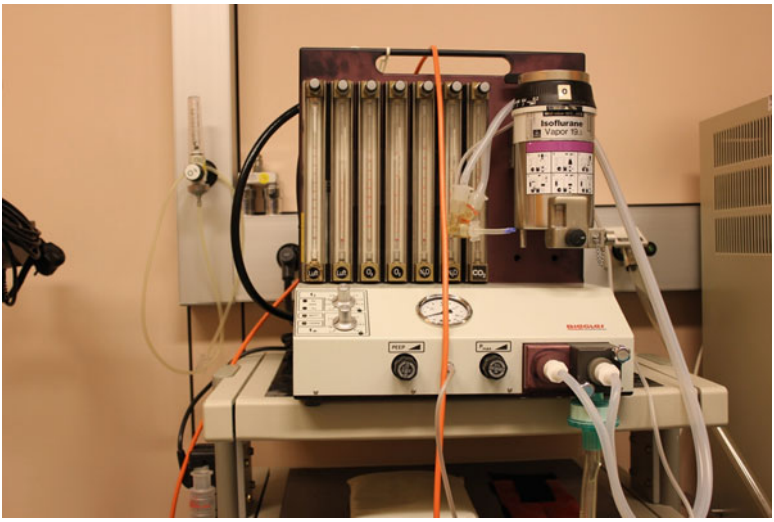

Fig. 10.1 Small animal ventilator (Biegler Medizin Elektronik)

Table 10.1 Ventilation management

Ventilation mode	Pressure control mode
Ventilation pressure (P_{max})	18 mbar
Positive end expiratory pressure (PEEP)	2–4 mbar
Single inspiration time	0.4 s
Singe expiration time	0.8 s
Ventilation frequency (f)	50/min
Flow (l/min)	1.5 l/min
Oxygen concentration (FIO_2)	0.7

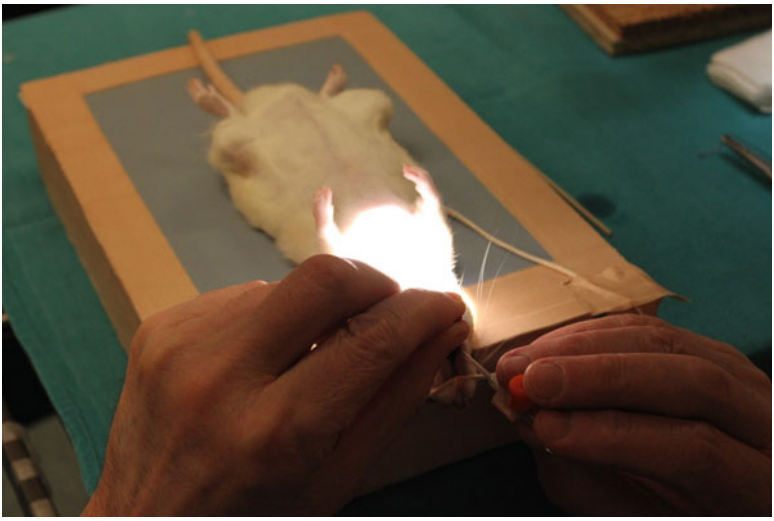

Fig. 10.2 Intubation of a Fischer Rat with a 14G catheter

Leica M651) donor animals are placed in a supine position (Fig. 10.3) under the operating microscope, recipient animals are placed in a right lateral position (Fig. 10.4) to allow good access to the complete left hemithorax.

10.2.3 Tips and Tricks

1. Over flexion of the rat head is essential to allow for direct vision of the glottis
2. Use a local anesthetic (xylocaine spray) to overcome gag reflex when inserting the tube

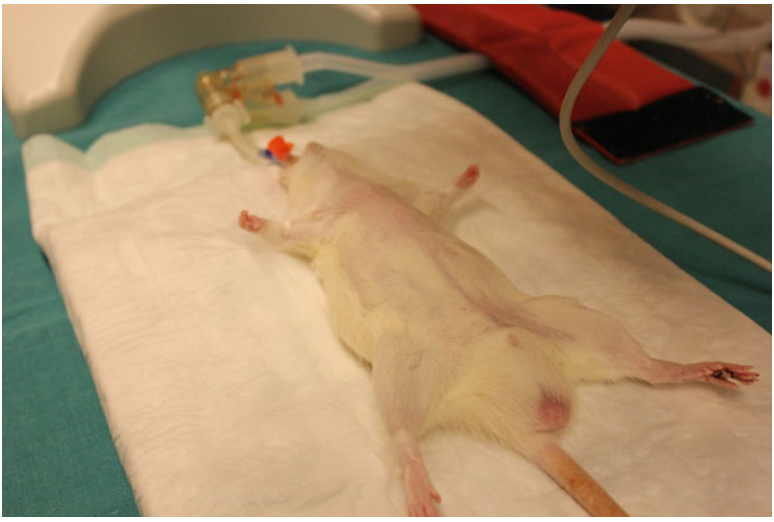

Fig. 10.3 Donor animal, supine position to allow midline sternotomy

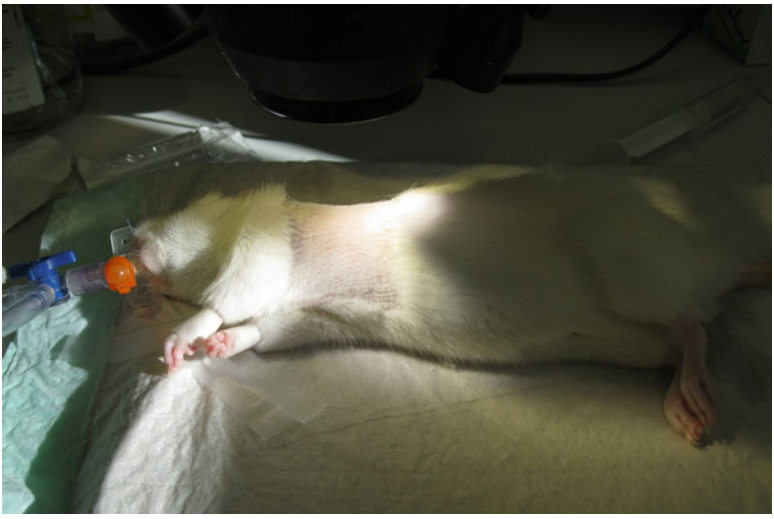

Fig. 10.4 Recipient animal is placed in a lateral position

3. When applying the local anesthetic, use a Q-tip to swab the inside of the rat's mouth for better results
4. Trans illumination of the rat neck markedly facilitates intubation
5. It is essential to avoid repeated tries of intubation. This could trigger swelling of the airways and is associated poor survival
6. Do not apply any force when inserting the intubation tube; it should come in fairly easily if in the correct position

7. The ventilation management presented in the Table 10.1 has been extensively tested and is associated with favorable outcome
8. It is strongly recommended to use an isoflurane induction box for induction of anesthesia prior to application of i.p. general anesthesia if the surgeon is not experienced in animal care and management
9. Using a supine position for the harvesting procedure and a right lateral position for subsequent implantation procedure guarantees excellent organ exposure and facilitates the procedure

10.3 Lung Graft Procurement

10.3.1 General Aspects and Lung Anatomy

The left lung is selected for transplantation because the lungs of the rat consist of one whole lung in the left hemithorax and four smaller lobes on the right side. Figure 10.5 schematically illustrates the thoracic anatomy in rats. Transplantation of four small lobes into the right hemithorax with complete bronchiovascular anastomoses is technically not feasible, especially not in a timely fashion to guarantee high recovery rates from anesthesia and excellent animal survival. However, transplantation of a left lung into the right side and replacing the four right lobes is surgically feasibly due to the relatively long hilar bronchovascular structures. Thus, preference is given to a left orthotopic transplantation protocol in order to keep a physiologic model.

10.3.2 Harvesting Procedure and Graft Preservation

As harvesting and dissection of the left bronchovascular structures is an extensive procedure and takes considerable time, we recommend doing both steps before opening the recipient animal. The recipient thoracotomy with exposure of both heart and left lung is probably the most extensive procedure that can be performed in a rat and should be done as fast as possible. As a consequence, perfect timing is essential.

10.3.2.1 Equipment and Material

Binocular operating microscope, small animal ventilator, 2 micro forceps, various larger forceps, 1 micro scissor, 1 large scissor, skin retractor, 7-0 silk suture cut to loops and ligatures, 1 20 ml syringe for perfusion solution, 1 5 ml syringe for heparin solution, 1 1 ml syringe for general anesthesia, 1 petri dish.

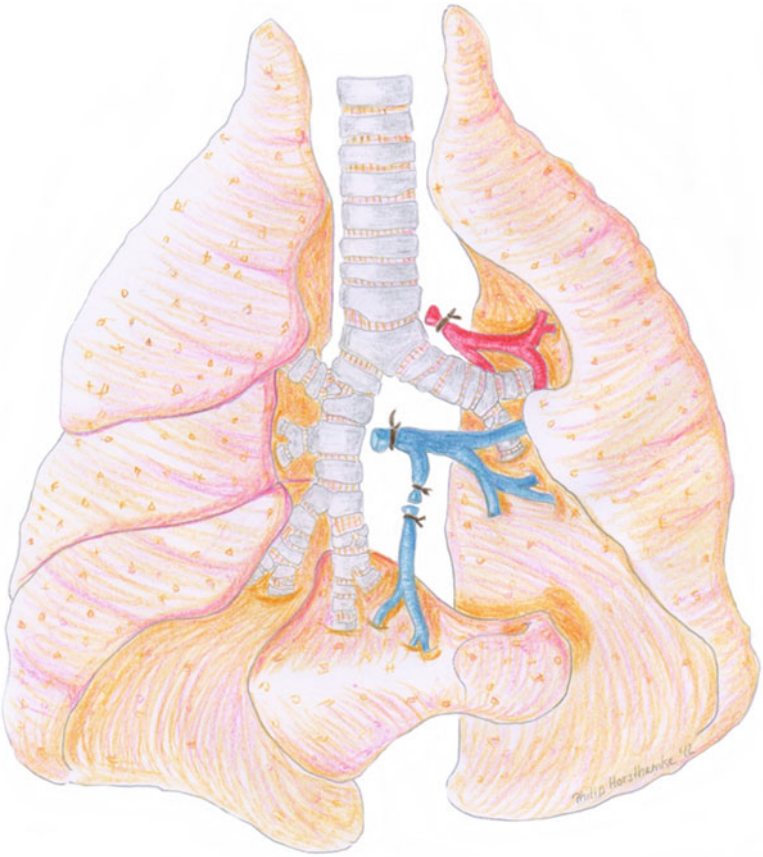

Fig. 10.5 Schematic illustration of the thoracic anatomy in rats. In the middle trachea, left and right main bronchus. On the *right side*, upper lobe, middle lobe, inferior lobe and postcaval lobe. Left lung and bronchovascular structures to the *left side*. Note: The postcaval segment functionally belongs to the right side, however, venous drainage is achieved via the left main pulmonary vein. Ligated vein of the right postcaval lobe to increase length of the left main pulmonary vein in order to facilitate venous anastomotic procedure

10.3.2.2 Procedure

1. Position the donor animal for median sternotomy. Shave the anterior thorax and abdomen and clean the incision sites with a 75 % alcohol solution.
2. Do a median skin incision reaching from the jugular notch to the pubic symphysis and extend the skin incision laterally from a mid-abdominal level to a T-shaped incision with subsequent cutting of subcutaneous tissue and anterior abdominal musculature.

3. Incise the peritoneum from the xiphoid to the pubic symphysis and, following the original T-shaped skin incision, extend the peritoneal incision laterally to both sides.
4. To allow for direct vision of the big ascending and descending retroperitoneal vessels, evert the abdominal viscera extraperitoneally.
5. Dissect the abdominal aorta and inferior vena cava and separate both between the inferior margin of the liver and the tributary renal vessels.
6. Cannulate the infrahepatic inferior vena cava using a 27G needle (27G x″ – Nr. 20, 0.4 mm × 19 mm, BD MircrolanceTM 3) and heparinize the donor animal systemically using 5,000 U Heparin.
7. Following anticoagulation, remove the thoracic diaphragm from its costal attachments and the tripartite of thoracic organs (consisting of heart as well as left and right lung) until it becomes visible.
8. Discontinue artificial ventilation intermittently to allow easy separation of the thymus and pericardial tissue from the posterior sternal wall as well as dissection of the inferior pulmonary ligaments.
9. Do a median sternotomy using conventional scissors. Apply conventional needle holders on each side of the transected sternum spreading apart the chest walls.
10. To properly observe the large central thoracic vessels and to allow separation of the ascending thoracic aorta from the pulmonary trunk, excise the thymus.
11. Cut the left atrial auricle to later allow drainage of the perfusion solution. As the left atrial auricle is cut, the donor rat starts to exsanguinate and ischemia time is initiated.
12. Next, fill the thoracic cavity with ice to accelerate cardiac arrest.
13. Place wet gauzes on the abdominal viscera to avoid dislocation of the intestines into the thoracic cavity.
14. Insert a 21G needle (21G × 1 1/2″ – Nr. 2, 0.8 mm × 40 mm, BD MircrolanceTM 3) into the pulmonary trunk via subvalvular myocard and pulmonary valve and perfuse the lungs with 20 ml of an anterograde cold preservation fluid of low potassium dextran glucose (LPDG) (Perfadex®) with 20 µl/20 ml of sodium bicarbonate (Fig. 10.6).
15. When the perfusion of the lungs is homogenous, extend the original median incision cranially to the level of the larynx.
16. Divide the infrahyoid muscles medially and ligate the trachea with the lungs fully inflated at 100 % of total lung capacity.
17. Cut the trachea proximal to the ligature while keeping the lungs fully inflated.
18. Excise the cardiopulmonary block by cutting the supra-aortic trunks consisting of brachiocephalic trunk, right common carotid artery and right subclavian artery and by transecting the thoracic aorta, superior vena cava, inferior vena cava and pulmonary ligaments.
19. Place the explanted cardiopulmonary block (Fig. 10.7) in a Petri dish with crushed ice and cooled, wet gauze.

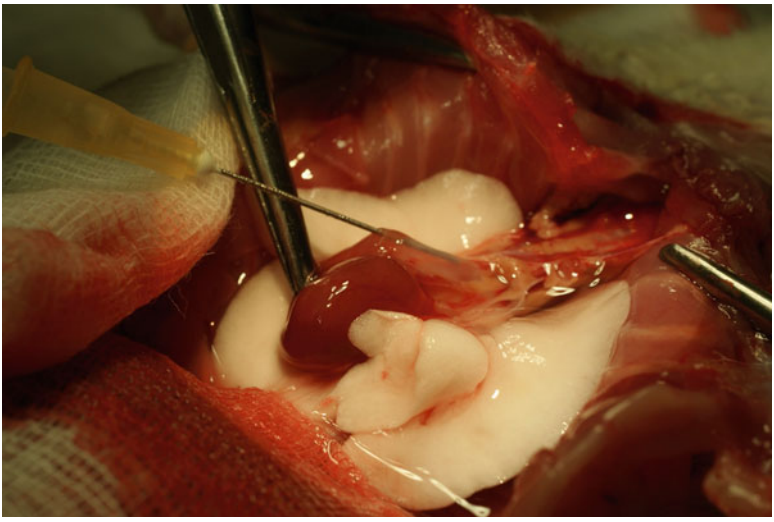

Fig. 10.6 Thoracic cavity in situ, cannulation of pulmonary trunk with 21G needle and perfusion of both lungs. Heart in the middle, aorta, supra-aortic trunks and pulmonary artery. To both sides perfused left and right lungs

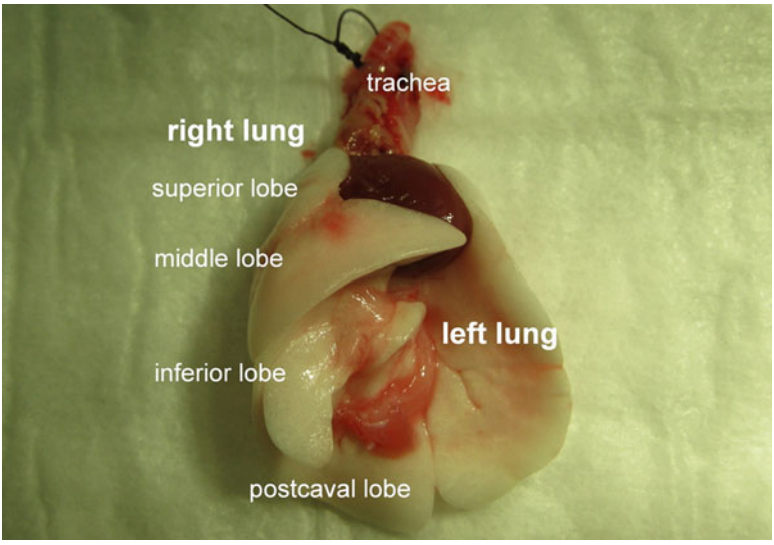

Fig. 10.7 Explanted cardiopulmonary block

10.3.2.3 Tips and Tricks

1. As with every surgical intervention, we recommend to prepare everything in advance, especially ligatures, loops, cuffs and preservation fluids.
2. Cut the ligatures to 2 cm and the loops to 3 cm
3. When doing the sternotomy, it is important to avoid broken ribs and loose bony fragments in order not to stab or injure the lungs
4. When flushing the lungs, do not apply any pressure on the syringe. This would damage the alveolo-endothelial interface of the lungs and trigger a pulmonary edema. We recommend using gravity flushing instead of it
5. The process of flushing takes about 5 min for 20 ml
6. It is recommended to cool the lungs with crushed ice from the moment of cardiac arrest is initiated

10.4 Left Lung Dissection

10.4.1 Procedure

1. Remove the remnants of the left inferior pulmonary ligament spanning between the left pulmonary vein and the right inferior pulmonary margin.
2. The first structure to dissect is the left pulmonary vein owing to its most anterior position. In our studies, we found that Fischer rats in particular are not prone to anomalies in the hilar region and we constantly observed two segmental veins, a left superior pulmonary vein crossing the left main bronchus on its anterior aspect and a left inferior pulmonary vein. Both branches are tributaries of the left main pulmonary vein close to the hilus forming a comparatively long venous trunk (Fig. 10.8). In contrast to human anatomy, the left venous pulmonary system also drains the right postcaval lobe.
3. To gain additional vessel length, ligate and transect the right inferior pulmonary vein draining the right postcaval lobe. By cutting the left main pulmonary vein medial to the ligated venous branch and closing the left atrium, a long donor left pulmonary vein is created.
4. Mobilize the artery carefully and dissect the artery from the pulmonary trunk to the hilus of the left lung. We have consistently observed a ligamentous fixation of the pulmonary artery on the distal left main bronchus. To facilitate the subsequent process of implantation and to gain a maximum of vessel length, we recommend transecting the ligament and cutting the artery as close to its origins as possible (Figs. 10.9 and 10.10).
5. Flush both vessels with 500 U of Heparin to prevent local thrombus formation.
6. As the left pulmonary artery crosses the left main bronchus anteriorly, we recommend making the left main bronchus the last structure to dissect (Figs. 10.11 and 10.12).

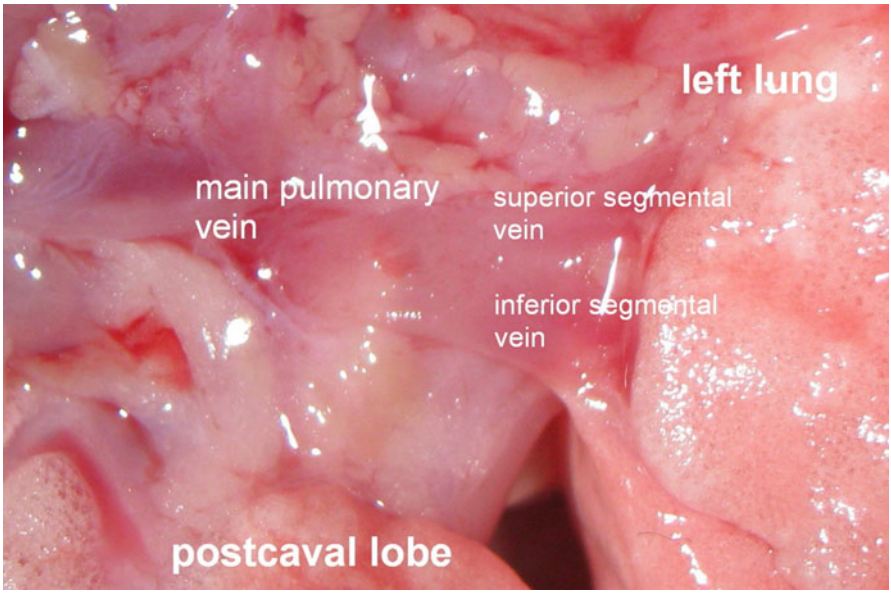

Fig. 10.8 Left main pulmonary vein with tributary segmental veins in Fischer Rats

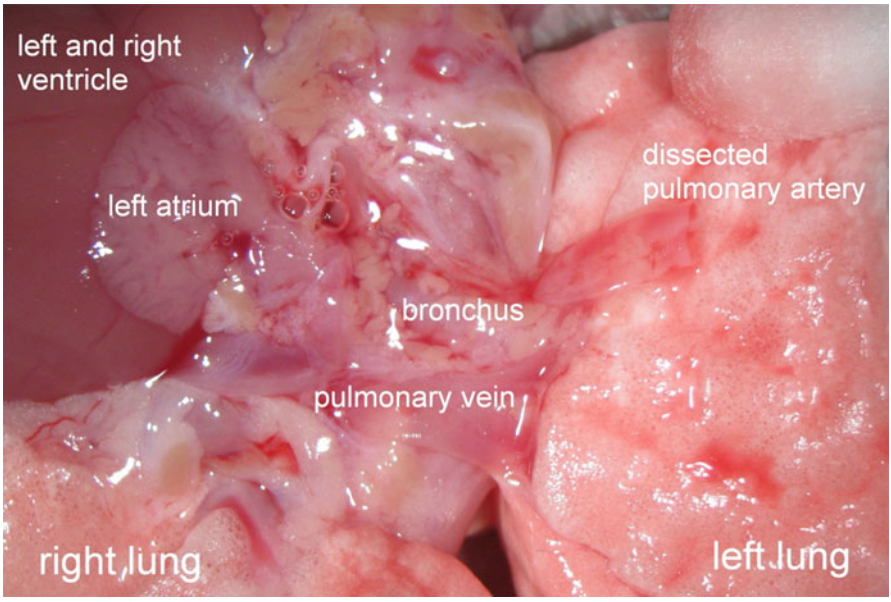

Fig. 10.9 Cardiopulmonary block with dissected and transected left pulmonary artery

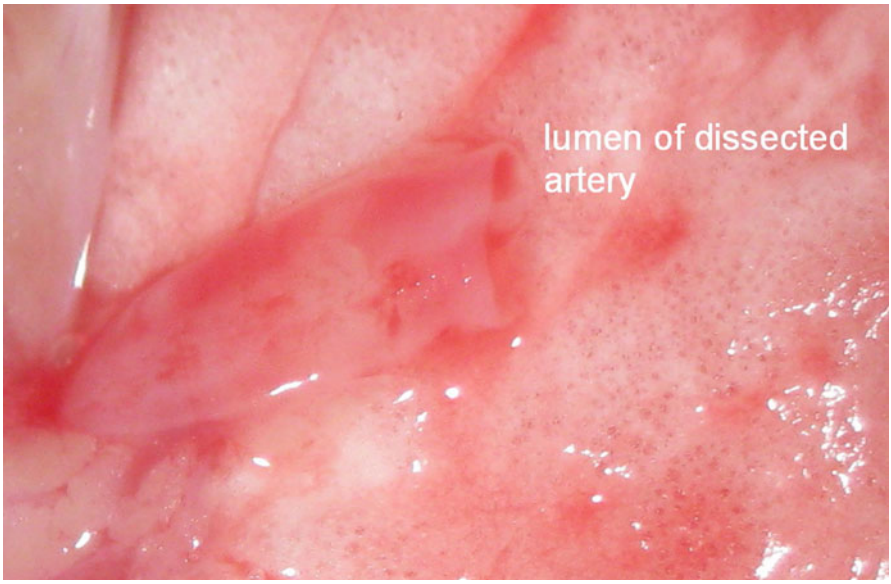

Fig. 10.10 Dissected left pulmonary artery

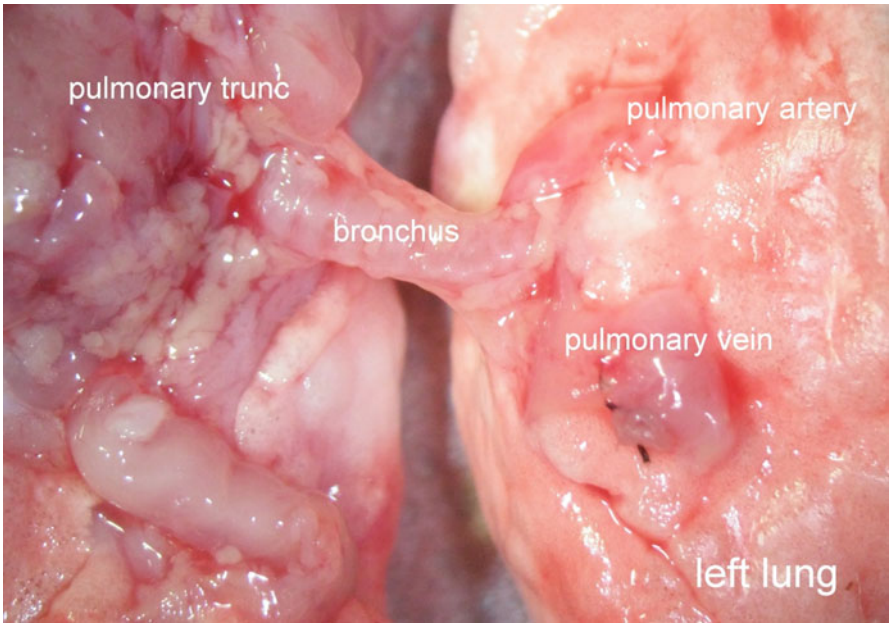

Fig. 10.11 Left main bronchus

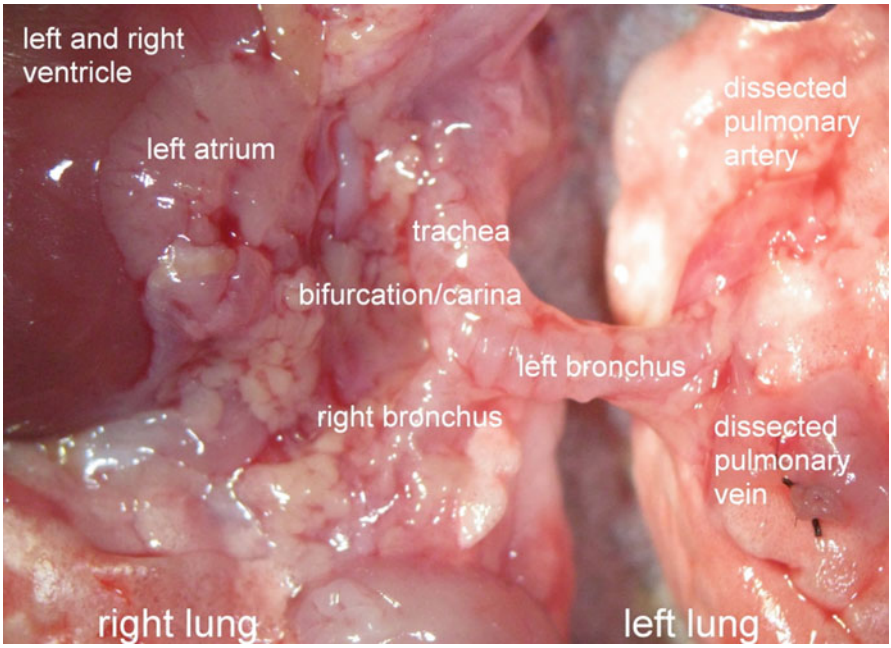

Fig. 10.12 Trachea with left and right main bronchus

7. Remove the peribronchial tissue, which mainly consists of fat. Otherwise, clear vision of the bronchial lumen is difficult to gain during anastomosis and suturing of the airway anastomosis is impeded.
8. Undertake efforts to keep the lungs fully inflated for as long as possible as very high positive end-expiratory pressure (PEEP) is necessary to remove atelectasis of a fully collapsed transplanted allografted lung. Thus dissect, ligate and cut both trachea and right main bronchus distally. Utilizing this modification, the left lung remains fully inflated until initiation of the bronchial anastomosis.
9. Figure 10.13 shows the entirely dissected left donor lung and displays the tripartite of bronchovascular structures consisting of a T-shaped left main bronchus with a ligated trachea and a ligated right main bronchus, a pulmonary artery and a pulmonary vein.

10.4.1.1 Tips and Tricks

1. To enable a successful transplantation all three bronchovascular structures consisting of left pulmonary artery, the left pulmonary vein and left main bronchus have to be meticulously dissected to an appropriate length and transected as centrally as possible to gain vessel length allowing subsequent anastomosis.

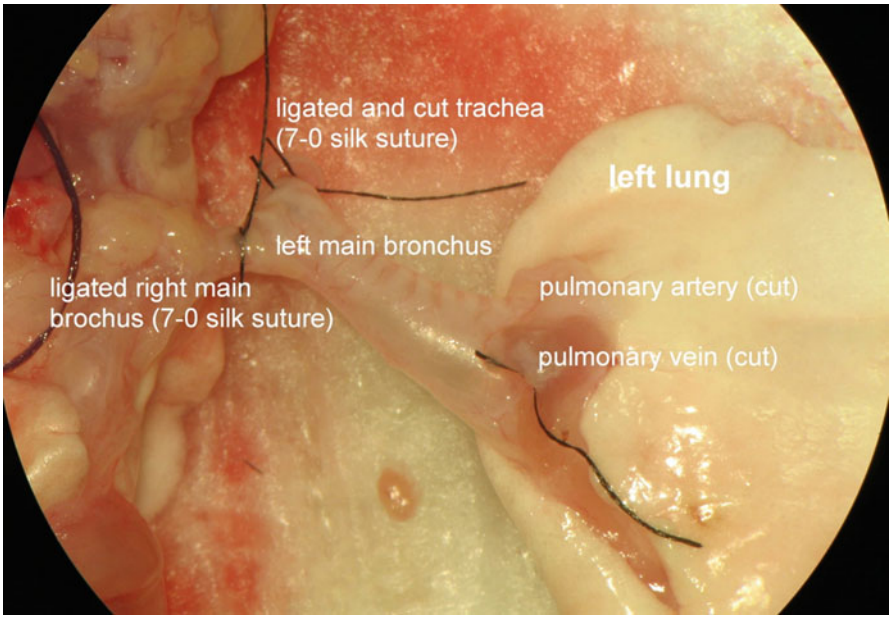

Fig. 10.13 Left lung with transected pulmonary artery and pulmonary vein, T-shaped bronchial appendage to keep the left lung fully inflated

2. A thorough dissection of the airways is equally important as a precisely displayed vasculature as perforation of the bronchus or leakage of the bronchial anastomosis would result in immediate pneumothorax.
3. Stick to the proposed sequence of dissection as this will facilitate the procedure.
4. It is strongly recommended to ligate the right main bronchus and the trachea rather than direct ligation the left bronchus as this procedure guarantees a long bronchus for the subsequent anastomosis.
5. Keep the lungs fully inflated for as long as possible as very high positive end-expiratory pressure (PEEP) is necessary to remove atelectasis of the fully collapsed transplanted lung

10.5 Orthotopic Lung Transplantation

10.5.1 General Aspects

All animals are orotracheally intubated and mechanically ventilated, using the same respirator and ventilation management as for our donor animals. With the recipient animal positioned for a left thoracotomy, the left lateral thorax is shaved and cleaned with a 75 % alcohol solution. A clean, non-sterile technique is used.

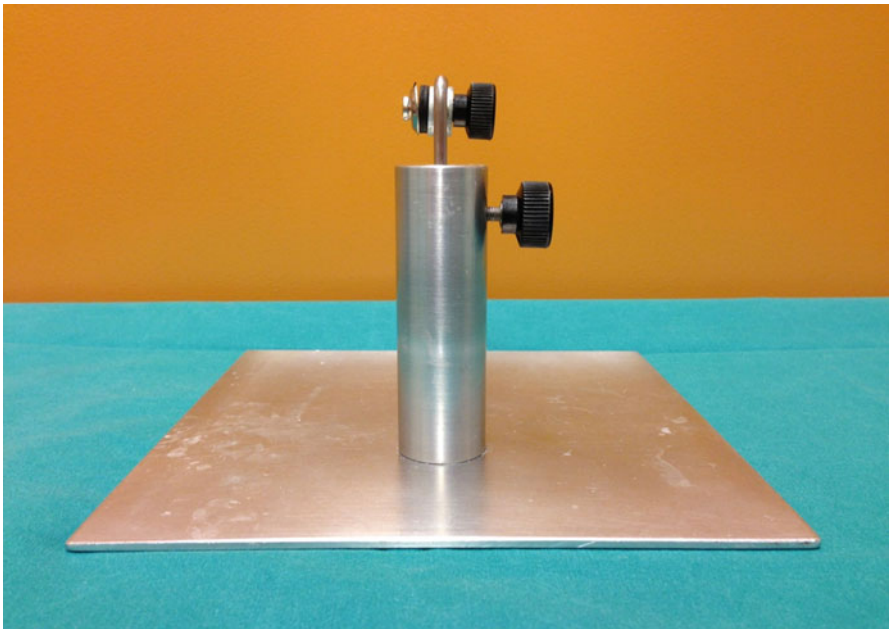

Fig. 10.14 Triple axis precision stabilizer (posterior view). Steel cylinder, probed with a L-shaped 2 mm wire and tapped on the side to allow for vertical movement

10.5.2 Triple Axis Stabilizer

An aluminum plate serves as base for a 15 cm long steel cylinder (Fig. 10.14) that is probed with a L-shaped 2 mm steel wire. The cylinder is tapped on the side to allow for vertical movement in the cylinder and fixation of the wire with a screw (Fig. 10.14). A commercial mosquito clamp is mounted on top and fixed with an articulated joint (Fig. 10.15). Intraoperatively, an aneurysm clip (Aesculap FE720 Miniclip) clamping the cuffed vessels and the recipient bronchus during anastomosis, respectively, is attached to the mosquito clamp (see below). This construction allowed for precise longitudinal movements of the clip on a vertical and horizontal axis as well as rotation on a vertical axis (Fig. 10.16).

10.5.3 Operating Procedure

1. Do a skin incision approximately 1 cm below the inferior margin of the scapula, cut the subcutaneous tissue and muscles exposing the lateral chest wall.
2. Use a bipolar cautery (Siemens Radiotom 904, Fig. 10.17) for hemostasis.

Fig. 10.15 Triple axis precision stabilizer (anterior view). Mosquito clamp fixed with an articulated joint

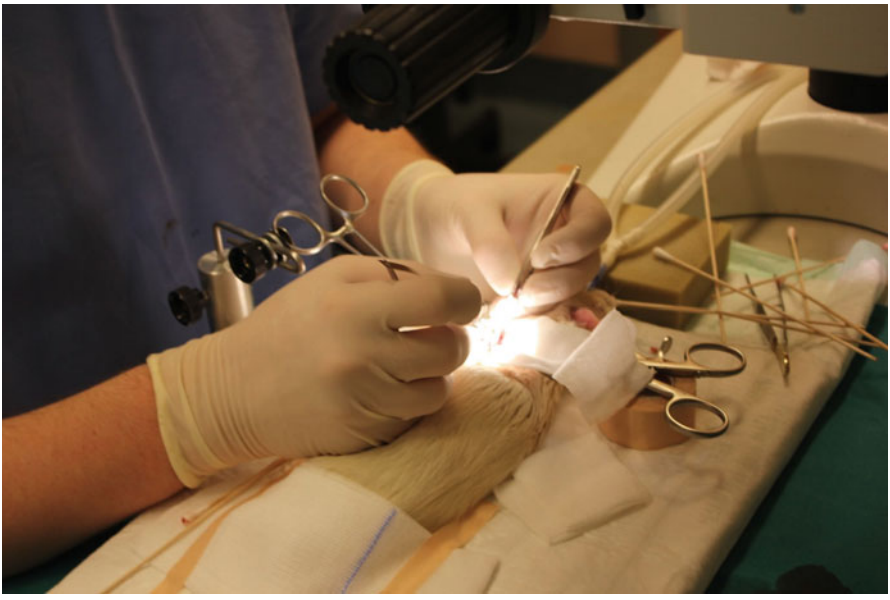

Fig. 10.16 Triple axis precision stabilizer (intraoperatively) placed between surgeon and rat, clamps cuffed vessels and the recipient bronchus during anastomosis, respectively. Wound retractor on the right side

Fig. 10.17 Siemens Radiotom 904 for bipolar cautery

3. To allow for maximum visibility of the hilar structures, open the thoracic cavity in the fourth interspace reaching from the sternum anteriorly to the thoracic vertebrae posteriorly.
4. Apply a common wound retractor in the 4th interspace between the 4th and 5th rib (Aesculap BV074R, 130 mm, blunt) to enable access to the left hemithorax.
5. Divide the left inferior pulmonary ligament using bipolar cautery and retract the left native lung outside the thoracic cavity.
6. Use conventional Q-tips (Figs. 10.18 and 10.19) to fix the retracted lung.
7. Figures 10.18 and 10.19 clearly illustrate that the left main pulmonary vein is the most anterior bronchovascular structure upon left thoracotomy. The left superior segmental vein crosses both the left pulmonary artery and the left main bronchus on their anterior aspects and together with the left inferior segmental vein constitutes a tributary of the left main pulmonary vein.
8. Dissection starts with mobilization of the left phrenic nerve using a non-touch technique and subsequent preparation of the right wall of the left inferior segmental vein and left main pulmonary vein from distal to central. The pulmonary vein is thin-walled and friable and needs to be treated carefully. Start on the right wall of the pulmonary vein and dissect the anterior aspect of the vein. If the correct layer of tissue is detected, it is possible to dissect the anterior bronchial wall at the same time moving from right to left.
9. Dissect the left pulmonary artery and mobilize the vessel as far distally as possible. A fibrous tissue connection between the distal pulmonary artery and the

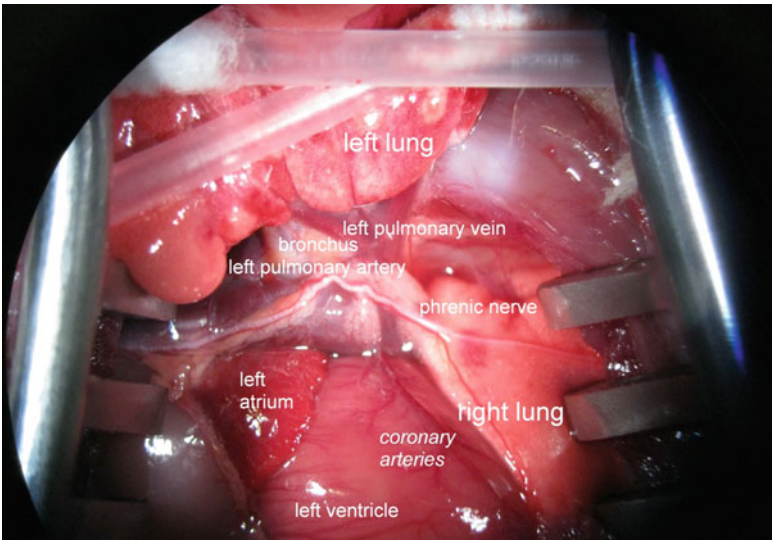

Fig. 10.18 Exposed left hemithorax via 4th interspace with left native lung, bronchovascular hilar structure, right postcaval lobe and heart

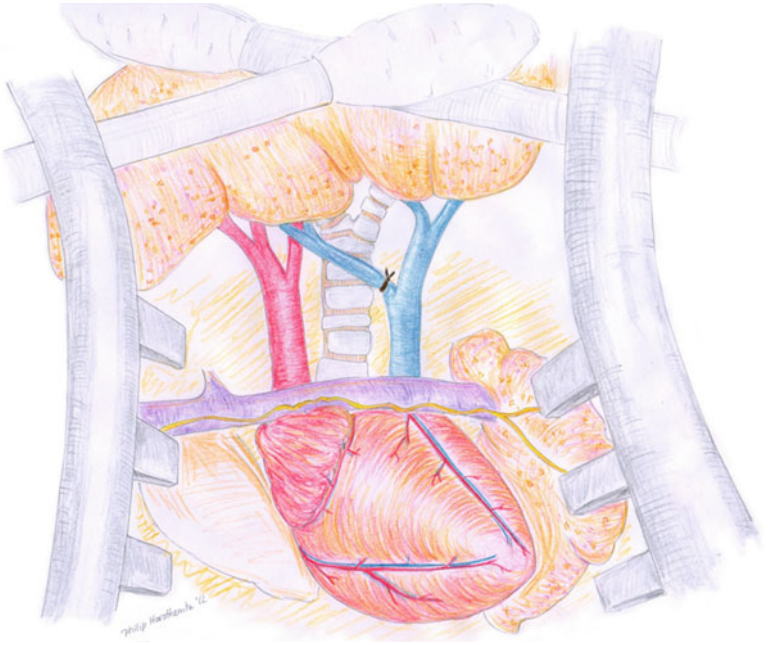

Fig. 10.19 Schematic illustration of the exposed left hemithorax. The left main pulmonary vein is the most anterior bronchovascular structure upon left thoracotomy

left main bronchus can be constantly observed. Cutting of this fibrous fixation results in extra vascular length and simplifies the subsequent process of cuffing.

10. In order to stop perfusion of the left native lung, ligate the distal pulmonary artery (7-0 silk suture, Catgut, Germany) and cut distal to the ligature. To keep blood loss to a minimum; the pulmonary artery must be clamped prior to the pulmonary vein as early ligation of the vein would result in a pooling of blood in the native lung.
11. Now dissect the left and posterior venous wall and partially mobilize the main pulmonary vein. To gain extra vessel length, which is of importance for the venous anastomosis, mobilize the superior and inferior segmental veins thereby completely releasing the pulmonary vein from its surrounding tissue.
12. Double-ligate the superior segmental vein as close to the venous trunk as possible using 7-0 silk suture and cut in-between close to the central ligature in order not to hamper venous cuffing (Fig. 10.20). The inferior segmental vein will be later used for anastomosis and is ligated as distal as possible and cut.
13. At this late point of hilus dissection, it is advisable to focus on small veins entering the inferior segmental vein on its posterior aspect proximal to the ligature as they can be torn apart as the main pulmonary vein retracts after cutting

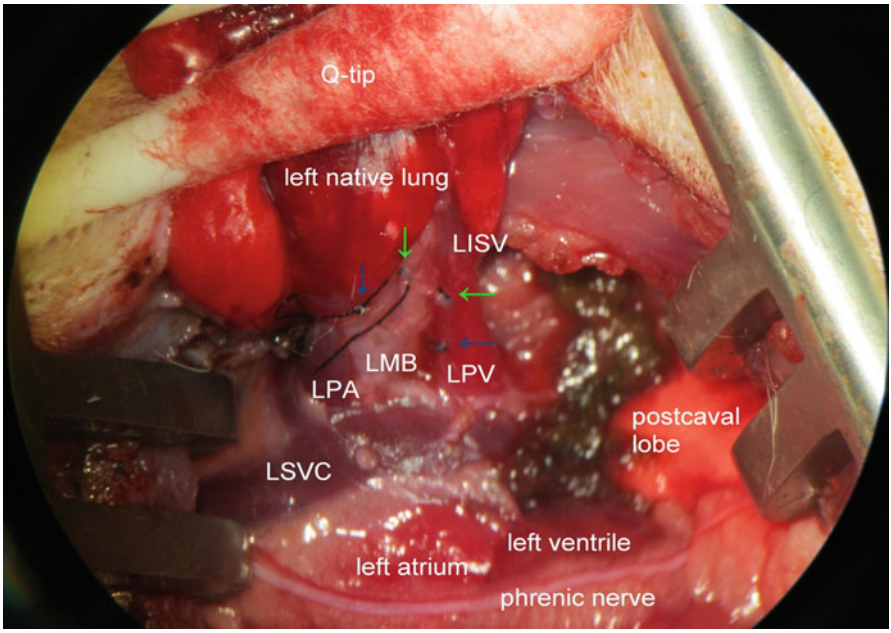

Fig. 10.20 In-situ left hemithorax with left native lung, fully dissected hilus. *LPA* left pulmonary artery, *LMB* left main bronchus, *LPV* left pulmonary vein, *LISV* left inferior segmental vein, *LSCV* left superior caval vein. *Green arrows* outline the extrapulmonary course of the left superior segmental vein, *blue arrows* outline the extrapulmonary course of an additional segmental pulmonary vein. Ligated left pulmonary artery prior to transection

leading to major retrograde bleedings from the left atrium. If you detect small tributary veins, cut them by using bipolar cautery.

14. All vascular structures have now been dissected and cut. The left main bronchus constitutes the remaining connection to the native lung.
15. Release the airway thoroughly from surrounding tissue and occlude vessels on the outer bronchial wall with bipolar cautery. This step is important in terms of hemostasis as transection of the bronchus for anastomosis would otherwise result in bleeding.
16. Clamp the left main bronchus using a microvascular aneurysm clip (Aesculap FE720 Miniclip), cut the bronchus distally and remove the native left lung.
17. Stabilize the aneurysm clip with the triple axis precision movement clip holder (Fig. 10.14 + Fig. 10.15) to reduce the movement of the heart and of the contralateral lung without touching the heart at all. Usually the cartilage ring on the level of transection is broken. We recommend re-cutting the whole bronchial circumference using microscissors, displaying the bronchial lumen and clear cutting edges.
18. Introduce the donor lung into the recipient thoracic cavity (Fig. 10.21) and cover the allograft with wet and cooled gauze throughout the subsequent process of implantation.
19. Remove the ligatures of the trachea and right main bronchus, respectively.

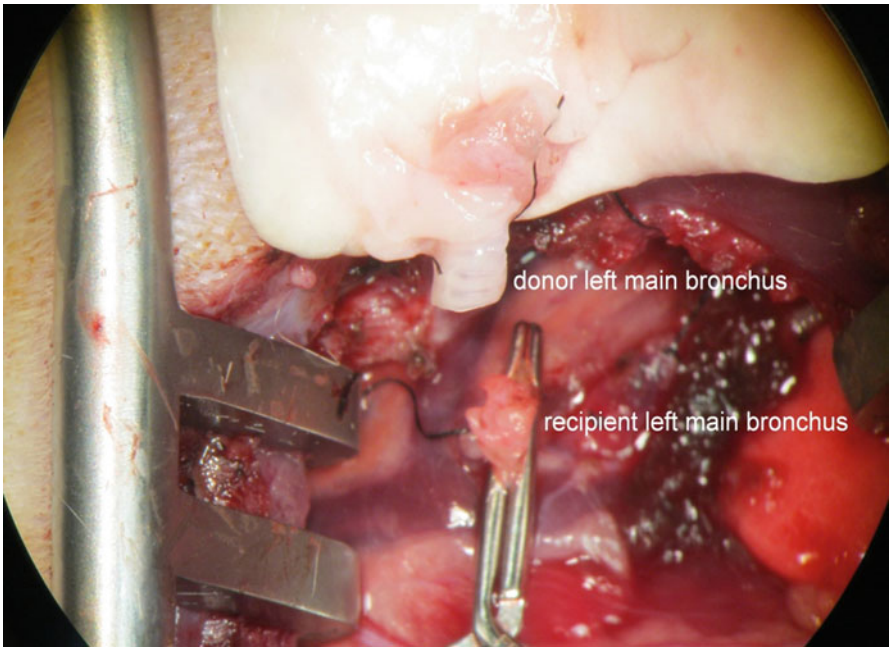

Fig. 10.21 Donor allograft positioned for implantation

20. Shorten the donor left main bronchus to an appropriate length and remove the remnants of the trachea and right main bronchus. The knowledge of the correct length of the left main bronchus is important in terms of kinking and judged upon the surgeon's experience and expertise. To facilitate suturing of the bronchial anastomosis, we recommend tapering the donor bronchial stump with a slanting cut from the membranous to the cartilaginous part.
21. Initiate the bronchial anastomosis with two interrupted stabilization-sutures (8-0 prolene) at 3 o'clock approximating the membranous part of the recipient and donor bronchial wall, respectively (Figs. 10.22 and 10.23).
22. Figure 10.24 schematically illustrates the microsurgical suture technique for airway anastomoses.
23. Continue the interrupted suture technique clockwise and anastomose the anterior half of the membranous part as well as the cartilaginous part of the bronchial wall using approximately 8 single sutures (8-0 prolene).
24. Then flip the lung over the heart to afford vision of the posterior bronchial wall and complete the airway anastomosis with approximately 8 interrupted sutures (8-0 Prolene) starting posterior to the initial stabilization sutures continuing anti-clockwise.
25. Check the airway for patency prior to the last stitch and remove intrabronchial fluid. Once bronchial continuity has been restored (Fig. 10.25), remove both the stabilization system and the aneurysm clip and re-inflate the lung. From this moment on, the transplanted lung is mechanically ventilated; however, it does

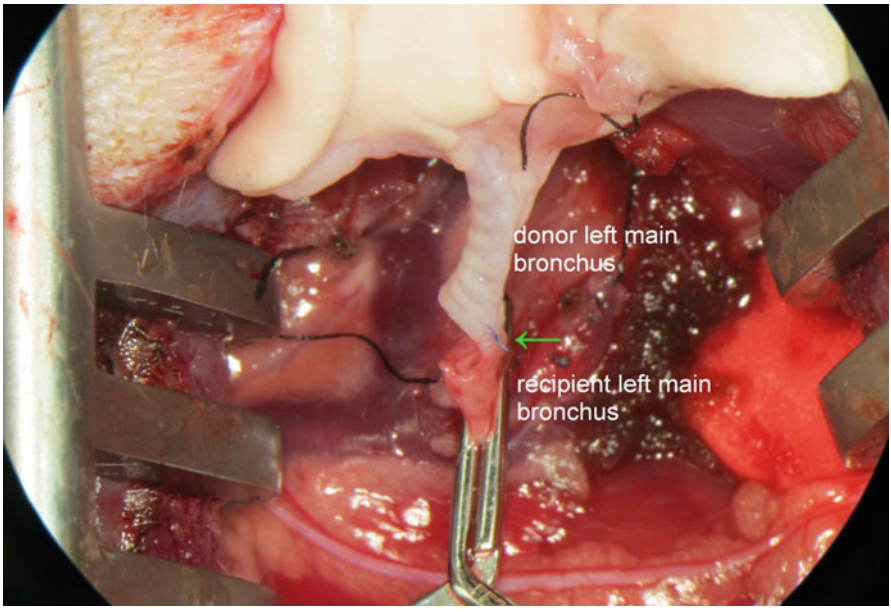

Fig. 10.22 Initiation of bronchial anastomosis by adapting donor and recipient membranous bronchial walls. *Green arrow* = 8-0 interrupted suture

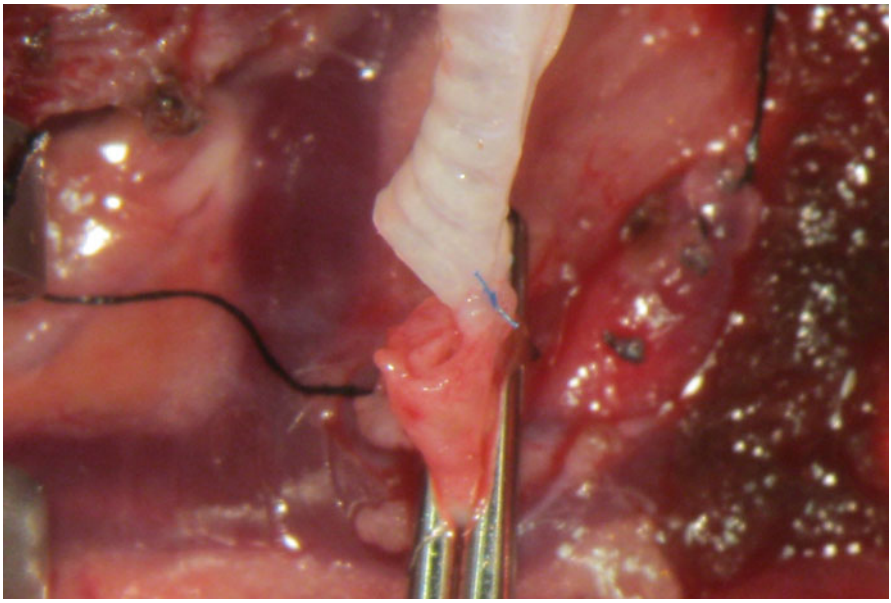

Fig. 10.23 Partially adapted membranous bronchial walls

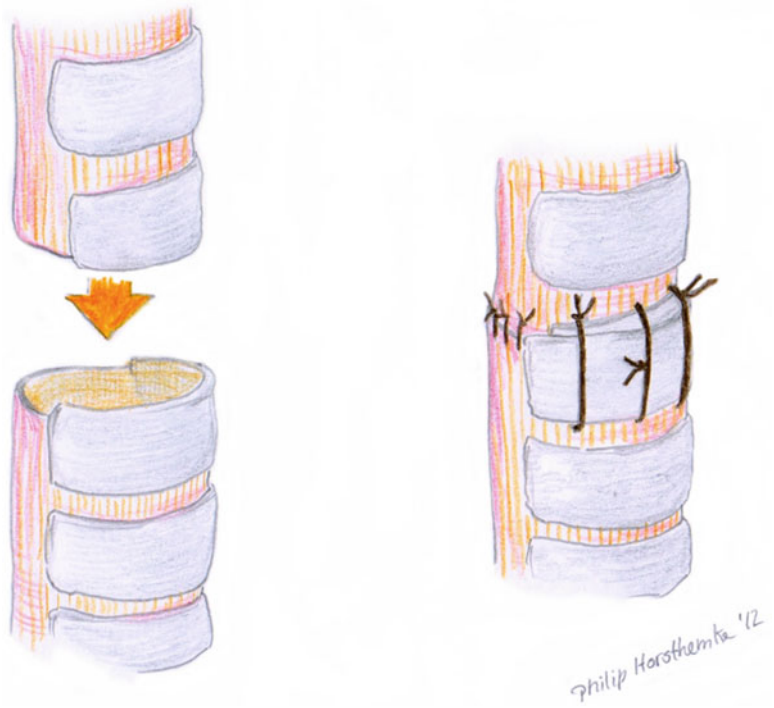

Fig. 10.24 Illustration of the bronchial suturing technique using 8-0 Prolene

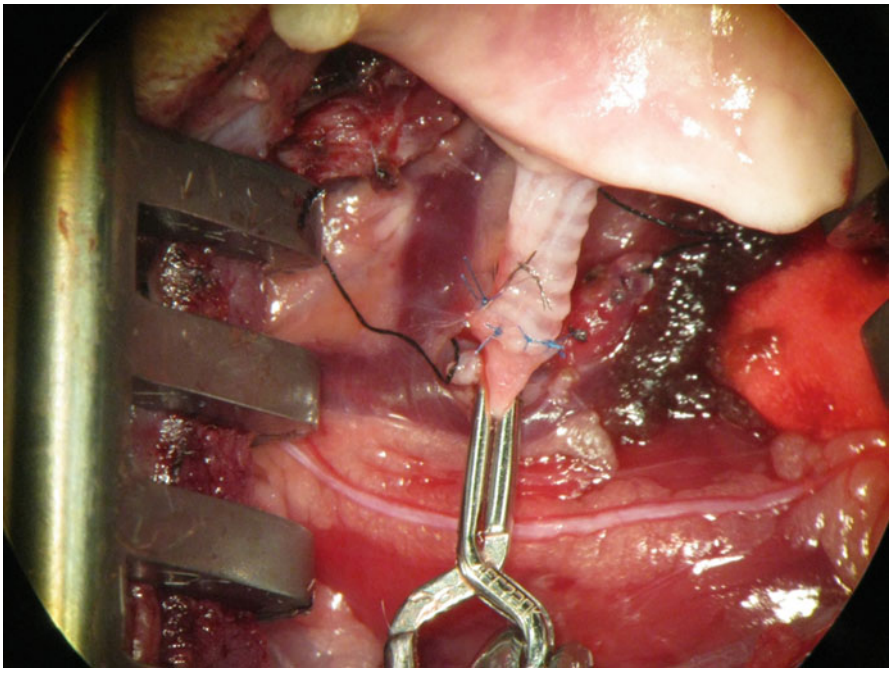

Fig. 10.25 Completed bronchial anastomosis

not take part in the process of oxygenation, as the recipient circulation is not yet re-connected to the donor lung.

26. Check the bronchial anastomosis for air leakage with 0.9 % sodium chloride at body temperature.
27. As for most surgical manipulations, flex the lung back with its costal surface placed on the rats back.
28. Re-warm the allograft for the subsequent reperfusion by placing wet gauze at body temperature on the lung.
29. Start the vascular anastomoses with reconnecting the pulmonary artery (Figs. 10.26 and 10.27) using the cuff technique.
30. Figure 10.28 schematically illustrates this technique for microvascular anastomosis.
31. After the initial process of vessel ligation (See Above) one thread was kept long so that it can now be "orchestrated" through an 18G polyethylene intravenous catheter together with the pulmonary artery.
32. Similar to bronchial clamping and stabilization, clamp the pulmonary artery by utilizing a microvascular aneurysm clip and stabilize both clip and vessel with the triple axis stabilizer.
33. Cut the ligature and apply heparin topically on the cut edge
34. Evert the vessel wall over the cuff and fix both using a 7-0 silk suture ligature (Fig. 10.29).

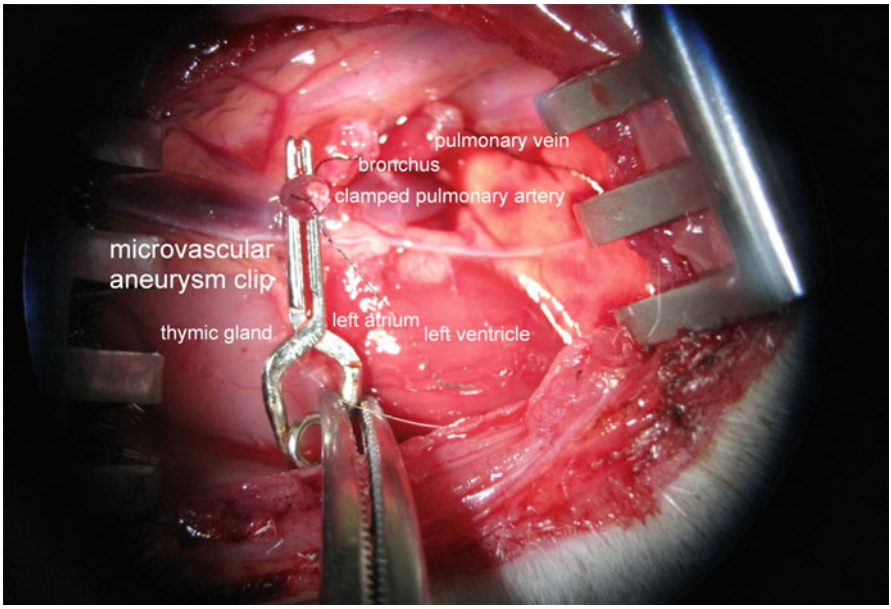

Fig. 10.26 Triple axis precision movements with a new aneurysm clip stabilization system (in situ position)

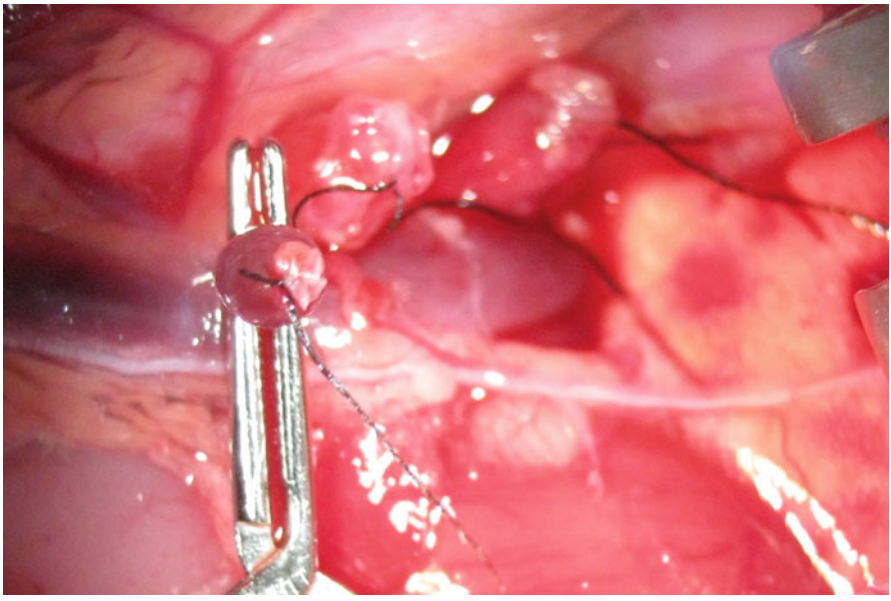

Fig. 10.27 Clamped pulmonary artery

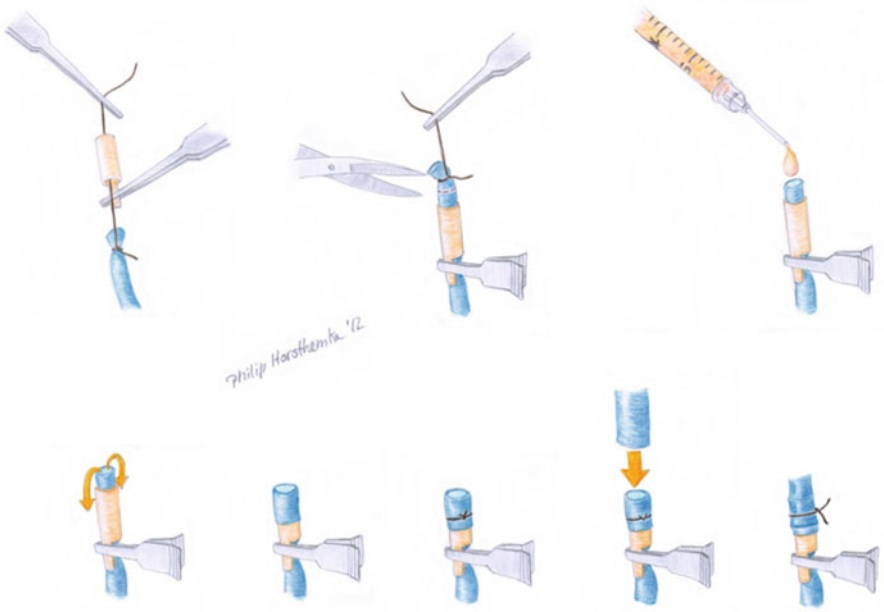

Fig. 10.28 Schematic illustration of the cuffing technique for vascular anastomosis

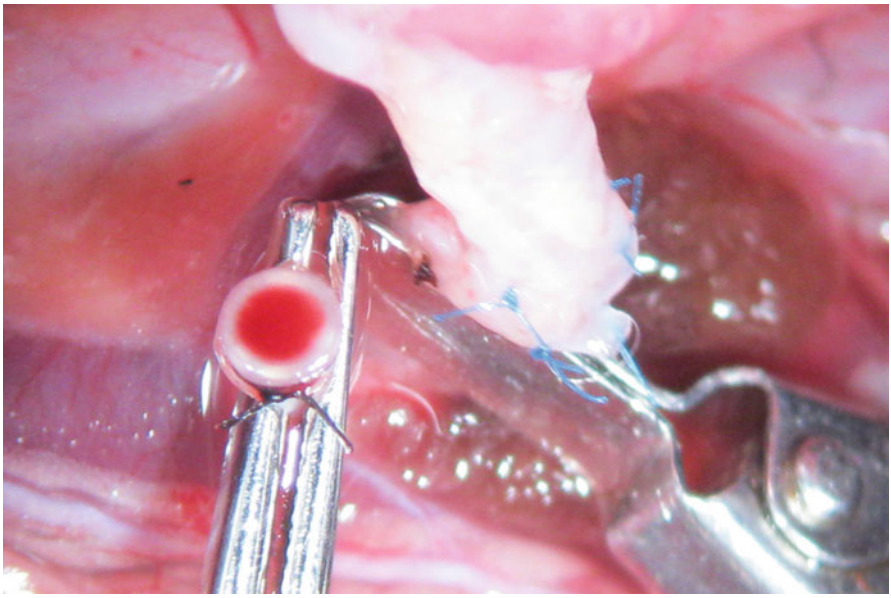

Fig. 10.29 Cuffed pulmonary artery

35. Then pull the corresponding donor pulmonary artery over this complex of cuff, evert the recipient vessel (Fig. 10.30) and secure the arterial anastomosis with a ligature (7-0 silk suture).
36. The continuity between recipient and donor pulmonary artery is now restored but can only be checked for patency and leakage after completion of the venous anastomosis by opening the clamps.
37. The vein is the last bronchovascular structure to be reconnected. Utilize the same techniques as for the arterial anastomosis (Figs. 10.28, 10.30 and 10.31) but use 16G polyethylene tube as cuff instead.
38. Replace the wet gauze at regular intervals to prevent dehydration of the allograft.
39. Remove the venous and arterial aneurism clips to initiate reperfusion of the transplanted lung (Figs. 10.32, 10.33, 10.34 and 10.35).
40. 25 mg of Cortisone (Urbason®) should be administered to all the recipient animals immediately after opening the clamps to prevent hyperacute rejection.
41. We recommend allowing the allografted lungs 10–15 min to re-perfuse.
42. If necessary, use bipolar cautery or an absorbable fibrillar hemostat (Tabotamp®) to stop minor bleeding.
43. Figure 10.33 schematically illustrates the thoracic situs upon transplantation.
44. Once a good reperfusion is obtained (Figs. 10.34 and 10.35) close the thoracic incision with 4-0 Prolene and use 4-0 Vicryl for subcutaneous tissue reattachment and skin closure, respectively.
45. Use an 18G intravenous catheter for thorax drainage.

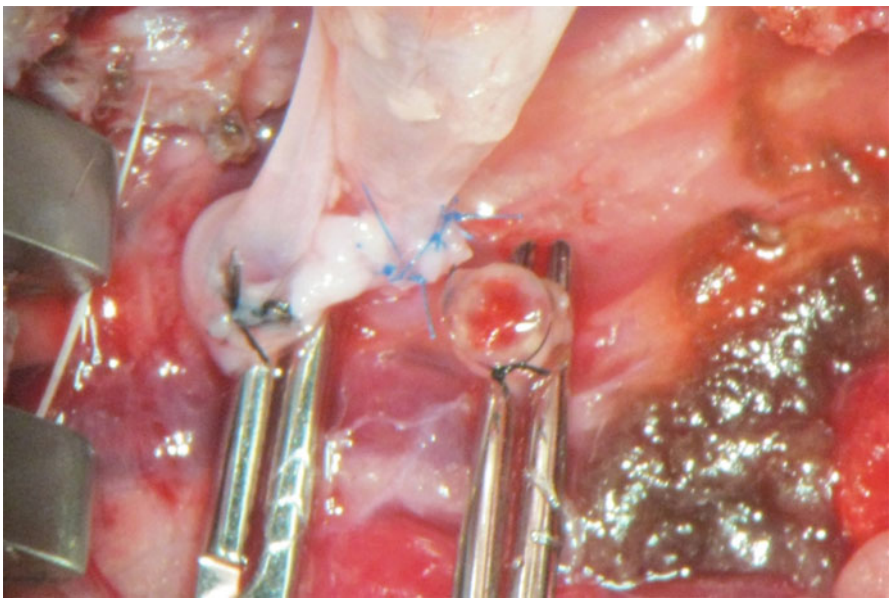

Fig. 10.30 Completed arterial anastomosis to the left and cuffed pulmonary vein to the right

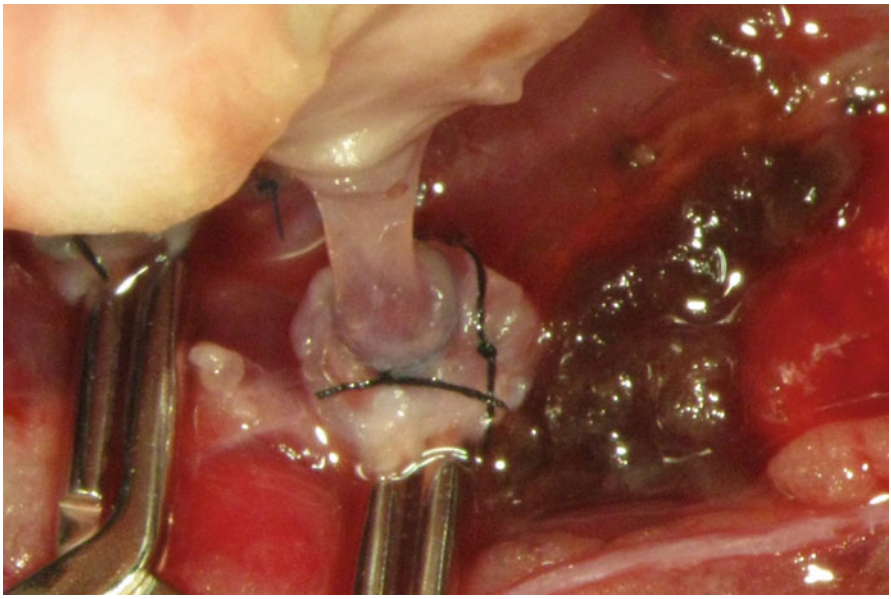

Fig. 10.31 Completed venous anastomosis

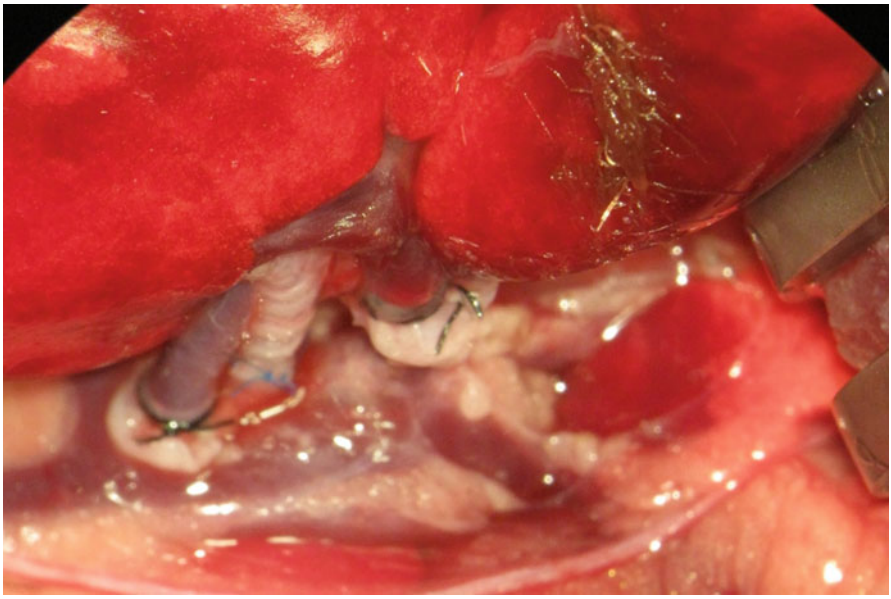

Fig. 10.32 Reperfusion of the allografted left lung upon orthotopic transplantation. Left main bronchus in the middle, pulmonary artery to the left, pulmonary vein to the right

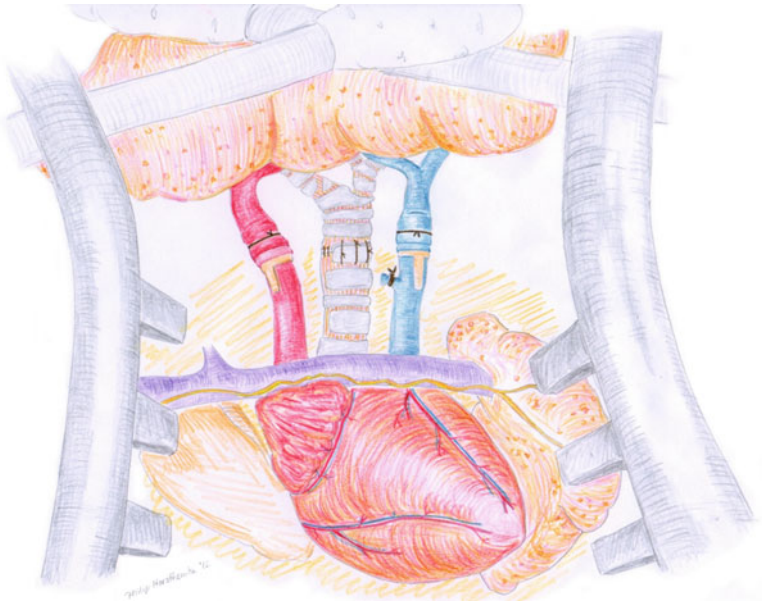

Fig. 10.33 Schematic illustration of the allografted left lung upon orthotopic transplantation

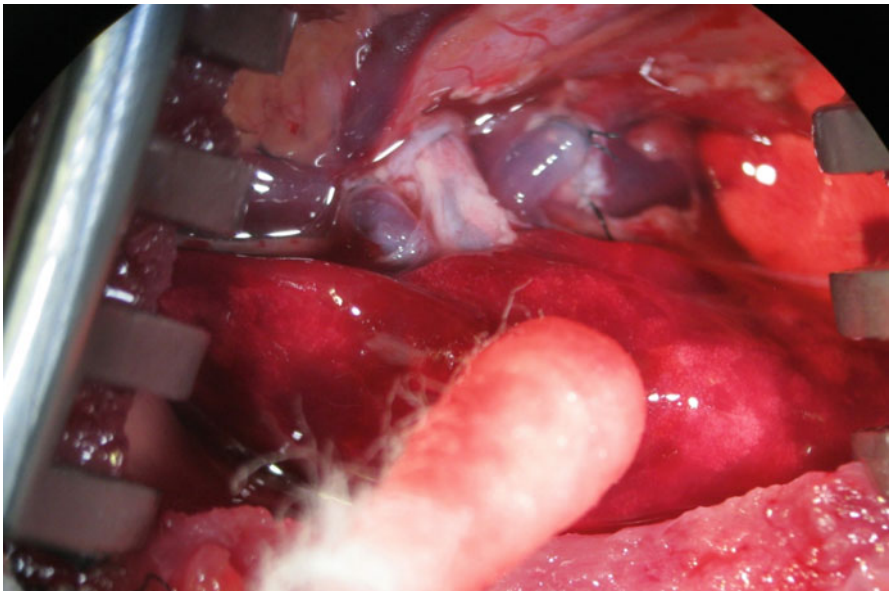

Fig. 10.34 Reperfusion of the allografted left lung upon orthotopic transplantation

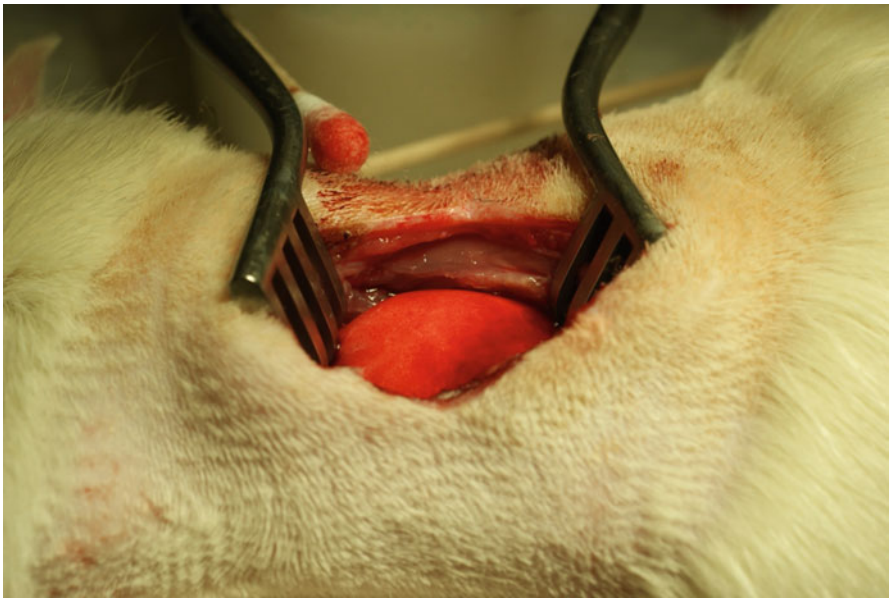

Fig. 10.35 Reperfused lung upon transplantation prior to thoracic closure

46. Wean the transplanted rat from mechanical ventilation and place the animal under a heat lamp until fully awake. Figure 10.36 demonstrates a post-transplant chest X-ray.

10.5.4 Tips and Tricks

1. When doing the thoracotomy, it is important to be aware of the natural course of the internal thoracic artery running immediately lateral and posterior to the lateral sternal margin as cutting of this vessel results in major, possibly fatal, bleeding.
2. Throughout the surgery, meticulous attention has to be paid to the hemostasis as every bleeding adversely effects the animal's survival.
3. Pulmonary tissue is highly vascularized and cutting through the lung while performing the thoracotomy or tearing of the lung while dissection of the hilar bronchovascular structures results in major bleeding as well as lancing of the airways and eventually renders mechanical ventilation inefficient.
4. Only use Q tips to manipulate the lung as pulmonary tissue is very friable
5. The hilus is highly vascularized with dozens of tiny vessels and capillaries passing through the inferior pulmonary ligament and the surrounding tissue of the bronchovascular tripartite. Thus, use bipolar cautery for most of the hilar dissection.

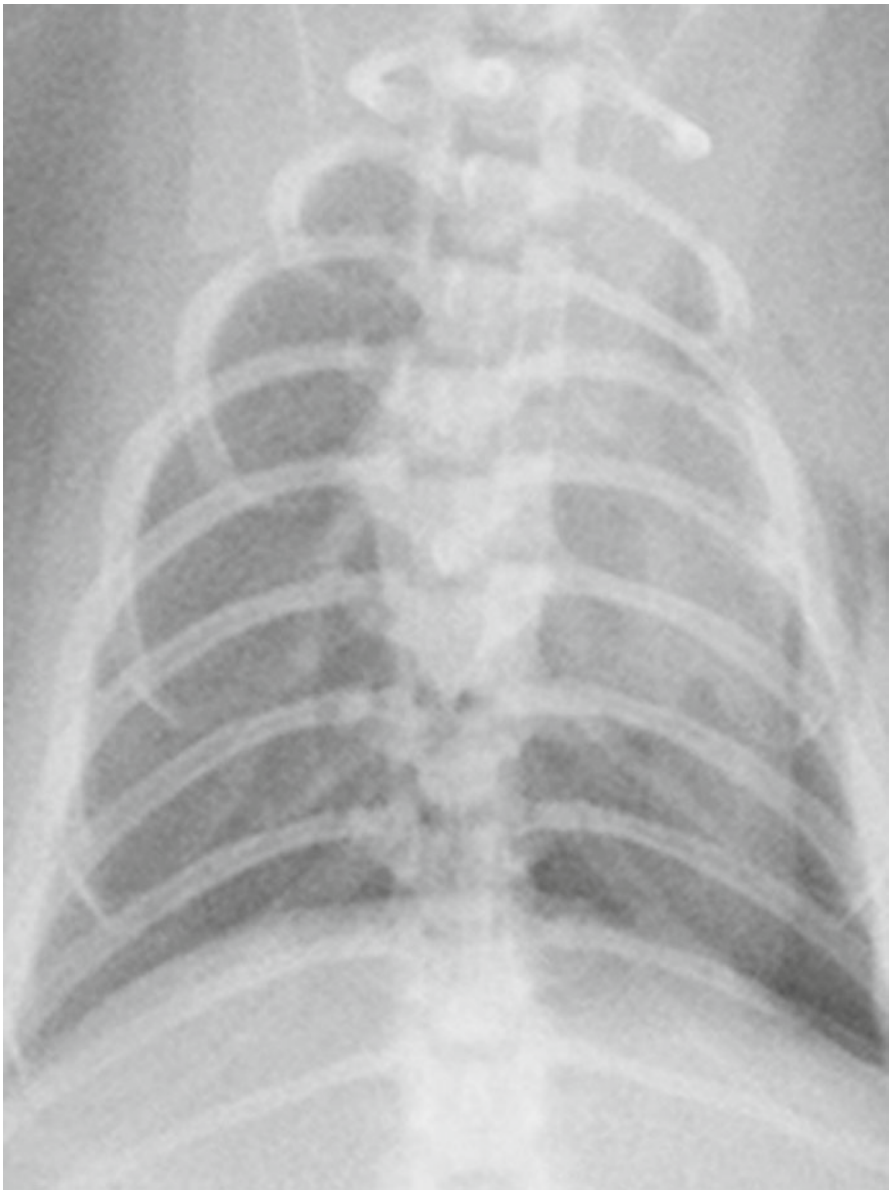

Fig. 10.36 Immediate post-transplant chest X-ray of a recipient Wistar Rat showing only minor pneumothorax and now pleural effusion

6. Blood perfusion of the left native lung should be stopped as soon as possible as monitoring of the rats circulation is only possible under great effort and not used routinely and the surgeon has no information on what systemic adverse effects maneuvering, luxation and compression of the lung might cause.

7. To keep blood loss to a minimum, the pulmonary artery must be clamped prior to the pulmonary vein as early ligation of the vein would result in a pooling of blood in the native lung. This condition would increase the recipient animal's blood loss.
8. When double-ligating the superior segmental vein, put one ligature as close to the venous trunk as possible cut in-between and again as close to the central ligature as possible in order not to hamper venous cuffing
9. The knowledge of the correct length of the left main bronchus is important in terms of kinking and judged upon the surgeon's experience and expertise. Long bronchial stumps technically facilitate the bronchial anastomosis yet bear the risk of kinking. Short bronchial stumps are technically more demanding to anastomose but guarantee good ventilation and patency.
10. We strongly recommend checking the bronchial anastomoses for air leakage with 0.9 % sodium chloride at body temperature as air leaking would inevitably lead to pneumothorax and result in the animal's death
11. Reconnect the bronchovascular structures in the exact inverse order as they were transected before. This makes the bronchus the first anastomosis to complete and the pulmonary vein the last structure to be reconnected. The bronchial anastomosis needs to be performed first to allow dislocation of to the donor lung in order to place the stitches as precisely as possible.

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Chapter 11
Experimental Liver Transplantation and Surgery

Firas Zahr Eldeen and Hynek Mergental

Abstract Experimental microsurgical procedures in liver research conducted on rat models include liver transplantation, liver resection and portacaval shunt. Transplantation is technically challenging and the first successful rat liver transplant was performed ten years after the first human liver transplant. The rat liver transplantation model has been frequently used for testing organ preservation solutions and Ischemia-reperfusion injury. A subsequently developed partial liver graft transplantation model, used to investigate liver regeneration in combination with portal hypertension, has been used in research supporting the development of live donor liver transplantation. The other relevant interventions described here are portal vein (PV) and/or bile duct ligation.

Keywords Liver transplantation • Liver segmental anatomy • Portocaval shunt • Liver resection

11.1 Introduction

Several liver transplantation variants have been described; orthotopic or heterotopic according to the position of the liver graft, and whole graft or partial transplant based on the graft size.

In orthotopic liver transplantation (OLT) the recipient liver is removed at the beginning of the surgery and replaced by the donor liver, which occupies the normal anatomical site. With an alternative heterotopic technique, the donor liver is first grafted into a space prepared in the recipient abdomen by unilateral nephrectomy, and the recipient liver is removed at the end of the procedure; in heterotopic grafting, only 30 % of the donor liver is transplanted. The recipient's own liver is not retained with either method (non-auxiliary technique, [1]). Heterotopic auxiliary grafts, in which the recipient liver remains in place, can also be performed [2, 3];

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their experimental use is limited, because they are invariably rejected, even in genetic strain combinations where non-auxiliary grafts are accepted [1].

Orthotopic liver transplantation in the rat was first reported by Lee et al. in [4], who used hand-suture techniques of all vessels [4]. Later the same author described a modified model without hepatic artery reconstruction with a temporal porto-jugular veno-venous bypass [5]. These procedures have not been widely adopted due to the high technical ability required [6]. The situation changed in 1979 when Kamada introduced a new method which connects vessels faster and more easily with a cuff technique [7]. This model does not include hepatic artery reconstruction, but it has become the most commonly adopted technique in experimental liver transplantation to date [6, 8, 9]. Unlike humans and large animals, rats can tolerate liver transplantation without re-establishing the arterial flow to the graft [7, 10]. The arterial reconstruction, however, may improve long-term animal survival [11], reduce biliary complications [12], modify hepatic microcirculation and alter immunological responses [11–13]. Liver arterialization combined with immunosuppression has an impact on post-transplantation rejection in some Syngenic combinations [14]. In addition, the re-established arterial blood supply may play an important role in carcinogenesis and progression of hepatocellular carcinoma [15, 16]. In view of these findings arterial reconstruction should be considered in research in these areas [17].

A short anhepatic phase is paramount in OLT. Portal vein clamping time should not exceed 25 min, while infra-hepatic inferior vena cava (IVC) clamping time should not exceed 30–35 min, or cardiovascular depression and acid base imbalances may ensue [9]. Microsuture technique may be associated with fewer complications, especially thrombosis, however, the cuff technique gives similar surgical outcomes with shorter clamping times [9, 18].

Transplantation using the microsurgical sewn technique for vascular anastomosing is described in this chapter. The infra-hepatic IVC and portal vein anastomoses can be also performed with cuff technique. This is briefly mentioned in this chapter and in detail in Chap. 6.

11.2 Segmental Anatomy and Volumetric Analysis of the Rat Liver

The rat liver consists of four distinct lobes of different size (Fig. 11.1). Each liver lobe has a separate hepatic artery and portal vein branch (Fig. 11.2). The main architecture of the hepatic venous outflow system is shown in Fig. 11.3 [19].

The left lateral lobe represents approximately 30 % of the total liver volume. It is located in the left lateral position and is connected to the superior caudate lobe by interlobular ligaments. The narrow pedicle containing the vessels is attached to the infra-hepatic IVC and the base of the left portion of the median lobe.

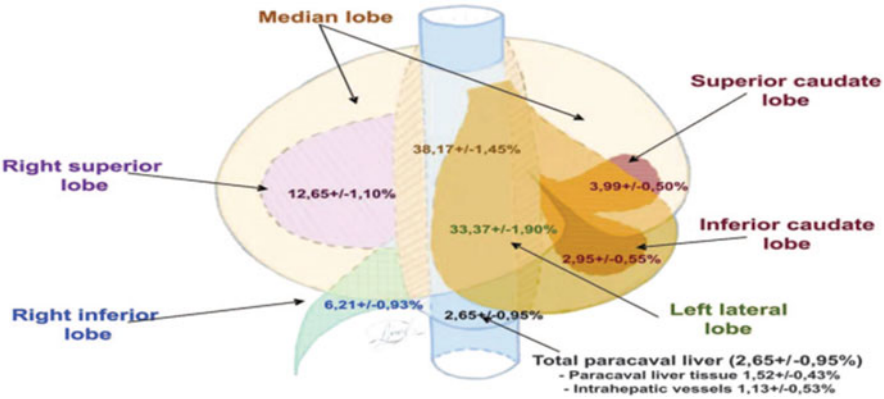

Fig. 11.1 Distribution of rat liver lobes in terms of volume

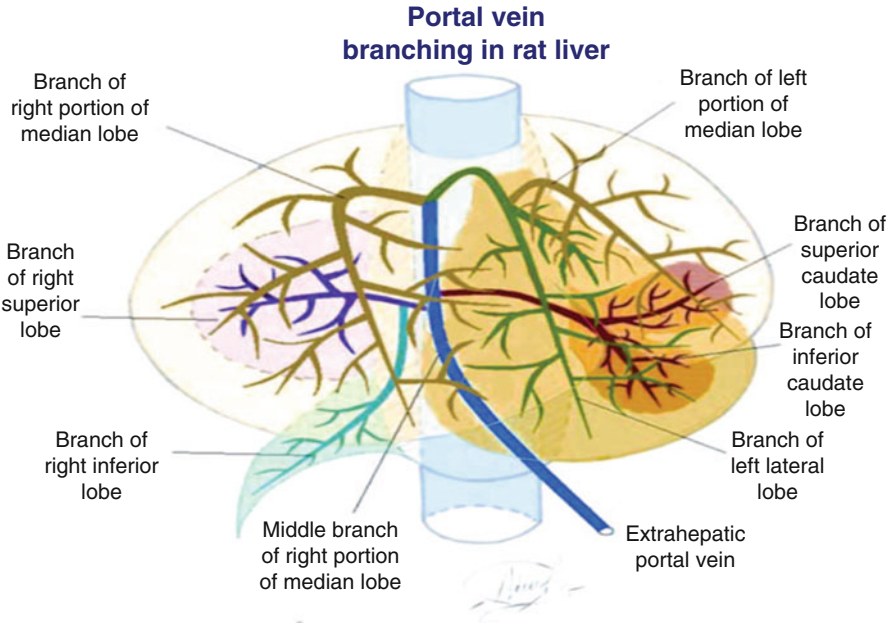

Fig. 11.2 Portal vein anatomy of the rat liver

The median lobe represents about 40 % of liver volume and consists of left (one third) and right (two thirds) portions separated by a deep fissure. It is located under the diaphragm and is fixed with the falciform ligament, which spans from the xyphoid and diaphragm to the liver forming the interlobular fissure. The median lobe has a wide base, surrounding almost half the circumference of the IVC.

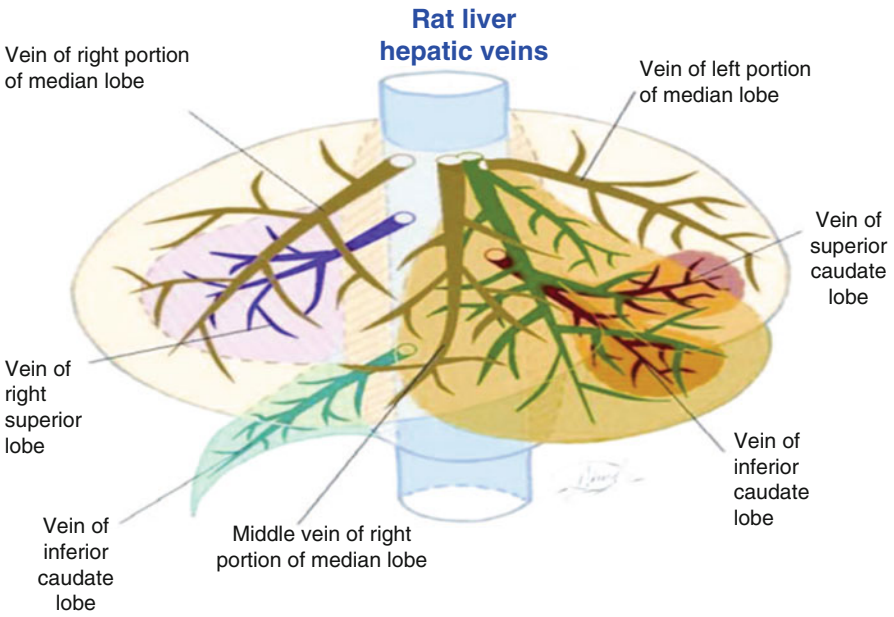

Fig. 11.3 Hepatic vein anatomy of the rat liver

The right liver lobe (20 % of liver volume) is located on the right side of the IVC and also consists of two distinct portions, an egg-shaped superior and a pyramid-shaped inferior lobe. The lobes are fixed dorsally with a hepatodiaphragmatic ligament to the diaphragm and ventrally towards the IVC.

The caudate lobe (7 % of liver volume) is located on the left side of the cava below the left lateral lobe; it is divided into two portions. The upper caudate lobe is connected to the left lateral lobe via a thin interlobular ligament and is fully covered by the lesser omentum, the lower caudate lobe is located behind the stomach in close proximity to the pancreas and spleen and is covered by a fibrous capsule, which is attached to the dorsal wall of the lesser omentum.

The paracaval liver portion (up to 3 % of the liver mass) does not represent a distinct anatomic unit, being the tissue at the dorsal parts of the bases of the right superior, right inferior, and caudate lobes, and covering the dorsal wall of the intrahepatic IVC, in addition to the liver tissue surrounding the main intrahepatic vessels which represents half of paracaval liver mass. This is the only liver tissue left in case of 97 % partial hepatectomy (PH).

Anatomical landmarks: [6] (Figs. 11.4, 11.5, 11.6).

- There is no gallbladder in the rat.
- The hepatic vein (HV) has no extra-hepatic margins for suture, therefore suprahepatic IVC (SHIVC) to SHIVC reconstruction must be performed.
- The esophagus is situated close to the liver, and the involvement of the esophagus at the total clamp of the SHIHC should be avoided.

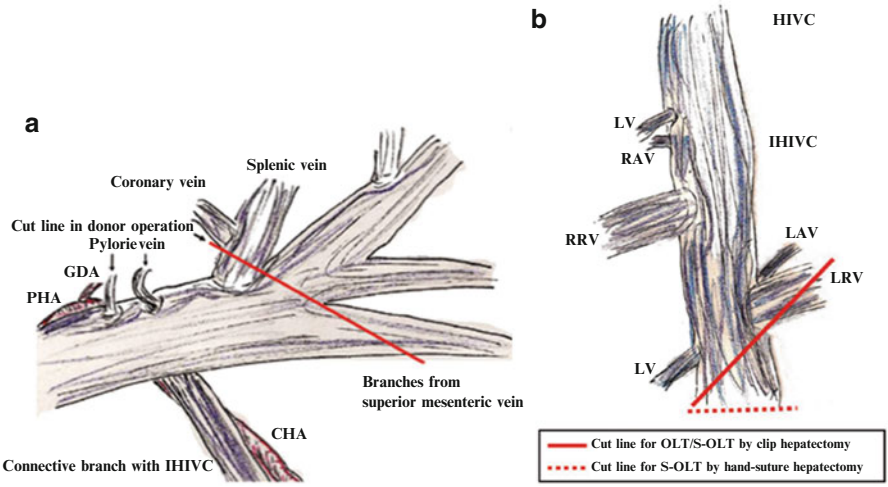

Fig. 11.4 (**a**) Anatomy of PV and HA (Source: [6]). (**b**) Anatomy of IHIVC

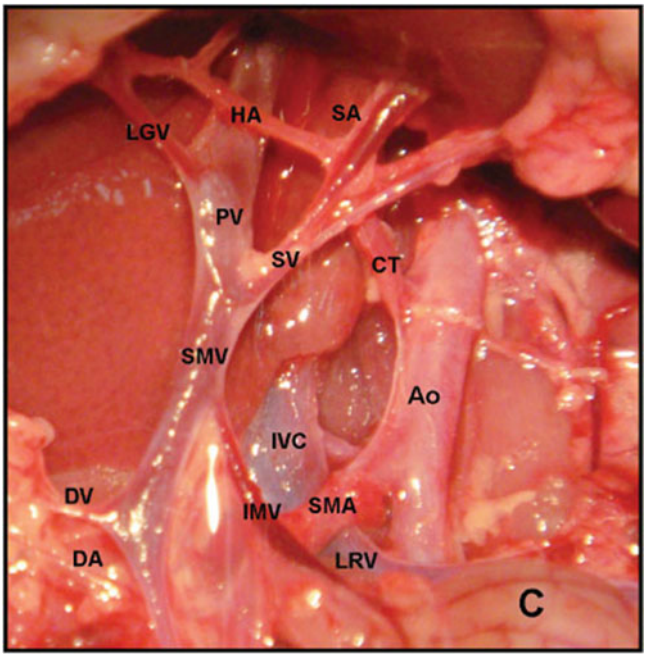

Fig. 11.5 Rat vascular anatomy from the upper abdomen (Source: [20]) *Ao* abdominal aorta, *C* colon, *CT* celiac trunk, *DA* duodenal artery, *DV* duodenal vein, *HA* hepatic artery, *IMV* inferior mesenteric vein, *IVC* inferior vena cava, *LGV* left gastric vein, *LRV* left renal vein, *PV* portal vein, *SA* splenic artery, *SMA* superior mesenteric artery, *SMV* superior mesenteric vein, *SV* splenic vein

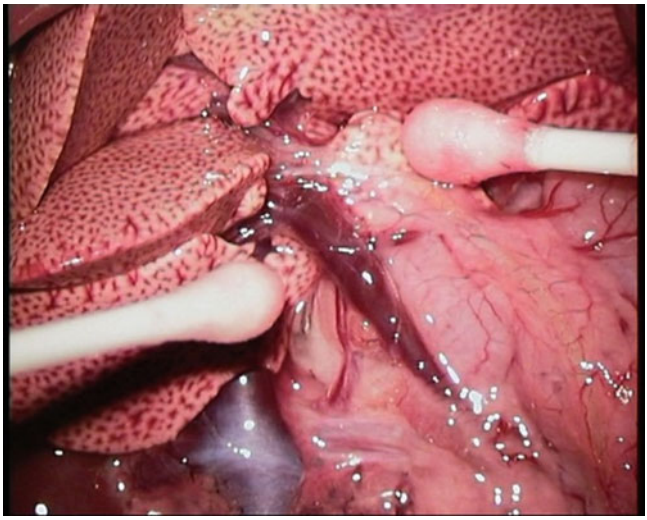

Fig. 11.6 Anatomy of the liver hilum

- Although the left median lobe is joined with the right median lobe, the predominant vessels of the left median lobe make a common channel with those of the left lateral lobe; this is important when preparing partial liver grafts.
- Large communicating vessels between the PV and the para-esophageal vessels can be detected on the superior caudate lobe.
- The right renal artery (RRA) is located behind the infra-hepatic IVC (IHIVC). The RRA usually branches into dual arteries, and the upper RRA branches into the right adrenal artery (Fig. 11.7).
- The right adrenal vein and the lumbar vein flow directly into the IHIVC at the point of the lowest edge of the right inferior lobe; these veins occasionally make a common channel before the IHIVC (Fig. 11.7).
- The left adrenal vein and the lower lumbar vein flow into the IHIVC at the junction of the left renal vein and the IHIVC (Fig. 11.7).
- The common hepatic artery (CHA) is located posterior to the PV trunk, and the branched proper hepatic artery (PHA) and the gastro-duodenal artery (GDA) are located at the left side of the PV trunk; the PV trunk and these arteries are encased together by a thin sheath (Fig. 11.4).

11.3 Orthotopic Liver Transplantation

11.3.1 Donor Operation: The Liver Graft Procurement [21]

- The procedure is performed under general intra-muscular anesthesia.
- After the introduction of anesthesia, shave the abdominal wall using electric clippers and scrub the skin with povidone-iodine.

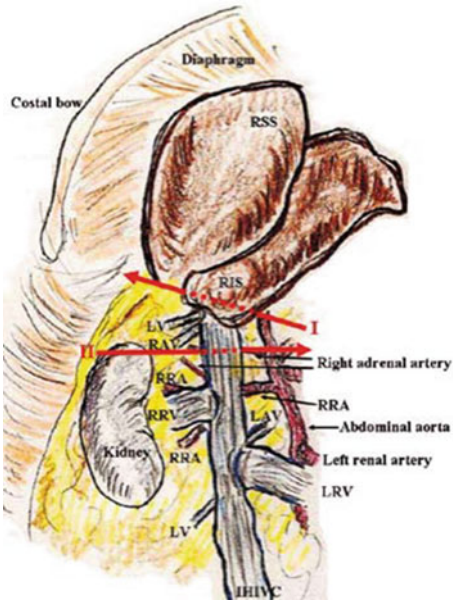

Fig. 11.7 Ligation of right adrenal vein (RAV) and lumbar vein (LV) (Source: [6])

- Make a midline xipho-pubic laparotomy incision, followed by a transverse incision. Retract the costal borders in a cranio-lateral direction to achieve the maximal liver exposure.
- Moisten the bowels with warm saline and position them on the outside of the left abdominal cavity, covering with gauze. Frequent dripping of warm Ringer-Lactate (RL) onto the liver and bowels during laparotomy is recommended.
- Divide the falciform, left triangular and gastro-hepatic ligaments; isolate and divide the left diaphragmatic vein between ligatures (Fig. 11.8).
- Divide the hepato-esophageal ligament and vessels between ligatures or with electrocautery (Fig. 11.8a).
- Divide the gastrosplenic vein.
- Divide the proper hepatic artery between the ties (Fig. 11.8b)
- Insert a 22G, 3.5 mm stent into the common bile duct through a small incision at approximately 1 cm distance from the hepatic hilum (Fig. 11.8c). Secure the stent in position with a 7–0 ligature (Fig. 11.8d).
- Clean the PV by separating it from the surrounding tissues, hepatic artery and bile duct (Fig. 11.8d).
- Isolate the IVC down to the left renal and right renal vein from surrounding tissues (Fig. 11.8e).
- Divide the right suprarenal vein between the ligatures and free the liver from its posterior ligaments by cutting under gentle traction (Fig. 11.7).
- Position a micro-clamp on the proximal part of the infra-hepatic IVC; this is needed to prevent bleeding after portal reperfusion during implantation (Fig. 11.8f).

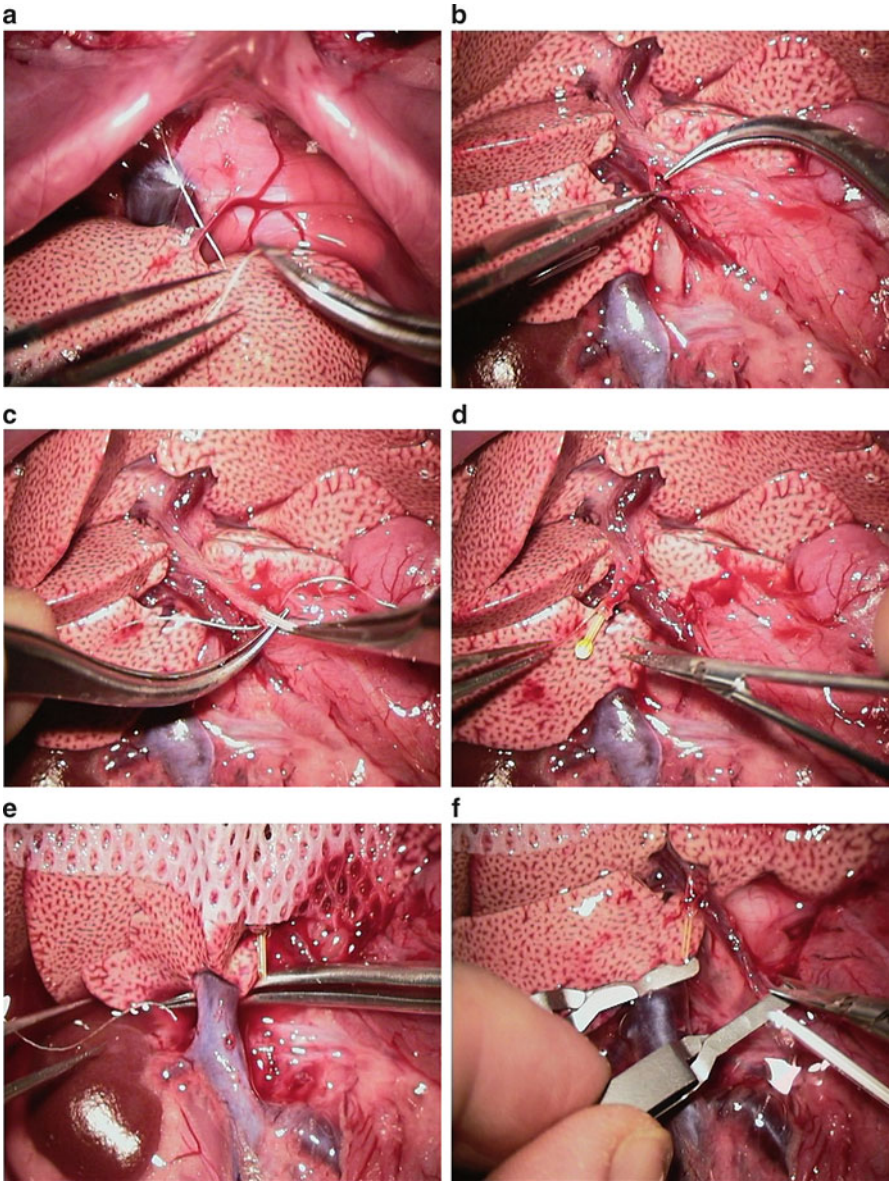

Fig. 11.8 Donor operation – liver graft procurement

- Position a micro-clamp on the proximal part of the PV and cannulate it with a 21G needle as far as possible from the hilum and gently flush the liver with 20 ml of a cold preservation solution or RL (Fig. 11.8f). Concurrently, divide the supra-hepatic IVC to allow an adequate washout. Divide aorta in the chest to euthanise the animal. The liver cold perfusion should last between 1 and 2 min.

- Cut the PV below at the level of cannula insertion (above the duodeno-pancreatic vein).
- Cut the common bile duct distally to the ligature around the stent.
- Cut the supra-hepatic IVC with a rim of the diaphragm; this tissue facilitates a safe supra hepatic IVC anastomosis during the implantation procedure.
- Remove the liver graft and place it into a basin filled with cold RL and lay the basin on an ice pad.
- Insert two Prolene 8–0 sutures at the opposite lateral edges of the supra-hepatic IVC.
- Inspect all three vascular orifices to be anastomosed during the implantation procedure and ensure the vessel walls are clean.
- Store the basin with the graft in the refrigerator at 0–4 °C.

11.3.2 The Recipient Procedure: The Orthotopic Liver Transplantation [21]

- The transplantation procedure is performed under general inhalation anesthesia with isoflurane on a cone mask. At induction, use 3 % isoflurane to anesthetize the animal, then decreased to 2 % until the recipient hepatectomy is completed. For the anhepatic phase, pause the anesthesia and restart at 1–2 % after the liver graft is reperfused. Maintain the airflow at 1 L/min with 70 % FiO2 throughout the whole procedure.
- Inject 10 ml of normal saline subcutaneously. Use antibiotic prophylaxis with 0.03 g of intramuscular piperacillin/tazobactam before laparotomy for procedures on immunosuppressed or immunocompromised animals.
- After the introduction of anesthesia, shave the abdominal wall using electric clippers and prepared with povidone-iodine scrub. Place the animal on a warm pad for the procedure.
- Perform a midline xipho-pubic laparotomy. Place retractors in latero-cranial and latero-caudal directions. Put a clamp on the xiphoid process and retract the rib cage cranially for adequate exposure. Check the breathing movements are not compromised by the retractor placements.
- Moisten the bowels with warm saline, position on the outside of the left abdominal cavity, covering with gauze. Check for an adequate arterial blood supply and venous outflow. Frequent dripping of warm normal saline onto the liver and bowels during laparotomy is recommended.
- Mobilize the liver as described in the liver procurement procedure (divide the liver ligaments, ligate and divide the left diaphragmatic vein, the hepato-esophageal vessels, the right suprarenal vein, the gastrosplenic vein and proper hepatic artery at the hilum)
- Mobilize the bile duct and divide it at its confluence in the liver hilum

- Isolate the IVC down to the left renal and right renal vein from surrounding tissues.
- Mobilize the posterior part of the liver, isolate the supra-hepatic IVC and place a silk tie beyond it to enable an retraction on the vessel
- Pause the inhalation anesthesia and continue ventilation with oxygen only.
- Position a micro-clamp on the infra-hepatic IVC at the level of the right renal vein.
- Position a micro-clamp on the PV at the level of gastrosplenic vein.
- Commence timing the anhepatic phase of the procedure
- Retract the supra-hepatic IVC in the caudal direction and place a Satinski clamp on the diaphragmatic rim around the vessel.
- Divide the supra-hepatic IVC at the liver parenchyma border level.
- Divide the portal vein in the liver hilum and the infra-hepatic IVC, both vessels just at the level of disappearing in the liver parenchyma.
- Remove the liver and place the graft into the abdominal cavity.
- Perform end-to-end supra-hepatic IVC anastomosis with a running 8–0 Prolene suture, fill the vessels with RL prior to finishing the anastomosis (Fig. 11.9).
- Anastomose the PV end-to-end with a running 9–0 Prolene suture (Fig. 11.10).
- Infuse warm RL into the abdominal cavity to ensure the supra-hepatic IVC anastomosis is immersed and remove the micro-clamp from the PV (Fig. 11.11) and the Satinski clamp from the supra-hepatic IVC.
- Stop the timing of the anhepatic phase of the procedure, restart the inhalation anesthesia
- Perform end-to-end anastomosis of the infra-hepatic IVC with a running 9–0 Prolene suture (Fig. 11.12).
- Repeatedly compress the recipient IVC stump to dissolve any blood clots which may have developed there
- Remove the micro-surgical clamps from the infra-hepatic IVC.
- Anastomose the bile duct by inserting the graft biliary splint into the recipient bile duct and secure the stent position with a circumferential tie fixation (Fig. 11.13).
- Inject 2–3 mL of RL into the distal IVC to cover the perioperative blood loss and the post-procedure fluid maintenance requirements.
- Close the laparotomy in layers with continuous 4–0 mono-filament suture.
- Stop the inhalation anesthesia, start intramuscular anesthesia and continue for 3–5 days after surgery.
- Maintain the recipient on a hot pad after surgery.
- Allow free oral intake after surgery.

11.3.3 Hepatic Artery Reconstruction (Fig. 11.14)

An atraumatic clamp is placed on the recipient's proper hepatic artery (as there is usually no back bleeding from the graft common HA (CHA) it is not necessary to clamp). Both HA edges are stripped from connective tissue and are sharply cut. An

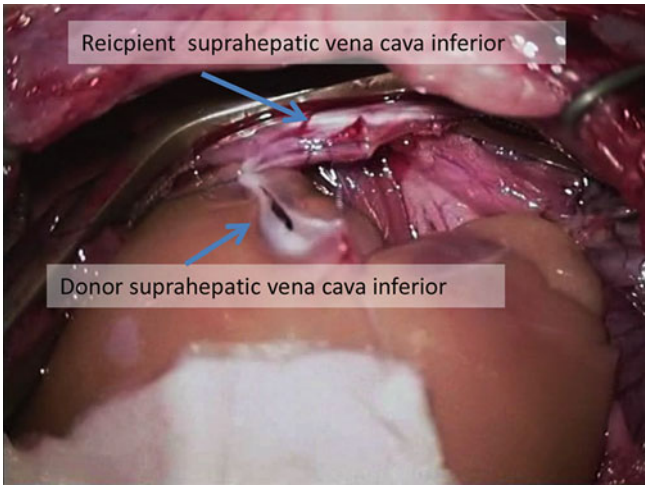

Fig. 11.9 End-to-end supra-hepatic IVC anastomosis

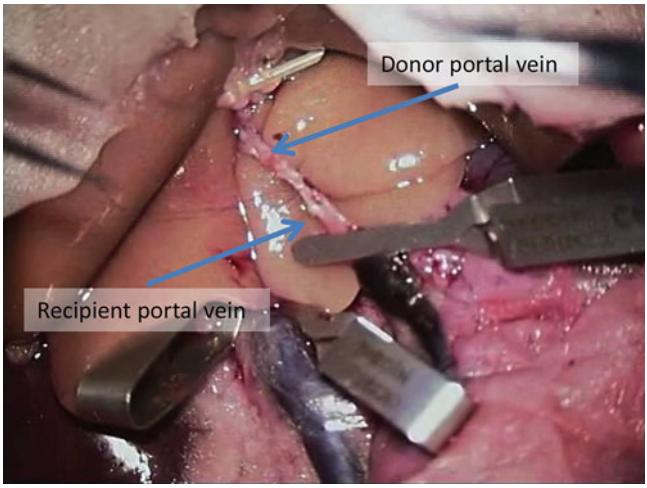

Fig. 11.10 Portal vein anastomosis, end-to-end

initial monofilament polypropylene suture (10–0) is passed through the whole layer of the CHA wall from the outside to the inside, and then through the whole layer of the PHA wall from the inside to the outside. A reverse thrusting from the PHA to the CHA is then made using the same thread (a horizontal mattress suture). Due to the difference in vessel diameter, the recipient PHA is then fed into the graft CHA using the "vest and pant method" and the vessel clamp is released. One or two superficial stitches are added if bleeding occurs. Alternatively, this anastomosis can also be completed using microsurgery in an end-to-end fashion. Another method of the liver graft arterialization is to procure the liver graft with a segment of abdominal aorta and preserved coeliac and proper hepatic arteries. The aortic segment is anastomosed end-to-side to the infra-renal recipient abdominal aorta.

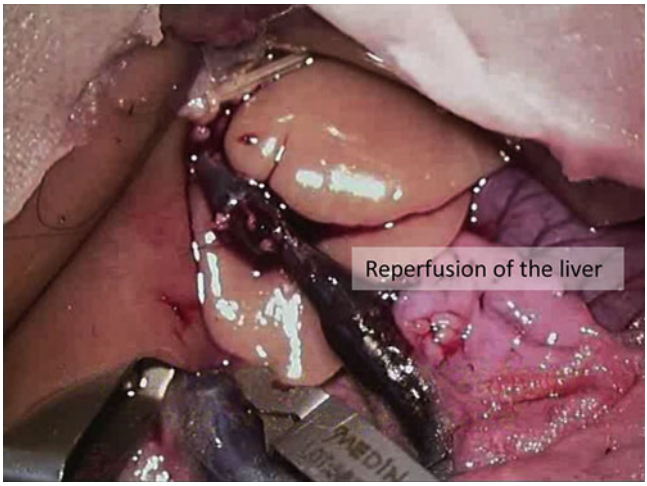

Fig. 11.11 Reperfusion of the liver graft after removing the clamps

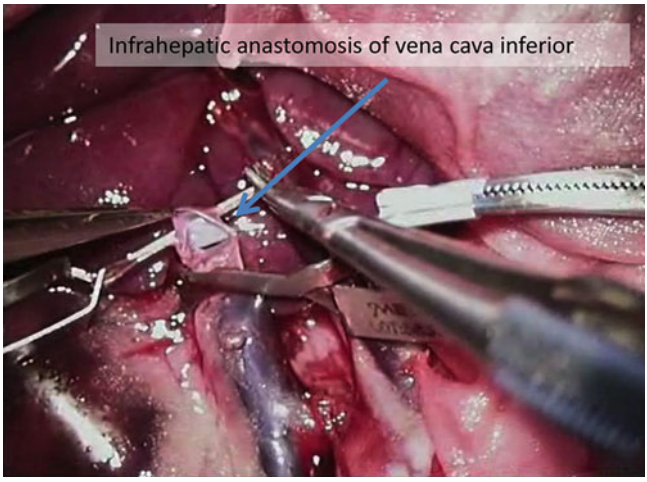

Fig. 11.12 End-to-end anastomosis of the infra-hepatic IVC

11.3.4 Liver Transplantation with the Cuff Technique and the Quick-Linker System [22]

To perform the cuff anastomosis of the PV and the infra-hepatic IVC the vessels need to be retrieved longer during the procurement procedure. This requires ligation and division of the right renal vein at the distal IVC and the duodenal vein on the proximal PV.

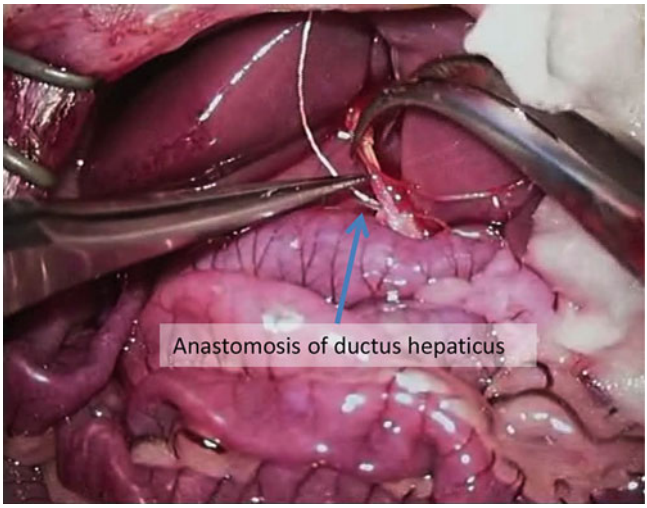

Fig. 11.13 Anastomose the bile duct by inserting the graft biliary splint into the recipient bile duct and secure the stent position with a circumferential tie fixation

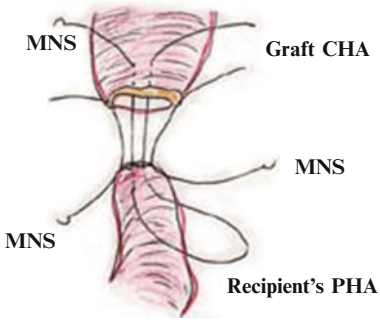

Fig. 11.14 Hepatic artery reconstruction (sleeve method)

11.3.4.1 PV Reconstruction (Fig. 11.15a)

Retention of the recipient PV is performed using mosquito forceps. Fine silk tie is set behind the recipient PV trunk. The PV is opened using the cut-down method at the point nearest the hepatic hilum and the patency of the inner side is confirmed by RL flush. The cuff is inserted into the recipient PV avoiding any torsion. The fixation of the PV cuff to the recipients' PV trunk is first made distally then proximally to avoid direct contact of thrombogenic silk tie to blood.

11.3.4.2 IHIVC Reconstruction (Fig. 11.15b)

The inner side of the IHIVC is easily detected by the adherent liver parenchyma. Stay sutures are made bilaterally, held with bulldog clamps and pulled cranially. The recipients' IHIVC is encircled beforehand with fine silk tie, and one knot is

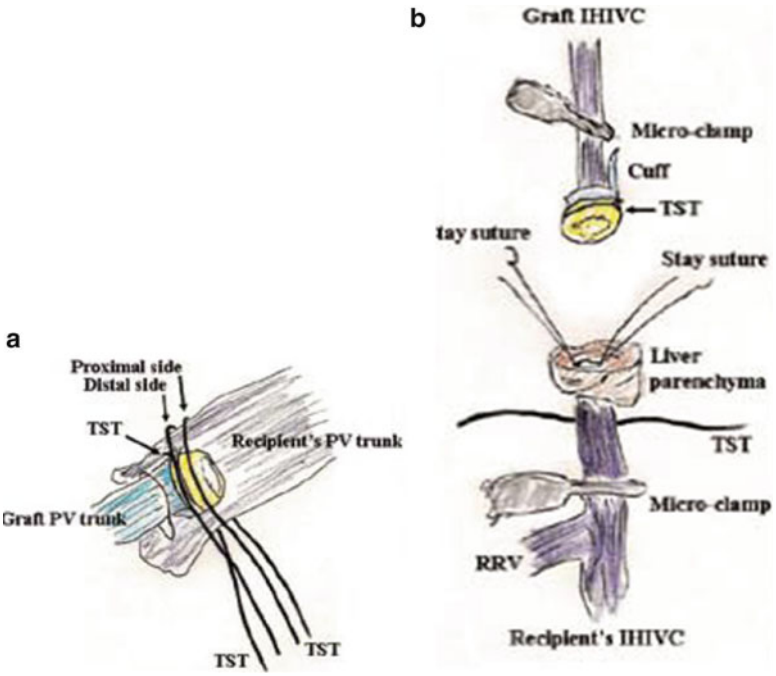

Fig. 11.15 Cuff technique, (**a**) portal vein reconstruction and (**b**) infra-hepatic IVC anastomosis

made for cuff fixation. The cuff is inserted into the recipient IHIVC avoiding any IHIVC torsion and fixed as proximal as possible, while fixation to the reversed IHIVC is performed at the distal side.

11.3.5 Tips and Tricks to Improve Outcomes After OLT

- Use 3–6 months old animals weighing 200–350 g; the donor should be 10–20 % smaller than the recipient.
- restrict a solid food intake for the recipient transplant animal to prevent bowel infarction; allow free access to water and glucose solution.
- Three caveats may guarantee successful surgery: (a) keep the anhepatic phase as short as possible, as duration greater than 20 min is associated with poor survival (b) avoid any blood loss, and (c) do not exceed a total operative time of 60 min (apart from HA reconstruction).
- Flush the anastomosis site prior to its completion as central air embolism can lead to sudden death.
- Minimize the manipulation of the intima and preserve its integrity, avoid aggressive graft flushing in the donor and ensure appropriate cold preservation of graft

to decrease risk of death due to liver failure or thrombosis of the vena cava. Use of anticoagulation after surgery is not recommended.
- For perioperative antibiotic prophylaxis a single dose of piperacilin/tazobactam (0.03 g) can be given before laparotomy. This minimizes the risk of common bile duct infection and subsequent deaths due to bacterial cholangitis.
- Death is a rare event after day 10. However, due to absence of arterial flow, some bile duct problems can be observed with cholestasis.
- Graft arterialization is ideal for studies focusing on liver regeneration; however, omission of HA reconstruction is acceptable for studies focusing on transplant immunology. HA reconstruction prolongs the procedure duration and may impact early survival.

11.3.6 Partial Hepatectomy Models

Hepatectomies in the rat allow the study of aspects of hepatic physiopathology, such as small for size syndrome, liver regeneration, or acute liver failure [8]. Partial hepatectomies (PH) ranging from 5 % to 95 % of total liver weight can be easily performed with high reproducibility using microsurgical techniques because the parenchymal mass of each lobe is relatively constant. Surgical removal of two-thirds (70 %) of the liver represents the most valuable and most extensively studied animal model of liver regeneration. Subtotal (90 %) hepatectomy represents an experimental model for studying acute liver failure [19].

11.3.6.1 70 % Partial Hepatectomy

Partial hepatectomy can be done by suture or clip technique (Fig. 11.16) [6].

The technique consists of the resection of the middle and left lateral lobes after "en bloc" ligation [23]. Sudden reduction in the liver vascular bed causes portal hypertension with subsequent development of portosystemic collateral circulation [24]. Twenty-one days after the 70 % PH in the rat, the liver completely recovers its

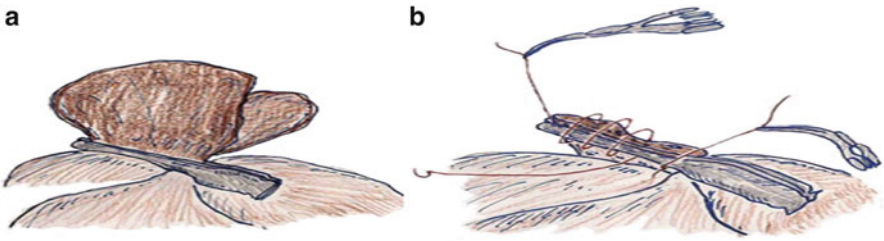

Fig. 11.16 Hepatectomy clip technique (**a**) and suture technique (**b**)

original volume [25] Regeneration is associated with an inflammatory process in which both the innate cellular immunity and growth factors are involved [26]. The 70 % PH is used to prepare a graft for heterotrophic liver transplantation in the rat.

11.3.6.2 Extended Partial Hepatectomy

This consists of the individualized dissection and ligation of the vascular and biliary branches from middle, left lateral and right lateral lobes, leaving the caudate process untouched. Following this procedure the animal needs a subcutaneous administration of glucose immediately after the operation and the addition of glucose to drinking water to prevent hypoglycemia. The one week survival rate varies from 100 % in a 90 % PH, 66 % in a 95 % PH and no survival in a 97 % PH [19]. These liver resection models are of great interest in studying the pathophysiological mechanisms leading to the failure of the remnant liver, as well as in studying new therapies that favor liver regeneration [27].

11.3.6.3 Total Hepatectomy

The total removal of the liver, while maintaining the portal and inferior vena cava circulation, provides a reproducible experimental model of acute liver failure. As rats can only tolerate an interruption in portal flow for a maximum of 20 min, total hepatectomy requires the construction of a microsurgical portacaval shunt [28]. Total hepatectomy in the rat can be performed in one, two or three stages. One-stage procedures consist of portacaval shunting and hepatectomy with prosthetic or vascular grafts [28, 29]. To avoid grafting, an initial partial constriction of both the portal vein and the vena cava is carried out to establish an adequate collateral circulation. Microsurgical techniques can be used to obtain an anhepatic rat, with a portacaval shunt and a total hepatectomy with preservation of the intrahepatic vena cava.

11.3.7 Portal Vein Ligation and Portal Hypertension Model

The most frequently used experimental model is achieved by simple partial portal vein ligation in the rat [30, 31]. This surgical technique was first described by Chojkier and Groszmann [32]. This can be achieved by calibrated stenosis of the portal vein using a single ligature (4/0 silk) tied around a 20 gauge blunt-tipped needle placed alongside the portal vein, which is removed after tying the ligature, [32], or triple ligatures maintained in position by a sylastic guide [8]. The portal vein ligation models enable studies of morphological and functional disorders presenting in the splanchnic and systemic tissues in prehepatic portal hypertension [8].

11.3.8 Bile Duct Ligation and Biliary Obstruction Model

Extrahepatic cholestasis models have been described based on interruption of the common bile duct (bile duct ligation/BDL) or selective interruption of lobar biliary drainage [33]. The experimental cholestasis models cause hepatomegaly with a ductular proliferation and fibrosis with preservation of the normal liver architecture [34]. As the experimental animals develop extrahepatic changes, jaundice, choluria, portal hypertension, splenomegaly, collateral portosystemic circulation, hepatic encephalopathy and ascites, the model is used for studying extrahepatic complications of portal hypertension [8].

11.3.9 Portacaval Shunts

End-to-side portacaval anastomosis in the rat was first performed by Lee et al. in 1961 [35]. In this procedure the portal vein is clamped at the confluence with the splenic vein and ligated (7/0 silk) at the level of its hilar bifurcation. A partial venotomy is then performed just below its hilar ligature. A 9/0 nylon thread with a loop at its distal end is used to perform the end-to-side shunt. The arterialization of the portal stump after portacaval shunt in the rat prevents hepatic atrophy and this technique is used in research of hepatic encephalopathy.

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Chapter 12
The Transplantation of Insulin Producing Tissue

Jan Kriz, Peter Girman, Zuzana Berkova, and Tomas Marada

Abstract The diabetes mellitus is a complex and polymorphic disease without simple and fully determined pathogenetic cause. Therefore there is not any complex and universal experimental model for studying of this disease. The experimental transplantation of insulin producing tissue and animal models employed in it allow to study just some of pathogenetic factors and some therapeutic aspects.

Within the chapter we shortly introduce some key models of diabetes mellitus, which can be used for experiments focused on microsurgical techniques, immunosuppressive protocols, graft imaging, development of bioarteficial pancreas and some others. They are not suitable for studying of pathogenetic mechanisms of both main types of diabetes.

Within the chapter, there are explained and clearly described step by step basic techniques for preparation, preservation, and transplantation of pancreas and isolation, cultivation and transplantation of pancreatic islets.

Keywords Pancreas transplantation • Pancreatic islet transplantation • Models of diabetes mellitus • Rat model

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12.1 Experimental Models of Type-1 Diabetes Mellitus

The history of experimental models of diabetes dates back to the end of nineteenth century when polyuria, hyperglycaemia and ketonemia were observed after total pancreatectomy. Since that time, several other models were described with features mimicking the relevant type of diabetes. The transplantation of insulin producing tissue is almost always indicated for type 1 diabetic candidates. Consequently, experimental studies with islet or pancreas transplantation are done on models which imitate this disease. For the purpose of the chapter, we will briefly describe the principles, advantages and disadvantages of two basic models of type-1 diabetes:

1. Chemical – streptozotocin or alloxan diabetes
2. Spontaneous – BB rats, LETL rats, KDP rats, Lewis.1AR1 rats

12.1.1 Streptozotocin Chemical Diabetes

Induction of experimental type-1 diabetes by intravenous administration of diabetogenic drugs is simple to use and provides consistent and reproducible hyperglycaemia in rats. Out of a wide variety of hyperglycaemic agents, streptozotocin and alloxan are preferably used as drugs with a specific toxicity to beta cells.

12.1.1.1 Mechanism of Action

Streptozotocin (2-deoxy –(3-metyl)-3nitrosoureido)-D-glucopyranosis is an analogue of N–acetyl glucosamine synthesised by Streptomyces achromogenes. The toxic effect requires intracellular uptake of the drug. The molecule enters the beta cell through the membrane via the low-affinity GLUT2 transporter. After internalisation, it exaggerates the toxic effects by the use of several mechanisms: DNA alkylation, exhaustion of beta cell ATP, production of reactive oxygen species, release of nitric oxide and inhibition of N-acetylglucosamine. It has been conjectured that alkylation of DNA is the main mechanism of STZ toxicity. Alkylation is caused by transferring the methyl group to DNA from the methyl-nitrosourea moiety of the streptozotocin molecule resulting in DNA strand breaks. Excessive DNA damage subsequently starts repair mechanisms which become exhausted early due to depletion of ATP as the indirect mechanism of STZ toxicity. The other mechanisms are still considered as alternatives and are not necessary for induction of hyperglycaemia [1].

12.1.1.2 Equipment and Materials

Ice, double distilled water, streptozotocin powder, syringe filter 0.22 mm, two sterile tubes 5 ml, fine scales, 27G needle, syringe 1 ml, pipette 5 ml, gun pipette, vortex, natrium citrate, hydrochloric acid.

12.1.1.3 Administration and Dosage

Streptozotocin is usually delivered as a white powder which should be stored frozen at −20 °C. Before administration, it should be dissolved in a citrate buffer solution with a pH = 4, 6 (adjusted with hydrochloric acid). Lower or higher pH leads to instability and decreases its pharmacological effect. The biological half-life of the streptozotocin is 30 min. A single intravenous dose of 55–75 mg/kg induces diabetes in more than 90 % of animals. Intraperitoneal administration is possible but requires a dose up to 80 mg/kg and it still could be ineffective. Multiple intravenous injections are usually used for mice but not for rats. The blood glucose concentration response to streptozotocin occurs in three phases:

First phase – the hyperglycaemic phase starts 60 min after STZ injection and is characterised by inhibition of insulin secretion and high blood glucose concentration. Morphological changes of beta cells include vacuolisation, mitochondria swelling, enlargement of area of Golgi apparatus and degranulation.

Second phase – represents the phase of hypoglycaemia which lasts from 1 to 4 h after STZ injection. Hypoglycaemia is caused by a robust influx of insulin into the blood stream from insulin granules released due to damaged beta cell membrane. Severity of hypoglycaemia may vary, but, it can reach extreme lows and cause animal death.

Third phase – this phase of chronic hyperglycaemia represents the status in which most of the beta cells are damaged while other endocrine cells remain intact. When glycaemia in the first week remains lower than 15 mol/l, diabetes may disappear and beta cells can regenerate. The restoration of normal blood glucose level is rarely permanent and diabetes can be diagnosed again at least in the next 3 months in its latent form [1].

12.1.1.4 Side Effects

Nesidiomas and liver cysts were described after STZ treatment. Extra-vascular application into the tail subcutaneous tissue may lead to necrosis which is the reason for animal exclusion from further experiments. Kidney toxicity can be observed but usually does not result in renal failure.

12.1.1.5 Drug Interactions with Respect to Metabolism of Immunosuppressive Drugs

Administration of streptozotocin may significantly affect the drug metabolism and pharmacokinetics. AUC levels of cyclosporine-A after intravenous dose of 5 mg/kg were lower by 30 % in a streptozotocin induced diabetes model of the Sprague-Dawley rat. It is supposed that the decrease resulted from up-regulation of CYP3A1 and 3A2 enzyme activity. On the other hand, 20 mg/kg of cyclosporine-A injected in a streptozotocin-induced diabetes model of the Fischer rat increased the AUC level at almost 130 % in comparison to controls. When using a Wistar rat model of

streptozotocin-induced diabetes, an application of 10 mg/kg of cyclosporine-A did not significantly change the AUC values in comparison with the control group. In our experiments, a daily 0.5 mg/kg intramuscular dose of tacrolimus resulted in 8–9 ng/ml mean blood through levels on 7 day in diabetic female Brown-Norway rats. Treatment with NPH insulin was reported to reverse the effects of streptozotocin on immunosuppressant metabolism.

12.1.1.6 Procedure

1. Into a 15 ml tube, add 5 ml of distilled water and reconstitute 0.147 g of sodium citrate powder
2. For better reconstitution, use a vibrating mixer (Vortex)
3. Adjust the pH to 4.6 using 1 M hydrochloric acid
4. Select the amount of solution according to number of animals
5. Add 25 mg of streptozotocin to the 1 ml citrate solution. Work carefully and use a face- mask.
6. For better reconstitution, use a Vortex
7. Filter the solution through a 0.2 μm filter
8. Store the solution in an ice drift and use it up to 30 min after reconstitution.
9. Administer intravenously as described in Sect. 6.2.

12.1.1.7 Tips and Tricks

1. Animals with glycaemia lower than 19 mmol/l should be excluded from experiment due to high rate of spontaneous diabetes reversal
2. Glycaemia over 23 mmol/l should be corrected with a low dose of long-acting insulin (2–4 IU s.c.) or insulin pellets
3. On days 1–3 after STZ injection, allow the animals free access to food and 5 % glucose with water ad libitum to prevent severe hypoglycaemia
4. Injection to peripheral vein can be painful so try short-term anaesthesia or be careful during application

12.1.2 Alloxan Chemical Diabetes

Alloxan (2,4,5,6 tetraoxypyrimidine) is an instable molecule with a biological half-time of about 1 min and stereo metrical structure similar to glucose.

12.1.2.1 Mechanism of Action

Mimicking glucose molecule, it enters the beta cell through the GLUT 2 transporter. Alloxan exhibits high affinity to molecules with –SH groups such as glutathione, serine enzymes and glucokinase. Oxidation of these groups results in inhibition of enzymes activity and represents one of the mechanisms responsible for toxic effects. Another effect leading to cell death are mediated through increased production of hydroxyl radicals and the disturbance of calcium homeostasis. An excessive amount of free radicals together with a decreased concentration of ROS scavengers (glutathione) causes DNA damage and consequently cell death. Calcium ions enter the cell after the change in membrane depolarisation and participate in both DNA strand breaks and an increase in insulin secretion [2].

12.1.2.2 Equipment + Material

Tubes 15 ml, syringe 1 ml, saline, alloxan powder.

12.1.2.3 Administration and Dosage

Alloxan can be administered intravenously, intra-peritoneally or subcutaneously, but, due to short biological half-time, intravenous injection is preferred to other methods. A single intravenous dose between 65 and 75 mg/kg induces diabetes in more than 70 % of animals. For intra-peritoneal application, the dose should be increased to 150 mg/kg. Subcutaneous doses fluctuate around 200 mg/kg and may be ineffective.

Blood glucose levels response to alloxan in four phases:

First phase: Hyperglycaemic phase – This phase is seen as early as 30 min after ALX injection and it is not observed after streptozotocin. Hyperglycaemia results from glucokinase inhibition in the beta cell and consequent diminished insulin secretion.

Second to third phases: Are similar to those observed after STZ injection.

Fourth phase: May run clinically with dramatic onset on 5–7 days when the animals are denied food and water and die due to hyperglycaemic coma in combination with renal failure [2].

12.1.2.4 Side Effects

Alloxan (ALX) is a toxic agent with a high mortality for animals. It frequently leads to renal failure and ketoacidotic coma.

12.1.2.5 Procedure

1. Reconstitute the alloxan in saline at the required dose
2. Inject intravenously into the tail vein of the restrained animal

12.1.2.6 Tips and Tricks

1. ALX diabetes can be prevented by food, so fast the animals at least 16 h before ALX application.
2. Due to toxicity, follow the manufacturers' recommendations when manipulating with alloxan.
3. Clamping of kidney vessels for 5 min can prevent renal damage. For amelioration of kidney failure, you can use external pressure on both kidneys for 5 min.
4. Correct hyperglycaemia similar as to the STZ diabetic animals
5. When animals deny food and water, change the long-acting insulin to rapid acting insulin in 4–5 doses per day and inject saline solution into the tail vein
6. For beginners or inexperienced researchers, we recommend inducing diabetes with streptozotocin.

12.1.3 Spontaneously Induced Diabetes

Animals with spontaneously developed diabetes are bred in order to understand the pathogenesis mechanism of autoimmune type-1 diabetes mellitus. In most cases, they are used for experiments focused on studying of immunologic and preventive strategies of beta cell damage. On the other hand, when used as transplantation models, they can serve for studying of autoimmune and alloimmune processes interaction in damaging of pancreatic allograft. The most widespread model comes from Canada and is called the Bio-Breeding rat. The less often used models included LETL, KDP or Lewis.1.AR1 rats.

12.1.3.1 Bio-Breeding Rats

The original outbred BB rat colony was established from Wistar rats at BioBreeding Laboratory in Ottawa, Canada. The rate of spontaneous diabetes among the original animals was about 10 %. Using selective breeding, the frequency of diabetic animals can exceed 90 % in some colonies. These animals are defined as "diabetic prone, DP".

BB rats can be obtained from Biomedical Research Models, Inc and are shipped worldwide. The animals obtained from this facility are inbred and genetically well-defined and served for establishment of many other colonies.

For maintaining BB rats, keeping certain precautions is recommended as viral infections could modify the frequency of spontaneously developed diabetes. All animals should be kept in specific pathogen free facility with autoclavable food,

materials and equipment. All surfaces should be repeatedly disinfected with chlorine dioxide based solutions or its equivalents. The pathogen status of the animals should be regularly tested serologically.

Diabetes develops suddenly between 50 and 100 days after birth with ketonuria, hyperglycaemia, weight loss and hypoinsulinemia. The frequency of diabetic animals may vary between 60 and 90 %, irrespective of sex. All diabetic prone animals have insulitis. Pancreatic islets are infiltrated with CD4, CD8 T-cells, NK cells, B-lymphocytes and macrophages. BB rats are characterised by peripheral lymphopenia which affects CD4, CD8 T- cell and B- cell populations.

A diet consisting of casein proteins, soya protein or lacking essential fatty acids can prevent diabetes in the BB rat. Other approaches, which divert diabetes, include immunosuppressive therapy with cyclosporine-A, tacrolimus or mycophenolate mofetil [3].

12.1.3.2 Tips and Tricks

1. Follow all recommendations concerning guidelines on the maintenance of BB rats
2. This type of model might not be suitable for all transplant experiments with the use of immunosuppression therapy
3. Treat all animals with insulin after diabetes development

12.1.3.3 LETL Rat

The Long Evan Tokushima lean (LETL) rat was inbred in Japan from Long Evan rats. The strain is characterised with an abrupt onset of diabetes, normal lymphocyte count and insulitis. The rate of diabetic animals varies between 16 and 20 %. Both sexes are affected equally [4].

Selective breeding of the LETL animal resulted in a colony with higher frequency of diabetes (80–90 %). The strain was named the Komeda Diabetes Prone rat (KDP rat). KDP rats share the same RT1 u/u/u haplotype with LETL and BB rat strains. Diabetes manifests between 3 and 4 months of age with sudden symptoms including polyuria, weight gain and hyperglycaemia. Lymphocyte infiltration of thyroid and salivary glands was reported. The mechanism inhibiting diabetes development has not yet been described [5].

12.1.3.4 LEW.1AR1 iddm Rat Strain

MHC haplotype RT1 AaBDu Cu arose from a LEW.1AR1 colony by genetic mutation. The rate of spontaneous diabetes ranges between 18 and 20 % and increases up to almost 60 % when both parents are diabetic. All diabetic animals are characterised with insulitis, undetectable autoantibodies, normal lymphocyte number and sudden onset of hyperglycaemia, ketonurie and hypoinsulinemia [6].

12.1.4 Surgical Anatomy of the Pancreas

The rat pancreas is localised in the abdominal cavity and is more lobular, dispersed, softer and less limited from the surrounding tissue than in humans. In 300 g rats, it typically weights approx. 1 g in average. The exocrine tissue is drained through two main and 15–40 smaller ducts which mostly enter the bile duct or sometimes directly penetrate into the duodenum. The splenic duct enters the bile duct typically about 10 mm from the duodenum and comes from the left lobe. The second large pancreatic duct (posterior pancreatic duct) enters the bile duct close to the duodenum (2 mm) and drains the duodenal portion of the pancreas. The organ can be divided into three main parts according to the flow of the pancreatic juice:

The biliary portion (right lobe or the head; Fig. 12.1) is located cranially within the mesoduodenum between the bile duct and descending duodenum. It consists of several small lobules. The duodenal portion (the body; Fig. 12.1) is located caudally, also within the mesoduodenum, specifically between the bile duct and the beginning of the mesojejunum.

The gastrosplenic portion (the left lobe = cauda; Fig. 12.2) is the largest one and continued along the dorsal wall of the stomach towards the splenic hilum and the transversal mesocolon. The tissue lies in the gastrosplenic ligament and is

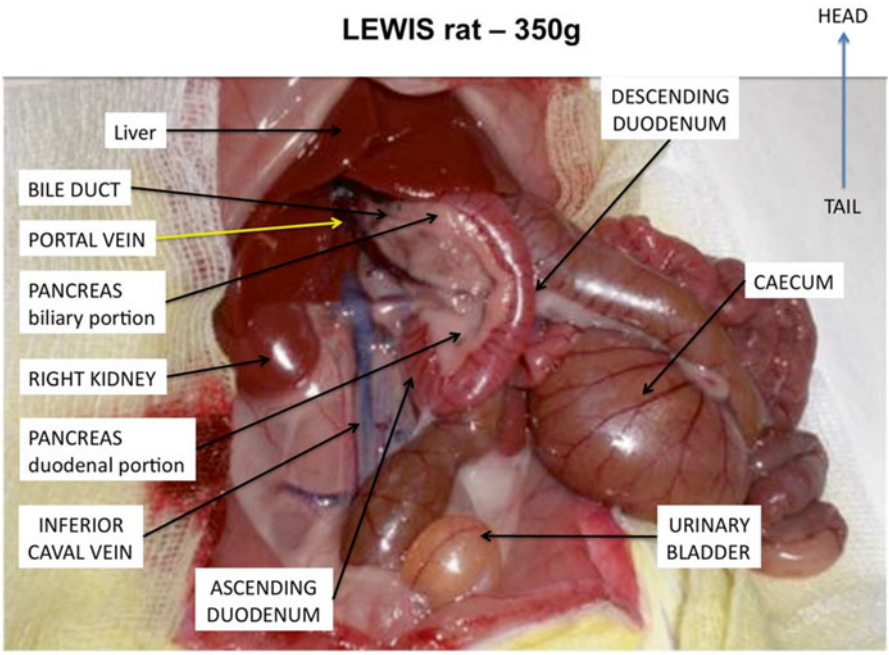


Fig. 12.1 Overview anatomy of the rat abdominal cavity – biliary and duodenal portions of pancreas

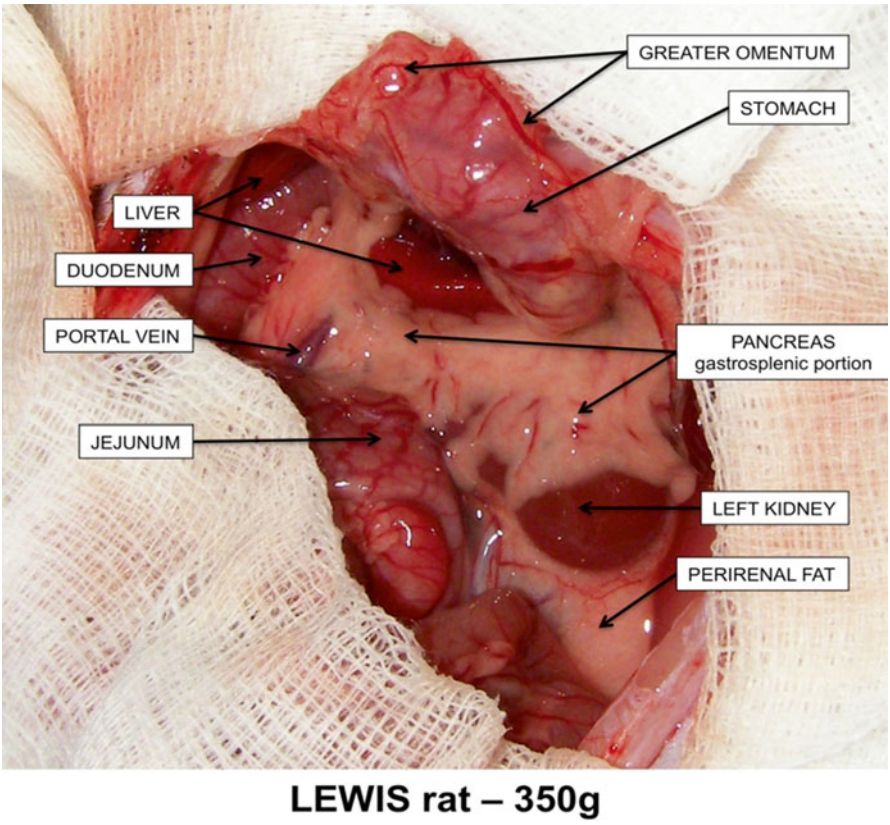


Fig. 12.2 The second overview of the abdominal cavity – gastrosplenic portion of pancreas

outlined with splenic and gastroepiploic blood vessels. It is drained by the anterior pancreatic duct which enters bile duct approx. 10 mm from the papila of Vater.

12.2 Preconditioning of the Animal Prior to Surgery of Pancreas

It is recommended to allow the animals to become familiar with the facility equipment and staff for at least 2 weeks after arrival from the supplier. This can provide the animals the necessary time to get accustomed to the environment and reduce stress. An added benefit is that the researcher can become familiar with individual animals, can identify those that are not in a perfect health condition and prevent them from inclusion into the experimental group. Ultimately, the animals used in chronic studies must be housed for at least 72 h prior to any procedures being performed.

Each animal must be clearly identifiable during the all phases of the study – they have to be explicitly marked before the first manipulation. Rats are unable to vomit, thus it is not absolutely required to let them fast before the surgery. But, in order to reduce the volume of intestinal content and therefore to make easier the surgical manipulations within abdominal cavity, overnight fasting is recommended. This fasting does not mean starvation. Thus, in order to prevent ketosis and hypoglycaemia, food pellets should be replaced by glucose solution and water freely accessible for the animal.

Both approaches for total anaesthesia induction and maintenance can be utilised according to the routine of the researcher. Ventilation anaesthesia used in our group is performed with a mixture (3–5 %) of isoflurane in the air throughout the duration of the surgery. Intramuscular anaesthesia can be based on ketamine (10 mg/kg)/xylazin (1.5 mg/kg) or ketamine (10 mg/kg)/medetomidin (0.25 mg/kg), both of which can be extended by the reinjection of 30 % of the initial dose. During the anaesthesia, temperature maintenance is crucial – the animal should be placed on safe heating pad, the room temperature should not be under 22 °C and the saline used for visceral moisturising must be warmed to 37 °C.

12.2.1 DO's Just Before the Surgery

1. Check the donor visually, weight it and calculate the dose of anaesthetic drugs
2. Inject the anaesthesia and let the donor sleep in the dark
3. Shave an area large enough to include a complete incision line and some surrounding skin allowing efficient disinfection.
4. Treat the skin using detergent ⇒ alcohol ⇒ disinfectant.
5. Cover recipient eyes with a proper unguent and put it onto the heating pad with the backside down
6. Drape the animal using the sterile cloth to provide a sterile field around the incision

12.3 The Pancreas Organ Transplantation

12.3.1 The Pancreas Procurement

12.3.1.1 Equipment + Material

Microsurgical Instruments Scissors, forceps, four vascular clamps, 9-0 nylon sutures, 7-0 and 4-0 silk suture, two micro-scissors (straight, curved), three microforceps (two straight, one curved), micro-needle holder, thermal cauterise unit, cotton swabs, gauze, saline solution, heparin solution, heating pad, needles, syringes (5 ml, 10 ml).

12.3.1.2 Procedure

1. Open abdominal cavity with a midline incision
2. Cauterise capillary bleeding from the incision edge
3. Enlarge the incision to subcostal space for better pancreas exposure
4. Retract the small bowel on the animal right side
5. Split up the ligament of Treitz (Fig. 12.3)
6. Disrupt the descending mesocolon (Fig. 12.4)
7. Put ligature on medium colic vessels (Fig. 12.5a) with 7-0 silk and divide (Fig. 12.5b)

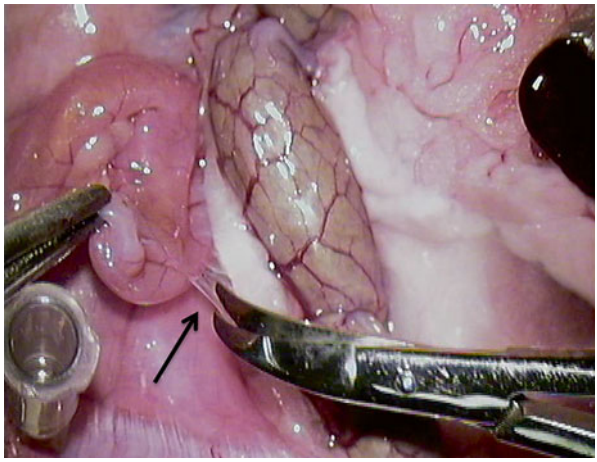


Fig. 12.3 Split up the ligament of Treitz

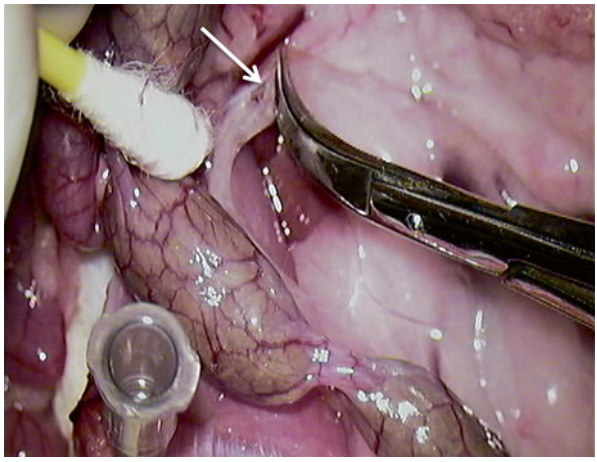


Fig. 12.4 Disrupt the descending mesocolon

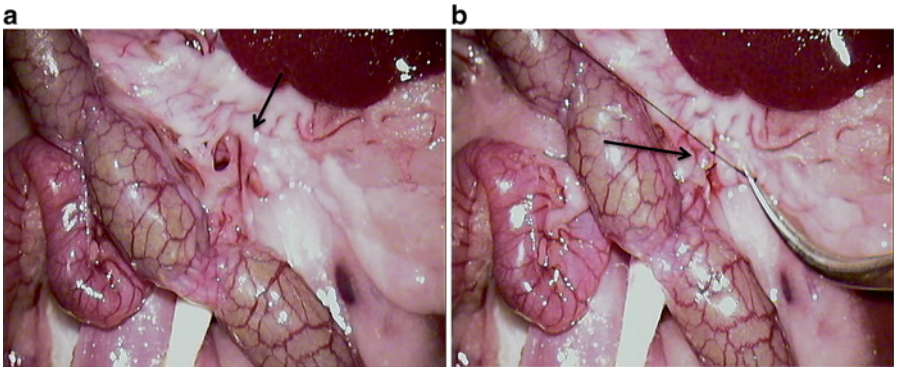


Fig. 12.5 Put ligature on medium colic vessels (**a**) with 7-0 silk and divide (**b**)

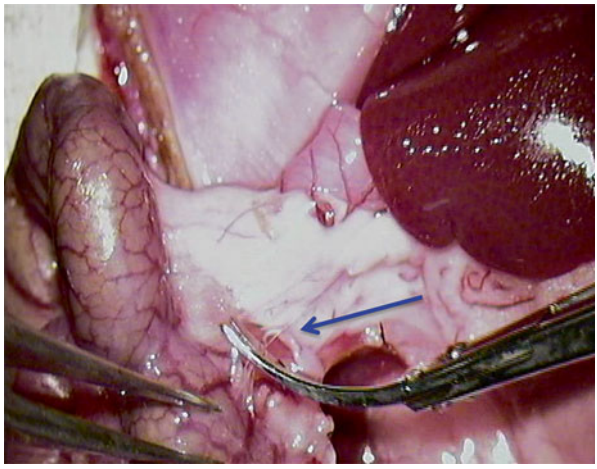


Fig. 12.6 Cut the "closer" layer of the mesocolon

8. Cut the "closer" layer of the mesocolon (Fig. 12.6)
9. Using cotton swab, gently divide pancreatic head from the mesocolon (Fig. 12.7)
10. Expose superior mesenteric vessels (Fig. 12.8a): m.v. = mesenteric vessels; P = pancreas; blue arrow = cut the first branch of the m.v. (Fig. 12.8b)
11. Tied mesenteric vessel with 7-0 silk using artificial aperture in the mesentery at the doudenojejunal flexure and cut it (Fig. 12.8c). P = pancreas; blue arrow = the cutting of mesentery containing vessels
12. Dissect the greater omentum from pancreas with cauteriser and open the omental bursa (Fig. 12.9a–c). P = pancreas; blue arrows = greater omentum
13. Ligate the splenic vessels; do not cut it – the spleen can be used as a holder for manipulation with the pancreas (Fig. 12.10) P1 = pancreas gastrosplenic portion; P2 = pancreas duodenal portion; blue arrow = splenic vessels
14. Retract the stomach up (Fig. 12.11)

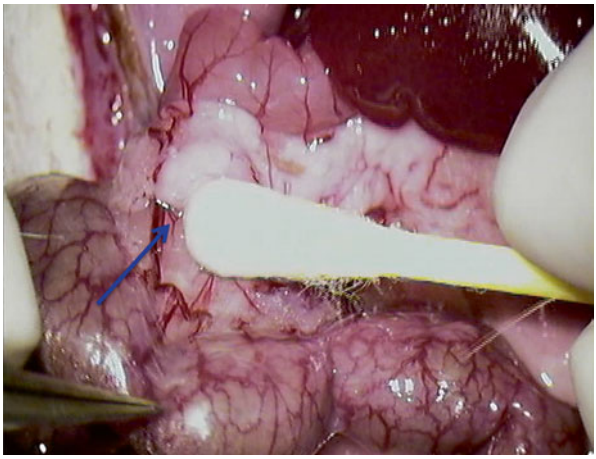


Fig. 12.7 Using cotton swab, gently divide pancreatic head from the mesocolon

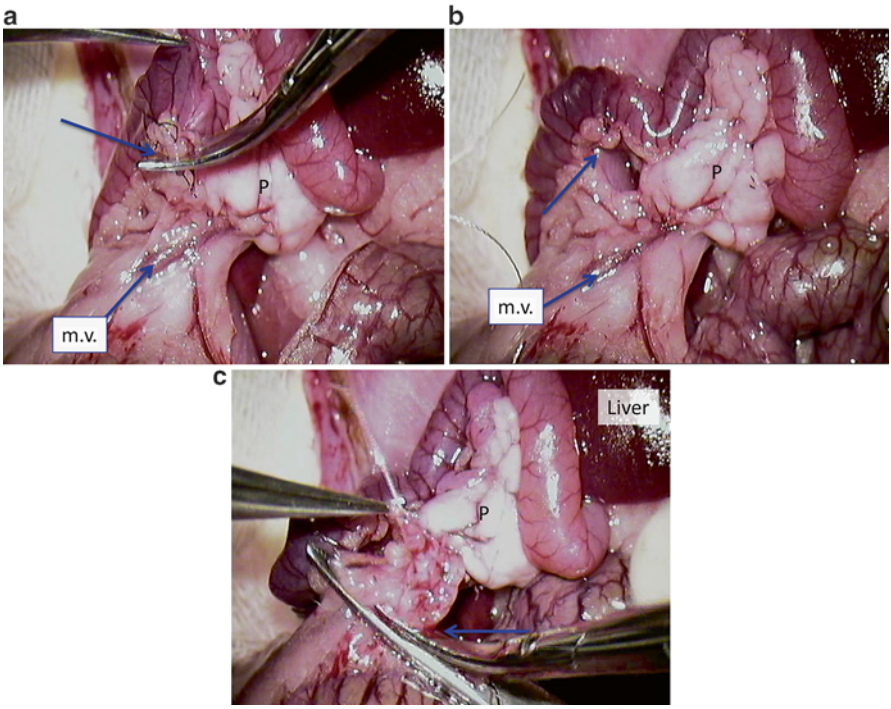


Fig. 12.8 Expose superior mesenteric vessels (**a**) m.v. = mesenteric vessels; P = pancreas; *blue* arrow = cut the first branch of the m.v. (**b**) Tied mesenteric vessel with 7-0 silk using artificial aperture in the mesentery at the duodenojejunal flexure and cut it (**c**) P = pancreas; *blue* arrow = the cutting of mesentery containing vessels

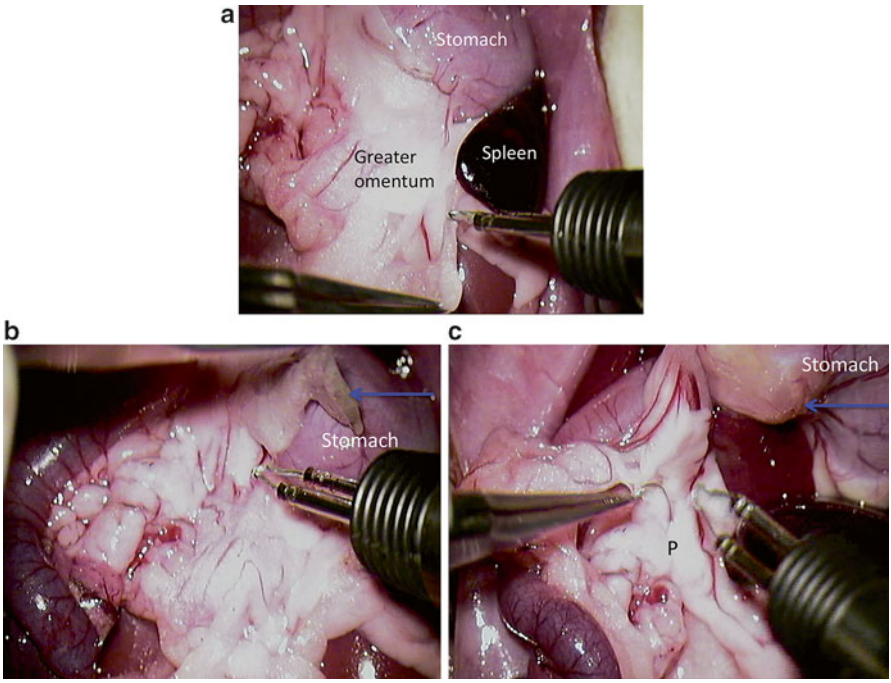


Fig. 12.9 Dissect the greater omentum from pancreas with cauteriser and open the omental bursa (**a**–**c**). P = pancreas; *blue* arrows = greater omentum

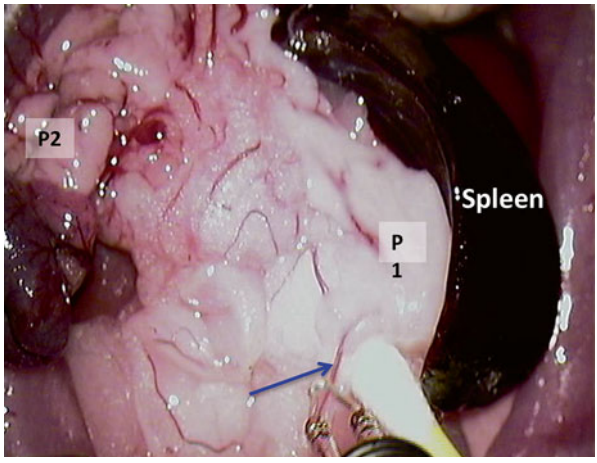


Fig. 12.10 Ligate the splenic vessels; do not cut it – the spleen can be used as a holder for manipulation with the pancreas. P1 = pancreas gastrosplenic portion; P2 = pancreas duodenal portion; *blue* arrow = splenic vessels

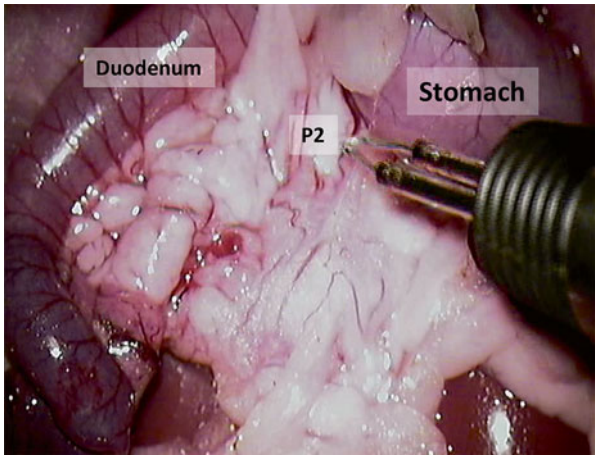


Fig. 12.11 Retract the stomach up

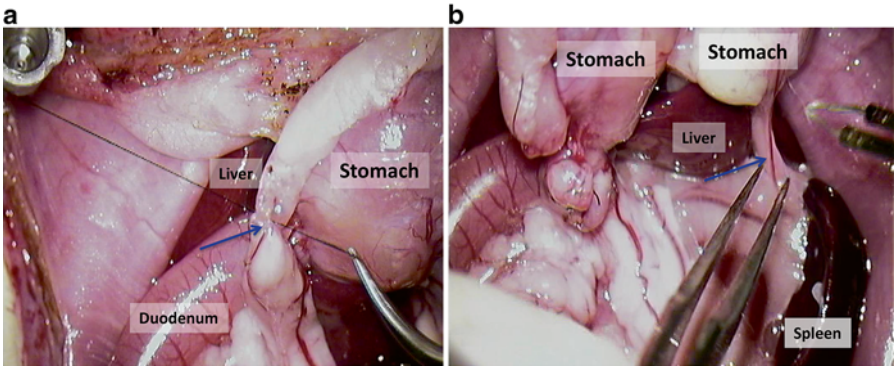


Fig. 12.12 (**a**) Tie and divide the right gastroepiploic vessels at the pylorus; *blue* arrow = gastroepiploic vessels. (**b**) Split up splenogastric ligament with vessels after the appropriate ligature; *blue* arrow = splenogastric ligament with vessels

15. Tie and divide the right gastroepiploic vessels at the pylorus (Fig. 12.12a); blue arrow = gastroepiploic vessels
16. Split up splenogastric ligament with vessels after the appropriate ligature (Fig. 12.12b); blue arrow = splenogastric ligament with vessels
17. Tie the pylorus and divide it (Fig. 12.13a); blue arrow = a suture over the pylorus; GO = greater omentum;
18. Ligate the right gastric vessels and divide (Fig. 12.13b); blue arrow = the right gastric vessels between two sutures
19. Tie the common bile duct (Fig. 12.14a); blue arrow = common bile duct with the suture; S = stomach; Pancreas – duodenal portion;

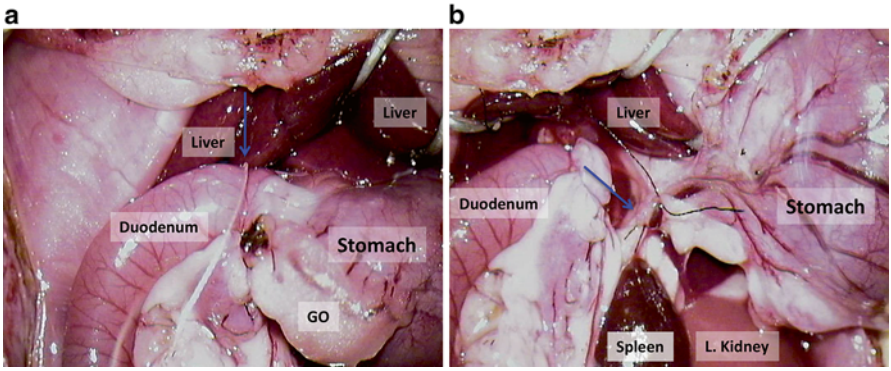


Fig. 12.13 (**a**) Tie the pylorus and divide it; *blue* arrow = a suture over the pylorus; *GO* greater omentum; (**b**) ligate the right gastric vessels and divide; *blue* arrow = the right gastric vessels between two sutures

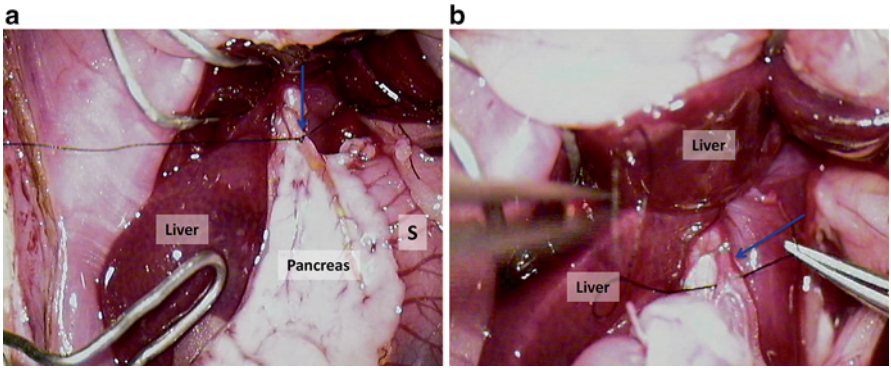


Fig. 12.14 (**a**) Tie the common bile duct; *blue* arrow = common bile duct with the suture; S = stomach; pancreas – duodenal portion; (**b**) put ligature on the proper hepatic artery; *blue* arrow = proper hepatic artery with the suture

20. Put ligature on the proper hepatic artery (Fig. 12.14b); blue arrow = proper hepatic artery with the suture;
21. Dissect the portal vein (Fig. 12.15a); D = duodenum; blue arrow = portal vein; green arrow = bile duct;
22. Retract the pancreas to the animals' right side (Fig. 12.15b); blue arrow = spleen, green arrow = left renal vein;
23. Divide pancreas from the backside tissue (Fig. 12.16a); LAG = left adrenal gland; blue arrow = portal vein;
24. Expose aorta, coeliac trunk and superior mesenteric artery using cotton swabs or a cauteriser (Fig. 12.16b)
25. Clamp the aorta above coeliac trunk (green arrow) and between mesenteric (yellow arrow) and left renal artery (Fig. 12.17a, b).

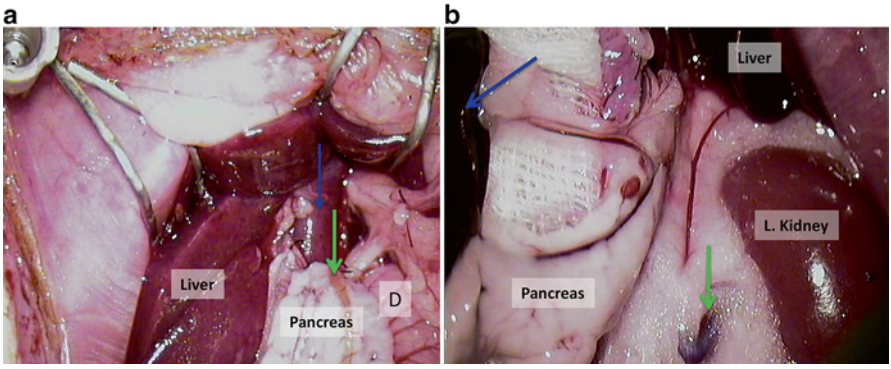


Fig. 12.15 (**a**) Dissect the portal vein; D = duodenum; *blue* arrow = portal vein; *green* arrow = bile duct; (**b**) retract the pancreas to the animals' right side; *blue* arrow = spleen, *green* arrow = left renal vein

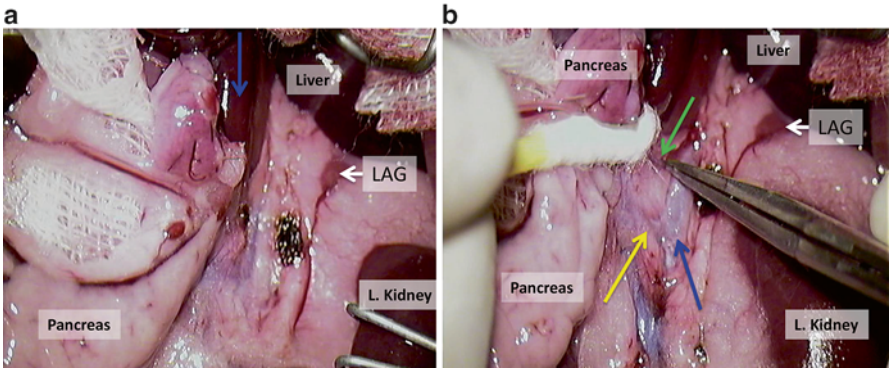


Fig. 12.16 (**a**) Divide pancreas from the backside tissue; LAG = left adrenal gland; *blue* arrow = portal vein; (**b**) expose aorta, coeliac trunk and superior mesenteric artery using cotton swabs or a cauteriser

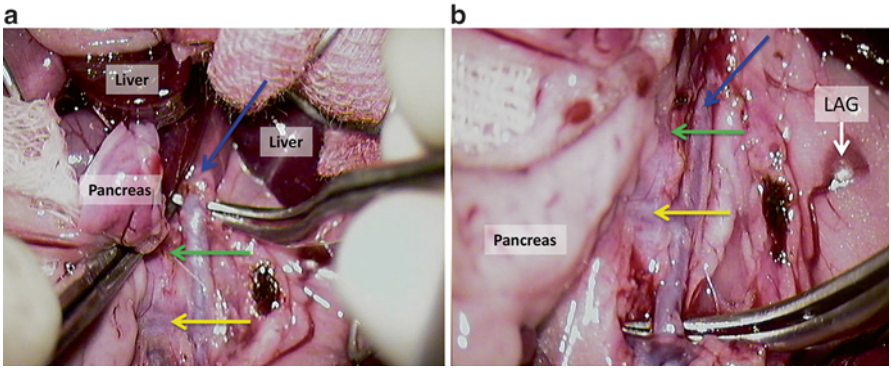


Fig. 12.17 (**a**, **b**) Clamp the aorta above coeliac trunk (*green* arrow) and between mesenteric (*yellow* arrow) and left renal artery

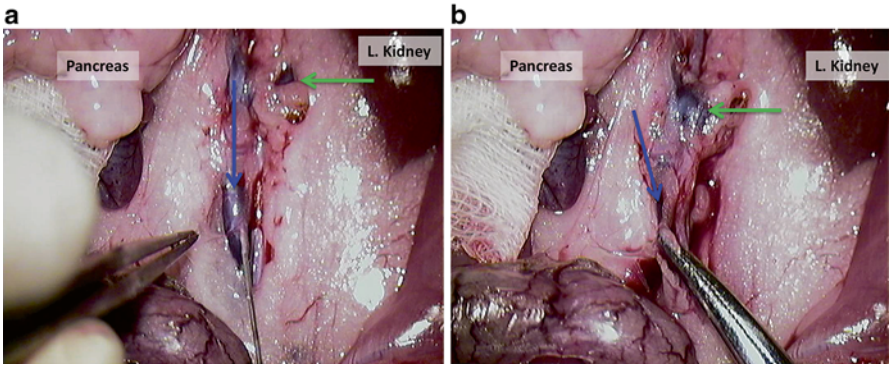


Fig. 12.18 Inject 3–5 ml heparin solution (10 i.u./ml of saline) into inferior caval vein; *blue* arrow (**a**); put the clamp on the inferior caval vein; *green* arrow = left renal vein (**b**)

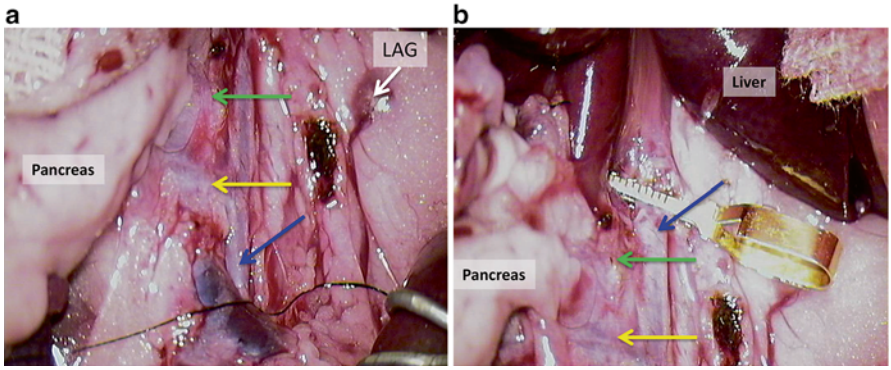


Fig. 12.19 Tie the aorta with 7-0 silk below mesenteric artery (**a**) and cross clamp the aorta above the celiac trunk (**b**); LAG = left adrenal gland; *blue* arrow = aorta; *green* arrow = coeliac trunk, *yellow* arrow = cranial mesenteric artery

26. Inject 3–5 ml heparin solution (10 i.u./ml of saline) into inferior caval vein (Fig. 12.18a);
27. Put the clamp on the inferior caval vein; green arrow = left renal vein (Fig. 12.18b);
28. Tie the aorta with 7-0 silk below mesenteric artery (Fig. 12.19a) and cross clamp the aorta above the celiac trunk (Fig. 12.19b); LAG = left adrenal gland; blue arrow = aorta; green arrow = coeliac trunk, yellow arrow = cranial mesenteric artery;
29. Cut the portal vein (Fig. 12.20a);
30. Wash the pancreas through the aorta with 5 ml of ice cold heparin solution (10 i.u./ml of saline; Fig. 12.20b); blue arrow = aorta; yellow arrow = cranial mesenteric artery.
31. Divide the aorta. Figure 12.21a = suture (yellow arrow) the aorta cranially to the puncture (blue arrow) after the needle; Fig. 12.21b = cut the aorta (blue arrow)

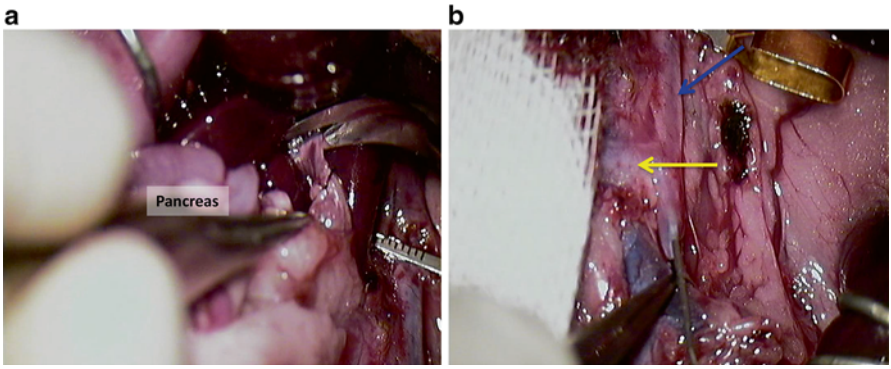


Fig. 12.20 Cut the portal vein (**a**); wash the pancreas through the aorta with 5 ml of ice cold heparin solution (10 i.u./ml of saline; (**b**) *blue* arrow = aorta; *yellow* arrow = cranial mesenteric artery

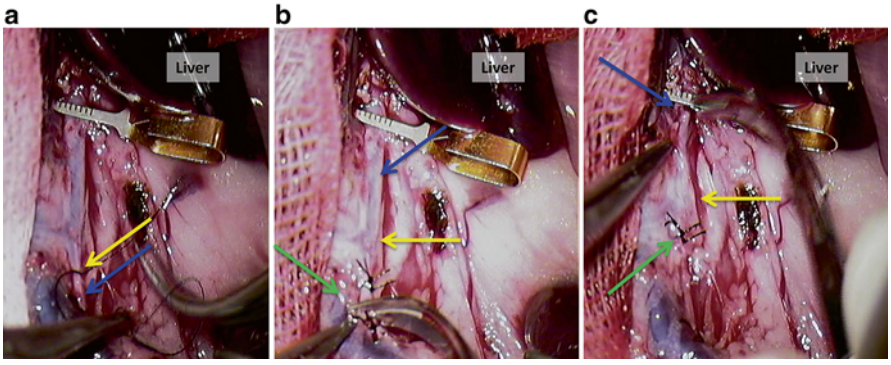


Fig. 12.21 Divide the aorta. (**a**) Suture (*yellow* arrow) the aorta cranially to the puncture (*blue* arrow) after the needle; (**b**) cut the aorta (*blue* arrow) between the sutures (*green* arrow), distally to cranial mesenteric artery (*yellow* arrow); (**c**) cut the aorta (*blue* arrow) close to the vascular clamp; *green* arrow shows the free end of aorta with sutures; *yellow* arrow shows the cranial mesenteric artery

between the sutures (green arrow), distally to cranial mesenteric artery (yellow arrow); Fig. 12.21c = cut the aorta (blue arrow) close to the vascular clamp; green arrow shows the free end of aorta with sutures; yellow arrow shows the cranial mesenteric artery;

32. Split up duodenum at the margin of tissue with an adequate perfusion Fig. 12.22a;
33. Divide the aorta from background tissue, interrupt the lumbar vessels and right renal artery using a cotton swab or cauteriser Fig. 12.22b; blue arrow shows the distal end of aortal segment of the graft; green arrow shows the bile duct;
34. Turn the pancreas with the cotton swab to the animals' left side
35. Finish the aorta preparation
36. Put the pancreatic graft onto the ice drift

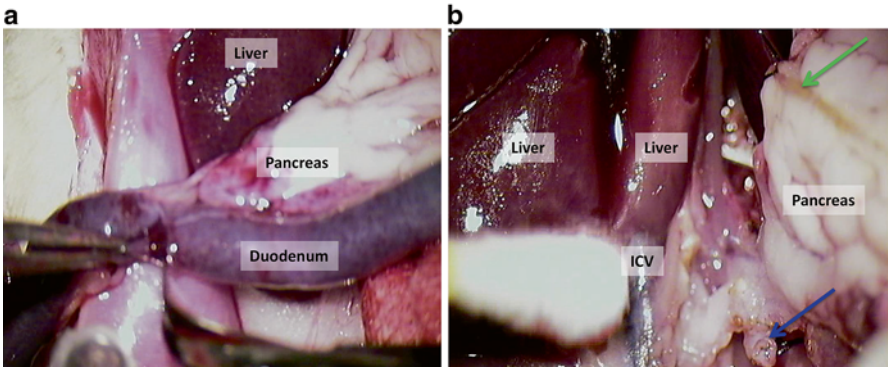


Fig. 12.22 Split up duodenum at the margin of tissue with an adequate perfusion (**a**); divide the aorta from background tissue, interrupt the lumbar vessels and right renal artery using a cotton swab or cauteriser (**b**); *blue* arrow shows the distal end of aortal segment of the graft; *green* arrow shows the bile duct

12.3.1.3 Tips and Tricks

1. Wash the serosa with warm saline (37 °C) so that the tissue will not be sticky for surgical instruments
2. Use a cauteriser for tissue dissection as it immediately stops small capillary bleedings and accelerates the work
3. Leave the spleen attached to the pancreas for further handling. It allows you to work with a no touch technique
4. Divide the duodenum at the site of the colour change which is apparent after graft perfusion
5. Leave the pancreas on a wet gauze square as it allows you to manipulate with minimal touching

12.3.2 Pancreas Organ Transplantation

12.3.2.1 Equipment + Material

Microsurgical Instruments Scissors, forceps, four single clamps, 9-0 nylon sutures, 7-0 and 4-0 silk suture, two micro-scissors (straight, curved), three micro-forceps (two straight, one curved), micro-needle holder, thermal cauter unit.

Material Cotton swabs, sterile gauze squares, saline, heparin solution, heating pad, needles, syringes (5 ml, 10 ml).

12.3.2.2 Procedure

1. Open abdominal cavity with a midline incision
2. Cauterise capillary bleedings at the wound edge
3. Put the small intestine aside
4. Start preparation of the inferior caval vein and aorta from the surrounding fat. Expose both vessels from the left renal artery to the external iliac vessels (Fig. 12.23)
5. Put ligature on all lumbar vessels and cut them (Fig. 12.24)
6. Put two microvascular clamps on aorta, place the first below the renal artery and the second above the left external iliac artery (Fig. 12.25)

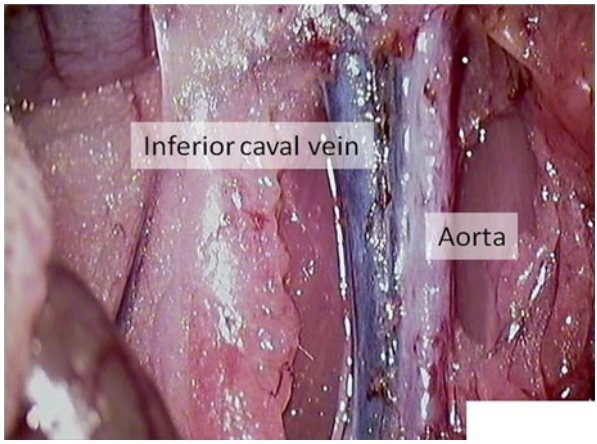


Fig. 12.23 Expose both vessels from the left renal artery to the external iliac vessels

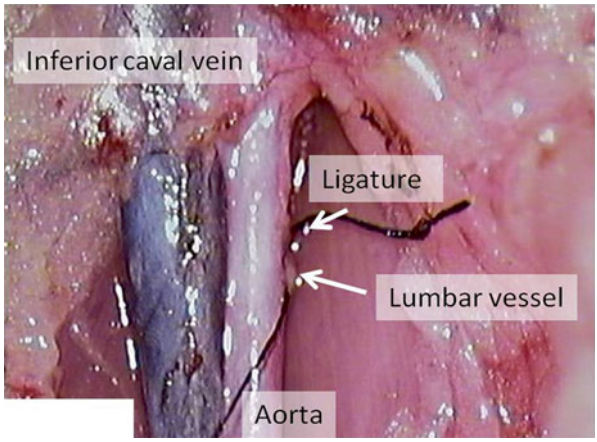


Fig. 12.24 Put ligature on all lumbar vessels and cut them

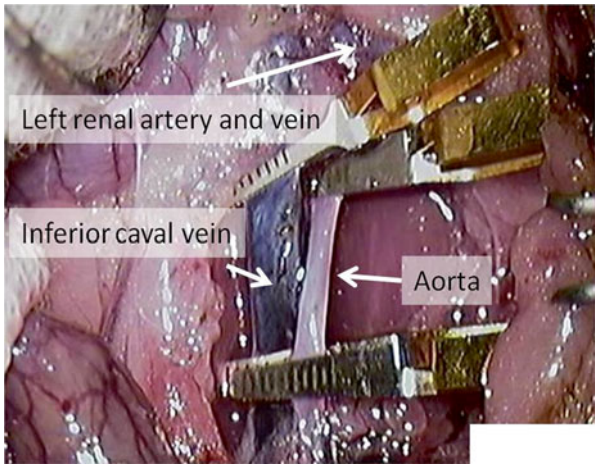


Fig. 12.25 Put two microvascular clamps on aorta, place the first below the renal artery and the second above the left external iliac artery

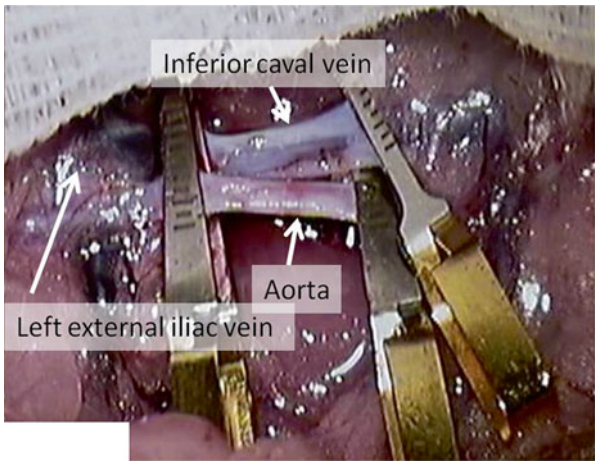


Fig. 12.26 Put the other two microvascular clamps on inferior caval vein, place the first below the left renal vein and the second above the left external iliac vein

7. Put the other two microvascular clamps on inferior caval vein, place the first below the left renal vein and the second above the left external iliac vein (Fig. 12.26)
8. The pancreatic graft should be wrapped within the wet gauze square exposing only the aortal patch and the portal vein
9. Make incisions in aortal and caval vein walls and flush the lumen with heparin solution (Fig. 12.27)
10. Start vascular anastomosis end to side with the closer side of the graft portal vein and recipient inferior caval vein (Figs. 12.28 and 12.29). Continue with aorto-aortal end-to-side anastomosis (Fig. 12.30), then turn the graft and finish the distant site of porto-caval anastomosis using a continuous 9-0 silk suture (Figs. 12.31 and 12.32).

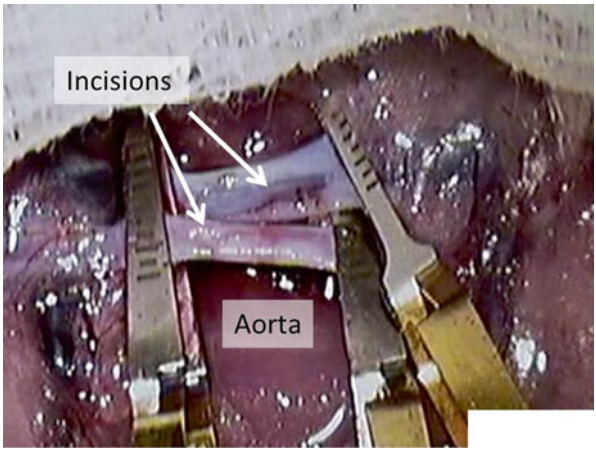
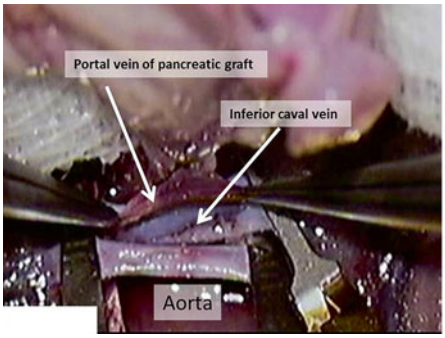
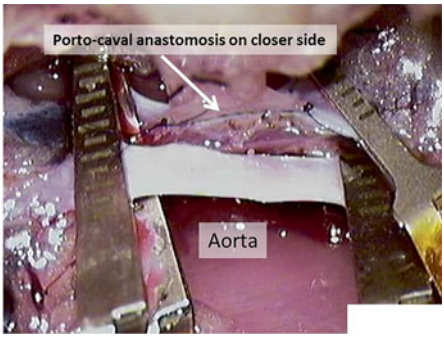


Fig. 12.27 Make incisions in aortal and caval vein walls and flush the lumen with heparin solution





Figs. 12.28 and 12.29 Start vascular anastomosis end to side with the closer side of the graft portal vein and recipient inferior caval vein

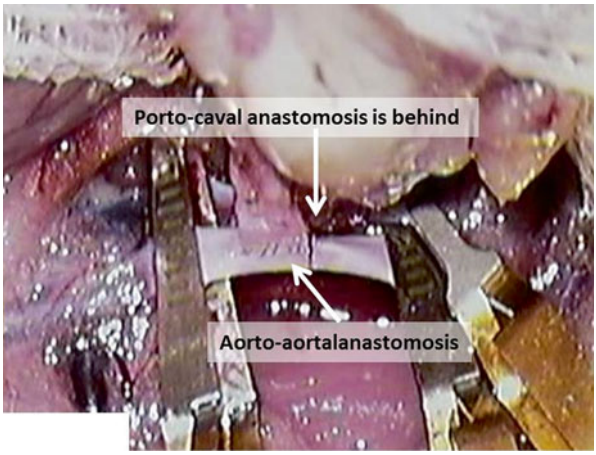
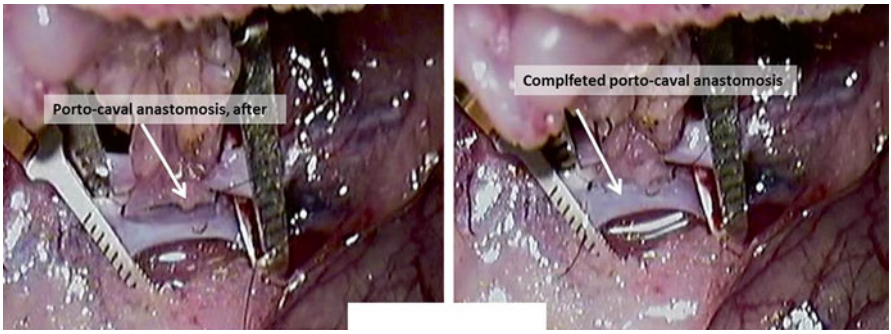


Fig. 12.30 Continue with aorto-aortal end-to-side anastomosis



Figs. 12.31 and 12.32 The distant site of porto-caval anastomosis using a continuous 9-0 silk suture

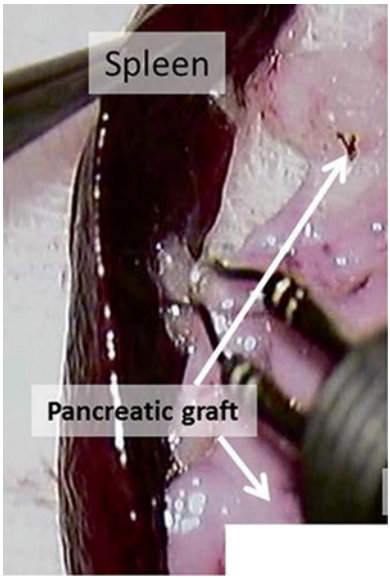


Fig. 12.33 Divide the spleen from the pancreatic graft using a cauterizer

11. Remove the clamps from large vessels (first remove the distally and then cranially located clamps on the caval vein and then aortic clamps in the same order)
12. Divide the spleen from the pancreatic graft using a cauteriser (Fig. 12.33)
13. Suture the aboral end of the duodenal graft (Fig. 12.34)
14. Continue with side to side duodeno-jejuno anastomosis using a continuous suture 8-0 silk (Sect. 6.1) (Fig. 12.35)

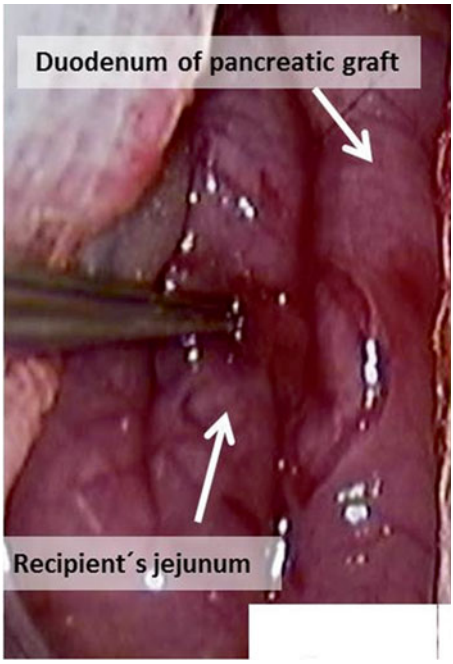


Fig. 12.34 Suture the aboral end of the duodenal graft

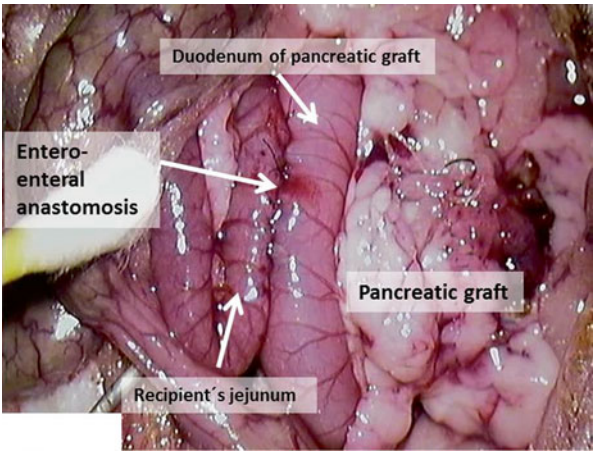


Fig. 12.35 Continue with side to side duodeno-jejuno anastomosis using a continuous suture 8-0 silk

12.3.2.3 Tips and Tricks

1. The pancreatic graft should be wrapped in sterile ice cold wet gauze squares
2. Thoroughly ligate all lumbar vessels to prevent bleeding during vascular anastomosis
3. Porto-caval anastomosis should be at least three times larger than the diameter of portal vein. It efficiently prevents a venous thrombosis of pancreatic graft

12.4 Transplantation of Isolated Pancreatic Islets

12.4.1 Pancreatic Islet Isolation

The pancreatic islets can be isolated from the whole organ by collagenase enzymatic digestion followed by the separation of islets from the exocrine tissue using centrifugation in density gradient.

There are two different approaches for instillation of collagenase. The first one uses the ligation of bile duct close to the duodenum and injection of the solution using a fine needle from the liver end. The second one consists of clamping of the bile duct at the liver end and catheterisation of the bile duct through the duodenum (Fig. 12.36).

The second one is more efficient, but carries the potential risk of the microbial contamination of the pancreas. Our own fairly extensive experience has shown no contamination at any time. The purpose of the intraductal administration of collagenase is the preferential localisation of the enzyme within the ducts and exocrine portion of the pancreas.

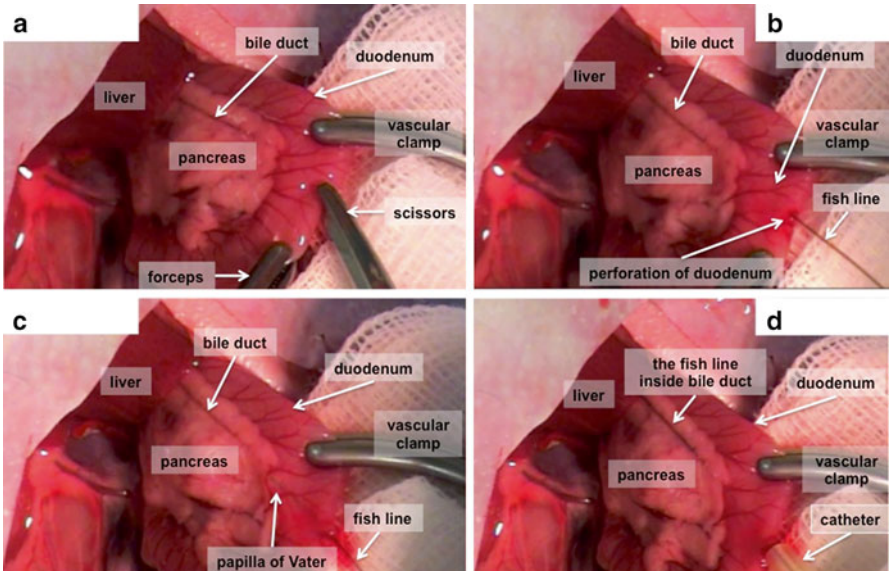


Fig. 12.36 Put the wet gauze square on the left side of the animal and, using wet cotton swabs, move the intestines onto it (**a**). Find the duodenal papilla, the intestinal end of the bile duct (**c**). Fix the duodenum using a vascular clip attached on the opposite side of the intestine, approximately 5 mm cranially to the papilla (**b**). Gently insert a fish line into the bile duct and use it as a loader for the 24G catheter (**c**, **d**)

12.4.1.1 Equipment + Material

Collagenase (i.e. Collagenase V; Sigma Aldrich), Hank Balanced Salt Solution, Fetal Calf Serum (FCS), Ficoll Islet Gradient 1.108, 1.096, 1.069, 1.037 g/ml; sterile gauze squares, saline, sterile cotton swabs, 24G i.v. catheter, fish line 10 kg, syringes 1, 10, and 20 ml, tubes 50 ml, pipettes 10 and 25 ml, Pasteur pipettes, stainless steel mesh (500 μm pore) with dish, Petri dish, dissection microscope with camera, crashed ice, culture media, culture flask, culture incubator.

12.4.1.2 Procedure

An experienced surgeon can reliably perform an isolation in four donors at once.

Preparation of solutions:

1. Prepare the HBSS solution: Hank Balanced Salt Solution supplemented with the 1 % Foetal Calf Serum
2. Dissolve the Collagenase in HBSS (1 mg/ml, 15 ml/animal).
3. Prepare the crushed ice – media and tissue should be cooled at all times.

Procedure itself:

1. Open the abdominal cavity by midline laparotomy
2. Apply surgical retractors on both sides of the cavity
3. Put the wet gauze square on the left side of the animal and, using wet cotton swabs, move the intestines onto it (Fig. 12.36a).
4. Cover the intestines with the wet gauze square
5. Find the duodenal papilla, the intestinal end of the bile duct (Fig. 12.36c)
6. Fix the duodenum using a vascular clip attached on the opposite side of the intestine, approximately 5 mm cranially to the papilla (Fig. 12.36a)
7. Open the duodenum on the opposite side of the intestine by small incision 2–3 mm distally to the papilla
8. Gently insert a fish line into the bile duct and use it as a loader for the 24G catheter (Fig. 12.36c, d)
9. Clip (or ligate) the bile duct at the liver end (Fig. 12.37)
10. Cut the inferior vena cava, allow for bleeding and remove all blood using sterile compresses. Then cut the diaphragm and aorta over the heart
11. Using forceps, gently hold the catheter on the site and pull the fish line out (Fig. 12.38a)
12. Connect the syringe with ice cold collagenase solution to the catheter (Fig. 12.38b) and inject 15 ml of the solution (Fig. 12.38c)
13. Gently pull out the catheter and clip (or ligate) the bile duct very close to the duodenum in order to prevent the solution from leaking (Fig. 12.38d)
14. Gently excise the pancreas from the surrounding tissues (Fig. 12.39). Put the pancreas into the 50 ml tube cooled within a crushed ice
15. In case of isolation from more donors at once, repeat steps 1–15. Clearly mark each tube with the organ

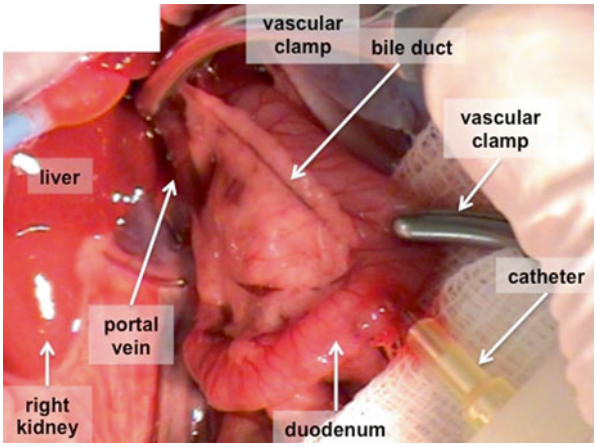


Fig. 12.37 Clip (or ligate) the bile duct at the liver end

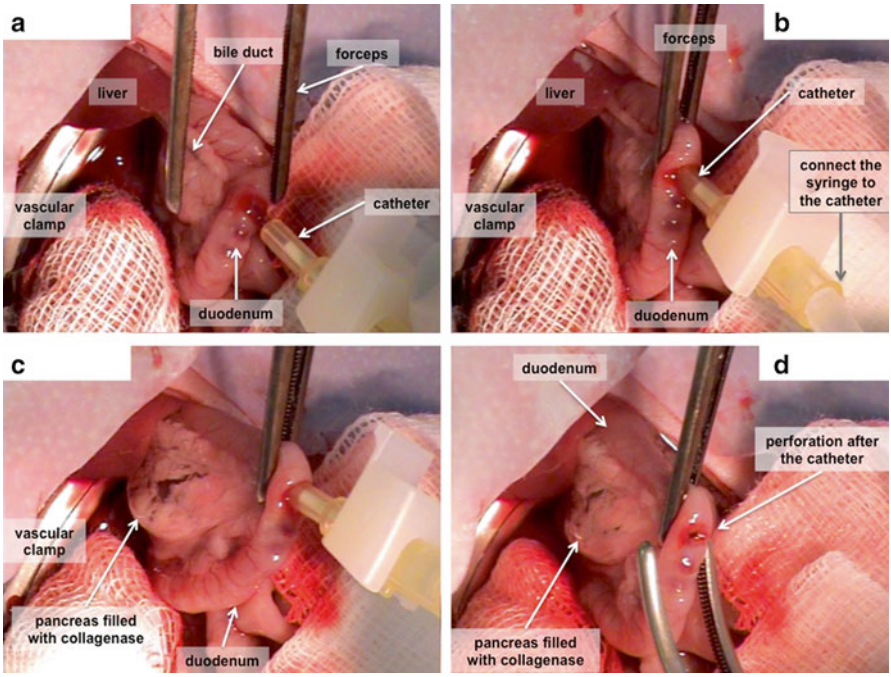


Fig. 12.38 (**a**) Using forceps, gently hold the catheter on the site and pull the fish line out; (**b**) connect the syringe with ice cold collagenase solution to the catheter and (**c**) inject 15 ml of the solution; (**d**) gently pull out the catheter and clip (or ligate) the bile duct

16. Place all tubes into the warm water shaker at 37 °C (8/min) and let it incubate for 10–25 min according to the collagenase activity. It should be optimised for each new lot of collagenase. Check the process of digestion visually – the integrity of tissue gets gradually eroded. When the pieces of tissue are not identifiable the digestion process is finished.

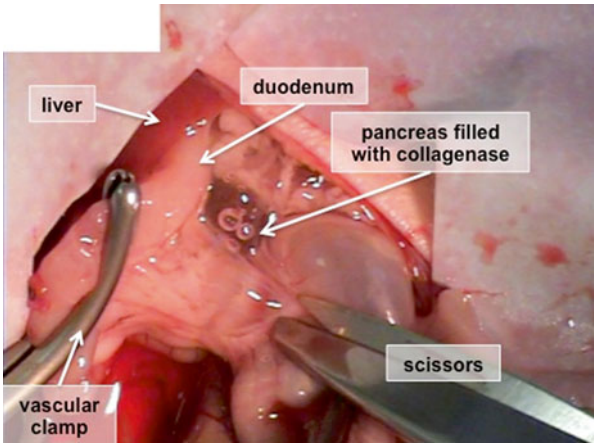


Fig. 12.39 Gently excise the pancreas from the surrounding tissues

17. After digestion, place the tube into the crushed ice inside a laminar flow box (hood)
18. The digestion should be terminated using 30 ml ice cold HBSS and mix the tissue gently using a pipette. Fill the HBSS up to 50 ml
19. Centrifuge the tube (100 G) for 5 min at 4 °C
20. Remove the fat and other floating tissue from the surface. Gently remove the supernatant and re-suspend the pellet in 30 ml ice cold HBSS. Fill in the HBSS up to 50 ml
21. Centrifuge the tube (100 G) for 5 min at 4 °C
22. Gently remove supernatant and re-suspend the pellet in 20 ml ice cold HBSS
23. Pass the tissue through the stainless steel mesh (500 mm) into the moistened stainless steel cap. Using ice cold HBSS, carefully wash the rest of tissue from the mesh and tube.
24. Transfer the tissue suspension immediately into a new sterile 50 ml tube (moistened) and fill it with the HBSS up to 50 ml.
25. Centrifuge the tube (100 G) for 5 min at 4 °C
26. Gently remove the supernatant and re-suspend the pellet in 30 ml ice cold HBSS. Fill the HBSS up to 50 ml
27. Centrifuge the tube (100 G) for 5 min at 4 °C
28. Gently remove the supernatant as much as possible
29. Create a bottom layer of the gradient by blending the tissue in 10 ml of the Ficoll Islet Gradient solution (1.108 g/ml)
30. Create the second layer of the gradient by gently overlaying with 10 ml of the Ficoll Islet Gradient solution (1.096 g/ml)
31. Create the third layer of the gradient by gently overlaying with 8 ml of the Ficoll Islet Gradient solution (1.069 g/ml)
32. Create the fourth layer of the gradient by gently overlaying with 8 ml of the Ficoll Islet Gradient solution (1.037 g/ml) – Fig. 12.40a

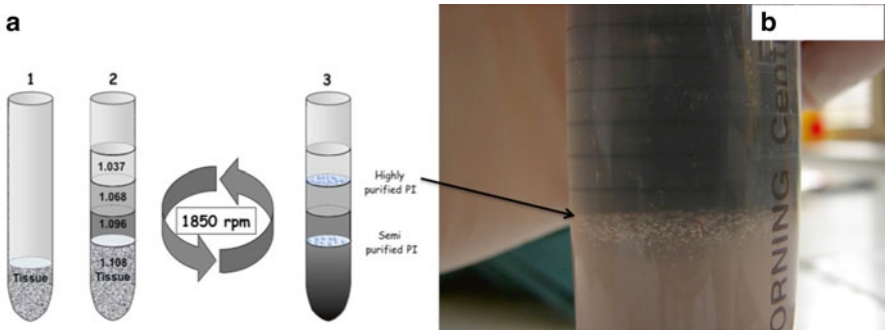


Fig. 12.40 (**a**) Create the fourth layer of the gradient by gently overlaying with 8 ml of the Ficoll Islet Gradient solution (1.037 g/ml), (**b**) centrifuge the tube (500 G) for 20 min at 4 °C ⇒ if the separation is optimal, islets and exocrine tissue will float at a different adequate interlayer

33. Centrifuge the tube (500 G) for 20 min at 4 °C ⇒ if the separation is optimal, islets and exocrine tissue will float at a different adequate interlayer Fig. 12.40b
34. Gently hold the tube and, using a sterile Pasteur pipette, aspirate islet from inter-layers (1.037/1.069 and 1.069/1.096)
35. Harvested islet/gradient suspension (15–20 ml) should be dissolved in ice cold HBSS (fill the tube up to 50 ml)
36. Centrifuge the tube (260 G) for 5 min at 4 °C
37. Gently remove the supernatant and re-suspend the pellet in 30 ml ice cold HBSS. Fill the HBSS up to 50 ml
38. Centrifuge the tube (100 G) for 5 min at 4 °C
39. The islets are now ready for quantification Fig. 12.41a, b, c and transfer to the tissue culture Fig. 12.41d or for transplantation. It is strongly recommended to take a picture of islet suspension for later analysis (documentation and eventually counting by digital image analysis)

12.4.1.3 Tips and Tricks

1. The ligature should be carefully located before the branching of the bile duct
2. Never ligate the portal vein – it can cause a diffuse haemorrhage into the pancreas
3. During the training period, the collagenase can be replaced with some surgical dye
4. An experienced researcher can put two organs to one tube ⇒ it can reduce number of manipulations and save some time
5. During the centrifugation of the gradient, withdraw the brake (set it to zero). It can prevent the destruction of the gradient.
6. Aspiration of islets from the gradient can be more easily performed by placing a black sheet behind the tube for clear visualisation.

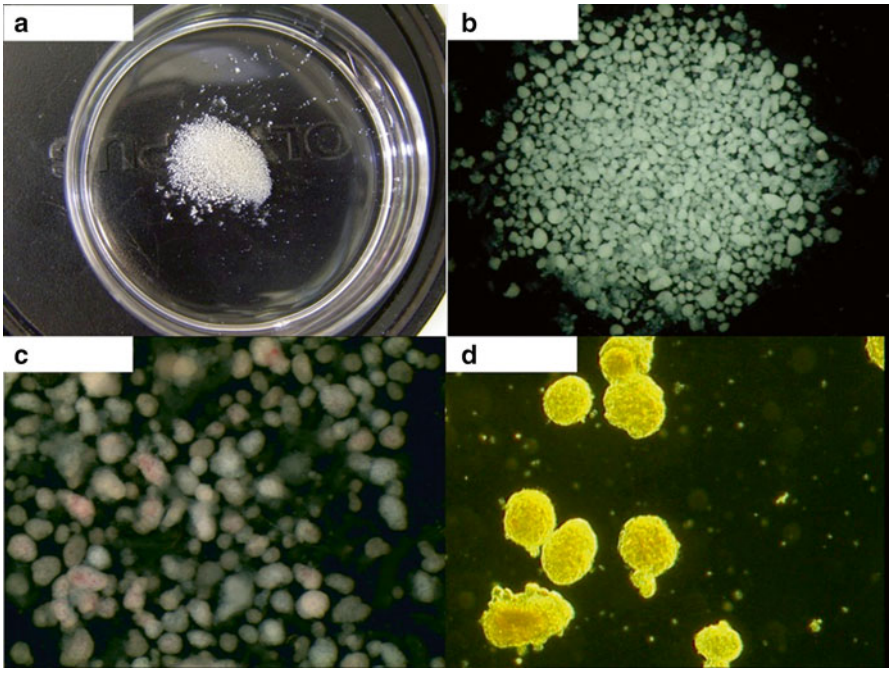


Fig. 12.41 (**a**, **b**, **c**) The islets in *black* field are now ready for quantification (**d**) islet in tissue culture

12.4.2 Preservation of Pancreatic Islets Within the Tissue Culture

Prepare the Basic Culture Media (BCM) – recipe for 10 ml of BCM. Place 1000 islets into a 25 cm^2 tissue culture flask and put it into the incubator.

12.4.2.1 Equipment + Material

1. Basic culture medium: 8.3 ml CMRL 1066; 1.0 ml Foetal Calf Serum; 0.5 ml HEPES; 0.1 ml L-glutamine; 0.1 ml penicillin/streptomycin;
2. Sodium hydroxide; Hydrochloric acid
3. Sterile tubes 50 ml; Pipettes 10 ml; Micropipettes 100 ml; Syringe micro-filter 0.22 mm; Tissue culture flasks 25 cm2
4. Laminar flow box (hood)
5. Tissue culture incubator – 5 % CO2, 37 °C, humidified,

12.4.2.2 Procedure

1. 5 ml of BCM is needed for each 1000 islets
2. The media should be completely fresh before use with the tissue culture
3. All components should be added to the CMRL-106, which is the first inserted into the tube
4. Mixture should be gently shaken for 20 min at 37 °C in a heating bath
5. Check the pH of the media. If necessary, it can be balanced with the hydrochloride acid or sodium hydroxide to 7.4

12.4.2.3 Tips and Tricks

1. Use a culture flask with HEPA filter in the lid
2. Insert 3 ml of well-balanced BCM (37 °C) into the 25 cm^2 tissue culture flask and moisten the whole bottom of the flask
3. Insert islets in minimal volume of HBSS into the flask
4. Wash the counting dish with 2 ml of BCM
5. Close the flask with the cap and put it into the incubator

12.4.3 Pancreatic Islet Transplantation

12.4.3.1 Equipment + Material

Hank Balanced Salt Solution, Fetal Calf Serum (FCS), sterile gauze squares, saline, sterile cotton swabs, 24G i.v. catheter, 27G i.v. butterfly needle, syringes 1, 20 ml, tubes 50 ml, Pasteur pipettes, Petri dish, dissection microscope with camera, crashed ice,

12.4.3.2 Procedure: Transplantation into the Right Liver Lobes

1. Open the abdominal cavity using a midline laparotomy
2. Apply surgical retractors on both sides of the cavity
3. Put a gauze square on the left side of the animal and, using a wet cotton swab, move the intestines onto it
4. Cover the intestines with a wet gauze square
5. Prepare a butterfly needle with cutting wings, connect it to a 1 ml syringe full of the saline and fill the tubing by saline
6. Using an magnification device (surgery microscope – magnification ten times), slowly pick up islets into the needle and tubing
7. Let an assistant to hold the syringe and butterfly needle in a vertical position
8. Find the ileo-caecal vein and cover the intestines with wet gauze square
9. Using a vascular clip, close the left hepatic branch of the portal vein Fig. 12.42a

10. Measure the total time of blood flow interruption
11. Insert the needle into the ileocaecal vein and ask the assistant to slowly push the plunger in order to inject islets into to vein Fig. 12.42b
12. When the total volume (transplant media containing the islets) is injected, cover the puncture with collagen foam and collagen flour. Overlay the collagen flour with a hemostatic foam square (5×5 mm), gently press it with the wet cotton swab (Q-tip) and remove the needle from the vein Fig. 12.43a. Hold the vein for 1 min in order to stop the bleeding Fig. 12.43b.
13. Gently remove the vascular clip from the hepatic branch of the portal vein.
14. Place the intestine gently back into the abdomen and inject saline (approx. 2 ml)
15. Suture the abdominal wall muscles and then the skin (Vicril 5-0)
16. Cover the abdomen with a gauze square with disinfectant and let the animal to recover on the heating pad

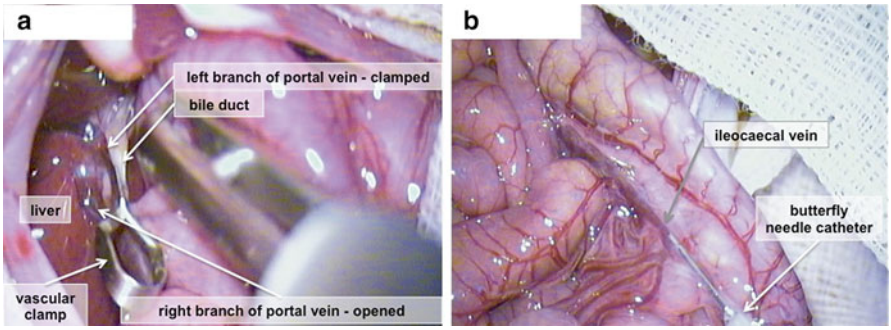


Fig. 12.42 (**a**) Using a vascular clip, close the left hepatic branch of the portal vein (**b**) insert the needle into the ileocaecal vein and ask the assistant to slowly push the plunger in order to inject islets into to vein

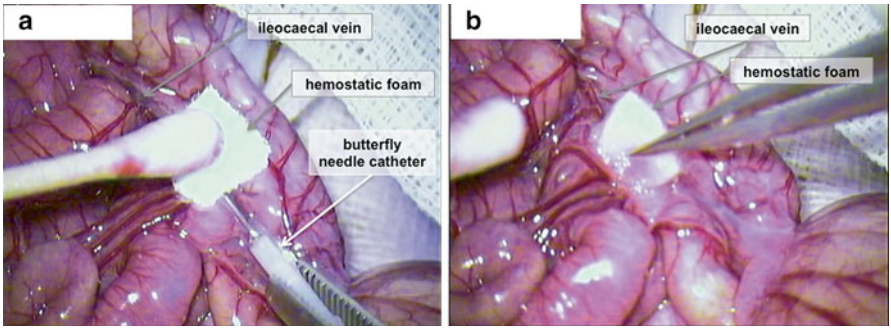


Fig. 12.43 (**a**) Remove the needle from the vein, (**b**) hold the vein for 1 min in order to stop the bleeding

12.4.3.3 Procedure: Transplantation into the Whole Liver

1. Open the abdominal cavity using a midline laparotomy
2. Apply surgical retractors on both sides of the cavity
3. Put a wet gauze square on the left side of the animal and using wet cotton swabs move the intestines onto it
4. Cover the intestines with a wet gauze square
5. Prepare a butterfly needle with cutting wings, connect it to a 1 ml syringe full of the saline and fill the tubing by saline
6. Using an magnification device (surgery microscope – magnification ten times), slowly pick up islets into the needle and tubing
7. Let an assistant to hold the syringe and butterfly needle in a vertical position
8. Find the ileo-caecal vein and fix it so that its clearly visible
9. Insert the needle into the ileocaecal vein and ask the assistant to slowly push the plunger in order to inject islets into to vein Fig. 12.42b
10. When the total volume (transplant media containing the islets) is injected, overlay the puncture with a hemostatic foam square (5×5 mm), gently press it with a wet cotton swab and remove the needle from the vein Fig. 12.43a. Hold the vein for 1 min in order to stop the bleeding Fig. 12.43b.
11. Place the intestine gently back into the abdomen and inject saline (approx. 2 ml).
12. Suture the abdominal wall muscles and then the skin (Vicril 5-0)
13. Cover the abdomen with a gauze square with disinfectant and allow the animal to recover on the heating pad

12.4.3.4 Procedure: Transplantation Beneath the Kidney Capsule

1. The rat should lie on its abdomen. Shave the hair on the left flank between the front leg and the hip.
2. Open the abdominal cavity using a transverse incision over the left kidney
3. Apply surgical retractors on both sides of the cavity
4. Expose and hold the kidney in position using a wet cotton swab Fig. 12.44a.
5. Cover it with a wet sterile gauze square
6. A 24GA catheter connected to a Hamilton syringe with screw plug should be filled with a saline.
7. Then using a magnification device (surgery microscope – magnification ten times) slowly pick up islets into the catheter
8. Have an assistant hold the syringe and catheter in a vertical position
9. On the distal end of the kidney, make a small incision (1 mm) into the kidney capsule Fig. 12.44b. The capsule can be held using fine tip microsurgical forceps.
10. Ask the assistant to screw the plug in order to approximate the suspension to the tip of the catheter.

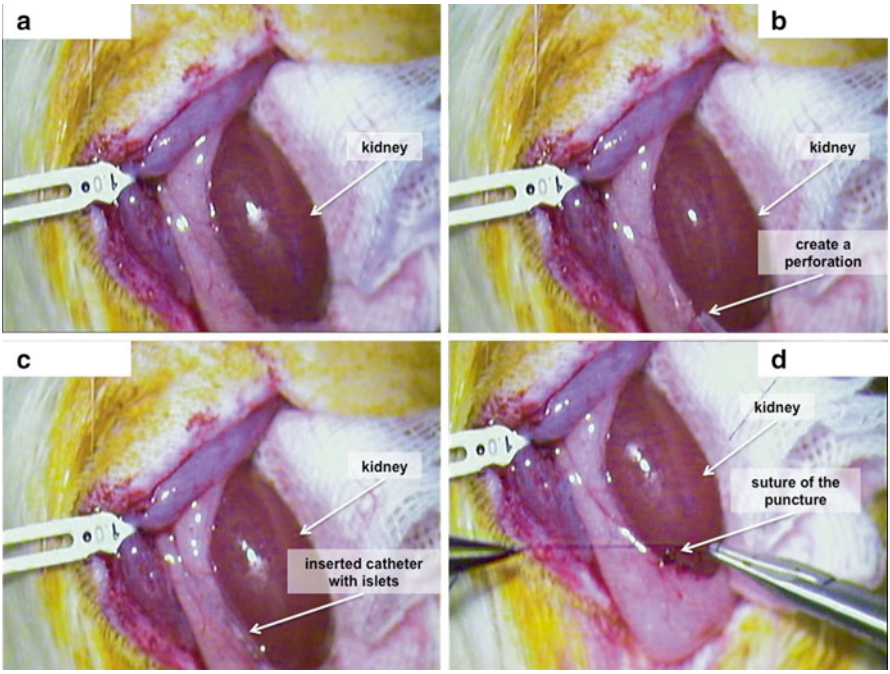


Fig. 12.44 (**a**) Expose and hold the kidney in position using a wet cotton swab, (**b**) make a small incision (1 mm) into the kidney capsule, (**c**) insert the catheter through prepared small incision, (**d**) slowly remove the catheter and close the incision

11. Insert the catheter through prepared small incision and push it gently over the surface to the cranial end of the kidney Fig. 12.44c. The capsule is really very delicate and vulnerable, therefore keep it wet and work extremely gently
12. If the islet pellet is already in, slowly remove the catheter and close the incision (a. with the collagen flour; b. using cauterisation; c. suture it; Fig. 12.44d)
13. Place the kidney back and suture the muscle wall and skin
14. Cover the wound with a gauze square with disinfectant and let the animal recover on a heating pad

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Chapter 13
Small Intestine Transplantation

Michal Kudla and Peter Balaz

Abstract Small bowel rat transplantation (SBT) is time-consuming and technically demanding procedure with high postoperative mortality in the first postoperative days. The SBT can be performed either heterotopic or orthotopic transplantation with portal or systematic venous drainage. Advantages and disadvantages of all procedures are also described. The main factor for surviving of animals is a time of vascular anastomosis (manipulation time). Threshold of manipulation time is recommended less than 45 min. In the end of chapter the tips and tricks are clearly described accomplished with pictures and schemes.

Keywords Small bowel transplantation • SBT • Manipulation time • Orthotopic SBT • Heterotopic SBT • Entero stoma • Surgical technique

13.1 Introduction

Small bowel rattransplantation (SBT) is time-consuming and technically demanding procedure. The success of the procedure is based on the correct choice of an appropriate model and the accurate implementation of the vascular connection with a short clamping time of the donor vessel (i.e. manipulation time). Monchik and Russell [1], in their pioneering study of heterotopicsmall bowel rat transplantation using portocaval anastomosis with terminal enteral stoma of both edges of the graft, achieved a manipulation time between 35 and 60 min. Postoperative mortality in the first 4 days after transplantation was assessed 19.7 % and the main factor of mortality was thrombosis of the graft, peri-operative and post-operative bleeding. Regarding nonsurgical complications, the authors noted the risk with anesthesia and fatal complications during enteral biopsy. A recent experimental study, confirmed the importance of decreasing manipulation time, which is the main factor of graft and animal survival. The threshold of manipulation time in rat SBT is defined as less than 45 min

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[2, 3]. Most animal deaths occurred in the early post-operation period and, for this reason, technical success is considered as a survival with functional graft more than 3 days. From the fourth day of survival, the risk of technical error is significantly decreased. The main causes of death are primarily thrombosis of the vascular anastomosis and bleeding from the anastomosis. However, relatively often we are not able to identify the cause of death even with autopsy. In this situation, the death of animal is results from anesthesia, loss of blood during surgery (more than 2 ml of blood is considered as fatal) or hypothermia during the procedure.

13.2 Models of Small Bowel Transplantation

The first small bowelrattransplantation described in the literature was the study of Monchik and Russell [1] in 1971. From then on, progress and improving of SBT technique continued as described in Table 13.1.

13.2.1 Heterotopic SBT

In this model, the recipient's own small bowel is left in the abdomen and the graft is not connected into the intestinal tract of recipient. The graft venous outflow is reestablished using the vena cava caudalis (Fig. 13.1). The main advantage of this model is relatively easy technical implementation contrary to other models and the possibility to easily perform enteral biopsy of the graft. Even if the heterotopic model of SBT does not imitate the physiological conditions as well as orthotopic transplantation, it is suitable for immunological study and the study of ischemic reperfusion injury.

13.2.2 Orthotopic SBT with Systemic Venous Drainage

Unlike heterotopictransplantation, in this model the small bowel of the recipient is removed and replaced with a donor's graft. Venous drainage is re-established with porto-caval anastomosis. Compared to heterotopic transplantation, this model is

Table 13.1 Models of experimental ratsmall boweltransplantation

Author	Year	Model
Monchik and Russell [1]	1971	Heterotopic SBT with systemic venous drainage
Kort [4]	1973	Orthotopic SBT with portal drainage
Schraut [5]	1983	Heterotopic SBT with portal drainage
Deltz and Thiede [6]	1985	Two-stage SBT (heterotopic-orthotopic)
Wallander [7]	1988	SBT with using "cuff technique" for vascular connection
Szymula von Richter [8]	1996	SBT with reconstruction of lymphatic vessel

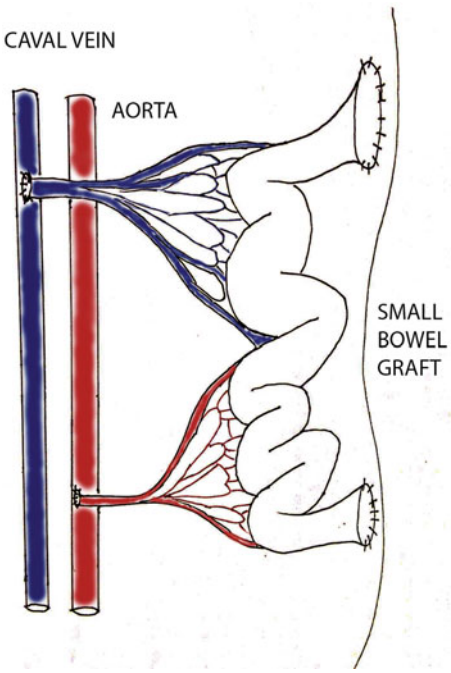

Fig. 13.1 Heterotopic SBT with two entero stoma (sketched by Dr. Petr Wohl)

associated with increased mortality though a skilled surgeon could achieve more than 85 % of animal survival [9] (Fig. 13.2). The main advantage of this model is that the entire graft is exposed to normal intraluminal environment of recipient, which consists of nutrition, gastrointestinal juice and germs and thriving of recipient absolutely depends on functional integrity of the bowel graft. Orthotopic SBT is suitable for physiological research of the transplanted graft, e.g. functional tests, growth of the animal and metabolic changes.

13.2.3 Orthotopic SBT with Portal Drainage

This technique is similar to the above described technique with the difference being the venous anastomosis performed as a porto-portal connection (Fig. 13.3). Ischemia time during the clamping of venous anastomosis is an important factor for success of the procedure. If the time is longer than 15 min venostasis results in increased mortality. Even if this model represents the physiological way of venous drainage, in some studies the superiority over the systemic venous drainage was not proven [10, 11]. This model is always intended for special research such as pharmacokinetics of drugs metabolized after bowel resorption in the liver [2].

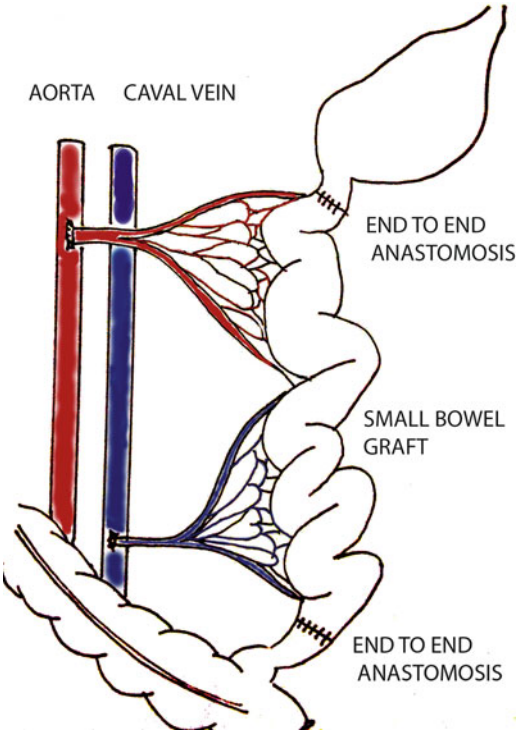

Fig. 13.2 Orthotopic SBT with systemic venous drainage (sketched by Dr. Petr Wohl)

13.2.4 SBT with Using "Cuff Technique"

The standard surgical technique of small boweltransplantation using a "handmade" vascular anastomosis is a technically demanding microsurgical procedure requiring an experienced surgeon with long-time training. For this reason, SBT is always frustrating for the beginner associated with high mortality of experimental animals. The cuff technique is a simple method of microvascular anastomosis where the vascular connection of the polyethylene cuffs are inserted to the donor and recipient vessel and are then connected together (Fig. 13.4). The main advantage of this technique is the easy and fast performance of an unexperienced surgeon. There are exist some different types of this technique where the cuff is used for both anastomoses (arterial and venous) or only for venous anastomosis [3, 12]. The cuff technique is described in detail in Chap. 6.1.5 Vascular Anastomosis with Using "Cuff Technique".

13.2.5 SBT with Reconstruction of Lymphatic Vessel

This part of small boweltransplantation is described in Chap. 14.

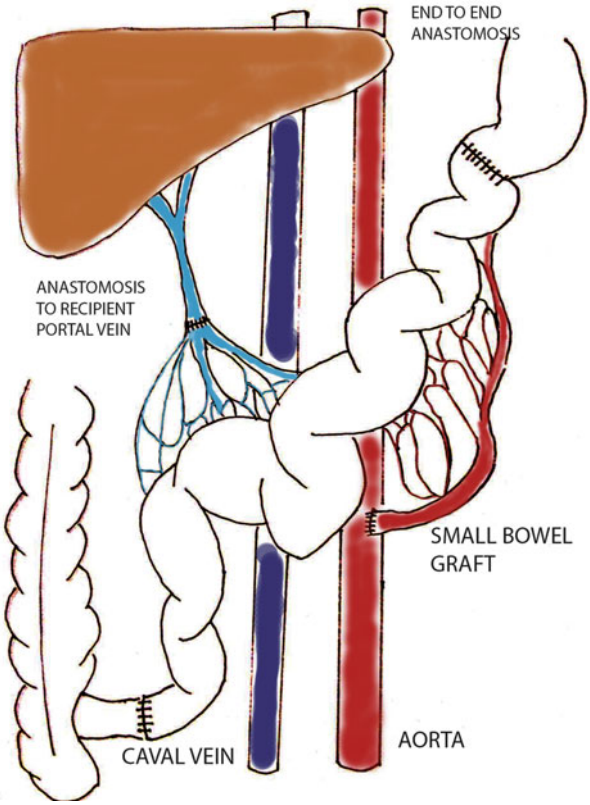

Fig. 13.3 Ortothopic SBT with portal drainage (sketched by Dr. Petr Wohl)

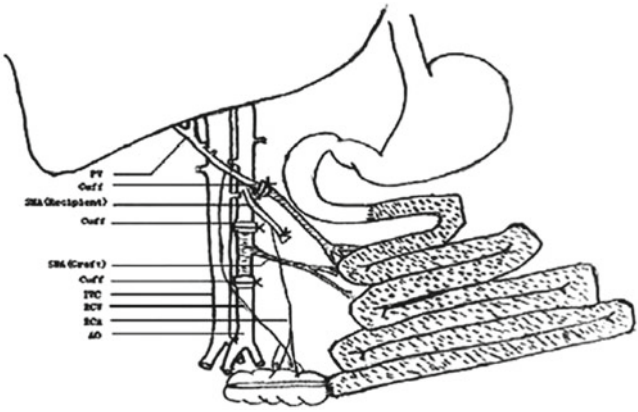

Fig. 13.4 Illustration of the complete reconstruction of the entire small bowel with using "cuff technique" [13]

13.3 Procurement of Small Bowel Graft

13.3.1 Surgical Equipment

Small bowel transplantation is performed under magnification using a surgical microscope. Instruments: scissors, forceps, micro-scissors (straight, curved), two micro-forceps (straight, curved), micro-needle holder. Material: 9-0 nylon suture, 7-0 and 4-0 silk suture, cotton swabs, gauze, saline solution, heparin solution, heating pad, needles, syringes.

13.3.2 Anesthesia

For general anesthesia a mixture of ketamine (10 mg/kg) with chlorpromazine (2.5 mg/kg) intramuscularly is recommended. Anesthesia offset can occur usually after 60–90 min and one third of the total dose should be administered to prolong it. The rat should be positioned on its back with easily extended extremities. The recommended magnification of surgical microscope is 12.5–20.0 times.

13.3.3 Surgical Procedure

1. Perform a midline laparotomy.
2. Retract the small bowel cranially toward the right side of the animal.
3. Sharply dissect the avascular retroperitoneal membrane and divide the colon from the small bowel.
4. Dissect the vasa ileocolica, vasa colica media and vasa colica dextra (in this step, separate the colon from the small bowel).
5. After repositioning the small bowel, identify the portal vein (Fig. 13.5).
6. Dissect the vena pylorica with two silk ligatures and with a cotton swab gently divide the portal vein from the surrounding tissue and from the liver hilus toward to vena lienalis (Fig. 13.6).
7. After assessment of appropriate length of small bowel, interrupt the mesenteric root so that the vascular supply for the graft will be preserved.
8. Aorta preparation starts proximally from the aortic bifurcation (2 cm) by bluntly dissecting towards the stump of arteria mesenterica cranialis (amc). Divide the aorta from the surrounding tissue and from the vena cava caudalis.
9. Ligate or coagulate the small lumbar arteries.
10. A few millimeters below the amc stump, dissect the left renal vein and artery.
11. Cranially from the amc, encircle the aorta and prepare it for ligation (Fig. 13.7).
12. Before cannulation and flushing of the graft, dissect the right renal artery (usually the stump is in the line with the amc).
13. Ligate the aorta above the bifurcation and place another ligature above the stump of the amc.

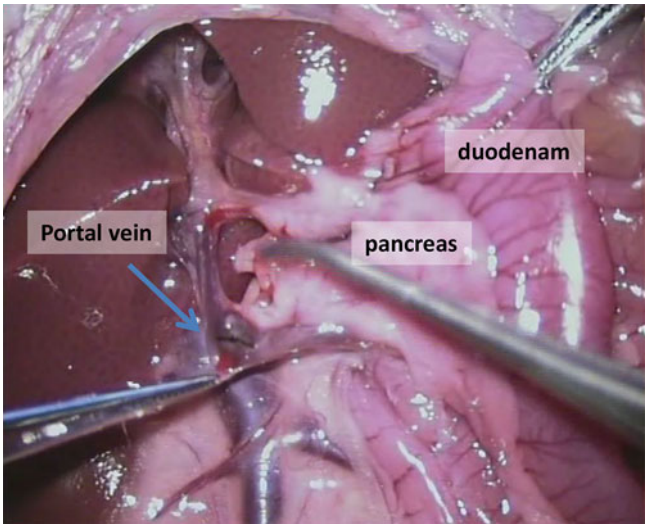

Fig. 13.5 Dissection of portal vein from the pancreas

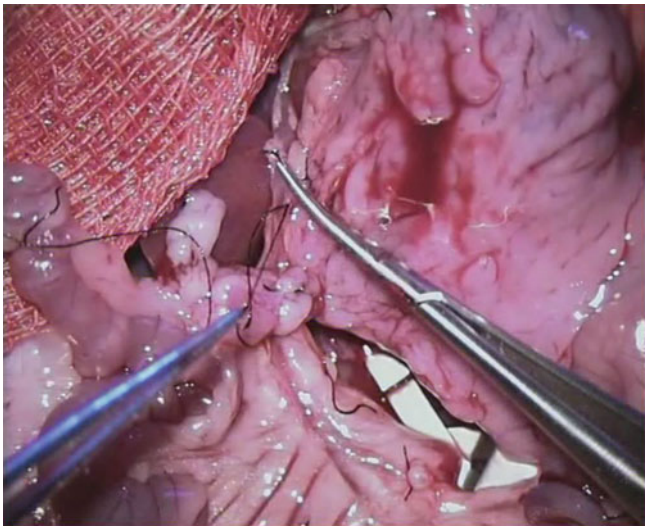

Fig. 13.6 State after dissection of the portal vein in the area of the liver toward to mesenterial root

14. Cannulation is usually performed with a thin syringe needle (25 G) which is passed through anterior wall of aorta into the lumen. Slowly flush the entire graft with heparinized cold solution (4 °C, 100 IU Heparin in 1 ml solution)
15. The aorta is dissected in the place where the needle is inserted and cranially from the ligature above the stump of the amc. The graft is removed from the body and placed in a container with preservation solution (Fig. 13.8).

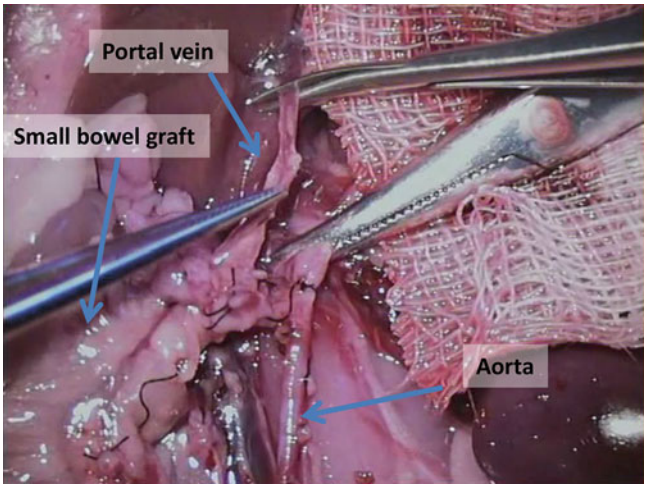

Fig. 13.7 Graft before perfusion

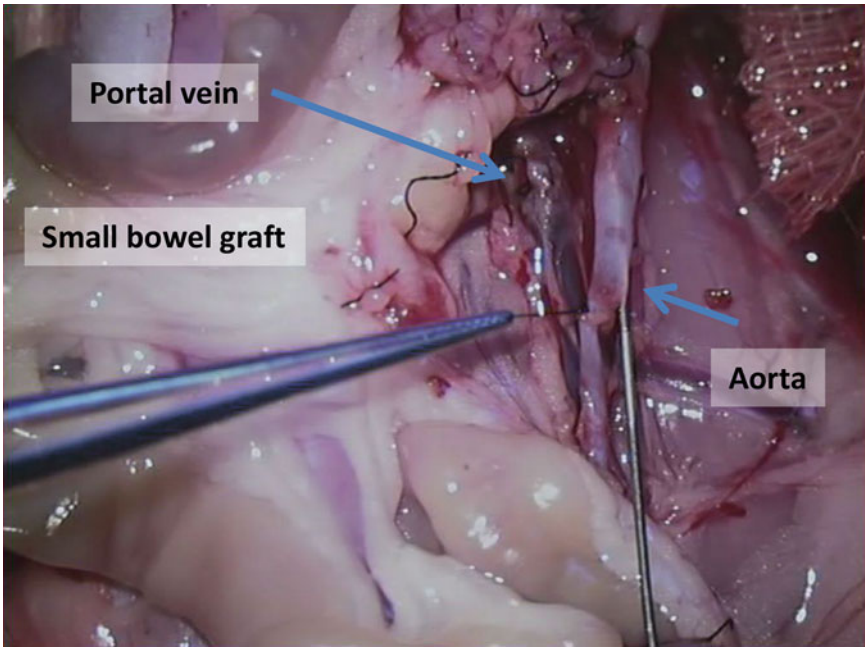

Fig. 13.8 Graft after perfusion

13.3.4 Tips and Tricks

- For better visualization in the surgical field, the laparotomy can be accompanied with bilateral transversal incision of the abdominal wall.
- Pack the small bowel in wet warm gauze.
- During manipulation of the bowel, pay attention to torsion of the mesenteric root to avoid occlusion of blood perfusion (warm ischemia) and injury of the bowel graft.
- To facilitate the outflow of venous blood in the graft perfusion phase, dissect the portal vein near its entry to the portal hilus.
- Assessment of quality perfusion is evaluated according the rate of discoloration of mesenteric vessels.
- For ideal flushing, 3–4 ml of perfusion solution is recommended.

13.4 Heterotopic SBT with Systemic Venous Drainage

Equipment, material and anesthesia are same used in procurement procedure of the small bowel graft listed above.

13.4.1 Surgical Procedure

1. Perform midline laparotomy.
2. After revision of abdominal cavity, turn the heating pad with rat so that the head of animal is turned to the left hand of the surgeon and the tail to the right hand. Pack the small bowel of the recipient in warm gauze and retract towards the left side of the animal.
3. Aorta preparation starts proximally from the aortic bifurcation (2 cm) with bluntly dissection toward to stump of arteria mesenterica cranialis (amc). Divide the aorta from the surrounding tissue and the vena cava caudalis.
4. Ligate or coagulate the small lumbar arteries
5. Mobilize, clamp and incise the vena cava caudalis. Flush the venotomy with heparinized saline.
6. Preparation of the graft. Pack the graft with cold gauze use caution with torsion and compression of the mesenteric root.
7. The first anastomosis is arterial. After aortic clamping, perform the incision and rinse the lumen with heparinized saline (Fig. 13.9).
8. After two polar stitches (Nylon 9-0) to the recipient aorta and aorta of the graft, perform a running suture of the posterior and after anterior wall (Figs. 13.10, 13.11).
9. The venous anastomosis between portal vein and vena cava caudalis is performed in a similar fashion (Figs. 13.12, 13.13).
10. After completion of both anastomoses, release the clamps from the vena cava caudalis (first remove the distal clamp) and then release the clamps from the

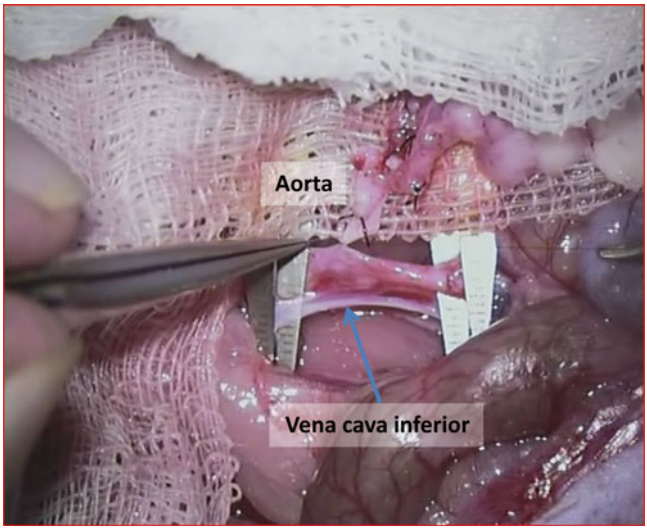

Fig. 13.9 Clamping of the aorta and vena cava caudalis

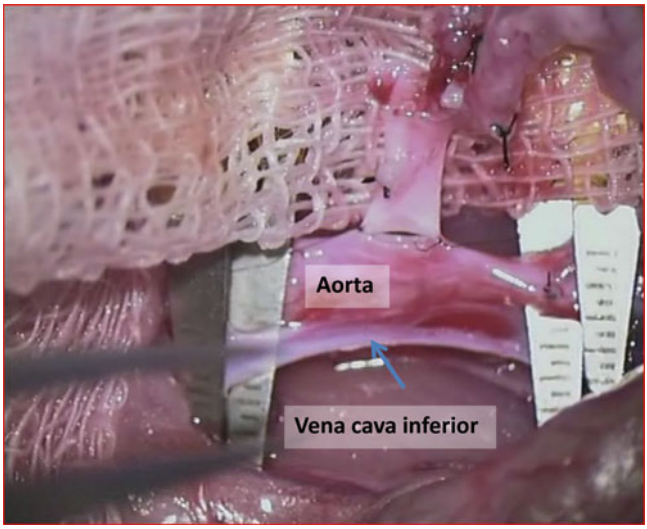

Fig. 13.10 Two polar stitches in arterial anastomoses

aorta (first remove the distal clamp). At this time, the reperfusion of the graft occurs (Fig. 13.14).

11. Cold ischemia time starts at the time of perfusion of the graft in the donor and finished at the time of reperfusion in the recipient body. Manipulation time is defined as a time of graft removal from preservation solution to the time of release of the arterial clamp.

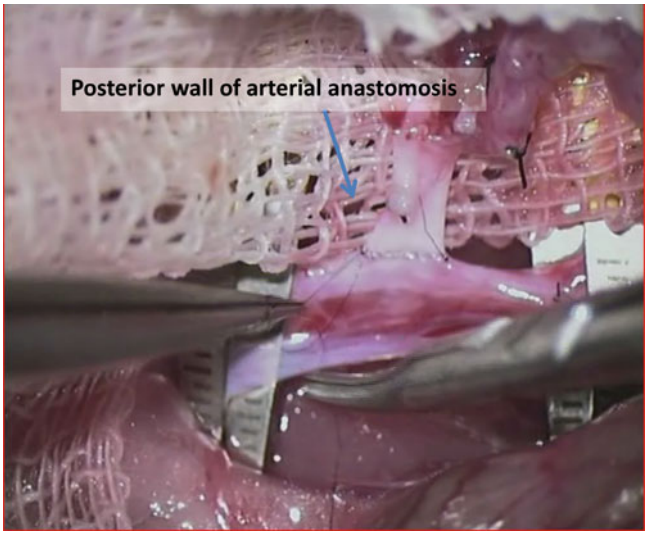

Fig. 13.11 Complete arterial anastomoses

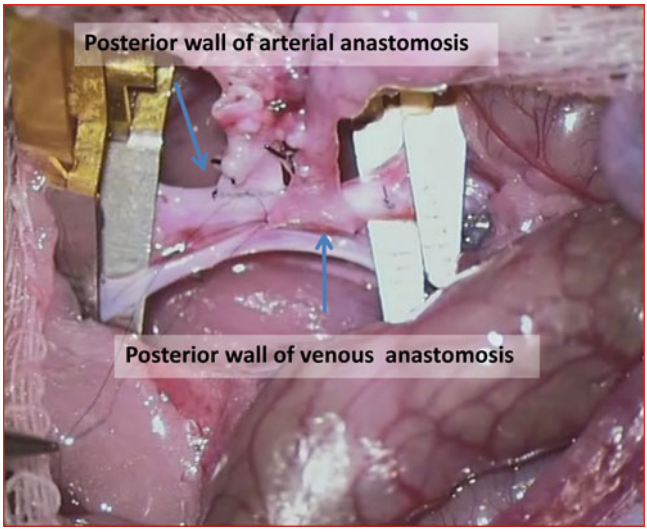

Fig. 13.12 Posterior wall of the venous anastomoses and arterial anastomoses

12. Check the patency of anastomosis and quality of graft reperfusion.
13. The oral end of bowel is ligated and the aboral end is used for entero stoma. Entero stoma (terminal) is fixed in the right distal quadrant of the abdomen (Fig. 13.15) with single stitches to the skin (Prolene 7-0).
14. The abdominal cavity is flushed with warm saline and abdominal wall is closed in two layers.

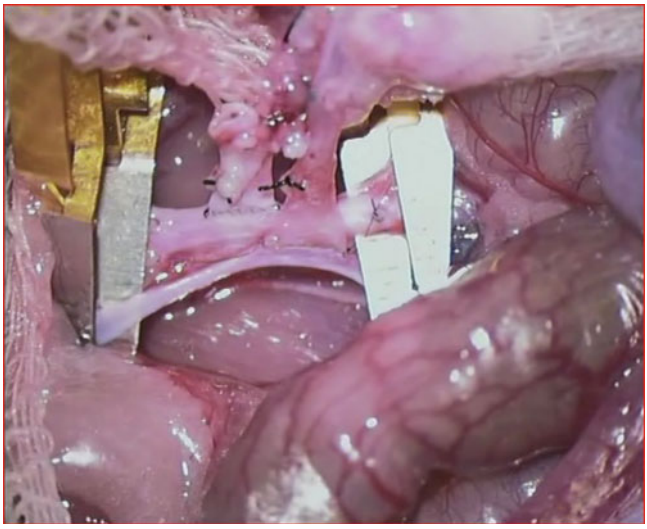

Fig. 13.13 Vascular anastomoses completed, status before reperfusion

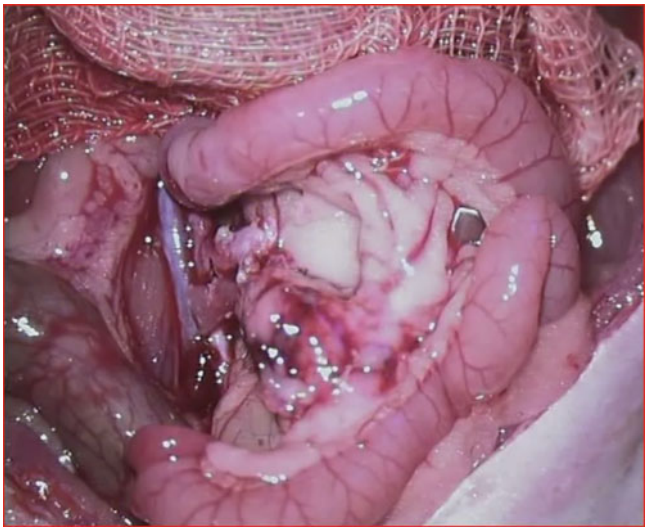

Fig. 13.14 Reperfusion of the graft

13.4.2 Tips and Tricks

- For the better visualization and avoid to contamination of the anastomotic area, put a strip of a silicon glove behind the aorta and vena cava caudalis.
- The place for venous anastomosis is positioned distally to the arterial anastomosis to avoid compression with arterial anastomosis and to avoid tension of venous anastomosis.

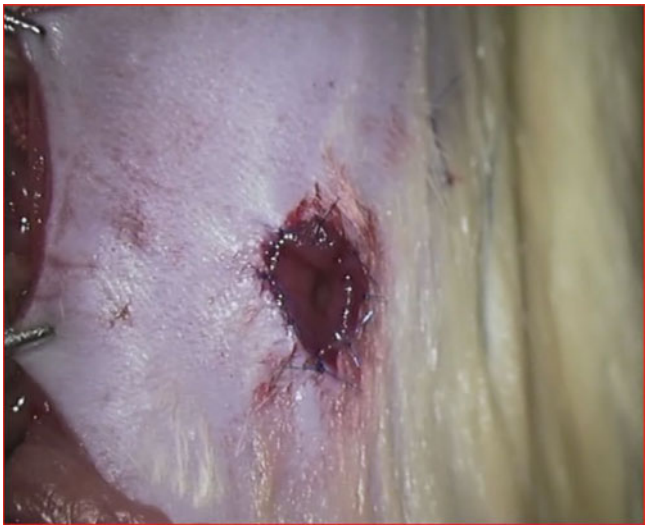

Fig. 13.15 The terminal entero stoma in right part of abdomen

- Before reperfusion, gently touch the anastomosis with a cotton swab for hemostasis and, in case of bleeding, add a new single stitch.
- Pack the graft at the time of vascular anastomoses in cold gauze and after reperfusion change it to warm gauze.

13.5 Orthotopic SBT with Systemic Venous Drainage

The surgical technique in steps 1–11 is identical with procedure of heterotopicsmall boweltransplantation.

1. Dissect and ligate the vessels in the mesentery so that the perfusion is preserved only for first 2 cm of oral jejunum and 2 cm of terminal ileum.
2. Cut the bowel in the vital zone and remove the small bowel from the recipient body.
3. Jejuno-jejunal entero anastomosis is performed with running or interrupted suture (Vicryl 7-0) and starts in the mesenterial side of the bowel.
4. Ileo-ileal entero anastomosis is performed in the same fashion.
5. To avoid stenosis of the anastomosis, put into the lumen small part of Macaroni, this serves as a stent. After completion of the bowel anastomosis, Macaroni can gently be shifted distally to the small bowel where will be soon absorbed. Do not worry about enteral obstruction (Fig. 13.16).
6. Flush the abdominal cavity with warm saline and abdominal wall close in two layers.

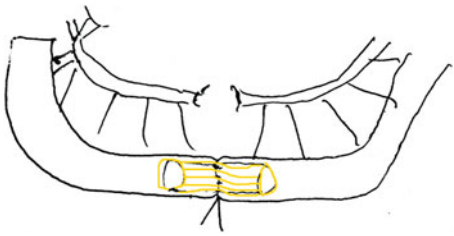

Fig. 13.16 Macaroni inserted into the lumen of the bowel during entero anastomosis

13.5.1 Types of Restoration Intestinal Tract in SBT Model

The oral and aboral ends of the bowel are used as terminal enteral stoma.

The oral end of the graft is used as terminal enteral stoma and the aboral end is anastomosed to the ileum of recipient.

Close the oral end of bowel and anastomose the aboral with the ileum of the recipient.

Close the oral end of bowel and the aboral end is used as terminal enteral stoma.

13.5.2 Postoperative Care

Immediately after procedure, transport the animal to the heated box at 37 °C. The recovery from the anesthesia usually takes 30–45 min. Water intake is immediate after the procedure and food intake with granules from the first day after surgery. Perform daily an assessment of the weight and clinical status of the animal. In the third and seventh day after transplantation, flush the stoma with 1–3 ml of saline as a prevention of occlusion with enteral clot. The analgesic regimen is standard.

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Chapter 14
Transplantation of Lymph Vessels

Michal Kudla

Abstract The lymphatic system possesses very good regeneration capability, but it has its limitations. When the regeneration is not complete, within months or years there might develop decompensation of the lymphatic system leading to swelling of the tissue which contributes to worsening of the graft function. Investigating lymphatic vessels transplantation requires an appropriate model. The small intestine possesses a very well developed lymphatic system due to the enormous surface of mucosa and to continuous contact with infectious bowel content. Small bowel transplantation accompanied with reconstruction of lymphatic vessels appears to be the most appropriate model to study lymphatic vessels function in solid organ transplantation.

Keywords Rat • Lymphatic vessels • Transplantation • Small bowel

14.1 Introduction

The lymphatic system possesses very good regeneration capability, but it has its limitations. When the regeneration is not complete, within months or years there might develop decompensation of the lymphatic system leading to swelling of the tissue which contributes to worsening of the graft function.

Investigating lymphatic vessels transplantation requires an appropriate model. The small intestine possesses a very well developed lymphatic system due to the enormous surface of mucosa and to continuous contact with infectious bowel content. Small bowel transplantation accompanied with reconstruction of lymphatic vessels appears to be the most appropriate model to study lymphatic vessels function in solid organ transplantation.

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14.1.1 Spontaneous Regeneration of Lymphatic Vessels After Small Bowel Transplantation

Up to now, the behavior of lymphatic vessels after solid organ transplantation has been studied solely in small bowel transplantation. The main reason is that lymphatic vessels play an extremely important role in the small bowel function as they participate in absorption of liquids and fats from the bowel and their effective function is also relevant for bowel allograft function. All the studies with small bowel transplantation show more or less that the same regeneration capability of lymphatic vessels as was observed in the experimental block of lymphatic drainage in a dog's limb [1, 2]. The bowel allograft presents all the symptoms of lymphostasis from the first postoperative day [3, 4]. Microscopy reveals dilatation of lymphatic vessels in all of the bowel wall thickness. Lymphatic angiography reveals dilatation of the lymphatic collectors in mesentery and retrograde flow back to the bowel wall and the absence of lymphatic drainage from the bowel allograft. Several hours after the transplantation, the spontaneous closure of lymphatic vessels can be seen which makes impossible even simple drainage of lymph into the abdominal cavity [5, 6].

New formation of many small lymphatic vessels around the suture of mesentery starts 3 or 4 days after autologous transplantation. They create anastomoses with the recipient's lymphatic system. In allogenic transplantation, this process takes much longer, usually starting 1 or 2 weeks later and the delay is probably influenced by rejection [4, 7]. The newly formed anastomoses grow slowly and become wider which leads to the regression of swelling. Lymphatic angiography can demonstrate lymphatic flow from the bowel into thoracic duct of the recipient. It can also confirm the transfer of fatty acids and chylomicrons from the bowel allograft [8].

Complete recovery of the truncus intestinalis, which is the largest lymphatic collector of the small bowel, has never been reported in any experimental study. Experimental work presented by Rotman has provided proof of the partial recovery of the lymphatic system. He also described an existing flow from the small bowel after 210 days after small bowel autotransplantation not only into thoracic duct but also through lymphatic-venous anastomoses into the portal vein and to the liver [9]. This situation under normal conditions does not occur and is present only in case of lymphostasis [10]. High demands on lymphatic drainage tend to cause chronic decompensation of the incompletely regenerated lymphatic system and this leads to the development of lymphedema. In our case, this can deteriorate the function of the small bowel.

The lymphatic system plays important role in the immunity response of the organism and its defense from infections, etc. Lymphocytes and antigen presenting cells travel from peripheral tissues through lymphatic vessels to the lymphatic organs where the immunity response to pathogens and alien antigens takes place [11]. The function of this system is significantly influenced when lymphatic vessels are damaged or disrupted.

During the solid organ transplantation, there is disruption of the vascular, nervous and lymphatic supply of the organ. The arterial and venous system in the small

bowel must be reconnected within 6–12 h from discontinuation of the blood circulation. The consequences of disruption and reconnection in the nervous and lymphatic system have not sufficiently explored yet.

Up to now, in the experimental studies focusing on the long term function of the small bowel allograft, there has been reported a malabsorption of lipids and increased bowel permeability for manitol and polyethylene-glycol [12]. The same changes occur also in autogenic transplantation. Therefore, we cannot blame this on immunity mechanisms but we expect participation of consequences of bowel denervation and poor regeneration of the lymphatic vessels.

Kanakogi demonstrated that mesenteric lymph nodes (MLNs) in the bowel graft play a very important role in the immune response of the organism especially through circulating leucocytes contained in the MLNs of the graft [13].

Thus far there exist two publications engaged in the relevance of lymphatic vessels reconstruction in the small bowel transplantation [14, 15]. Both cases established the statistically relevant extension of survival in experimental animals with reconstruction of lymphatic vessels (lymph-lymphatic anastomosis) and treatment with a sub-therapeutic dose of Cyclosporine A. There were no significant differences in the length of survival in the group without immunosuppressive treatment.

Szymula was the first who revealed significant prolongation of survival in rats with reconstruction of the lymphatic vessels. He used a model of allogenic transplantation (Brown Norway to Lewis) and he administered Cyclosporine A in dose 10 mg/kg/day only in the first 5 days. The survival of animals without immunosuppression was about 10 days. With immunosuppression, the survival length increased to 20 days. Animals with immunosuppressive treatment with Cyclosporine A and lymphatic vessels reconstruction survived longer than 200 days.

Kellersman in his work divided the rats into four groups after small bowel transplantation. It was an allogenic orthotopic small bowel transplantation of Brown Norway to Lewis rats. He divided the rats according to dose of administered immunosuppression, Cyclosporine A (CsA) in dose 0, 2, 5 and 10 mg/kg/day, treatment was given from postoperative day 0 until day 6. In each group, he evaluated the relevance of the lymphatic anastomosis according to the length of survival of the animals. In the group without immunosuppression and in the group with the highest dose of CsA, there was no influence of lymphatic reconstruction on survival. In contrast, the group of animals with subtherapeutic dose of Cyclosporine A (CsA) revealed a significantly longer survival of the rats with the reconstruction of lymphatic vessels anastomosis. The histology was evaluated 100 days after transplantation. In the surviving animals, there was a significant difference only in the extent of the mucosal erosions but, on the other hand, there were no significant differences in the damage of goblet cells, in bleeding into intestinal villi, in lymphatic infiltration, vasculitis or even cryptitis.

In contrast to these studies, another calcineurin inhibitor – tacrolimus in dose 0.15 mg/kg/day - has also been used for immunosuppression. Tacrolimus is nowadays an integral part of most of the immunosuppressive regimens after bowel transplantation. As the main criteria for assessment of the importance of lymphatic vessels reconstruction, the histological signs of acute rejection on postoperative day

7 were chosen. On the third postoperative day in the biopsies, there were no signs of acute rejection. On the seventh postoperative day, biopsy revealed four cases of modes rejection and one case of medium intensity acute rejection. In both previous studies, the main criteria was survival of the animals and they used model of chronic rejection after small bowel transplantation (SBT). In our study, we practiced model of heterotopic SBT, Brown-Norway (BN) as donor and inbred males of strain Lewis (LEW) as recipient, while in previous referred studies a model of orthotopic SBT [16] was used.

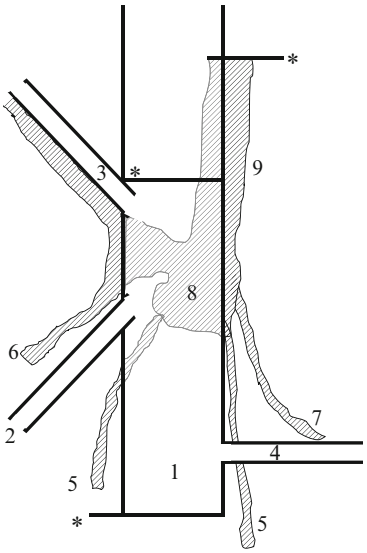
14.2 Anatomic Notes

14.2.1 Derivation of Lymph in the Rat Bowel

In every intestinal villus, there are some blind endings of lymphatic vessels and their amount is specific for every strain. With rats, this amount corresponds to 3–10 lymphatic vessels per one villus [17]. In the base of the villus there is a confluence of lymphatic vessels into a wide sinus which releases another two or three minor vessels. These small vessels penetrate through lamina muscularis mucosae and they develop a lymphatic plexus in submucosa. Mucosal and submucosal lymphatic vessels do not possess any valves as the lymphatic flow is maintained only by contractions of the intestinal wall. In the tunica muscularis, one more lymphatic system exists which is less developed when compared to the previous one and these two systems are not connected either in anatomy or in function. Both systems are connected right in the mesentery and develop larger collectors already containing valves and presenting rhythmic contractions. In the mesentery, there are always two or three lymphatic collectors accompanying the artery and the vein. Collectors continue through the mesentery into regional lymph nodes around cranial mesenteric artery. Here, the collectors merge into a big trunk called truncus intestinalis which is so firmly attached to arteria mesenterica communis that separation is not possible even by extremely gentle preparation. Truncus intestinalis merge with two other collectors (trunci lumbales) and the lymphatic collector from the left kidney on the dorsal side of abdominal aorta and generate cysterna chyli. This is further collects lymph from the celiac trunk (truncus coeliacus) and generating thoracic duct. The thoracic duct goes through diaphragm into the chest on the backside of aorta (Picture 14.1).

14.3 Lymph Vessels Graft Procurement

Animals The experiment was carried out on inbred males of strain Brown-Norway (BN) as donors and inbred males Lewis (LEW) as recipients.



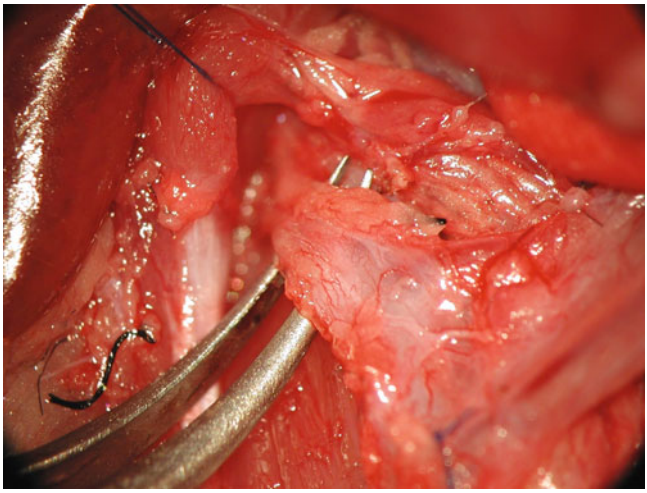
Picture 14.1 *1* aorta abdominalis, *2* arteria mesenterica cranialis, *3* arteria celiaca, *4* arteria renalis lat. sin., *5* truncus lumbalis, *6* truncus intestinalis, *7* lymphatic collector of left kidney, *8* cysterna chyli, *9* ductus thoracicus. *The area where aorta was interrupted and eventually the thoracic duct at organ removal (see Chap. 13, small bowel transplantation)

Equipment + Material Scissors, forceps, micro-scissors (straight, curved), two micro-forceps (straight, curved), micro-needle holder, Operating microscope, 9–0 nylon suture, 7–0 and 4–0 silk suture, cotton swabs, gauze, saline solution, heparin solution, heating pad, needles, syringes.

14.3.1 Operation of Donor

Operation of donor differs from isolated small bowel transplantation in some steps. The initial phase of operation, position of animal, anesthesia and instruments are identical with the description in Chap. 13. After release of aortic caudal part above the bifurcation, and after release of portal vein and both ends of jejunum, start with operation of the lymphatic vessels.

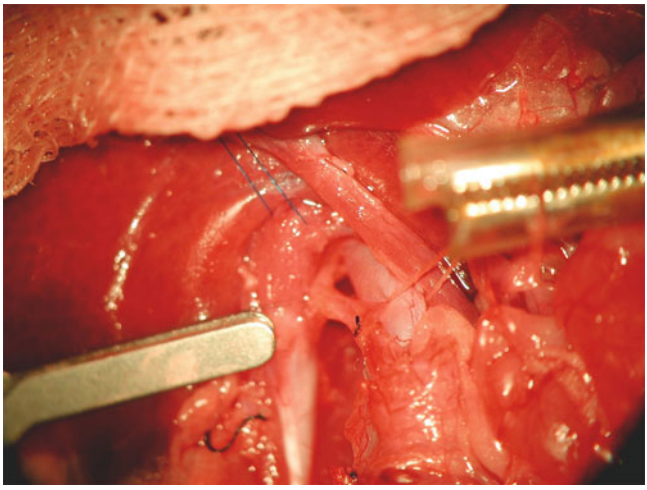
1. Gently separate the aorta from the retroperitoneum up to the base of arteria mesenterica cranialis
2. Between the ligatures, look up and cut up both trunci lumbales and the lymphatic collector of the left kidney
3. Dissect the ductus thoracicus from animal's right side (Picture 14.2). Follow the ligation of the right renal artery which deviates from the aorta on the same level as the arteria mesenterica cranialis and lymphatic collector of the right kidney
4. Release the aorta from the mesentery up to above the base of the celiac artery. Be extremely careful at this point in order to avoid damage of the cysterna chyli, which is attached to the dorsal side of the aorta in the place of the base of mesenteric artery and the thoracic duct on the dorsolateral side of the aorta



Picture 14.2 Detail of thoracic duct

5. Above the base of the celiac artery, separate the thoracic duct from the aorta and continue the blunt dissection of thoracic duct from the aorta and continue up to diaphragm. Ligate the small lumbar arteries coming out of the lumbar aorta
6. Ligate the celiac artery together with the connecting lymphatic vessels
7. After blunt dissection and separation of the left part of aortal segment from retro-peritoneum, the graft is ready to be rinsed with preservative solution. The graft consists of a 7–10 cm long segment of jejunum with the corresponding part of the mesentery and portal vein, aortal segment from bifurcation up to the base of the celiac artery and the lymphatic vessels collecting lymph from the small bowel graft and opening out into the thoracic duct (Picture 14.3)
8. Ligate the aorta above the bifurcation and above the celiac artery base
9. Insert the cannula for perfusion into the aorta just above the bifurcation
10. Interrupt the portal vein before the insertion into liver and start with perfusion with approximately 3–4 ml of physiological saline solution with heparin
11. After the perfusion is finished, interrupt the aorta in the site of cannula insertion and above the ligature of the aorta below the diaphragm
12. Remove the graft and place it into the cold saline solution
13. Euthanize the animal

Preparation of lymphatic vessels prolongs the time of donor operation by about 45–60 min.



Picture 14.3 Situation before rinsing the graft with preservative solution, separated aorta, portal vein and thoracic duct

14.3.2 Tips and Tricks

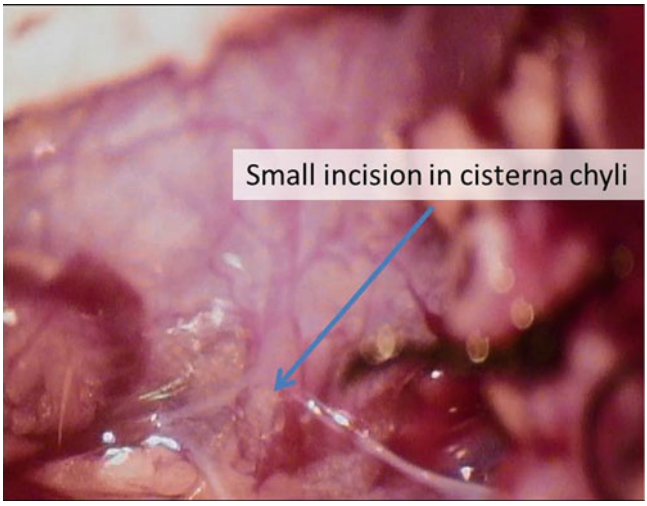
- Twelve hours before the surgery, the animals (donors and recipients) should not get any food. Access to water is not limited until the surgery.
- Work under the maximum possible magnification of microscope.
- The amount of the saline solution for perfusion depends on the quality of perfusion. Rinse the graft until the solution coming out of portal vein becomes limpid.

14.4 Lymph Vessels Graft Transplantation

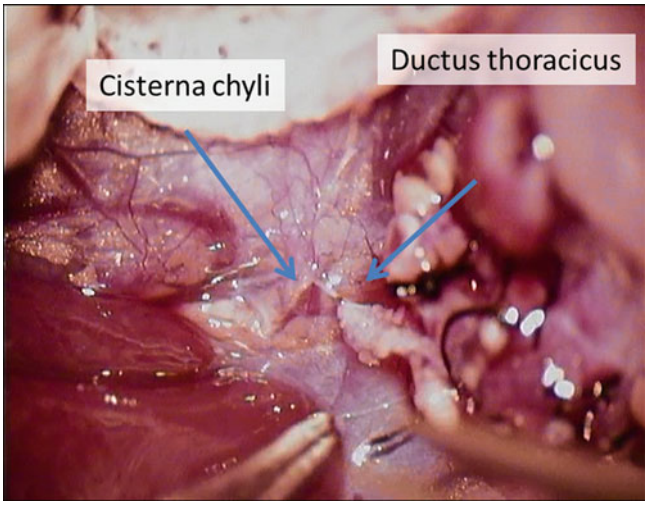
Equipment + Material Scissors, forceps, vascular clamp, micro-scissors (straight, curved), two micro-forceps (straight, curved), micro-needle holder, operating microscope, Vicryl suture 10–0 or 11–0, 9–0 nylon suture, 7–0 and 4–0 silk suture, cotton swabs, gauze, saline solution, heparin solution, heating pad, needles, syringes.

The initial phase does not differ from procedure described in Chap. 13.

1. Start with lymphatic vessels reconstruction only after the arterial and venous anastomoses are ready and after reperfusion of the transplanted small bowel.
2. Gently release cysterna chyli below the base of the arteria mesenterica cranialis from the aorta. On its ventral side, carry out a small longitudinal incision which becomes oval due to retraction. Dry up the lymph from surgical field in order to keep clarity.

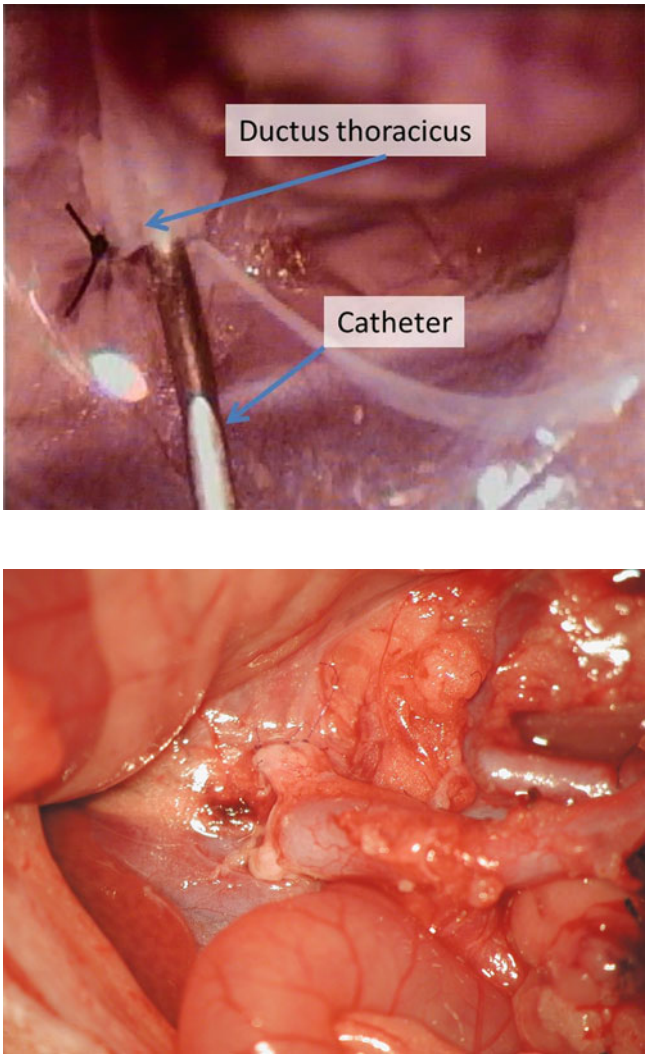


3. Now connect the donor's thoracic duct with the recipient's cysterna chyli. Use Vicryl 10–0 or 11–0 suture. First, put a stitch on the right upper margin of the aperture in the cisterna chyli.



4. Continue anticlockwise stitch by stitch and place the nodes outside the anastomosis. Usually 4–6 stitches are enough (Picture 14.4)

Reconstruction of lymphatic vessels prolongs the time of transplantation by about 30–45 min.



Picture 14.4 Surgical field after completion of arterial, venous and lymphatic anastomosis

14.4.1 Tips and Tricks

- Twelve hours before the surgery the animals (donors and recipients) should not get any food. The access to water is not limited until the surgery.
- Again work under the maximum possible magnification microscope.
- Be very careful to keep the suture without tension to avoid rotation of the thoracic duct.
- Before establishing the last two stitches, check the passage of the anastomosis with a tiny cannula

- The anastomosis does not have to be completely waterproof because the pressure in the lymphatic system is much lower than in the venous system and, in the case of leak, it will spontaneously close in short time.
- During the stitching, it is advisable to rinse the surgical field and thoracic duct regularly in order to avoid sticking the vascular walls which could cause stenosis of the anastomosis.
- At the end of suture, add an adaptation stitch from the cranial end of donor's aorta to the retroperitoneum. This decreases the traction in the lymph-lymphatic anastomosis.

14.4.2 Postoperative Care

After the surgery the animals should be placed into heated cages with access to water. They should be given standard laboratory food on the first postoperative day. Measure the rats' weight and monitor the health condition.

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Chapter 15
Transplantation of Vascular Allograft

Ivan Matia, Martin Varga, and Peter Fellmer

Abstract The implantation of syngeneic and allogeneic veins and arteries is used in clinical cardiac and vascular surgery in broad spectrum of indications. Experiments on animals represent an important part of cardiovascular research of vessel grafts. The arterial and vein allografts models are used for both studying the pathology of their acute rejection and to test different immunosuppressive regimens in vivo. Moreover, the aortic allograft model in the rat was originally introduced as a model for chronic rejection in transplant experimental medicine. Syngeneic vein-to-artery graft models are widely used for both studying the pathology of vein graft disease and to test therapeutic strategies in vivo. In this chapter, we describe in detail abdominal aortic to abdominal aortic and iliolumbar vein to abdominal aortic transplantation models between Brown-Norway (RT1n) and Lewis rats (RT1l). These genetically inbred rats differ at major and minor histocompatibility loci resulting in massive rejection of both arterial and vein allografts. Moreover, the iliolumbar vein to abdominal aortic transplantation model between syngeneic animals is useful to study vein graft intimal hyperplasia which represents a significant clinical problem in vascular medicine.

Keywords Arterial allografts • Venous allografts • Arterialisation • Aortal grafts • Arterial transplantation • Vessel transplantation • Intimal hyperplasia • Experimental immunosuppression • Graft rejection

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15.1 Transplantation of Abdominal Aortic Allograft

15.1.1 Introduction

Rat aortic allograft is an attractive experimental model of vascular rejection. Vascular rejection remains the major factor impeding long-term survival of transplanted organs in humans and the arterial wall seems to be the main target in the process of chronic rejection [1].

The rat aortic allograft model allows a specific focus on the arterial wall immunological targeting and the response induced by allotransplantation because of the plain structural organization of the arterial wall in three easily identifiable, differentiated cell layers: the endothelium, the media and the adventitia. In contrast with the rejection of other transplanted, more complex functional organs, untreated rejection of arterial allografts is associated with injury compatible with the maintenance of the main elementary arterial wall functions (blood contention and conductance). Chronic arterial wall rejection could therefore be studied in the absence of immunosuppressive treatment [1].

A lot of rat aortic transplants experiments are performed between Brown-Norway rats (BN) (RT1n) as donors and Lewis rats (LEW) (RT1l) as recipients. These genetically inbred rats differ at major and minor histocompatibility loci resulting in massive rejection of donor aortic grafts [2]. Arterial wall allograft rejection is characterized by three major components: an adventitial inflammatory infiltration followed by fibrosis, the disappearance of medial smooth muscle cells and an intimal proliferation [1].

In summary, the arterial allograft represents a useful experimental model to study due to:

1. Response of the arterial wall to allotransplantation [3]
2. Different cells [4] and alloantibodies [5] involved in the chronic rejection
3. The influence of immunosuppression [6] and other pharmacological regimens [7] on the vascular rejection
4. The influence of different other modalities [8–10] on vascular rejection

Our group used the BN to LEW aortic transplantation model to study the possibility delaying the use of immunosuppressive therapy with tacrolimus in recipients of arterial allografts [11].

15.1.2 Preconditioning of the Animal Prior to Surgery

For aortic allograft transplantation, we used Brown-Norway rats (BN) (RT1n) weighting 220–320 g as donors and Lewis rats (LEW) (RT1l) weighting 240–380 g as recipients. Each animal was housed in a separate cage. Food was taken away 2 h before and water just before the operation.

The donor animals were anaesthetized by an intramuscular injection of ketamine (Narkamon®, Spofa a.s., Prague, Czech Rep) at 100 mg/kg and xylazine (Rometar®, Spofa a.s., Prague, Czech Rep) at 10 mg/kg.

The recipient animals with planned follow-up were anaesthetized with less invasive anesthesia (intramuscular injection of sufentanil (Sufenta®, Janssen Pharmaceutica Inc., Beerse, Belgium) at 20 μg/kg and azaperone (Stresnil®, Janssen Pharmaceutica Inc., Beerse, Belgium) at 1 mg/kg) to ensure more natural awakening. If necessary, an additional dose (1/3 of the first dose) was injected intramuscularly to upper limbs because of the interruption of the blood supply to the lower half of the body by the aortic clamp.

15.1.2.1 DO's Just Before the Surgery

Evaluate the rat to ensure that it is apparently healthy, weight it and calculate the dose of anesthetic drugs. The ideal weight of the donor should be 200–300 g.

The animal should be prepared in an area away from the surgical area (Note: animal preparation includes anesthetic induction, hair clipping and initial scrub).

Induce anesthesia and check anesthetic depth after the required induction time by verifying lack of withdrawal with a firm toe pinch (the toe pinch method).

After the animal is anesthetized, apply a bland sterile ophthalmic ointment to the eyes to prevent drying, which could result in development of corneal ulcers. (Note: Animals do not close their eyes when anesthetized and they do not blink.)

Remove fur from the surgical site using electric clippers.

Scrub the shaved skin with a chlorhexidine or povidone iodine soaked gauze/cotton.

15.1.3 Abdominal Aortic Allograft Procurement

15.1.3.1 Equipment + Material

Plastic foil to cover visceral organs during the surgery (Fig. 15.1a), gauze to cover the plastic foil (Fig. 15.1b), the gauze should be wetted at intervals with warm saline solution to avoid temperature decrease of animal body.

Heating apparatus to keep the saline solution warm.

Small fluid containers with saline solution to clean the microsurgical instruments during the surgery if necessary (Fig. 15.1c).

Cotton swabs (Fig. 15.1k), 5/8th inch (16 mm) orange needle, syringes (2 ml, 5 ml, 10 ml), heparinized saline solution (concentration 100 IU/ml), thiopental (Thiopental®, Spofa Czech Rep).

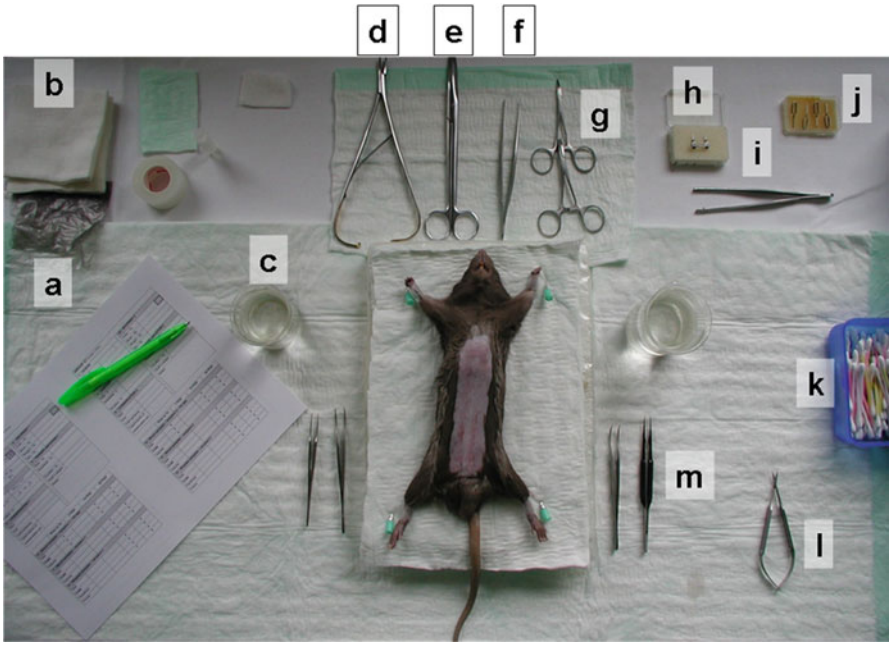

Fig. 15.1 Equipment and material for aortic graft procurement

15.1.3.2 Surgical Instruments

One scissors (Fig. 15.1e), one forceps (Fig. 15.1f), 2 × small dissecting forceps (Pean) (Fig. 15.1g).

15.1.3.3 Microsurgical Instruments

One micro-scissors (curved) (Fig. 15.1l), two micro-forceps (one straight, one curved) (Fig. 15.1m), 8-0 silk suture.

15.1.3.4 Procedure

1. Place animal on a clean pad (Fig. 15.2a)
2. Position with tape. Do not overstretch the legs or bind them in such a way as to restrict circulation (Fig. 15.2b).
3. Check level of anesthesia again using the toe pinch method (verifying lack of withdrawal with a firm toe pinch).
4. Make the midline laparotomy from urinary bladder to xyphoid using surgical scissors and forceps (Fig. 15.2c).
5. Pull away wall flaps with an abdominal retractor (Fig. 15.2d)

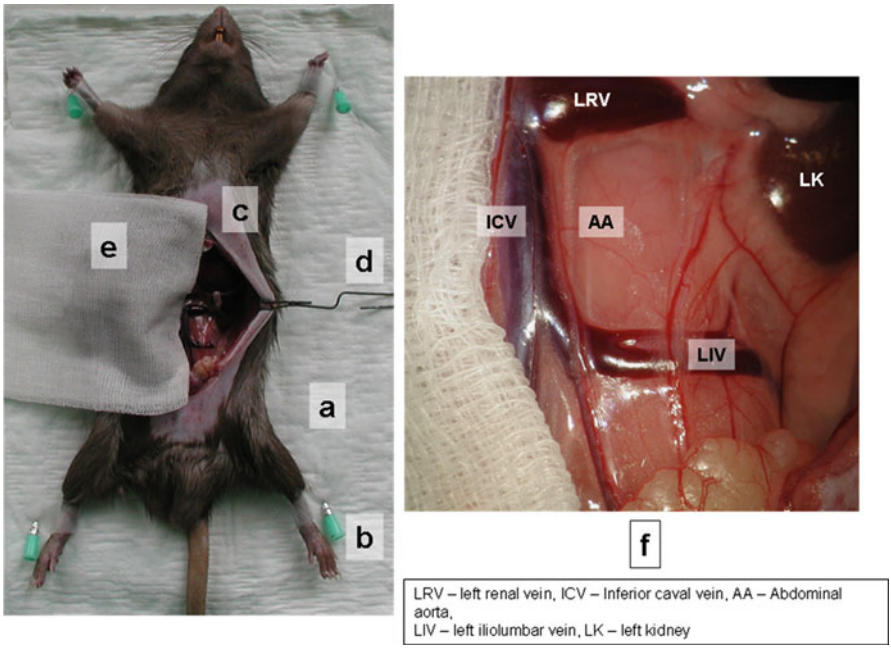

Fig. 15.2 Position of animal donor and focusing of microscope field

6. Retract the small bowel on the animals' right side (from the view of animal) and cover it with plastic foil and warm gauze (Fig. 15.2e).
7. Install the operation microscope (Fig. 15.2f).
8. Dissect retroperitoneum and retroperitoneal fat and carefully identify inferior caval vein and abdominal aorta (Fig. 15.3a)
9. Gently separate inferior caval vein from abdominal aorta using cotton swabs and microsurgical forceps (Fig. 15.3b). Be careful with the inferior caval vein manipulation with forceps as the vein wall is very fragile.
10. Ligate the lumbar arteries using 8-0 silk suture (Fig. 15.3b, arrow) to obtain a long mobile aortic graft reaching from renal arteries to aortic bifurcation.
11. In some anatomical variations, is it necessary to ligate iliolumbar vein crossing the aorta from ventral (Fig. 15.3a, arrow).
12. Make a puncture of the inferior caval vein with 5/8th inch (16 mm) orange needle (Fig. 15.3c, arrow) and apply 2 ml of heparinized saline solution (concentration of 100 IU/ml)
13. Use the same application method for applying of lethal doses of thiopental
14. Apply small dissecting forceps on aorta and inferior caval vein both in the region of renal arteries and aortic bifurcation and dissect the aorta between both dissecting forceps (Fig. 15.3d, arrows)
15. Flush ex situ the aortic lumen with heparinized saline solution (concentration of 100 IU/ml) using 5/8th inch (16 mm) orange needle and 2 ml syringe (Fig. 15.4a).

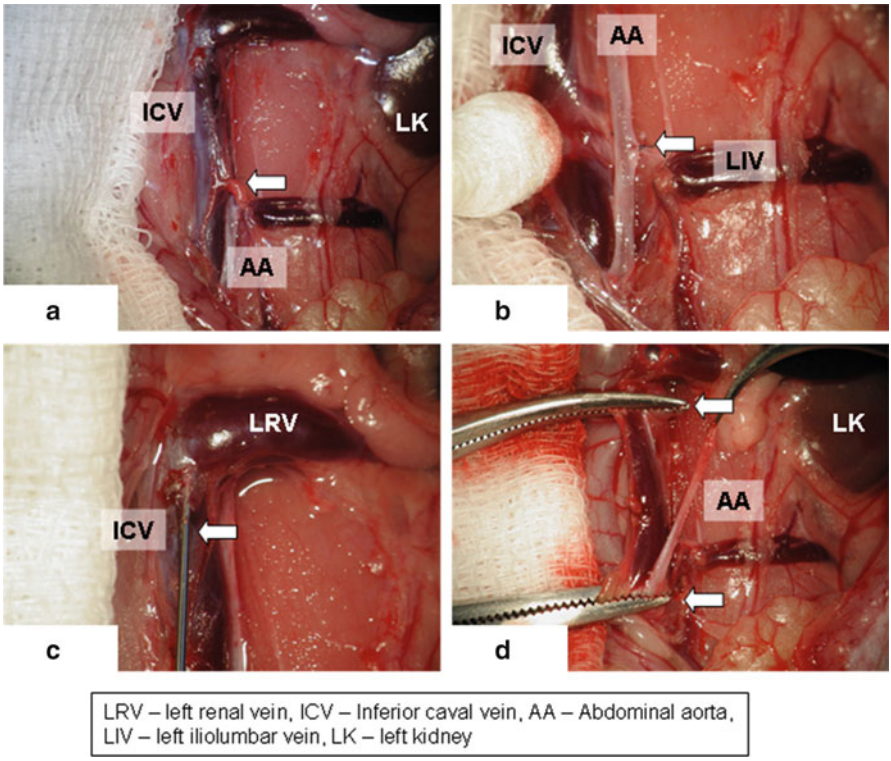

Fig. 15.3 Identifying of inferior caval vein (**a**), its separation from aorta (**b**), perfusion of caval vein with heparinized solution (**c**), clamps on aorta and inferior caval vein (**d**)

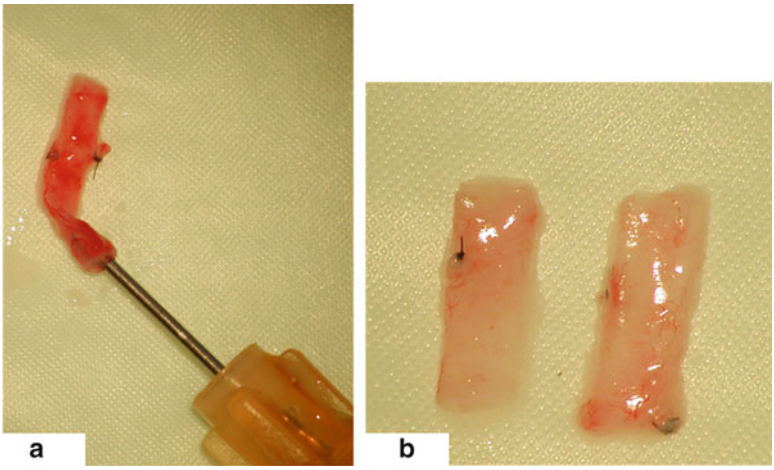

Fig. 15.4 Flushing of the graft in situ (**a**), cutting of the graft on two pieces (**b**)

16. Cut the aorta into two pieces of an identical length for the use in two recipient rats (Fig. 15.4b)
17. In according to study protocol, use the conservation solution for aortic pieces (saline solution, specific transplantation solution, cryoprotective solution etc.)
18. Check the death sign of donor animal and handle with the body in accordance with the dead animal disposal act

15.1.3.5 Tips and Tricks

1. Be very careful with the forceps manipulation of inferior caval vein as the vein wall is very fragile. Venous bleeding is hard to handle and is frequently lethal.

15.1.4 Abdominal Aortic Allograft Transplantation

15.1.4.1 Equipment + Material

Plastic foil to cover visceral organs during the surgery (Fig. 15.5a), gauze to cover the plastic foil (Fig. 15.5b), the gauze should be wetted at intervals with warm saline solution to avoid temperature decrease of animal body.

Heating apparatus to keep the saline solution warm.

Small fluid containers with saline solution to clean the microsurgical instruments during the surgery if necessary (Fig. 15.5c).

Cotton swabs (Fig. 15.5k), cutaway 5/8th inch (16 mm) orange needle, one, syringes (2 ml, 5 ml, 10 ml), saline solution.

15.1.4.2 Surgical Instruments

One scissors (Fig. 15.5e), one forceps (Fig. 15.5f), one needle holder (Fig. 15.5d), absorbable 3-0 sutures.

15.1.4.3 Microsurgical Instruments

One micro-scissors (curved) (Fig. 15.5l), two micro-forceps (one straight, one curved), (Fig. 15.5m, l) micro-needle holder (Fig. 15.5n), four vascular clamps (Fig. 15.5j), approximator vascular clamp (Fig. 15.5h), applying forceps for approximator vascular clamp (Fig. 15.5i), 10-0 monofilament sutures, 7-0 silk suture.

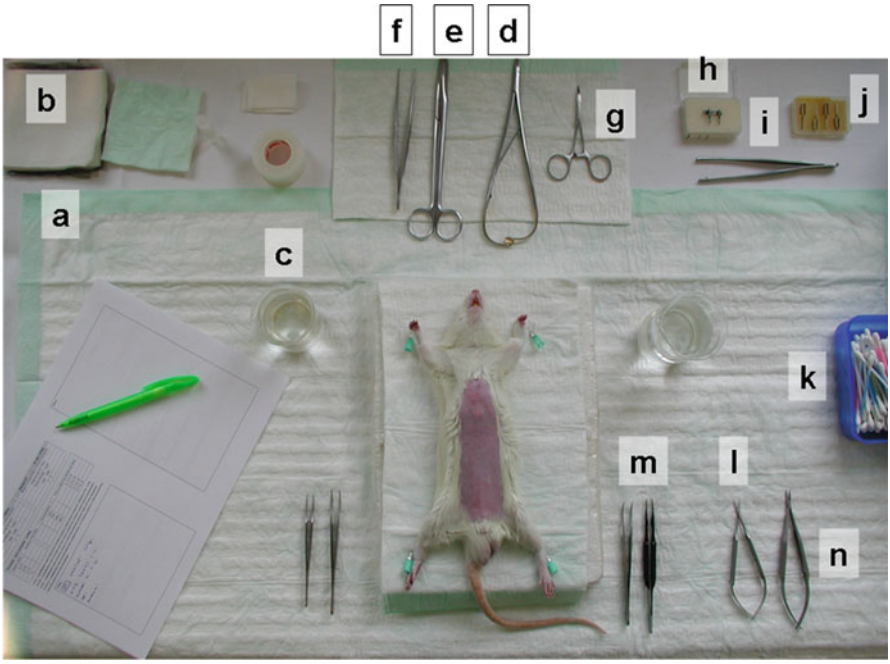

Fig. 15.5 Equipment for aortic graft transplantation

15.1.4.4 Procedure

1. Place animal on a clean pad (Fig. 15.6a)
2. Position with tape. Do not overstretch the legs or bind them in such a way as to restrict circulation (Fig. 15.6b).
3. Check level of anesthesia again using the toe pinch method (verifying lack of withdrawal with a firm toe pinch).
4. Make the midline laparotomy from urinary bladder to xyphoid using surgical scissors and forceps (Fig. 15.6c).
5. Pull away wall flaps with an abdominal retractor (Fig. 15.6d)
6. Retract the small bowel on the animals' right side (from the view of animal) (Fig. 15.6e) and cover it with plastic foil (Fig. 15.6f) and warm gauze (Fig. 15.6g)
7. Install the operation microscope (Fig. 15.7a)
8. Dissect retroperitoneum and retroperitoneal fat and carefully identify inferior caval vein and abdominal aorta (Fig. 15.7b)
9. Gently separate inferior caval vein from abdominal aorta using cotton swabs and microsurgical forceps (Fig. 15.7c). Be careful with inferior caval vein manipulation with forceps as the vein wall is very fragile.

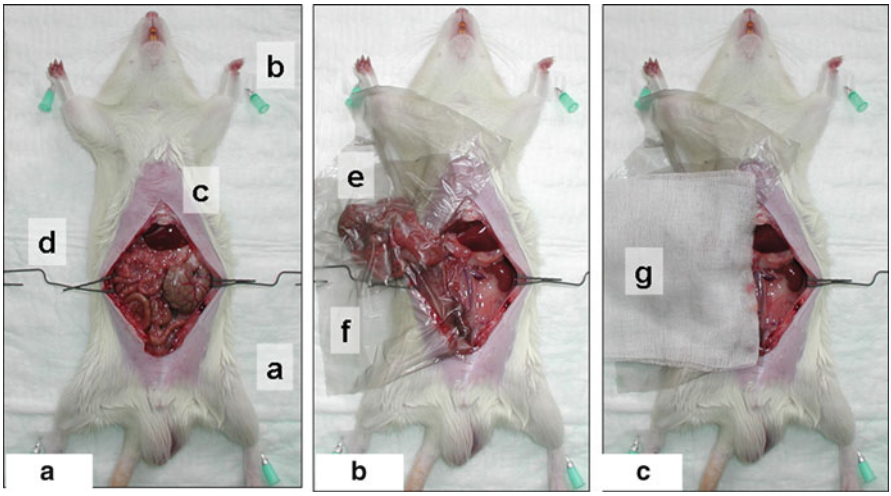

Fig. 15.6 Position of the recipient animal (**a**), operation field with plastic foil (**b**), midline laparotomy (**c**)

10. Ligate the lumbar arteries using 8-0 silk suture (Fig. 15.7c, arrow, Fig. 15.7d, arrow) to obtain a long mobile aortic graft reaching from renal arteries to aortic bifurcation (Fig. 15.7d).
11. Clamp the aorta with an approximator vascular clamp using the applying forceps (proximal clamp first) (Fig. 15.8a). No heparinization of recipient animal is necessary during the surgery.
12. Cut the aorta in the middle part with scissors (Fig. 15.8b). It comes to retraction of both aortic stumps due to the elasticity of the aortal wall. Use the approximator to adjust the required distance of both stumps. This distance depends on the length of aortic allograft (Fig. 15.8c, d).
13. Wash out blood coagula by flushing both aortic lumens with saline solution using the 2 ml syringe and a cutaway orange needle (Fig. 15.8c)
14. Clean carefully the 1 mm broad margin of both aortic stumps from adventitial tissue using microsurgical forceps and scissors (Fig. 15.8d)
15. Carefully clean the 1 mm broad margin from adventitial tissue of both aortic ends of arterial allograft (Fig. 15.9a) using microsurgical forceps and scissors (Fig. 15.9b)
16. Rotate the pad with the animal 90° counterclockwise to get the animal head on your left side (Fig. 15.10a).
17. Put the aortic graft between the both clamped aortic stumps (Fig. 15.10a)
18. Connect all four edges of aortic allograft to edges of both aortic stumps using a separate 10.0 suture and hang the microsurgical clamps on every one of the four sutures. The aortic allograft is now fixed in a suitable position for disrupted suture (Fig. 15.10d, arrows).

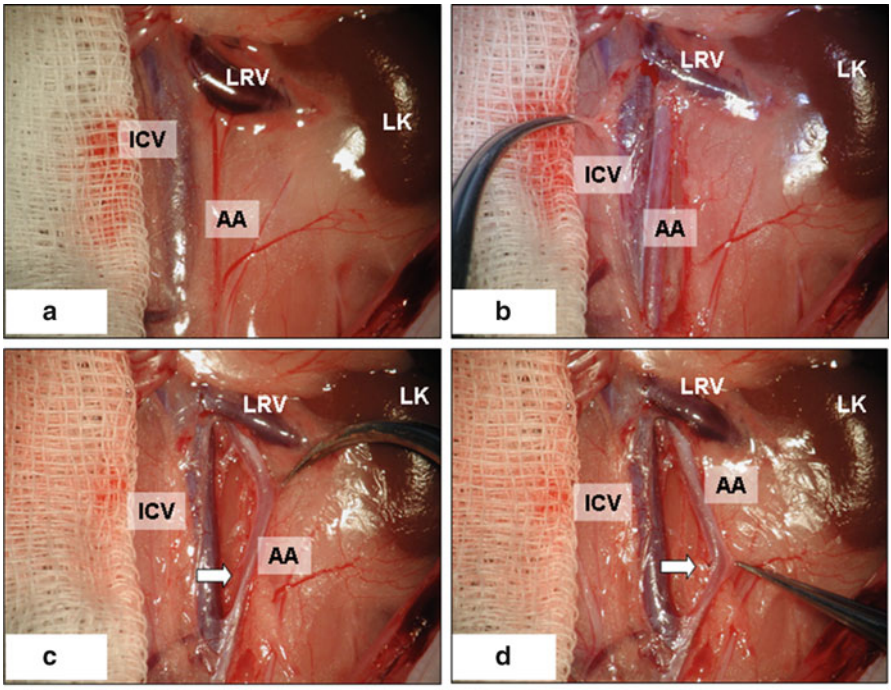

Fig. 15.7 Position of microscope field (**a**), identifying of inferior caval vein (**b**), separation of caval vein from aorta (**c**), ligation of lumbar arteries and mobilization of aorta (**d**)

19. Now use 10.0 monofilament separate stitches to make proximal and distal anterior anastomosis (Fig. 15.11)
20. Make the first middle stitch between the both edge-stitches and the next one between the middle and one of the edge-stitches.
21. Continue with this "middle stitch technique" until the anastomosis is finished (Fig. 15.11b). You will need approximately 7–8 stitches for one side of the anastomosis (Fig. 15.11c).
22. After finishing ventral side of both proximal and distal anastomosis, rotate the approximator vascular clamp for 180° from left to the right side of the animal (Fig. 15.12a). This maneuver will make dorsal part of both anastomosis accessible (Fig. 15.12a, arrows).
23. Now construct the dorsal part of both proximal and distal anastomosis in the same way as the ventral one. You will need approximately 6–7 stitches for this dorsal side of the anastomosis (Fig. 15.12b, c)
24. Cut all four edge-stitches and rotate the approximator vascular clamp to the initial position (Fig. 15.13a)

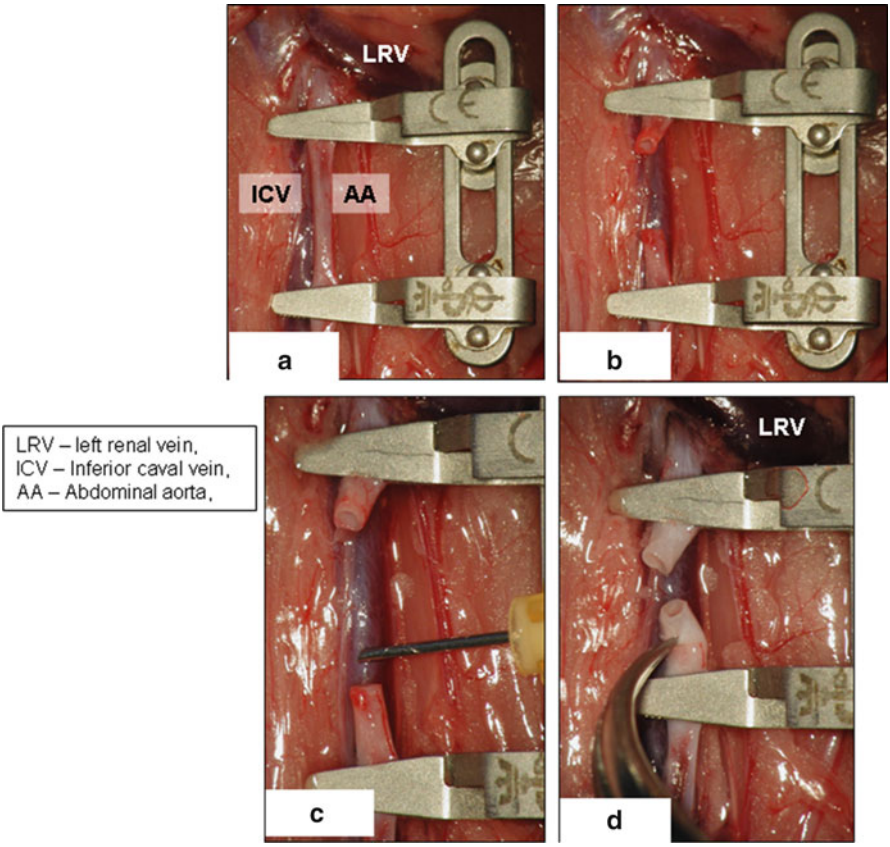

Fig. 15.8 Clamping (**a**) and cutting of the recipient's aorta (**b**, **d**), flushing the coagula from aortic stumps (**c**)

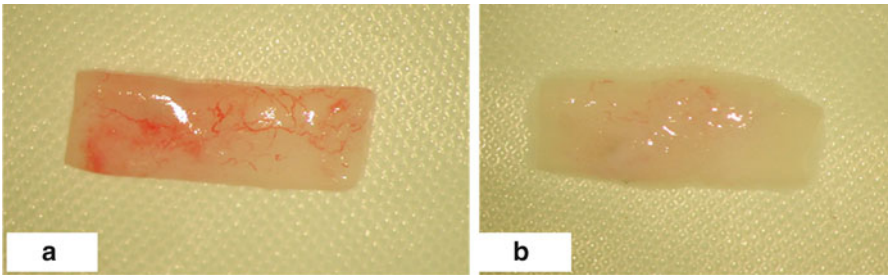

Fig. 15.9 Cleaning the margins of aortic graft from adventitial tissue (**a**, **b**)

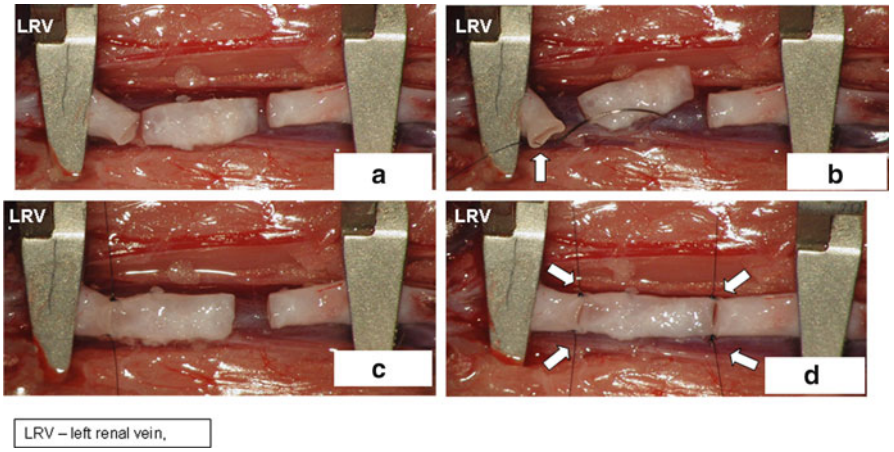

Fig. 15.10 Rotation of recipient animal 90° clockwise (**a**), put aortic graft between clamped aortic stumps (**b**), connect aortic graft to recipient's aortic stumps by separate sutures on both ends (**c**, **d**)

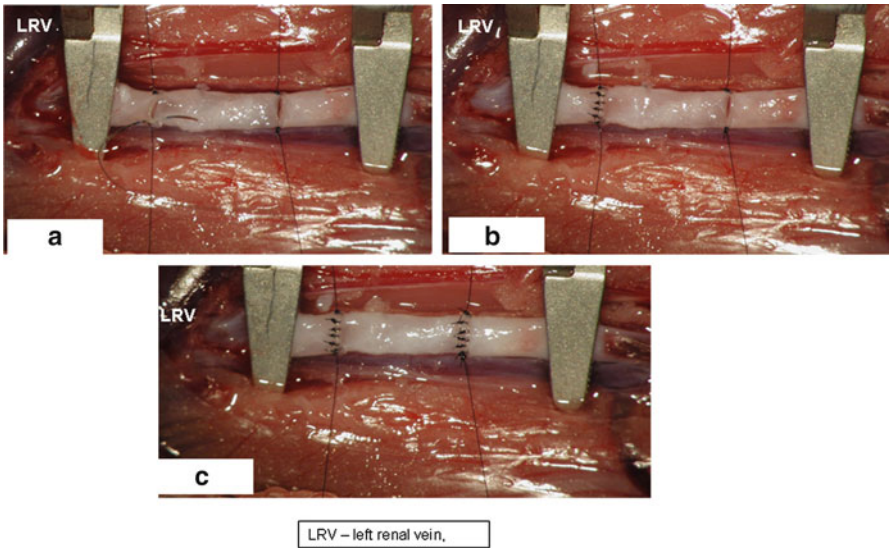

Fig. 15.11 Makes separate stitches to make proximal and distal anastomosis (**a**), finished the anastomosis with middle stitch technique (**b**, **c**)

25. Rotate the pad with the animal 90° clockwise to get the animal to the initial position as well (Fig. 15.13a)
26. Now release the distal and then the proximal clamp of the approximator by using the applying forceps (Fig. 15.13a, arrow)
27. Small bleeding from anastomosis can be stopped with a warm swab (Fig. 15.13b)

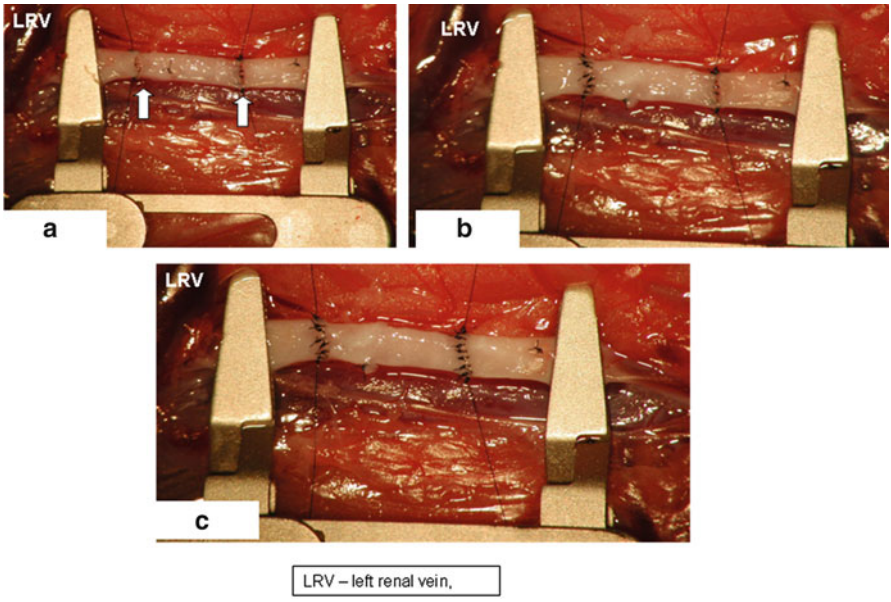

Fig. 15.12 After finishing ventral side, rotate the clamp for 180° from left to the right animal side (**a**), construct the proximal and distal anastomosis (**b**, **c**)

28. Check the both anastomosis for patency and tightness. Restore the visceral situs of the animal and rinse out the retroperitoneum and abdomen with warm saline
29. Suture the abdominal wall with absorbable 3-0 sutures
30. Suture the skin with absorbable 3-0 sutures (Fig. 15.14)
31. Let the animal recover on a heating pad

15.1.4.5 Tips and Tricks

1. It is very important that the recipient rats be kept warm during and after surgery
2. Be very careful with forceps manipulation of inferior caval vein as the vein wall is very fragile. Venous bleeding is hard to handle and is frequently lethal.
3. Carefully clean the 1 mm broad margin of both aortic stumps and aortic allograft from adventitial tissue using microsurgical forceps and scissors to ensure a comfortable suture

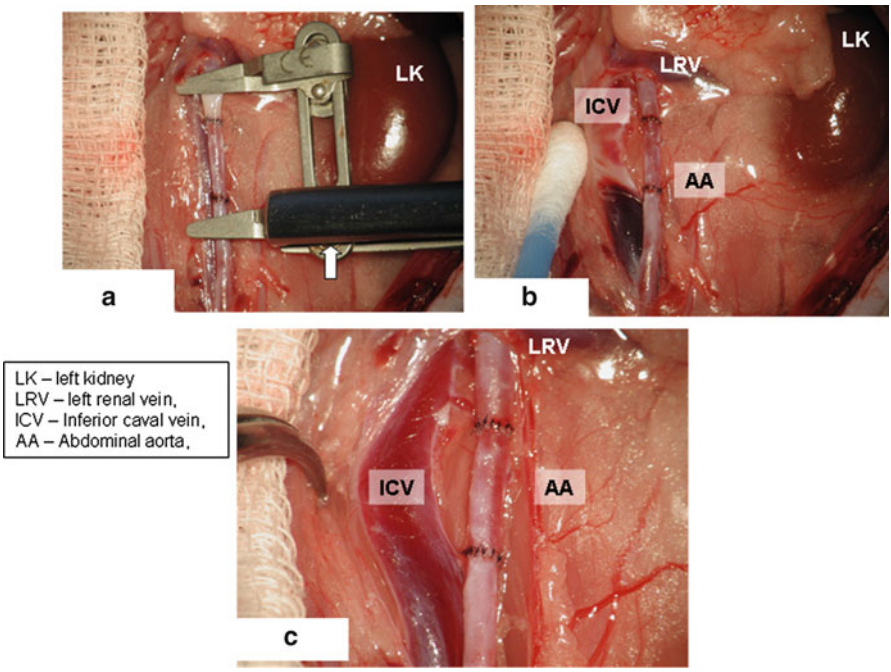

Fig. 15.13 Cut all four fixating stitches (**a**), rotate the clamp to the initial position as well as animal to the initial position, release the clamps (**b**), stop the small bleeding (**c**)

15.2 Transplantation of Iliolumbar Vein Allograft

15.2.1 Introduction

Interposition of syngeneic or allogeneic vein into the arterial circulatory tree is used in cardiac and vascular surgery. This results in an adaptation of the venous wall to the new biomechanical conditions of arterial system with its subsequent remodeling. Vein remodeling is a complex biological process that requires alterations in cell proliferation, cell death, cell migration and degradation and/or production of extracellular matrix components [12]. These adaptive interactions among vascular cells, their substrates and the local environment leave in the vein syngeneic graft to the typical anatomical changes associated with vein arterialization: intimal hyperplasia, circumferential medial thickening with well-organized concentric layers of smooth muscle cells and adventitial neovascularization [13].

The process of allogeneic vein arterialization is possible only under suppression of the host immune system [14]. Otherwise allogeneic non immunosuppressed veins are rejected with no adaptation on the arterial system conditions and with a high risk of thrombosis or rupture [14].

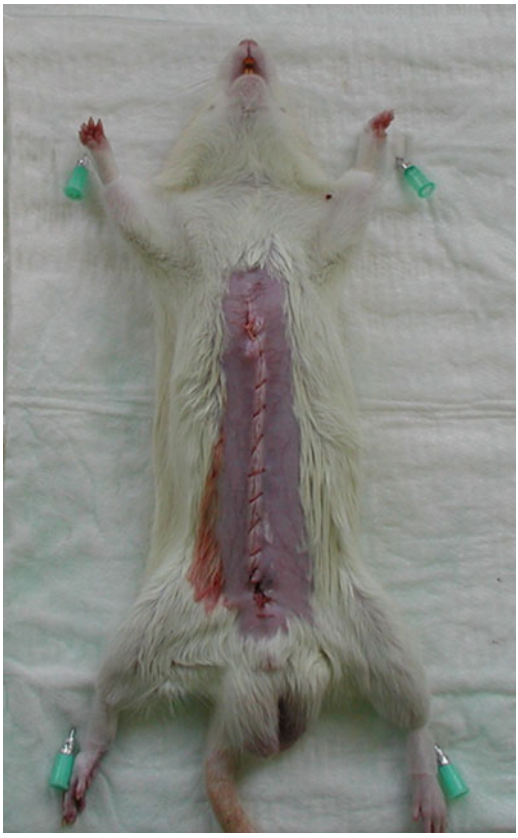

Fig. 15.14 Suture the abdominal wall

Rat iliolumbar vein to abdominal aortic implantation model is useful for studying the arterialization in both syngeneic and allogeneic conditions.

The syngeneic model is used for both studying the pathology of vein graft disease [15], and to test therapeutic strategies in vivo [16, 17].

In addition, the allogeneic model is used for both studying the pathophysiology of vein rejection [18] and to test the suppression of host immune system with different immunosuppressive regimens [19].

With allogeneic experiments, it is useful to use Brown-Norway rats (BN) (RT1n) as donors and Lewis rats (LEW) (RT1l) as recipients of iliolumbar veins. These genetically inbred rats differ at major and minor histocompatibility loci resulting in massive rejection of donor vein allografts [2].

15.2.2 Preconditioning of the Animal Prior to Surgery

The principles of animal preconditioning prior to vein-to-aorta surgery are same as described previously for aorta-to-aorta surgery.

15.2.2.1 DO's Just Before the Surgery

The do's are same as described previously for abdominal aortic transplantation.

15.2.3 Iliolumbar Vein Graft Procurement

15.2.3.1 Equipment + Material

Plastic foil to cover visceral organs during the surgery, gauze to cover the plastic foil, heating apparatus to keep the saline solution warm, small fluid containers with saline solution to clean the microsurgical instruments

Cotton swabs, gauze, 5/8th inch (16 mm) orange needle, syringes (2 ml, 5 ml, 10 ml) (Fig. 15.1).

15.2.3.2 Surgical Instruments

One scissors, one forceps (Fig. 15.1).

15.2.3.3 Microsurgical Instruments

One micro-scissors (curved), two micro-forceps (one straight, one curved), 7-0 and 4-0 silk suture, thermal cauterize unit (Fig. 15.1).

15.2.3.4 Procedure

1. Place animal on a clean pad (Fig. 15.2),
2. Position with tape. Do not overstretch the legs or bind them in such a way as to restrict circulation (Fig. 15.2).
3. Check level of anesthesia again using the toe pinch method (verifying lack of withdrawal with a firm toe pinch).
4. Make the midline laparotomy from urinary bladder to xyphoid using surgical scissors and forceps (Fig. 15.2).
5. Pull away wall flaps with an abdominal retractor (Fig. 15.2)

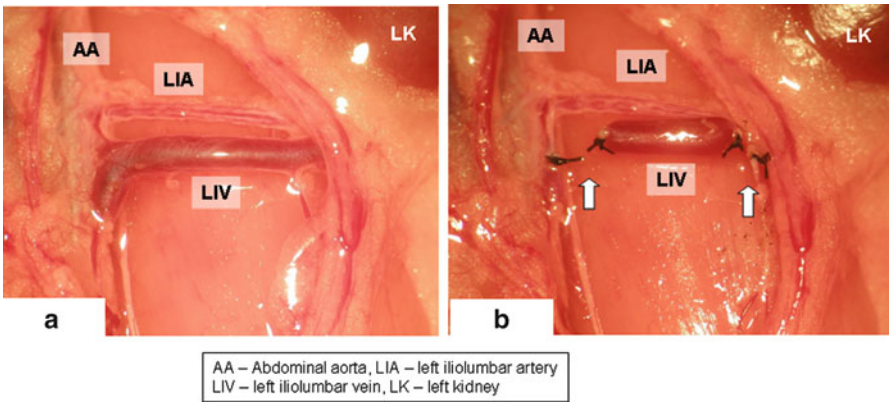

Fig. 15.15 Separate both iliolumbar veins from the arteries (**a**), ligate the veins (**b**)

6. Retract the small bowel on the animals' right side (from the view of animal) and cover it and right side of the animal with plastic foil and warm gauze (Fig. 15.2)
7. Install the operation microscope (Fig. 15.2)
8. Dissect retroperitoneum and retroperitoneal fat and carefully identify inferior caval vein and abdominal aorta
9. Separate both iliolumbar veins from iliolumbar arteries and retroperitoneum by using two tweezers to their peripheral branching (Fig. 15.15)
10. Ligate the vein of adequate length twice on both ends (Fig. 15.15b, arrows). Cut the graft out and transferred it to saline solution
11. The graft must be kept in saline solution until the time of transplantation. If the ends of graft are left tied, no rinsing is necessary.
12. Make a puncture of the inferior caval vein with 5/8th inch (16 mm) orange needle and apply a lethal dose of thiopental
13. Check the death sign of donor animal and handle with the body in accordance with the dead animal disposal act

15.2.3.5 Tips and Tricks

1. Disruption of the descending mesocolon will lead to better visualization of central part of right iliolumbar vein
2. Small veins could be coagulated by using the cauter for vascular prosthesis
3. Primary central ligation of the vein leads to its dilatation which could be beneficial during transplantation
4. Sometimes the iliolumbar vein doubled, each of 1⁄2 in diameter. If this is the case of a particular donor, it is better to quit this procurement as it is quite problematic to implant with high risk for occlusion of the reconstruction
5. Preparation must be very thorough. Venous bleeding is hard to handle and is frequently lethal.

15.2.4 Iliolumbar Vein Graft Transplantation

15.2.4.1 Equipment + Material

Plastic foil to cover visceral organs during the surgery, gauze to cover the plastic foil, the gauze should be wetted at intervals with warm saline solution to avoid temperature decrease of animal body (Fig. 15.5).

Heating apparatus to keep the saline solution warm.

Small fluid containers with saline solution to clean the microsurgical instruments during the surgery if necessary

Cotton swabs, gauze, 5/8th inch (16 mm) orange needle, syringes (2 ml, 5 ml, 10 ml), heparin.

15.2.4.2 Surgical Instruments

One scissors, one forceps, one needle holder, absorbable 3-0 sutures (Fig. 15.5).

15.2.4.3 Microsurgical Instruments

One micro-scissors (curved), two micro-forceps (one straight, one curved), microneedle holder, four vascular clamps, approximator vascular clamp, applying forceps for approximator vascular clamp, 10-0 monofilament sutures, 7-0 silk suture (Fig. 15.5).

15.2.4.4 Procedure

1. Place animal on a clean pad (Fig. 15.6)
2. Position with tape. Do not overstretch the legs or bind them in such a way as to restrict circulation (Fig. 15.6).
3. Check level of anesthesia again using the toe pinch method (verifying lack of withdrawal with a firm toe pinch).
4. Make the midline laparotomy from urinary bladder to xyphoid using a surgical scissors and forceps (Fig. 15.6).
5. Pull away wall flaps with an abdominal retractor (Fig. 15.6)
6. Retract the small bowel on the animals' right side (from the view of animal) and cover it and right side of the animal with plastic foil and warm gauze (Fig. 15.6)
7. Install the operation microscope
8. Dissect retroperitoneum and retroperitoneal fat and carefully identify the inferior caval vein and abdominal aorta (Fig. 15.7)

9. Gently separate the inferior caval vein from abdominal aorta using cotton swabs and microsurgical forceps.(Fig. 15.7b) Be careful with inferior caval vein manipulation with forceps as the vein wall is very fragile.
10. Ligate the lumbar arteries using 8-0 silk suture to obtain a long mobile aortic graft reaching from the renal arteries to aortic bifurcation (Fig. 15.7c, arrow, Fig. 15.7d, arrow).
11. Clamp the aorta with an approximator vascular clamp using the applying forceps (proximal clamp first) (Fig. 15.8a). No heparinization of recipient animal is necessary during the surgery.
12. Cut the aorta in the middle part by scissors (Fig. 15.8b). It comes to retraction of both aortic stumps due to elasticity of aortal wall.
13. Wash out blood coagula by flushing both aortic lumens with saline solution using the 2 ml syringe and orange needle (Fig. 15.8c)
14. Carefully clean the 1 mm broad margin of both aortic stumps from adventitial tissue using microsurgical forceps and scissors (Fig. 15.8d)
15. Put the iliolumbar graft between both clamped aortic stumps
16. Use four 9.0-10.0 sutures and place each stitch to one of four edges of aorta
17. Subsequent to that, fix the adjacent four edges of vein with the same suture (Fig. 15.16a)
18. Wash out blood coagula by flushing fixed venous graft lumen with saline solution using a 2 ml syringe and orange needle
19. Then cut off the ligatures of venous graft and tie the sutures
20. Hang the microsurgical clamps on the fibers, used as a holder which stretches the ends of vessels (Fig. 15.16a, arrows)
21. The first construct the front walls of both anastomosis starting from left holder stitch (Fig. 15.16a)
22. After that, turn the approximating clamps for 180° back to the front- this maneuver will make dorsal part of anastomosis accessible (Fig. 15.16b, arrow)
23. Construct the suture in the same way as on the front wall

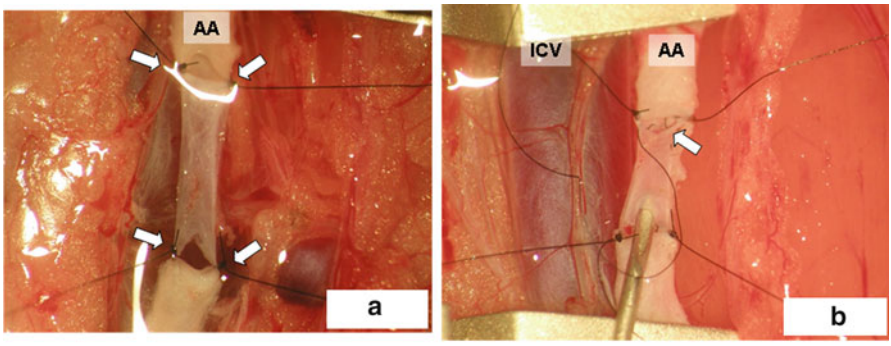

Fig. 15.16 Fix venous graft edges with suture and make the anastomosis on proximal and distal ends on ventral (**a**) and dorsal side (**b**)

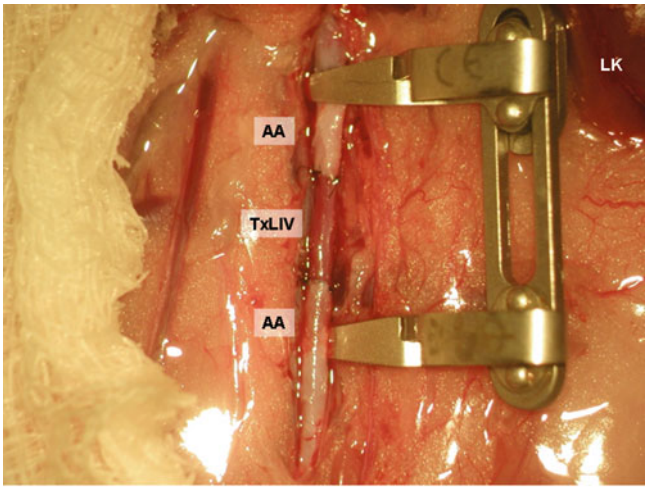

Fig. 15.17 Release the clamps, distal one as the first

24. After tying at the end, cut the sutures and holding sutures
25. Turn the approximating clamps back to normal position and release- distal clamp first (Fig. 15.17)
26. Small bleeding from anastomosis can be stopped with a warm tampon
27. Check the both anastomosis for patency and tightness. Restore the visceral situs of the animal and rinse out the retroperitoneum and abdomen with warm saline
28. Suture the abdominal wall with absorbable 3-0 sutures
29. Suture the skin with absorbable 3-0 sutures (Fig. 15.13)
30. Let the animal recover on a heating pad

15.2.4.5 Tips and Tricks

1. In small rats (under 200 g), the procedure more technically challenging but not infeasible
2. An additional dose of intramuscular anesthesia during the procedure will not work if applied in lower limb muscles due to transected aorta and limited blood supply
3. Clear the ends of the cut aorta of the adventitial layer to simplify the suture
4. Special attention must be paid not to involve the back wall in to suture. A rinse of venous graft with saline could be very helpful
5. Approximately six stitches are used on one side
6. Careful adjustment of the stitch is crucial in stenosis prevention

15.2.5 Postoperative Care After Arterial and Venous Transplantation

Postoperative care after arterial and venous transplantation has no specific requirements. A heating blanket or light is used to warm the animal during the recovery period. In this period, each animal should be in a separate cage with clean paper towels inside the cage until it is sterile. This is to avoid aspiration of bedding while the animal is still anesthetized.

Water and food supply should be available immediately after recovery from anesthesia. Following surgery, all cages must be clearly identified and appropriate and complete records of the surgical procedure, anesthesia and pre- and postoperative care are needed to be kept.

We used no anti- aggregation or anti-coagulation drugs after both allogeneic and syngeneic abdominal aortic replacement with aortic or vein grafts. No thrombosis has been observed using this practice.

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Abbreviations

ALI	Acute lung injury
ALX	Alloxan
ARDS	Acuter respiratory distress syndrome
ATG	In general the term used for several polyclonal antibodies, obtained by immunization of rabbit or horse by immune cells. Thymoglobulin – is obtained by immunization of rabbit by human thymocytes. ATG Fresenius – is obtained by immunization of rabbit with Jurkatt cells
ATP	Adenosine triphosphate
AUC	Area under the curve
BB rat	BioBreeding rat strain
BC	Before Christ
BN	Brown Norway rat strain
BUFF	Buffalo strain rats
CRF	Chronic renal failure
CYP	Cytochrome P
DA	Dark Agouti rat strain
ECG	Electrocardiography
F344	Fischer rat strain
FiO2	Inspiratory fraction of oxygen
FKBP	FK binding protein
GLUT 2	Glucose transporter
HBSS	Hanks balanced salt solution
I/R	Ischeamia reperfusion injury
IL –2	Interleukin 2
IMP	Inosinmonoposhate
KDP	Komeda diabetes prone rat strain
Lc	Lymphocenter
LE rat	Long Evans rat
LELT	Long Evans Tokushima Lean rat strain
LEW	Lewis rat strain

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Ln	Lymphonodus
Lnn	Lymphonodi
LP	Lipopolysaccharide
LPDG	Low potassium dextran glucose
MAC	Minimal alveolar concentration
MHC	Major histocompatibility complex
MMF	Mycophenolate mofetil
MN	Membranous nephropathy
MPA	Mycophenolic acid
mTOR	Mammalian target of rapamycin
NF-AT	Nuclear factor of activated T cells
NK	Natural killer cells
NPH insulin	Neutral Protamine Hagedorn
NSAID	Non steroidal anti-inflammatory drugs
OLT	Orthotopic liver transplantation
PEEP	Positive end-expiratory pressure
PRPP	Phosphoribosyl pyrophosphate
PV	Portal vein
PVG	Piebald Viral Glaxo
RTA	Rat MHC system
SBT	Small bowel transplantation
SD	Sprague Dawley rat strain
STZ	Streptozotocin
TGF beta	Transforming growth factor beta
TNF alpha	Tumor necrosis factor
WAG	Wistar albino Glaxo rat strain
WAG	Wistar albino Glaxo rat strain
WF	Wistar Furth rat strain
WF	Wistar Furth rat strain
WKY	Wistar Kyoto rat strain

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