ARTERIAL MICROANASTOMOSIS WITH SIZE MISMATCH.

A trial of two techniques.

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"Doctrine once sown strike deeply its root,

and respect for antiquity influences all men."

William Harvey, 1628

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For Annette, Imogen, Alice, Thomas,

and for my parents, Roger and Anne Rickard

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Introduction

Use of perforators as recipient vessels in microvascular reconstruction has led to arterial diameter discrepancy becoming an increasingly common finding. Experimental and clinical evidence confirms that patency rates decrease with increasing diameter mismatch, but no good evidence is available to direct the choice of end-to-end microanastomotic technique where a small-to-large discrepancy exists.

A programme of research has been conducted comparing two techniques of end-to-end arterial microanastomoses, where a small-to-large diameter discrepancy exists of between 1:1.5 and 1:2.5. These techniques are; 45º oblique section of the smaller vessel, and; invaginating the smaller vessel inside the larger.

Materials and Methods

Three-dimensional computational modelling of haemodynamics was conducted, followed by characterization of a paired Wistar rat Superficial Caudal Epigastric Artery / Femoral Artery model. Using this model, a large, randomized, paired experiment using two investigators was performed, examining anastomotic patency at one hour, one week, and six weeks. The time taken to complete each method was recorded.

Flow through each technique up to one week was studied using transit-time ultrasound flow measurement. Vessel cross-sectional area at ten months, determined by corrosion casting, was used as a proxy for longer-term flow rate. These casts were also used to examine anastomotic stenosis.

A semi-quantitative histological assessment of anastomotic healing and intimal hyperplasia was also performed.

Results

Areas of complex flow separation were found in both methods in the *in silico* study, and although shear stresses were distributed differently, their values were similar. No patency advantage between the two was found in the rodent model used, although the invagination was faster and simpler to perform. Flow through the invagination was at least equal to that through the oblique end-to-end anastomosis, with some experimental evidence suggesting that flow may be greater in the longer term. No difference in the degree of anastomotic stenosis was found. Invagination was less traumatic, inducing less vessel wall necrosis. Intimal hyperplasia was present in both techniques to a similar degree.

Conclusions

These studies suggest that the invagination technique is the better method. Further, clinical investigation is warranted.

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1 INTRODUCTION

Outline

1.1 Introduction

Since initial clinical reports over three decades ago, the transplantation of autologous tissues by microvascular technique has enjoyed increasing application and refinement, and can now be considered an everyday reconstructive option (Gottlieb and Krieger, 1994, Bennett and Choudhary, 2000). Clinical success rates have increased from 75% to 95% (Davies, 1982, Harashina, 1988, McGrouther and Soutar, 1993, Wei *et al*., 2002, Wei, 2005), and are consistent in most centres (Wei *et al*., 2002).

However, the corollary of a success rate in the region of 95% is that one transplant in twenty fails. Anastomotic thrombosis remains at the core of transplant failure, and among the causes of thrombosis is vessel size mismatch (Godina, 1979, Monsivais, 1990, Chuang *et al*., 1992). Abrupt changes in vessel diameter may cause flow separation and vortex formation. In turn, these flow disturbances can promote platelet aggregation (Karino and Goldsmith, 1979a) and deposition (Karino and Goldsmith, 1979b), encouraging thrombus formation and subsequent anastomotic failure.

Godina (1979) advocated the use of an end-to-side anastomosis in the situation of size mismatch. Indeed, this technique has long been proposed to overcome size mismatch (Carrel, 1902), but should this option be unavailable, an end-to-end anastomosis will necessarily be employed.

Minimal size mismatch in end-to-end anastomosis may be dealt with relatively simply by judicious dilatation of the smaller vessel and by taking differentially spaced suture bites. Where the size discrepancy is greater

than can be managed by these manoeuvres, techniques are described, each of which either increases the circumference of the cut end of the smaller vessel, or decreases the circumference of the cut end of the larger vessel.

However, few controlled studies of techniques have been performed, and no controlled experiments of adequate sample size are available to direct clinical decision-making.

1.2 A History of Vascular Surgery

Until the late 1800's, the only vascular operations were ligations. By the turn of that century, surgeons had sutured only nine human arteries successfully, success defined as patency at the conclusion of surgery. By July 1902, however, twenty-one successful arterial repairs in man had been reported and in 1903 this had increased to thirty. In 1910, it was reported that successes numbered more than one hundred (Guthrie, 1912).

1.2.1 Reconstructive Vascular Surgery

Eck is credited with performing the first documented anastomosis of two blood vessels in 1877 in Leningrad, when he reported successful side-to-side portocaval anastomosis in dogs (Eck, 1877, Child, 1953). Jassinowsky of Odessa, using an ovine carotid arteriotomy model, first demonstrated that injured arteries could be repaired with preservation of patency (Jassinowsky, 1891). In 1896, Jaboulay and Briau of Lyon, France, reported successfully uniting the ends of a transected carotid artery in a donkey by means of everting mattress sutures, ensuring intima-to-intima contact and exclusion of suture material from the vessel lumen (Jaboulay and Briau, 1896). Later that year, Murphy of Chicago, USA, performed the first successful arterial repair in man (Murphy, 1897).

Dörfler, in Rostock, Germany, described his principles for the successful repair of vessels in 1899, penetrating all layers of the vessel

wall (Dörfler, 1899). In 1900, Payr of Leipzig, Germany described the use of extra-luminal magnesium tubes to invaginate the proximal cut end of a blood vessel inside the distal (Payr, 1900). Bouglé reported the successful anastomosis of canine carotid arteries by means of an invagination method in 1901 (Bouglé, 1901).

Murphy's report of the first successful clinical vascular repair is particularly germane to this thesis and is worthy of greater scrutiny. On 7 October 1896, Murphy operated on a 29-year-old Italian pedlar, three days after he had been shot in the anterior abdominal wall and right groin. Murphy resected a through-and-through injury of the right common femoral artery, taking "*one-half inch*" (13mm) of the vessel. He re-anastomosed the artery using an invagination technique, employing four double-needled silk sutures to invaginate the proximal artery inside the distal "*for a distance of one-third inch*" (8mm), followed by "*a row of sutures*" around the end of the distal vessel. Despite formation of a post-operative wound abscess, the repair remained clinically patent until the report was written three months later.

Prior to clinical application, between 4 March and 5 December 1896, Murphy had performed 31 experimental arterial repairs on 22 animals (14 dogs, 5 sheep and 3 calves). Of these repairs, 18 were of complete transections of the common carotid (n = 15), aorta (n = 2) and femoral arteries (n = 1). Two animals died from haemorrhage and seven of the eighteen became clinically infected. Retrospective analysis of his results does not show any significant patency advantage of invagination

technique over direct end-to-end suture. Marked stenosis was found in three of the six patent invaginated anastomoses, compared with '*slight diminution*' of one of the two end-to-end anastomoses. Murphy concluded, however, that, '*By this method of approximation fewer sutures are necessary to secure blood-proof apposition*'. Guthrie (1912) reports that Murphy later modified his invagination technique by temporarily everting the distal vessel over a cylindrical instrument to allow more direct visualisation of suture placement.

Prior to this report, venous repair seems to have been an accepted surgical procedure. Murphy's main contribution was to implore surgeons to attempt arterial reconstruction rather than to ligate large and important arteries, citing mortality for ligation of larger vessels of up to 100% as the reason to attempt repair.

Bouglé (1901) and Watts (1907) each cite a report presented in 1897 by Dr Djemil Pasha of Constantinople at an international medical congress in Moscow of two cases of axillary artery repair using Murphy's technique. Watts describes one further by Krause, and two more by Kümmel in 1900. Watts notes that Payr described successful clinical application of his magnesium cuff invagination technique in 1901. A further report of successful arterial repair in man using Murphy's invagination technique was published by Brougham in 1906.

Carrel of Lyon, France, began (or perhaps continued (Sade, 2005)) research on an operative technique for vascular anastomosis whilst a prosector in the Anatomy Department at the University of Lyon (Carrel,

1902). Carrel initially tried the invagination method described by Murphy (Carrel, 1912) and the cuff technique of Payr. Carrel also tried using tubes or rods of caramel, designed to facilitate a running, circumferential suture without producing stenosis. However, he found these techniques too difficult to master, and working on cadaveric vessels, started to develop his triangulation technique using an over-and-over running suture between three stay sutures (Carrel, 1902).

Carrel continued his work in dogs, and together with Morel, described the arteriovenous fistula (Carrel and Morel, 1902a, 1902b). By 1903, however, Carrel had twice failed the entrance exam for a faculty position, and in 1904 he left France and sailed to Montreal. Here, a paper he presented (Carrel, 1904) led to an assistantship in physiology at the Hull Physiology Laboratory of the University of Chicago, where Carrel was assigned to work with Guthrie, a physiologist seven years Carrel's junior. Carrel and Guthrie's experimental work included perfection of Carrel's anastomotic technique, using finer and finer needles and sutures, and their collaboration appears to have been the start of consistent success in arterial anastomosis. Guthrie later claimed that before their collaboration, '[Carrel] *reported that his experiments did not always yield good results*' (Guthrie, 1909).

Around this time, much debate existed over whether the intima should be included in a vascular repair. Burci (1890), Jassinowsky (1891), and Carrel and Morel (1902a) each favoured omitting the intima, while Jaboulay and Briau (1896), Dörfler (1899), Tomaselli (1902), Jensen (1903) and Höpfner (1903) insisted upon including the intima in the arterial

anastomosis. In his monograph of 1912, Guthrie states that '*The technique previously studied by Berard and Carrel and by Carrel and Morel in 1902 was first tried, but very soon the endeavour to avoid penetrating the intima was discontinued, and its inclusion in the stitches as recommended by Dörfler was practiced. Other modifications were developed, until 'finally we developed a technique which is equally well adapted for arterio-arterial, veno-venous, or arterio-venous anastomoses, and which yields uniformly successful results. This new technique has been used since 1905*'.

Watts (1907) followed Carrel's methods of dissecting out the vessels, the use of soft, rubber-shod haemostatic clamps, the careful removal of adventitia and the prevention of tissue dessication, and was able to report long-term success in 13 of 13 canine carotid artery repairs, a remarkable result when judged in the light of later experience (Jacobson, 1997).

Subsequent to perfection of their end-to-end anastomotic technique, Carrel and Guthrie published work on the use of vein grafts in the arterial system, on the preservation of tissues, the transplantation of kidneys, ovaries, thyroids and hearts. They reported together the heterotopic autotransplantation of a kidney into a dog's neck, which Carrel had performed whilst a prosector in Lyon (Carrel and Guthrie, 1905). In 1906, they gave an account of the replantation of a dog's limb above the junction of middle and lower thigh (Carrel and Guthrie, 1906b), a technique which would not be employed clinically for another 53 years (Tamai, 1993). The dog's limb became congested soon after surgery and the animal was

sacrificed fifty hours after operation. It was found that the arterial and venous anastomoses were patent, and that loss of the limb had been due to a constricting dressing.

Guthrie left Chicago in 1906 to take up a chair in physiology and pharmacology at Washington University. Carrel took up a position at the new Experimental Surgery Unit at the Rockerfeller Institute for Medical Research in New York, where he remained, bar service in the French Army during World War One, until enforced retirement in 1938. Throughout his life, Carrel published 63 articles on experimental vascular surgery (Comroe, 1978) and was awarded the Nobel Prize for Medicine in 1912.

Despite Carrel and Guthrie's work, reconstructive vascular surgery largely remained an experimental curiosity until after World War Two. During the Balkan War (1912) and World War One (1914-1918), numerous attempts were made to reconstruct arterial injuries by direct anastomosis or by vein grafting. Weglowski, of present-day L'viv, Ukraine, then part of the Second Polish Republic, reported on 51 vein grafts used to repair traumatic arterial defects in Polish and Russian casualties of World War One (1914-1918) and of the subsequent Polish-Bolshevik War (1919-1921). Forty cases were successful and were returned as serving soldiers to their units (Weglowski, 1925). This experience would appear to be unique around this time - results were generally disappointing due to frequent wound infections. Amongst the British during World War One, the treatment of acute arterial injuries remained ligation. Surgeon-General Sir

George Makins (1916) reported on over 1200 cases of vascular injury and stated that: '[He regretted] *not being able to give any information regarding the treatment of gunshot wounds of the arteries by direct suture. In the cases I myself have seen, the nature of the defect in the arterial wall, the condition of the surrounding tissues, or the fact that septic condition of the primary tract afforded small hope of performing an aseptic operation, militated against the choice of this method'*. Bernheim (1920), who was a devotee of the techniques of primary anastomosis in civilian practice, recorded the experiences of American military surgeons thus: '*Opportunities for carrying out more modern procedures for the repair or reconstruction of damaged vessels were conspicuous by their absence… not that the blood vessels were immune from injury or that gaping arteries and veins could not be sutured, but it would have been foolhardy to have tried to suture arteries or veins in the presence of such infections as were the rule in practically all the battle wounded* '.

Perhaps influenced by these results, few advances in civilian practice were made during the peacetime interval of the 1920s and 1930s, (Fisher, 1959) and during World War Two, long evacuation times (of an average in the region of 12.5 hours), and extensive tissue loss following particularly high-energy trauma precluded many vascular repairs. In a review of 2471 vascular injuries, DeBakey and Simeone (1946) found only 81 instances of suture repair, of which only three were direct end-to-end suture. Repairs resulted in a limb salvage rate of 64 percent. No sutured vein grafts were carried out, although 'non-suture' vein grafting, using vitallium tubes in the

manner of Payr and Höpfner were tried in 40 cases, with a salvage rate of 50 percent. The rate following ligation was 51 percent.

In 1945, Crafoord and Nylin, and Gross each reported the successful treatment of coarctation of the aorta by resection with end-to-end anastomosis by continuous over-and-over suture and by continuous mattress suture respectively (Crafoord and Nylin, 1945, Gross, 1945). The first clinical use of saphenous vein bypasses for atherosclerosis was reported in 1949 (Kunlin, 1949). These reports stimulated a revival of interest in reconstructive vascular surgery, resulting in the employment of anastomotic techniques in the treatment of aneurysms and occlusive arterial disease (Seidenberg *et al*., 1958). The successes achieved with these techniques in large vessels were facilitated by advances in ancillary aspects of surgery such as anaesthesia, blood transfusion, antibiotics and the introduction of heparin.

It was not until 1952, during the Korean War, that restoration of vascular continuity following ballistic trauma was attempted on a large scale. With the routine use of helicopters, casualty evacuation times were much shorter than in World War Two, averaging 9.2 hours overall, and 4 to 6 hours for those with a recognized major arterial injury. In a review of 304 major arterial injuries, Hughes (1958) reported a limb salvage rate of 87 percent following direct end-to-end repair, vein or artery graft, compared to 49 percent following ligation. This success continued into the Vietnam War, when a review of 1000 acute arterial injuries also demonstrated an 87 percent salvage rate (Rich *et al*., 1970).

1.2.2 Small Vessel Surgery

Despite these successes in war, results from the experimental anastomosis of smaller vessels remained poor. Shumaker and Lowenberg (1948), in one of the first well-designed, paired animal studies examining suture technique, found thrombosis in 9 of 70 (13%) arteries of greater than 3.2mm, compared to 8 of 26 (30%) in arteries of 3.2mm or less. Thal and colleagues (1956) demonstrated long-term patency in only 7 of 17 vessels (41%) of less than 3mm internal diameter, anastomosed using a running, everting 6-O silk mattress suture. Contemporary opinion in the 1950s was that the anastomosis of vessels of less than 5 or 6mm led to inevitable thrombosis, so whilst these results were an improvement on this view, the technique at this calibre remained unreliable (Thal *et al*., 1956, Jacobson, 1997).

Seidenberg and colleagues, working in the Montefiore hospital in New York, USA studied the anastomosis of arteries varying in diameter from 1.5 to 4mm in a series of experiments on mongrel dogs from 1950, reporting success as "*the rule rather than the exception*", although no figures are given in their report (Seidenberg *et al*., 1958). This article is noteworthy also for its inclusion of three clinical cases using small vessel anastomosis, and in particular one case which is described more comprehensively in a subsequent paper (Seidenberg *et al*., 1959). On 30 July 1957, the authors performed an immediate reconstruction of the cervical oesophagus of a 63-year-old man by the autotransplantation of a segment of jejunum, the first clinical "free tissue transfer". Seidenberg *et al*. performed an end-to-end anastomosis of a branch of the superior

mesenteric artery to the inferior thyroid artery, both measuring 2.5 - 3mm in diameter. The mesenteric vein was anastomosed to the anterior facial vein using a siliconized titanium ring prosthesis after the method of Payr. Unfortunately, the patient suffered a cerebrovascular accident and died on the seventh post-operative day. However, at autopsy the jejunal segment was judged to have been viable *ante-mortem*. In their discussion, the authors forecast the use of small vessel anastomoses in reconstructive surgery.

Roberts and Douglass (1961) reported two similar jejunal autotransplants two years later, again using a prosthesis to effect the venous anastomosis. Hiebert and Cummings (1961) performed the transplantation of a gastric antrum in 1960, and Nakayama *et al.* (1962) transplanted the sigmoid colon to replace a similar oesophageal defect in a further case in 1961.

Much effort around this time was spent in the quest for successful revascularization of severely injured extremities and the replantation of amputated parts. Kleinert *et al*., of Louisville, USA, reported the successful revascularization of four cases of devascularized upper limbs in 1963, including a case of a right hand and wrist incompletely amputated in a power saw accident in 1958 (Kleinert *et al*., 1963). The authors also reported three unsuccessful attempts at the replantation of digits. In 1959, in Nara, Japan, Onji and Tamai successfully replanted the leg and thigh of a 47-year-old woman whose limb had been amputated by an electric saw (Tamai, 1993). Malt and McKhann, in Boston, USA, performed the first

successful arm replantation on a 12-year-old boy in May of 1962 (Malt and McKhann, 1964). Reportedly, the patient was caught shoplifting using the hand several years later (Tamai, 1993), and eleven years later was in employment as a car mechanic (Harris and Malt, 1974). Ch'en and colleagues at the Sixth People's Hospital, Shanghai, China, replanted a hand and forearm amputated one inch proximal to the wrist in January of 1963. The authors used polyethylene cuffs after the method of Payr for two venous and two arterial anastomoses (Ch'en *et al*., 1963). Inoue and colleagues in Osaka, Japan, succeeded in replanting the left hand of a 26-year-old man in October of 1963 (Inoue *et al*., 1967). In 1965, Ch'en *et al*. reported two subsequent Chinese cases from 1963; one a replantation of an arm using vein grafts and titanium coupling devices, and one case where the patient's right hand had been amputated by an electric motor through the mid palm. In this latter case, performed in December 1963, the authors carried out suture anastomoses of the superficial palmar arch and third common digital artery using monofilament Capron® (nylon) of 0.04mm diameter, equivalent to USP 8-O. The vessels' internal diameters were less than 1.2mm (Ch'en *et al*., 1965). The authors used a x3 binocular loupe (Buncke *et al*., 1973) and it would seem that an operating microscope was not available at the Sixth People's Hospital until after 1973 (Meyer, 1985). By 1967, without a microscope, the team at this hospital had successfully replanted 20 digits (Ch'en, 1967). By 1971, their experience totalled 351 attempted finger replantations, with a success rate of 51 percent (Meyer, 1985).

1.2.3 Microvascular Surgery

Microvascular surgery arose as the natural development of small vessel surgery, made possible by the employment of the operating microscope, and the development of finer instruments, needles and suture materials.

The operating microscope had been introduced into otological practice by Nylén in 1922 and its use was popularised within that discipline by Holmgren (Nylén, 1954). It was introduced into ophthalmic practice in 1946 (Nylén, 1972).

The introduction of the operating microscope into small vessel surgery in 1960 by Julius Jacobson, then of the University of Vermont, USA, has been the single most important advance in improving outcomes in small vessel surgery. Jacobson was collaborating in a pharmacological study that required denervation of the canine carotid artery. He realised that the only way to achieve this was to divide and then re-anastomose the artery, even though, at that time, it was thought that arteries of less than 6mm could not be anastomosed with consistent success (Jacobson, 1997). Jacobson writes that it became obvious that the problem was one of a lack of visual acuity, rather than manual dexterity, and following unsuccessful attempts using magnifying lenses over the operating site, and a surgical loupe, he borrowed an operating microscope from ENT surgery. Jacobson refined instruments and sutures, and in conjunction with Litmann of the Carl Zeiss company, in 1961 he developed the first double binocular microscope, which was named a diploscope (O'Brien, 1977). In 1960, Jacobson reported 100 percent patency at four months in the anastomosis of the carotid arteries of 20 dogs and 6 rabbits, the vessels averaging 3.2

and 1.4mm in diameter respectively (Jacobson and Suarez, 1960). Jacobson and colleagues subsequently went on to demonstrate the significant clinical advantages of microsurgical technique in the nascent speciality of coronary artery surgery and in neurosurgery and peripheral vascular surgery (Jacobson *et al*., 1960, Jacobson *et al*., 1962, Jacobson, 1963, Yasargil *et al*., 1970).

The advantage of magnification when anastomosing small arteries was confirmed two years after Jacobson's initial report by Chase and Schwartz, who claimed a 100% patency rate in a series of 34 anastomoses in the brachial arteries of dogs, ranging in external diameter from 1.2 to 1.7mm (Chase and Schwartz, 1962). The authors used a 4× binocular loupe, but comment that in their opinion greater magnification would be necessary for vessels of less than 1mm (Chase *et al*., 1963).

Perhaps the most striking example of the improvement in patency achieved by the use of an operating microscope is the unique experience of Ch'en and colleagues in Shanghai, China. Before the introduction of an operating microscope at their facility, their success rate in digital replantation surgery was 51 percent. After its introduction in 1973, this rose to 91.5 percent (Meyer, 1985).

Despite Jacobson's successes, consistent patency at or below 1mm remained a challenge. This diameter was considered important because this was the size of digital vessels, and of vessels supplying the subdermal plexus (Buncke and Schulz, 1966, Strauch and Murray, 1967). In 1960, Buncke had started an experimental programme, aiming at consistent

patency in vessels of this size. Having initially, and unsuccessfully, tried intraluminal stents and extraluminal cuffing devices, in 1964 Buncke and Schulz reported the successful replantation of a rabbit's ear using nylon sutures metallized in a linear accelerator to form a needle, and an oblique, continuous suture anastomosis technique between two stay sutures (Buncke and Schulz, 1966, Randall, 1990). The central artery of the rabbit's ear measured 0.8mm. In 1965, Buncke and Schultz reported the successful replantation of the thumb and index fingers of a single Rhesus monkey, (Buncke and Schulz, 1965) and the following year, Buncke *et al.* reported the first successful toe-to thumb transplant in a Rhesus monkey (Buncke *et al*., 1966).

The first successful clinical replantation of a completely amputated digit was performed in Nara, Japan, on 27 July 1965, and reported some three years later. Komatsu and Tamai (1968) replanted the left thumb of a 28-year old man, the thumb having been amputated at the metacarpophalangeal joint in a steel-cutting machine. Two arteries and two veins were repaired end-to-end using interrupted 7/O silk and 8/O nylon.

It is difficult to know to whom precedence should be awarded for the idea of tissue reconstruction by the transplantation of tissue composites using microvascular technique. As noted above, Seidenberg and colleagues discussed the possibility in 1958, but Buncke, who worked as a senior house officer and registrar at the Royal Infirmary in Glasgow, Scotland, from January to June of 1957 attributes the idea to Tom Gibson

(Buncke and Schulz, 1966). Gibson postulated that, in order to avoid the many disadvantages of the tube pedicle, it might be possible to transplant a large flap of skin immediately by supporting its circulation with a temporary, small, extracorporeal oxygenator, whilst neovascularization occurred at the edges of the flap. Gibson (1986) and Buncke *et al*. (1991) report that the project was plagued by technical problems with the oxygenator, and by inadequate funding, and was abandoned. Gibson suggested that immediate anastomosis between flap and recipient vessels would of course solve the problem, but this was not technically feasible at the time.

In 1963, Goldwyn and Lamb, who had spent a '*profitable afternoon*' with Jacobson, reported, with White, an unsuccessful experimental series of five dogs, in which they had attempted to isolate large abdominal island flaps on the superficial caudal epigastric vessels, and to divide and re-anastomose these vessels in situ. The arteries measured between 0.6 and 1.0 mm and the veins between 1.2 and 3.0 mm. Goldwyn and Lamb performed end-to-end anastomoses using a Zeiss operating microscope, and 8-O monofilament nylon. One dog died on the first postoperative day, and one other flap was ischaemic five hours after re-anastomosis. The remaining three flaps survived only to 48 hours (Goldwyn *et al*., 1963, Goldwyn, 2006).

Two years later, Krizek *et al*., in a larger and more successful series using a similar canine model, achieved long-term success in 14 of 15 in situ re-anastomoses, and 19 of 20 heterotopic autotransplantations into defects created in the animals' necks. In the former group, the arterial

anastomoses were accomplished by means of a Carrel patch to the femoral artery (Carrel and Guthrie, 1906a). The venous anastomoses were constructed in an end-to-side fashion to the femoral vein, using continuous 7-O silk sutures. The heterotopic transplants were anastomosed to the common carotid artery and external jugular vein using an end-to-side technique (Krizek *et al*., 1965).

In 1966, Green *et al.* achieved patency to three weeks in 18 of 20 rat aortic anastomoses, averaging 1.3mm in external diameter, and 19 of 20 vena caval anastomoses averaging 2.7mm in external diameter (Green *et al*., 1966).

In 1967, Strauch and Murray reported successful Superficial Caudal Epigastric Artery flap transfer to the neck in 10 of 13 rats (77%) by end-to-end anastomosis of the femoral artery to the common carotid, and femoral vein to a branch of the external jugular vein, using interrupted 10-O nylon sutures. External diameter of the Femoral Artery measured 0.6 to 0.8 mm and of the Femoral Vein 0.8 to 1.0 mm (Strauch and Murray, 1967). In 1971, O'Brien and Shanmugan (1973, O'Brien, 1977) achieved 100% survival of 27 groin flaps in rabbits. Although vessel external diameter was not detailed, it was described as in the region of 1mm.

In April 1968, Cobbett, in East Grinstead, England, performed the first clinical toe-to-thumb transplant to the non-dominant hand of a 31-year-old woodworker who had lost his thumb, index and middle fingers in a circular saw accident. (Cobbett, 1969). In 1971, Antia and Buch in Bombay, India, attempted transfer of a de-epithelialised adipocutaneous superficial inferior

epigastric artery (SIEA) flap to the right side of the face of a 35-year-old woman. The authors anastomosed the SIEA to the external carotid artery, and the SIEV to the internal jugular vein by means of Carrel patches (Antia and Buch, 1971). Postoperatively, much of the flap was lost, and it is unclear if the anastomoses had remained patent.

Also in 1971, at Oak Knoll US Naval Hospital, California, USA, McLean and Buncke autotransplanted the omentum of a 29-year-old man into a scalp defect measuring 6 x 8 inches. The authors anastomosed the gastroepiploic artery (1.2 mm) to the superficial temporal artery (1.6mm), and their corresponding veins, using a commercially-available 10-O nylon suture on a straight needle. The omentum was then skin grafted (McLean and Buncke, 1972). Anastomotic patency was demonstrated by arteriography at three weeks. Although Seidenberg *et al.* (1958) had reported jejunal autotransplantation thirteen years previously, this was the first reported autotransplantation in man using microvascular technique.

O'Brien (1974, 1977) states that the first successful microvascular composite tissue transplant in man was performed in September 1972, by Harii and colleagues from Tokyo, Japan, who transplanted a free scalp flap based on the superficial temporal vessels. Harii *et al.* state that they had been performing free tissue transfer from the summer of that year, but do not detail their first case (Harii *et al*., 1974b, 1974a). Two cases from Melbourne, Australia, and one from Wasan Hospital in Shanghai, China were reported in 1973. Daniel and Taylor transferred a SIEA flap to the right ankle of a 21-year-old man on 20 January, 1973 (Daniel and Taylor, 1973). McDowell reports another case by Don-Yoa of Shanghai,

performed on 26 March 1973 (McDowell, 1973). O'Brien quickly followed with the transplantation of a groin flap to the ankle on or about 28 March (McDowell, 1973, O'Brien *et al*., 1973). Success in these individual cases was soon confirmed by case series from several groups (O'Brien *et al*., 1974, Harii *et al*., 1974a, Ikuta *et al*., 1975, Sharzer *et al*., 1975). A new era of reconstruction had begun.

1.3 Arterial Size Discrepancy

1.3.1 Clinical Scenarios

In contrast to the repair of severed vessels encountered when replanting amputated parts, microvascular tissue transplants often require the anastomosis of arteries with dissimilar diameters. Different discrepancies (i.e., large-to-small and small-to-large (described with the upstream vessel size first)) are encountered in different disciplines, and their relative frequencies have changed with changes in reconstructive practice.

Early transplants were limited largely to the deltopectoral flap, frontotemporal flaps, scalp flaps and, by far the most commonly used, the groin flap (O'Brien *et al*., 1974, Harii *et al*., 1974a, Ikuta *et al*., 1975, Baudet *et al*., 1976a). The groin flap, as described by McGregor and Jackson (1972), and anatomically delineated by Smith *et al.* (1972), is supplied by the superficial circumflex iliac artery, which has a mean external diameter of 1.2mm at its origin. Recipient vessel diameter was often much larger, and this led to a large-to-small discrepancy of 3:1 or 4:1 (Harii *et al*., 1974a, Godina, 1979, Chuang *et al*., 1992).

Harii *et al.* (1974) and O'Brien *et al.* (1974) used an oblique-cut anastomotic technique in this situation. Chuang *et al*. (1992), reporting on their extensive experience with the free groin flap, used dilatation, oblique cut end-to-end, or a fish-mouth incision of the smaller vessel, and end-to-side to manage discrepancy, as well as a Carrel patch or a longitudinal

split of the vessel bifurcation at its origin. These authors attribute their few failures specifically to arterial diameter mismatch.

Godina did not describe his end-to-end technique, but attributed the improvement in his success rate to the introduction of an end-to-side anastomotic technique to manage this discrepancy, although he changed his preferred flap to the latissimus dorsi at about the same time. The relative demise of the groin flap and the introduction of the latissimus dorsi and scapula flaps are also given as the reason, by McGrouther and Soutar, for a rise in their overall success rate from 76% in the first five years, to 88.5% in the second five years of microsurgical reconstruction at Canniesburn Hospital in Glasgow, Scotland (McGrouther and Soutar, 1993).

The introduction into microsurgical practice of the latissimus dorsi flap (Baudet *et al*., 1976b, Maxwell *et al*., 1978), and the description of the free fibula (Taylor *et al*., 1975), the lower abdominal (deep inferior epigastric) flap (Holmstrom, 1979), radial forearm (Yang *et al*., 1981, 1997), and anterolateral thigh flaps (Song *et al*., 1984), among others, led to the introduction of donor tissues with longer and larger-diameter pedicles. Nakayama *et al.* (1987) found that this led to a small-to-large size discrepancy when using the facial or superior thyroid arteries as recipient vessels in head and neck reconstruction, the opposite of the discrepancy found when using the groin flap. Çakir *et al.* (2003) found discrepancy in 24% of 103 arterial and 50% of 125 venous anastomoses in 99

consecutive microsurgical cases, discrepancy defined as a diameter ratio of greater than 1:1.5.

The recently described use of perforators as transplant recipient vessels is an attractive development in microvascular surgery, reducing operative time and morbidity (Koshima *et al*., 1998, Park *et al*., 2003, Hong and Koshima, 2010). However, unless a perforator-to-perforator anastomosis can be effected (Koshima, 2005, Hong and Koshima, 2010), the use of perforators as recipients can result in a small-to-large discrepancy in arterial diameter. This is illustrated by relatively recent refinements in microsurgical breast reconstruction, which have described the use of internal mammary artery (IMA) perforators as recipient vessels for the deep inferior epigastric artery (DIEA), removing the requirement to excise costal cartilage in order to access the internal mammary vessels themselves (Park *et al*., 2003, Haywood *et al*., 2003, Hamdi *et al*., 2004, Saint-Cyr *et al*., 2007). Haywood (personal communication) has managed the discrepancy by a variety of techniques: differential suture bites, obliquely sectioning the IMA perforator, suturing part of the larger DIEA longitudinally after excision of a wedge, or by using the DIEA more distally where it is smaller. Saint-Cyr *et al.* (2007) prefer the IMA perforators as recipients for the smaller superficial inferior epigastric artery because of the better size match.

Even in the situation where a perforator is not used as the recipient, it may hold true that the longer the flap pedicle, the greater the vessel diameter, and the longer the recipient vessel, the smaller the vessel

diameter. Thus, an increasing small-to-large diameter discrepancy may be encountered with increasing pedicle length.

1.3.2 Described Techniques

Daniel and Terzis (1977), considered the end-to-end anastomosis of arteries of different diameters the *sine qua non* of free flap transfers. More recent developments in free tissue transfer means that the management of discrepancy remains a technical challenge.

Most authors would manage a small difference in diameter by differential mechanical dilatation of the vessel ends. Daniel advises this method up to a diameter discrepancy of less than 50%, i.e., 1:1.5, or 1.5:1, and he describes its use in his first clinical case from 1973. In this case, Daniel and Taylor anastomosed the superficial inferior epigastric artery (1.8mm) to the posterior tibial artery (2.4mm), thus dealing with a 1.3:1 (large-to-small) discrepancy by this method (McDowell, 1973, Daniel and Terzis, 1977).

Beyond this mismatch ratio, a geometric manoeuvre is necessary to effect the successful anastomosis of two arteries. A variety of constructs is described:

1.3.2.1 End-to-side

End-to-side anastomotic technique was first mentioned as a means of managing arterial size discrepancy by Carrel, in his description of his triangulation technique of vessel anastomosis (Carrel, 1902).

In microsurgical practice, Ikuta (1975) used an end-to-side anastomotic technique to anastomose five free groin flaps, and his success was repeated by Serafin *et al.* in the latter six cases of their series (Serafin *et al*., 1977). Godina (1979) advocated the use of an end-to-side technique following a high failure rate with end-to-end technique in his early microsurgical experience. Scrutiny of his data, however, shows the predominance of a large number of flaps in his early series, especially the groin flap, which had small vessels anastomosed end-to-end to much larger recipient vessels. Godina describes a large-to-small diameter discrepancy of 3:1 or sometimes 4:1. His latter, end-to-side series, however, includes a majority of latissimus dorsi flaps (32 of 41), the use of which removed any marked discrepancy, and makes any direct comparison of technique difficult.

Subsequent analyses, including a large retrospective clinical series of 921 flaps by Samaha *et al.* (1997), shows no patency advantage of end-to-side technique over end-to-end where no size discrepancy exists, and end-to-side technique is often selected for its undisputed advantage of preservation of distal arterial flow. However, where a significant vessel size discrepancy does exist, the technique has utility. (Daniel and Terzis, 1977, Bas *et al*., 1986).

1.3.2.2 End-to-end

End-to-end anastomotic techniques to manage diameter discrepancy may be divided into those that increase the circumference of the cut end of

the smaller vessel, and those that decrease the circumference of the cut end of the larger vessel.

Techniques to increase the circumference of the smaller vessel include a fish-mouth incision (Hurwitt *et al*., 1953, Harashina and Irigaray, 1980, Harashina, 1988), or oblique section (Brener *et al*., 1974, Harii *et al*., 1974a, Fukui, 2003). Those that decrease the circumference of the larger vessel include differential suture bites, as illustrated by the open-loop suture technique described by Lee *et al.* (1984) and used in IMA perforator anastomoses by Park *et al.* (2003). Mattress sutures may be used to 'gather' the larger vessel (Boeckx *et al*., 1998, De Lorenzi *et al*., 2005). Alternatively, a wedge of the larger vessel may be removed, or excluded from the lumen, to taper the vessel and reduce its circumference to approximate it to that of the smaller (Hurwitt *et al*., 1953, Harashina and Takaki, 1983, Ueda *et al*., 1994, Suri *et al*., 2009).

Where the upstream vessel is smaller, the mismatch has also been dealt with by invaginating it inside the larger downstream vessel to a varying degree (Xiu and Song, 1993, de la Peña-Salcedo *et al*., 2000).

1.3.3 Experimental Data

Apart from the findings of Godina (1979) and Chuang *et al*. (1992), no clinical or experimental studies into the direct effect of vessel size discrepancy on patency rates in free tissue transfer are found. Four experimental studies examining patency rates in relation to interposition vein or artery graft size are available.

1.3.3.1 Effect of Size Discrepancy

Büchler and Buncke (1979) performed a series of arterial and venous micrografts into various vessels in a rat model. The authors interposed artery grafts into arteries, and vein grafts into both arteries and veins. In interpreting their data, it seems logical that the artery grafts into arterial defects would have most correlation with arterial size discrepancy in end-to-end anastomosis, as marked compliance differences between artery and vein are excluded. The authors imply a decreasing patency rate with increasing diameter discrepancy, although they admit that their numbers are small where a large discrepancy exists (Table 1.1.). In addition, scrutiny of their data shows a decreasing patency rate with decreasing

		Graft donor		
		Carotid artery 1.34 ± 0.07mm	Femoral artery 0.9 ± 0.14mm	Saphenous artery 0.48 ± 0.06mm
Graft recipient	Carotid artery		1 : 1.69* 5 of 5†	1 : 2.86 1 of 3
	Femoral artery	1.57 : 1 5 of 5	1 : 1 30 of 30	
	Saphenous artery	3.03 : 1 1 of 3		1 : 1 5 of 10

* = graft : recipient vessel diameter ratio
† = patency

Table 1.1. Vessel discrepancies and patency rates from Büchler, U. and Buncke, H. J. (1979) Experimental microvascular autografts. In Serafin, D. & Buncke, H. J. (Eds.) Microsurgical composite tissue transplantation. St. Louis, CV Mosby.

vessel and graft size, independent of discrepancy, which may confound these findings (saphenous artery *vs* femoral artery, $p < 0.0005$, Fisher exact test). Despite these limitations, Büchler and Buncke conclude that, in clinical practice, graft diameters should not exceed a diameter ratio of 1.5:1 or 1:1.5.

Monsivais (1990), in a study more specifically designed to examine the effect of diameter mismatch, interposed vein grafts of various diameters into one femoral artery in one hundred rats, their series being divided into five equal groups (Table 1.2). As with Büchler and Buncke, the author's end-to-end anastomotic technique is not described. Patency was assessed at four to six days. A significant reduction in patency is observed between groups with a diameter ratio of 1:1 and 1:2 ($p = 0.0324$, Fisher's exact test), 1:1 and 2:1 ($p = 0.0324$), and between 1:1 and 1:4 ($p < 0.0001$). In addition, a significant difference in patency is also seen between both 1:2 and 2:1, and 1:4 ($p = 0.0112$). Monsivais' conclusion, that a diameter discrepancy of greater than 1.5:1, or 1:1.5 produces a significantly lower patency rate, concurs with that of Büchler and Buncke.

	graft : vessel diameter ratio				
	1 : 1	1 :1.5	1 : 2	1 : 4	2 :1
Patency	18 of 20	16 of 20[ns]	12 of 20[†]	4 of 20[†]	12 of 20[†]

ns = not significant at 5% level
† = significant patency disadvantage ($p<0.05$, Fisher's exact test).

Table 1.2. Patency rates of interposition vein grafts of differing diameter discrepancies. From Monsivais, J. J. (1990) Microvascular grafts: effect of diameter discrepancy on patency rates. Microsurgery, 11, 285-7.

Harris *et al.* (1999) studied patency seven days after interposing vein grafts into the femoral arteries of rats in a superficial caudal epigastric flap model (n = 10 per group). Of the vein grafts of equal diameter to the femoral artery, nine of the ten were patent, compared to only three of the ten when a vein graft of twice the arterial diameter was used (p = 0.0198, Fisher's exact test).

In contrast to these three studies, Xiu and Song (1994), performed grafts of tail artery (0.35 ± 0.05mm) into the right carotid artery (1.16 ± 0.22mm) of 130 rats, giving a marked diameter discrepancy of 1:3 to 1:4 in each animal. The anastomoses at both ends of the graft were performed using a technique that draws the smaller vessel very slightly into the larger vessel by differentially sized interrupted suture bites (Watanabe and Makino, 1978, Xiu and Song, 1993). Patency was examined in groups at seven different intervals up to three months. A remarkable overall patency rate of 95% is reported. This high patency rate is difficult to explain in the light of the previous studies. Two differences with previous studies are noted – the anastomotic technique, and the use of arterial grafts rather than venous. The technique cannot be compared to other work, which does not give this detail. The numbers in this study, however, are much greater than those in Büchler and Buncke's experiment (Büchler and Buncke, 1979), and it may be that the use of artery grafts produces better results than vein grafts. From the available evidence, however, it is difficult to draw this conclusion with any conviction.

1.3.3.2 Effect of Technique

Three studies are found which compare microvascular anastomotic techniques where a diameter discrepancy exists (Ryan *et al*., 1988, Gumley *et al*., 1989, Ahn *et al*., 1994). (Table 1.3.) Each of these groups have used an animal model where a vein graft has been interposed into an artery to produce a suitable anastomotic diameter discrepancy.

Ryan and colleagues studied anastomotic patency across both 1:5 and 5:1 diameter mismatch in a rabbit model, using inferior vena cava (IVC) grafted into one femoral artery. These investigators used an IVC tributary to remove diameter mismatch at one of the two anastomoses. Five different anastomotic techniques were used at the other end, with ten animals in each group. No significant patency differences between techniques were observed, although *post hoc* power calculations reveal a high likelihood of a type 2 (beta) error in this study (see Table 1.3.).

Gumley *et al*. used rabbit external jugular vein grafted into one femoral artery to produce a 3 : 1 diameter mismatch. These authors used a similar method to Ryan *et al.* – that of using a tributary to remove the discrepancy at one end. As with Ryan *et al.*, the authors conclude that there is no significant patency difference between techniques. Compared to that of Ryan *et al*., a slightly higher chance of a type 2 error is found in this study

Ahn and colleagues studied anastomotic patency in three techniques, grafting an isogeneic IVC into one femoral artery in the rat to produce a 1 : 4.5 and 4.5 : 1 diameter mismatch at each end of the vein graft. Sixteen animals were used in each group, and patency was studied by '*standard patency tests*', and by a 20MHz Doppler probe, at three, seven

and fourteen days. Actual patency figures from this study are not presented, but extrapolating from a small graph of percentage patency shows no statistically significant patency advantage of any technique at any time point.

study	***n* / gp**	**vessel diameter ratio**	**seven day patency**					***post hoc* power***
			direct end-to-end	oblique or spatulate end-to-end	tapered end-to-end	side-to-end	end-to-side	
Ryan *et al*., 1988	10	1 : 5	10^{ns}	10^{ns}	9^{ns}	10^{ns}	10^{ns}	20%
Ryan *et al*., 1988	10	5 : 1	9^{ns}	9^{ns}	10^{ns}	10^{ns}	9^{ns}	20%
Gumley *et al*., 1989	10	3 : 1	9^{ns}	10^{ns}	7^{ns}	9^{ns}	10^{ns}	10%
Ahn *et al*., 1994	16	1: 4.5 + 4.5 : 1	7^{ns}	5^{ns}	9^{ns}	-	-	10%

* = for patency difference of 10%
ns = not significant at 5% level

Table 1.3. Patency rates from studies of anastomotic techniques to manage size discrepancy.

1.3.4 Conclusions

Although available experimental data are potentially confounded by the compliance mismatch introduced by the use of vein grafts, it can be concluded that reasonably good experimental evidence exists to support

the theory that anastomotic patency varies with vessel size mismatch. Some clinical reports are available which corroborate this finding.

In terms of the geometric manoeuvres available to manage the size mismatch, however, only experimental evidence of low statistical power is available. None of this has provided good evidence for either the presence or the absence of a difference between techniques.

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2 REFINING THE SUBJECT AND THE EXPERIMENTAL APPROACH

Outline

2.1 Introduction

Recent advances in microvascular composite tissue transplantation have led to a need to chose an anastomotic technique to manage a small-to-large diameter discrepancy. A review of the evidence leads to the conclusions that size discrepancies result in decreased patency, but that no good evidence is available to direct a choice of technique where a diameter discrepancy exists.

Many reports of microvascular techniques are either a description of the technique followed by a few case reports of success, or are small animal series of low statistical power. Many describe 'no difference' from small numbers: these almost certainly create a high risk of a type 2 (beta) error, i.e. a high false-negative rate.

An exploration was therefore made into the sample size necessary to comprehensively examine patency in an experimental animal model. A scientifically robust, paired model, where each technique provides a matched control, and which examines categorical outcomes (e.g. patency), is based on the McNemar test for matched case-control studies (McNemar, 1947). In searching for small differences in patency (below five percent), an experiment will necessarily involve hundreds of animals. These numbers increase exponentially into the thousands when a patency difference of one percent is sought. It is not, therefore, justifiable to look at many different techniques in an animal model in a single study, nor to mix venous and arterial anastomoses. Doing so simply weakens the

conclusions that may be drawn. In turn, this negates the point of sacrificing so many animals, making such a study ethically unjustifiable.

The decision was therefore made to compare only two techniques, and only in arteries. This single comparison could then be addressed comprehensively.

2.2 Choice of Techniques

In choosing which techniques to examine, it was concluded that the conventional, obliquely-sectioned end-to-end anastomosis used clinically to manage size discrepancy by Harii *et al.* (1974) and O'Brien *et al.* (1974), and commonly described in microvascular texts as the method of choice (Daniel and Terzis, 1977, Fukui, 2003, Wei and Suominen, 2006, Sabapathy, 2009) would necessarily be one of those under scrutiny.

The other technique would need to possess several advantages, whilst equating to the first in terms of:

1. Patency, and;
2. Blood flow through the anastomosis, in both the short and the long term.

The advantages sought would include:

3. Technical simplicity, especially in very small vessels (≤ 0.8 – 1mm in external diameter), to allow clinical application to perforators as

recipients and a growing interest in supermicrosurgery as a technique;

4. Speed of execution, and;
5. A reduction in vessel trauma caused by fewer sutures and less frequent handing of the vessels.

The invagination technique, described in the anastomosis of equal-sized vessels by Murphy (1897) and Bouglé (1901), and introduced into microvascular surgery by Lauritzen (1978) and Meyer (1980), has the potential to meet many of these criteria. This technique is not widely accepted in the clinical microvascular anastomosis of equal-sized vessels. However, some researchers have recommended it for clinical use in situations where a small-to-large discrepancy exists (Meyer *et al.*, 1980, Sully *et al.*, 1982, Stamatopoulos *et al.*, 1982, Duminy, 1989). Two short clinical series using this technique in small-to-large arterial size discrepancies have been reported (Nakayama *et al.*, 1987, de la Peña-Salcedo and Lopez-Monjardin, 2000).

2.3 Review of Chosen Techniques

2.3.1 The Invaginating Anastomosis

As noted above, this technique of end-to-end anastomosis has been investigated in the microanastomosis of equal-sized vessels. Meier, a cardiothoracic surgeon in Zurich, Switzerland, studied the technique in canine arteries measuring 2 – 4mm in diameter from the mid 1970s, and reported his work in 1978 (Meyer, personal communication, (Meier, 1978).

Lauritzen, from Göteborg, Sweden, also conducted a series of experiments into an invagination or 'sleeve' technique, publishing his findings from 1978 to 1980 (Lauritzen, 1978, Lauritzen and Bagge, 1979, Lauritzen *et al.*, 1979, Lauritzen and Hansson, 1980, Lauritzen *et al*., 1980).

Meyer, previously a student of Meier's, independently started studying an invagination or 'telescoping' technique in rodent femoral vessels in 1978. He reporting his findings in the spring of 1979 at the third meeting of the International Society for Reconstructive Microsurgery in Guarujá, Brazil (Meyer, personal communication), subsequently publishing them in 1980 (Meyer *et al.*, 1980).

Lauritzen placed two sutures proximally at 180º from one another, invaginating the proximal vessel by then manipulating it into the distal vessel (Figure 2.1.b.). Thus the end of the proximal vessel was free within the distal (Lauritzen, 1978). In later work, he describes the use of three sutures rather than two (Figure 2.1.d.)(Lauritzen and Bagge, 1979, Lauritzen and Hansson, 1980). Lauritzen purports the advantages of his 'sleeve' technique in rodent and rabbit vessels to be ease of execution and speed, whilst maintaining patency and a flow rate comparable to a conventional end-to-end technique (Lauritzen and Bagge, 1979).

Meyer *et al.* (1980) concur with many of the findings of Lauritzen, these authors attaining a high (93%) patency rate in both conventional and 'telescoping' techniques in rodent femoral arteries (*n* = 80 each). Meyer's technique differed from Lauritzen's in that the upstream, proximal vessel

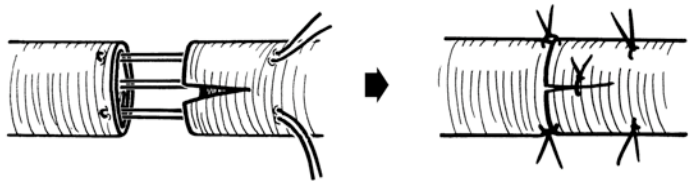
was invaginated by means of two distal, indrawing or invaginating sutures placed diametrically opposite each other (Figure 2.1.c.). Despite the high patency rate in this model, Meyer comments that the technique would have limited application in equal-sized vessels in humans because of the relatively greater thickness of the arterial wall. He suggests that, '*For free composite tissue transfer, telescope anastomosis could be considered if the recipient artery is smaller than the donor artery*.'

The relative ease with which the invagination, telescoping or sleeve technique was performed in equal-sized vessels made it an attractive option in the relatively nascent speciality of microsurgery. This prompted widespread investigation and many modifications of the technique.

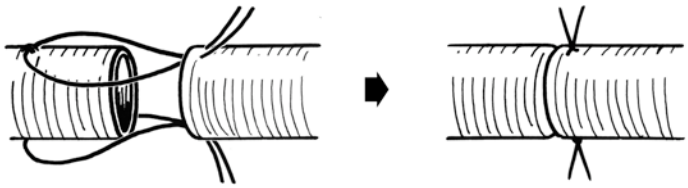
Hyland *et al.* (1981) added a 'traction' suture to Lauritzen's method. This suture was used to invaginate the proximal vessel inside the distal, and was removed after flow was restored (Figure 2.1.c.). In a study in 20 rodent femoral arteries, these authors also found the technique simple and quick, and they obtained a high (90%) patency rate at one week.

Stamatopoulos and colleagues (1982), in a small study in rat carotid arteries (*n* = 13) used two distal, full-thickness suture bites to draw the proximal end into the distal (Figure 2.1.f.). These authors studied the anastomoses at five months by scanning electron and light microscopy, concluding that no anatomical or functional stenosis was seen. All anastomoses were patent.

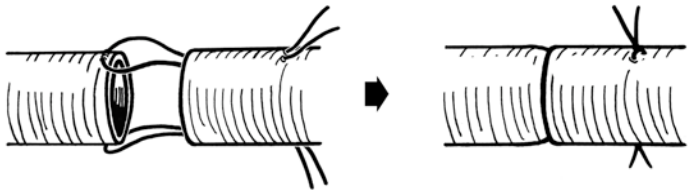
Chen and colleagues in Shanghai, China, reported a three suture method similar to Lauritzen's later method. These investigators examined



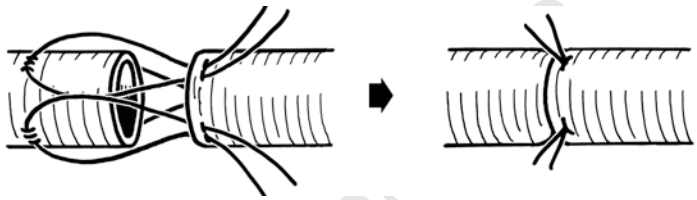
a. Murphy, 1897.



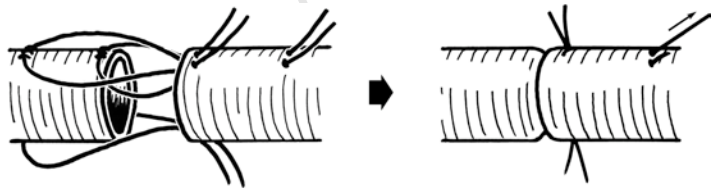
b. Bouglé, 1901; Lauritzen, 1978.



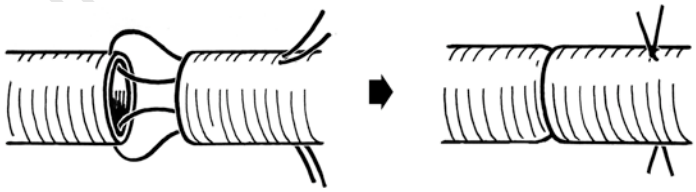
c. Meier, 1978; Meyer, 1980.



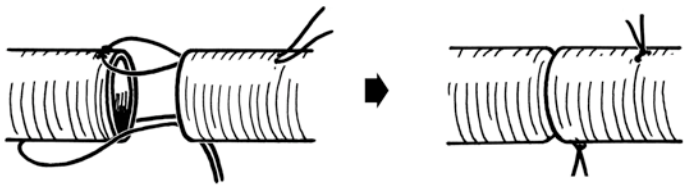
d. Lauritzen & Bagge, 1979; Chen, 1983.



e. Hyland, 1981



f. Stamatopoulos, 1982; Sully, 1982.



g. Duminy, 1988.

Figure 2.1. Described techniques of invaginating anastomoses in equal-sized vessels. See text for descriptions.

the technique in a paired study in 100 femoral arteries of rats, attaining a 98% patency rate (Chen *et al.*, 1983). Following from this work, Zhang and colleagues report good experimental results from a variety of 'sleeve' techniques using longitudinal side cuts in the distal vessel, and varying numbers of sutures. (Zhang *et al.*, 1991, 1995, 1996).

Duminy (1988, 1989) described a further modification of Lauritzen's method, placing one suture proximally, and one suture distally, diametrically opposite the first. By this technique, Duminy attained a high (>98%) patency rate in a study of 77 rat femoral arteries at various time points ranging from one to sixty postoperative days. Duminy noted stenosis in the majority of vessels anastomosed by the invaginating technique. Like Meyer, he comments that because of the higher wall thickness in human arteries, the technique was unlikely to find wide acceptance in the direct anastomosis of equal-sized, small human arteries, and that it might best be used where a favourable small-to-large diameter discrepancy exists.

Despite the advantages of speed and ease of execution, clinical acceptance of the invagination technique in equal-sized vessels has been limited by studies from four groups in particular.

Sully *et al.* (1982) failed to find equivalent patency in a paired study in the femoral arteries of 50 New Zealand white rabbits. These authors used a technique similar to that of Stamatopoulos *et al.* Anastomoses were examined at 10 undefined time points ranging from two hours to six weeks. Patency in the 'sleeve' technique used by Sully *et al.* was

significantly lower in comparison to the matched control of the conventional end-to-end technique on the opposite side (42/50 *vs* 49/50, *p* = 0.031, Fisher's exact test). These authors, however, comment on the potential methodological failing in this model of the relatively high tension encountered at the anastomoses performed by invagination. Whilst no data was available from this study, like Meyer and Duminy, the authors suggest that the technique should be used in the situation of a small-to-large size discrepancy.

Wieslander and colleagues, in a series of studies in the central artery of the rabbit ear, noted stenosis at the anastomosis, and a lower flow rate through the invagination or 'end-in-end' technique when compared to a conventional end-to-end anastomosis (Wieslander and Åberg, 1980, 1982, 1983, Wieslander and Rausing, 1984).

Nakayama and Soeda, in studies performed in rabbit carotid arteries, also found stenosis and reduced flow in the invaginating technique when compared to conventional end-to-end suture technique in equal-sized vessels. (Nakayama and Soeda, 1981, 1984).

Further concerns were raised by Kanaujia *et al.* (1988). These researchers examined the technique by corrosion casting in a paired study in the carotid and femoral arteries of rats (*n* = 25 each). They conclude that a significantly greater number of aneurysms were found in carotid arteries anastomosed by an invagination technique, although scrutiny of their data does not confirm this statistically (*n* = 3/25 *vs* 0/25, *p* = 0.098, Fisher exact test).

In summary, a wide variety of suturing methods has been described to anastomose equal-sized vessels by invaginating the proximal end inside the distal. These numerous modifications have not significantly altered the outcome in these experimental models. Use of an invaginating technique has consistently demonstrated greater speed and ease of execution when compared with conventional end-to-end microanastomosis performed with interrupted full-thickness sutures.

However, some advocates of the technique in experimental animal models have commented on its doubtful use in small human vessels of equal diameter. Concerns about lower patency rates found in some experimental studies, and the presence of anastomotic stenosis and reduced flow through an invaginating technique have limited clinical acceptance as a replacement for conventional technique in equal-sized vessels.

Aware of these limitations, Nakayama and colleagues used the technique of invagination successfully in fifteen clinical cases of varying diameter mismatch (Nakayama *et al.*, 1987). Vessel size and diameter mismatch are not detailed, and the authors comment that the cases are a mix of equal-sized and small-to-large diameter discrepancy. They confirm that stenosis and reduced flow are inevitable when vessels of an equal size are anastomosed by invagination. However, this did not compromise flap survival in their series. Nakayama *et al.* conclude that the indication for the invagination technique is a favourable small-to-large diameter discrepancy.

De la Peña-Salcedo and López-Monjardin (2000) report a clinical series of 28 invaginating anastomoses in the head and neck. Exact vessel measurements are not detailed, but vessel size discrepancies are reported as small-to-large, with diameter ratios between 1:2 and 1:4. The technique used was similar to that of Sully *et al.* (1982). No failures were reported from this series.

2.3.2 The Oblique End-to-End Anastomosis

In stark contrast to the invagination technique, little is written about the oblique-cut end-to-end technique. As previously noted, Harii *et al.* (1974) and O'Brien and colleagues (1974) used the technique clinically in the large-to-small diameter mismatch often encountered in pioneering clinical microvascular surgery.

Brener *et al.* (1974) describe the geometry of oblique end-to-end anastomoses between small (4 to 6mm) arteries and prosthetic grafts of dissimilar diameters, highlighting a number of principles. These authors illustrate that abrupt changes in flow direction can be avoided by appropriate angles of section of both vessel ends. In order to approximate the circumferences, the smaller vessel must be cut more obliquely than the larger. The resulting angle between the axes of the vessels can be reduced by longer, more oblique sections of each vessel.

In microvascular surgery, together with a spatulate technique, oblique cut end-to-end anastomosis is described by Daniel and Terzis (1977) as their preferred method for managing size discrepancy greater than 50%.

These authors comment that an angle of greater than 30º should be avoided, postulating that this leads to turbulence at the microanastomosis. However, they do not provide any evidence to support this theory, attributing it to Harii *et al.* (1974). Harii and colleagues, however, make no mention of it in the reference that Daniel and Terzis provide. Sabapathy (2009) reiterates this recommendation, although again, no evidence is provided to support it.

As discussed in section 1.3.3.2, despite noting no significant patency difference between techniques, Gumley *et al.* (1989), in examining a 3:1 large-to-small size discrepancy in their experimental animal model, conclude that the end-to-end method with a 45º oblique cut of the smaller vessel is technically straightforward, quick, and requires least vessel length. These authors also found that the technique was less liable to kinking or to compression than an end-to-side technique, and conclude that it was their preferred method for clinical use.

2.4 Research Programme

2.4.1 Design Considerations

A research programme was formulated to compare these two techniques – invagination of the smaller vessel inside the larger, and a 45 degree oblique cut end-to-end anastomosis after the method of Gumley *et al.* (1989).

In designing the experimental series, an attempt was made to address the author's and others' methodological criticisms of previous experimental work into microanastomotic techniques to manage size discrepancy, and into the invaginating microanastomosis in equal-sized vessels. An attempt was also made to address potential confounding factors found in these studies. Specifically, the design considerations included:

1. A lack of previous study into, or understanding of, the likely haemodynamic differences between the two techniques under scrutiny;
2. Use of an animal model that, in comparison with previous work, more directly mirrors clinical practice by replicating perforator size and which produces a clinically relevant size discrepancy without the use of grafts. This size ratio was judged to be between 1:1.5 and 1:2.5;
3. The avoidance of the use of arteriovenous anastomoses, or of vein grafts, in an attempt to remove the potential confounding factor of compliance mismatch;

4. Use of a sample size large enough to robustly compare patency rates.
5. An experimental series conducted by more than one investigator, in order to allow greater generalisation of the findings into microsurgical practice, and;
6. A paired method of measuring flow rates using contemporary technology with an acceptably small inherent measurement error.

In addressing the sought-after advantages on one technique over the other (section 2.2.), two further, complementary and amplifying pieces of work were designed. These were:

7. A study which used vessel remodelling as a proxy for long-term flow through the anastomoses, and which also addressed the degree of stenosis found at the anastomosis over a longer term, and;
8. A histological study to compare the trauma induced by the two techniques, and to look for differences in intimal hyperplasia that might arise from the differing geometries and their resulting haemodynamics.

2.4.2 Study Outline

From these design considerations, the following studies arose (Figure 2.1):

1. *In silico* (computational) modelling of haemodynamics to look for laminar flow separation and to model wall shear stresses in each method.
2. *In vivo* animal work:
    a. Establishment of a model that used arteries of a size similar to that of perforators (≤ 0.8 – 1mm in external diameter), and which produced a size discrepancy of clinical relevance without the use of arteriovenous anastomoses or of interposition vein or artery grafts;
    b. A paired patency and timing experiment shared between two investigators;
3. Amplifying *in vivo* and *ex vivo* experiments:
    a. A paired flow experiment, using the parameters of mean volume flow rate, relative resistance and waveform analysis by pulsatility index;
    b. A longer-term, paired experiment using luminal diameter as a proxy measurement of flow, and which quantified anastomotic stenosis, and;
    c. A semi-quantitative histological study of endothelial and medial necrosis, and which also quantified intimal hyperplasia in each technique.

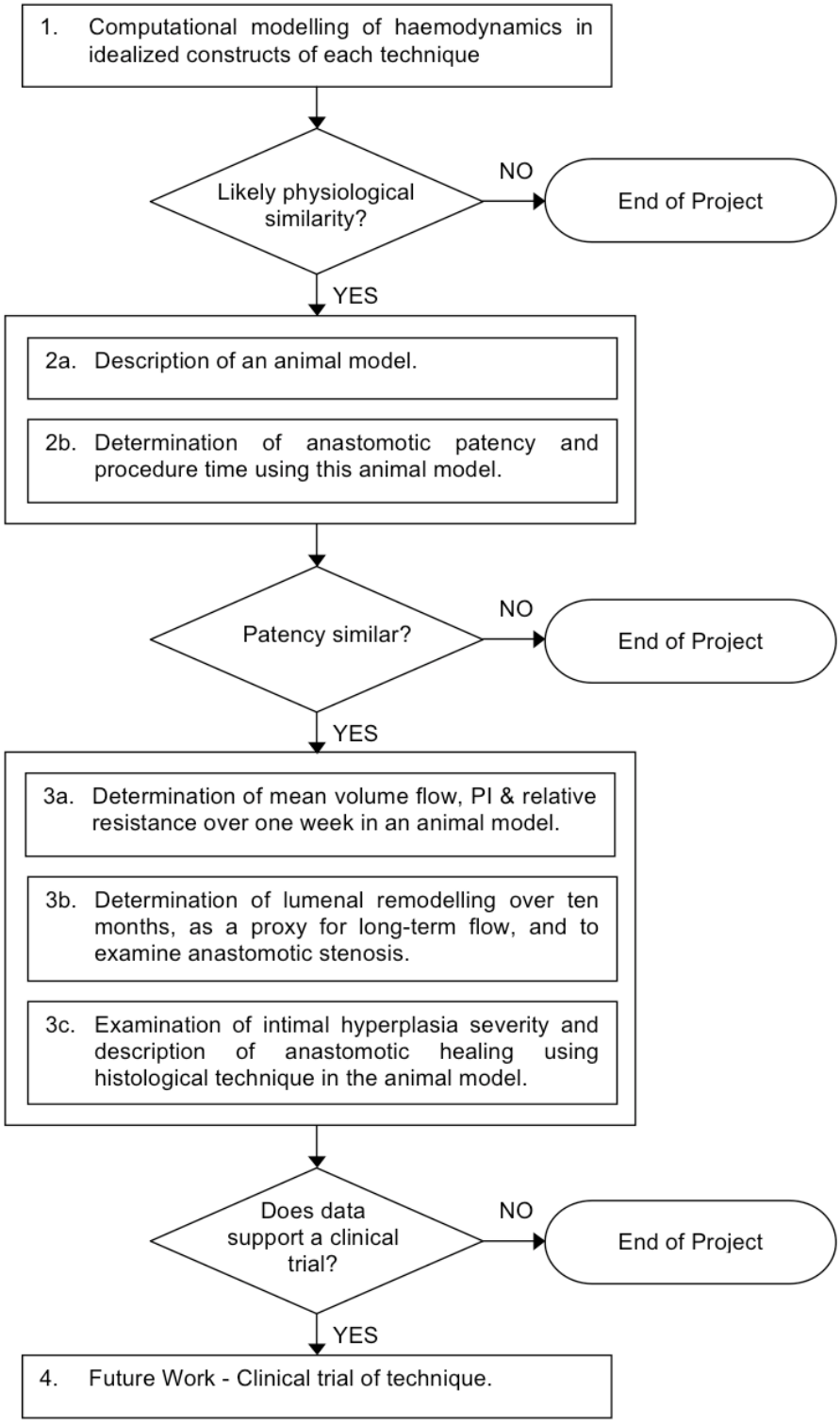


Figure 2.2. Flowchart of the experimental programme. PI = pulsatility index.

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3

COMPUTATIONAL MODELLING OF HAEMODYNAMICS

Outline

3.1. Introduction

Abrupt changes in vessel diameter and direction can lead to flow separation and to vortex formation. These flow disturbances in turn lead to an increase in the transit time of thromboactive substances at these areas (Leonard, 1972), leading to platelet aggregation and deposition (Karino and Goldsmith, 1979a, 1979b). Thus thrombus formation is encouraged, and where flow disturbances occur at an anastomosis, this may lead to anastomotic failure. It follows, therefore, that the geometry of an anastomosis may influence its patency.

In addition, endotheliocytes act as transducers of wall shear stresses, and orchestrate vessel wall remodelling in the presence of shear stress changes (Kamiya and Togawa, 1980, Langille and O'Donnell, 1986). Shear stress gradients adjacent to anastomoses will be affected by anastomotic geometry and may therefore influence vessel remodelling after the construction of an anastomosis between vessels of dissimilar diameters.

The aim of this study was to numerically model flow patterns and wall shear stresses in the two anastomotic techniques under investigation. Visualization of these factors might reveal physiological differences that would favour one technique over the other.

3.2. Materials and Methods

Modelling of haemodynamic factors was carried out using commercially available fluid modelling software. The two constructs were modelled geometrically in three dimensions. The smaller vessel was set at an internal diameter of 1mm, and the larger at 2mm (Figure 3.1).

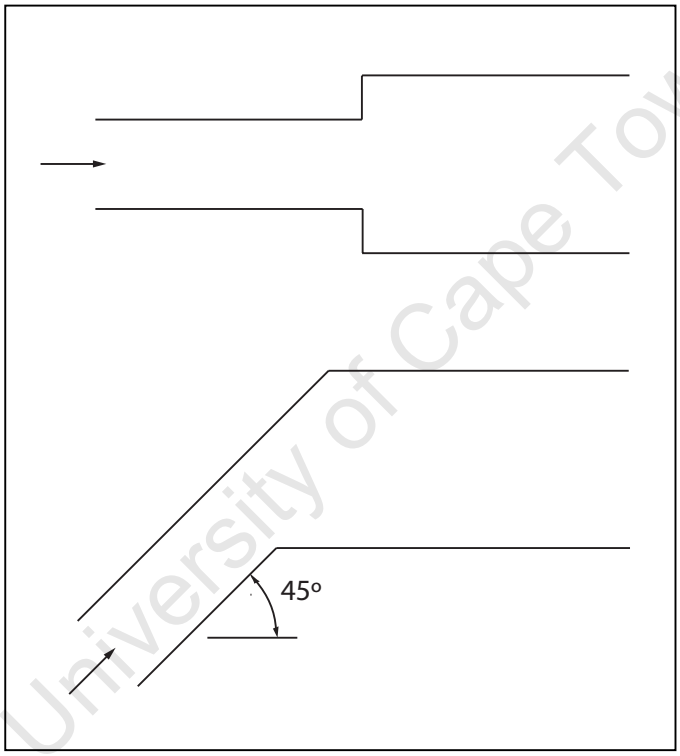

Figure 3.1. Approximate two-dimensional geometries of the two idealized end-to-end anastomotic constructs. Top figure; invaginating anastomosis model. Bottom figure; oblique anastomosis model. Arrows indicate direction of flow (upstream vessel on the left). Upstream vessel diameter 1mm, downstream vessel diameter 2mm.

Walls were deemed non-compliant. Flow was considered laminar and Newtonian. Flows of this nature are mathematically characterised by the Navier-Stokes equations (White, 1991), which in turn are resolved using

the so-called control volume formulation (Versteeg and Malalasekera, 2007). This is implemented in the commercially available Computational Fluid Dynamics (CFD) package Fluent™ (Fluent Inc., Lebanon, NH, USA. http://www.fluent.com).

3.2.1. Computational Grid

In order to numerically resolve flow through these models, it was necessary to define a calculation domain and appropriate boundary conditions.

The calculation domain was then divided or discretized into a number of finite computational elements or cells, which together form the computational grid (Figures 3.2 and 3.3). Once resolved, a computed solution provides flow-variable values such as magnitude and direction of the flow velocity vector at the centroids of each of these cells. The number of cells used in the grid is a compromise between the computational resources available and the desired resolution, because the required computational power increases almost exponentially with an increase in the number of cells.

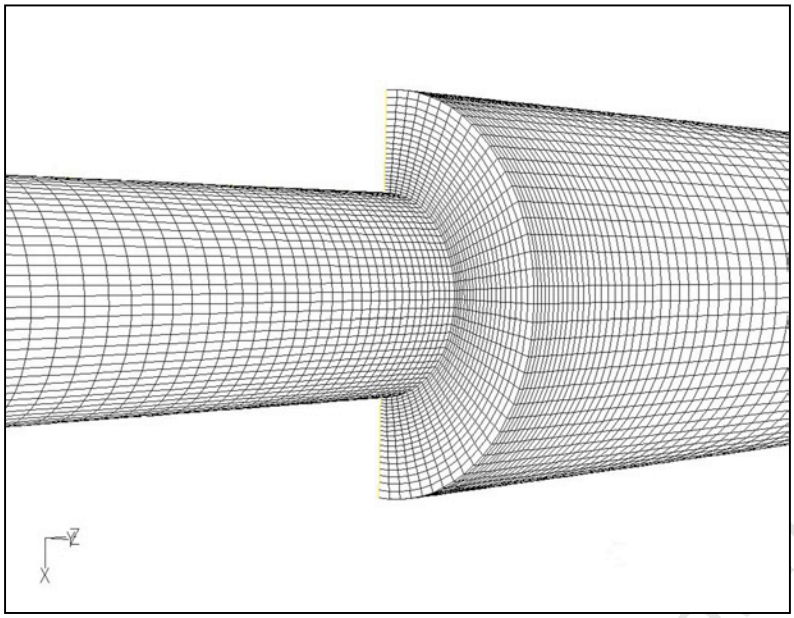

Figure 3.2. Surface computational grid at the junction of the two arteries in the invaginating anastomosis model.

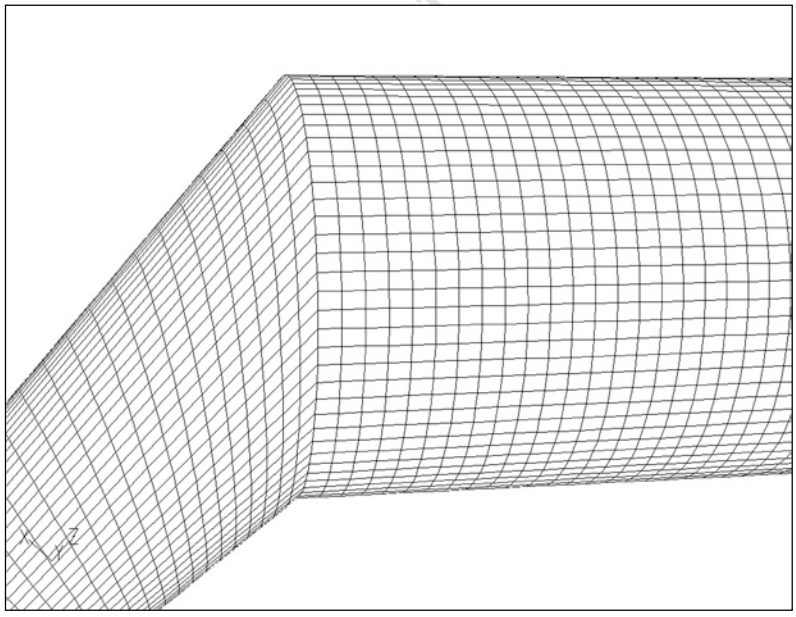

Figure 3.3. Surface computational grid at the junction of the two arteries in the oblique anastomosis model.

3.2.2. Boundary Conditions

Boundary conditions needed to be specified for all surfaces that bound the computational grid. Four types of boundary condition were defined during the course of this investigation (Figure 3.4):

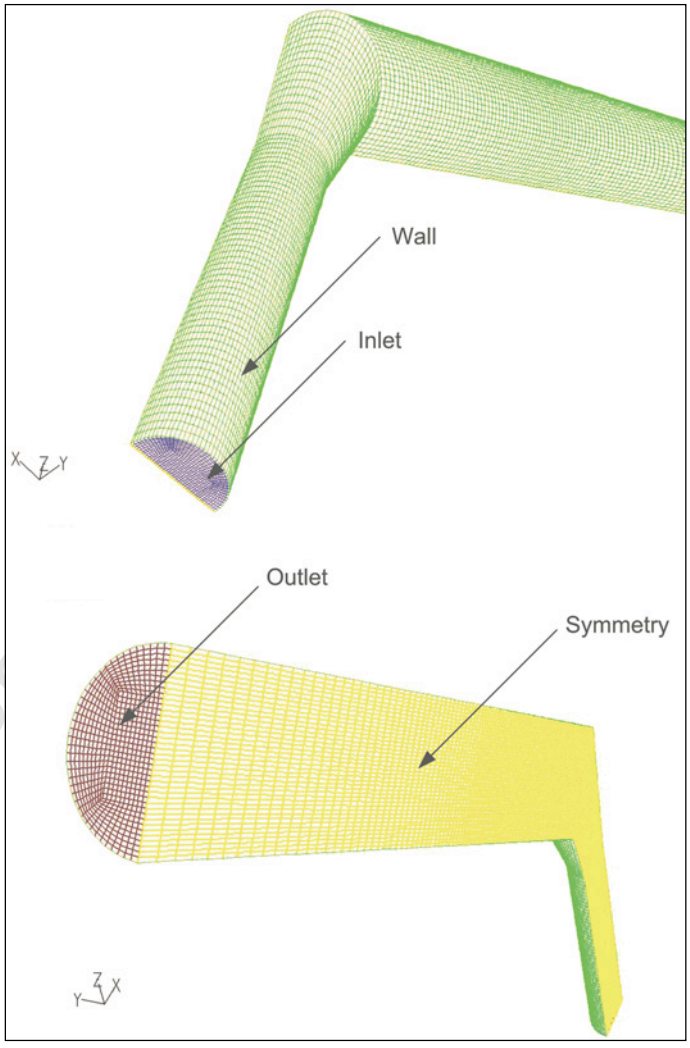

Figure 3.4. Location of boundary conditions on the computational grid used for the oblique anastomosis model.

(i) Velocity Inlet Boundary Condition

The magnitude and direction of the velocity vector at the inlet boundary surface are derived from volume flow rate (Q). For modelling purposes, flow rate through the left femoral artery of a single (413g) outbred male Wistar (HsdOla:WI) strain rat was measured. Ethical review was conducted by the Animal Ethics Committee of the University of Cape Town (Rec Ref 03/04), and animal care was conducted according to University protocols. The animal was terminally anaesthetised using a combination of parenteral ketamine HCl (Anaket-V®, Centaur Laboratories, Bryanston, South Africa) and xylazine HCl (Rompun®, Bayer (Pty) Ltd., Isando, South Africa. http://www.bayer.co.za). Core body temperature was monitored by a digital rectal thermometer and was maintained between 37.0 and 38.0°C by the use of a warming mat and a radiant heat source.

The left femoral artery was exposed and a transit-time ultrasound flow probe (Model 1RB, Transonic Systems Inc., Ithaca, NY, USA. http://www.transonic.com) was placed around the vessel. Flow was measured by a transit-time ultrasound flow meter (T204, Transonic Systems Inc.). Flow waveform was digitized using an analogue-to-digital interface (PowerLab® 400, AD Instruments Pty Ltd., Castle Hill, NSW, Australia. http://www.adinstruments.com) linked to an Apple® iBook® computer (Apple Computer Inc., Cupertino, CA, USA. http://www.apple.com) running Chart® v5.2 software (AD Instruments Pty Ltd.).

Because flow rate is influenced by respiration, a representative sample of flow taken over one respiratory cycle was selected. Flow values were

taken every 0.01s. This gave 71 discrete volume flow measurements over five pulses.

For the idealized geometries it was assumed that flow at the velocity inlet boundary displayed a laminar distribution so that:

$$u = \frac{2Q}{\pi r_o^4}\left(r_o^2 - r^2\right)$$

where u is the axial velocity, r_o is the inside radius of the inlet section in question (0.5 mm) and r is the radial location as measured from the centre of the boundary surface.

(ii) Outlet Boundary Condition

An outlet boundary is appropriate where fluid leaves the calculation domain through a surface. It is important that the surface is located sufficiently far downstream of obstacles or disturbances that might cause recirculating flow, as the flow should be directed outwards everywhere across that boundary. All variable-value gradients perpendicular to the surface at the outlet boundary were set to zero.

(iii) Wall Boundary Condition

All velocity values were set to zero at the surface where a wall boundary condition was applied.

(iv) Symmetry Boundary Condition

Symmetry boundary conditions may be used when the physical geometry of interest, and the expected pattern of the flow solution show mirror symmetry. The flow fields and computational domains under investigation both exhibit mirror symmetry (see Figure 3.4), in that the geometries can be divided axially through the centre of the inlet and outlet surfaces, effectively reducing the computational grid by one half.

3.2.3. Blood Density and Viscosity

For modelling purposes a constant density of 1059 kg/m^3 and a molecular viscosity of 0.005 kg/ms were specified.

3.3. Results

Figure 3.5 displays the transit-time ultrasound-determined blood flow rate used during the course of the investigation. A time step size of 0.01s was used for all simulations conducted. Flow rate in the rat femoral artery during this sampling period varied between a minimum diastolic flow rate of 2.54mL/min and a maximum systolic flow rate of 12.54 mL/min, and averaged 5.14mL/min.

The complete solution for a particular geometry consists of the resolved flow field at 71 discrete time points from 0.01s to 0.71s. Simulations of flow lines by velocity magnitude, and of wall shear stresses for the entire respiratory cycle shown in Figure 3.5 were performed. In order to better

visualize the resulting solutions, these images were animated (see accompanying CD). A single pulse from 0.27s to 0.40s is used for analysis and discussion purposes.

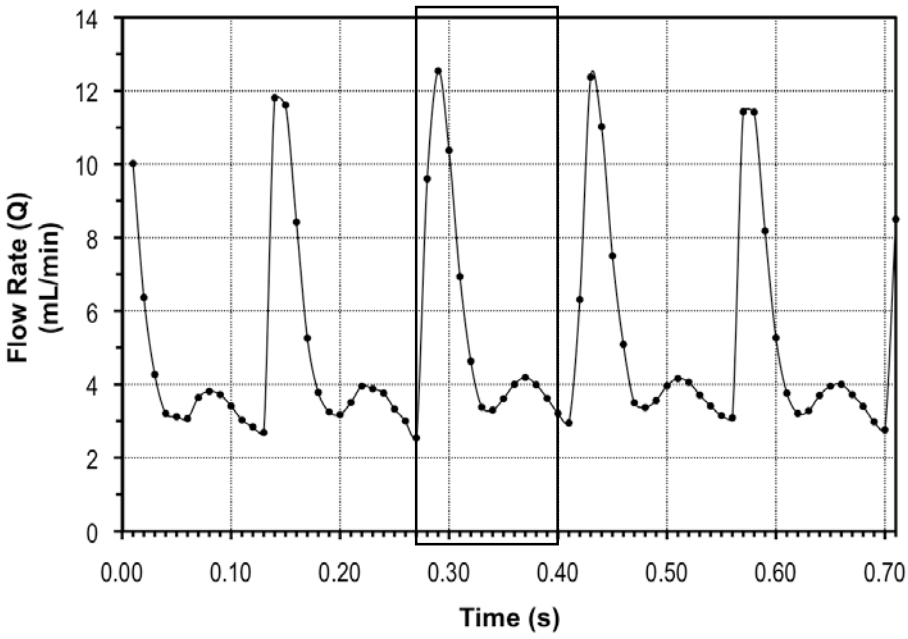

Figure 3.5. Time evolution of flow rate measured over one respiratory cycle. A single pulse from t = 0.27s to t = 0.40s is used in analysis

3.3.1. Invaginating Anastomosis

Flow line and shear stress results for the entire respiratory cycle are at Figures 3.8 and 3.9 respectively. Animations of flow lines and of shear stress contours are on the accompanying CD (files Invaginating_Flow_Lines.mov and Invaginating_Shear_Stresses.mov).

Figure 3.6 shows flow lines for time points t = 0.27, 0.28, 0.29, 0.31, 0.33, 0.35, 0.36, 0.37, 0.39, and 0.40s in greater detail. The flow field is characterized by the formation of a corrugated ring vortex, or torus, within the larger diameter artery immediately after the fluid moves through the junction between the two vessels. From t = 0.27 to 0.29s the flow accelerates from a minimum of 2.54 to a maximum of 12.54 mL/min. From Figure 3.6b., it can be seen that the vortex size is smallest as the flow maximally accelerates.

From t = 0.29 to 0.34 s the flow rate reduces to 3.30 mL/min. It can be seen that as the flow decelerates from t = 0.29, the ring vortex stretches in the downstream direction, reaching a maximum diameter at t = 0.33s. (Figure 3.6e).

From t = 0.34 to 0.37s the flow rate increases to reach a secondary maximum of 4.19 mL/min. This change from a decelerating to an accelerating flow causes a rapid reduction in the diameter of the vortex, which appears to fragment and dissipate in parts of the vessel circumference. The size of the ring vortex rapidly stabilises and is seen to remain virtually unchanged as the flow slowly accelerates.

From t = 0.37 to 0.40s the flow rate decreases again to 3.21 mL/min. As earlier the deceleration of the flow causes an increase in the distance that the ring vortex extends in the downstream direction, although it is much smaller than at the end of the more severe deceleration period from t = 0.29 to 0.34s.

Figure 3.7 shows the wall shear stresses at the same time intervals as Figure 3.6. Shear stress in the smaller vessel ranges from 2.3 to 14.0 N/m^2, and in the larger from 0.47 to 2.3 N/m^2. Shear stress scales with flow rate, and for both the low and high flow rates (at t = 0.27s and t = 0.29s respectively), the maximum wall shear stress occurs at the inner edge of the junction of the two sections. At this point, shear stress ranges between 2.8 and 14.0 N/m^2. The point that experiences the lowest average shear stress throughout the cycle is in the larger vessel immediately following the rapid expansion.

Of further interest are concentric shear stress contours, seen in the larger vessel, which correspond to the presence of ring vortices during high flow rates between t = 0.28 and t = 0.35 s.

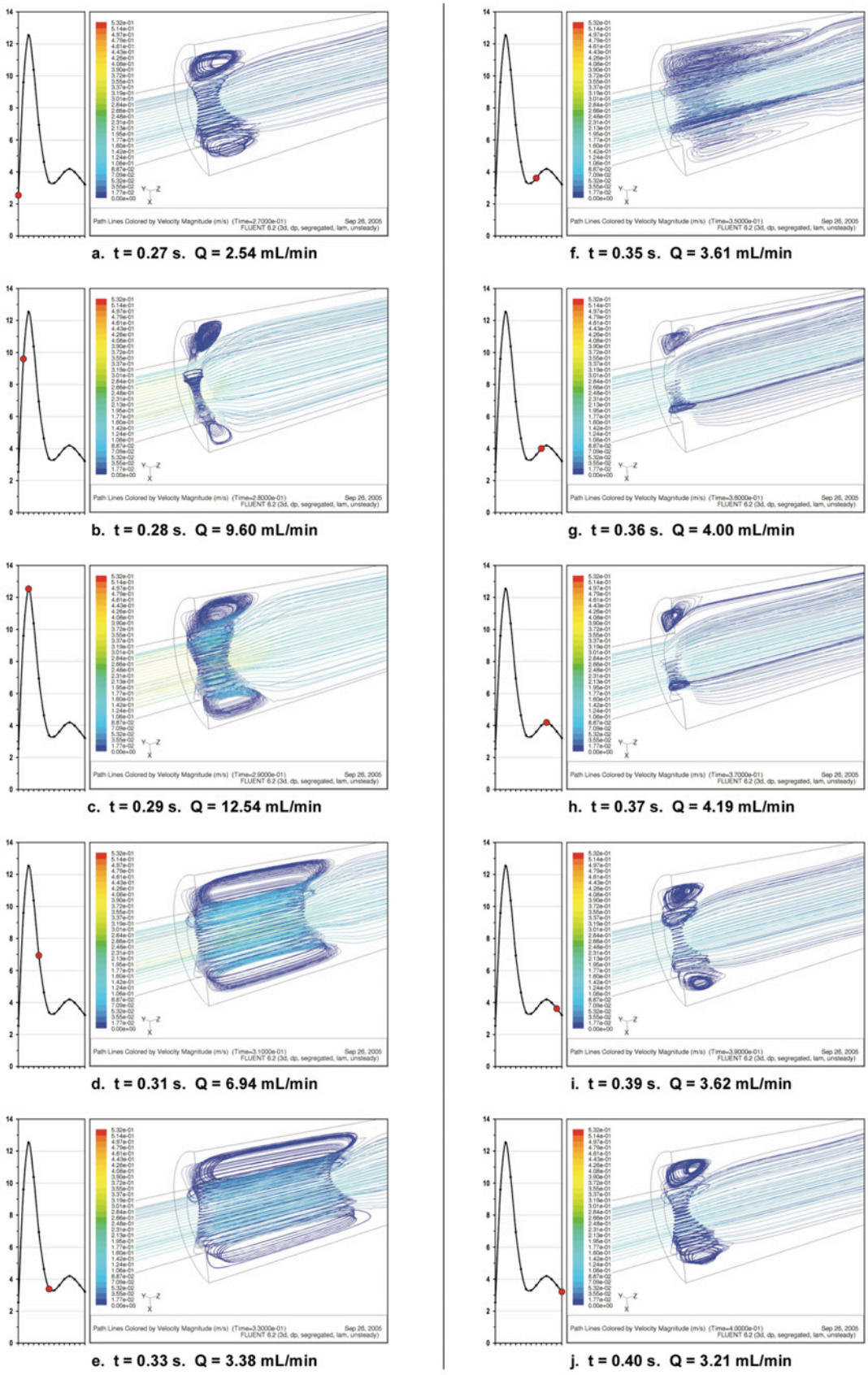

Figure 3.6. Flow lines through the invaginating anastomosis model at t = 0.27, 0.28, 0.29, 0.31, 0.33, 0.35, 0.36, 0.37, 0.39, and 0.40s. Lines coloured by velocity magnitude (ms^{-1})

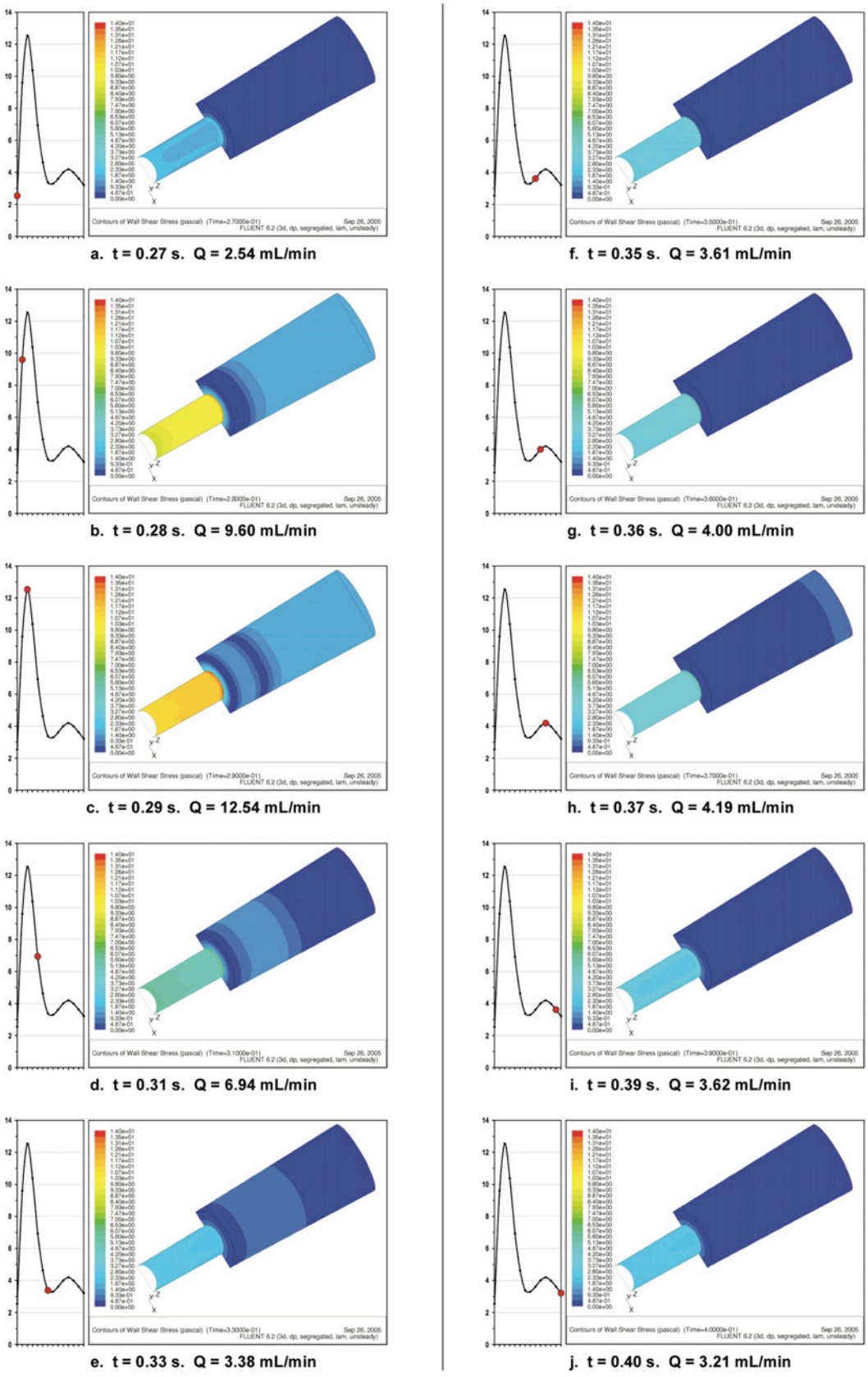

Figure 3.7. Wall shear stress contours in the invaginating anastomosis model at t = 0.27, 0.28, 0.29, 0.31, 0.33, 0.35, 0.36, 0.37, 0.39, and 0.40s. Lines coloured by shear stress magnitude (Pascal)

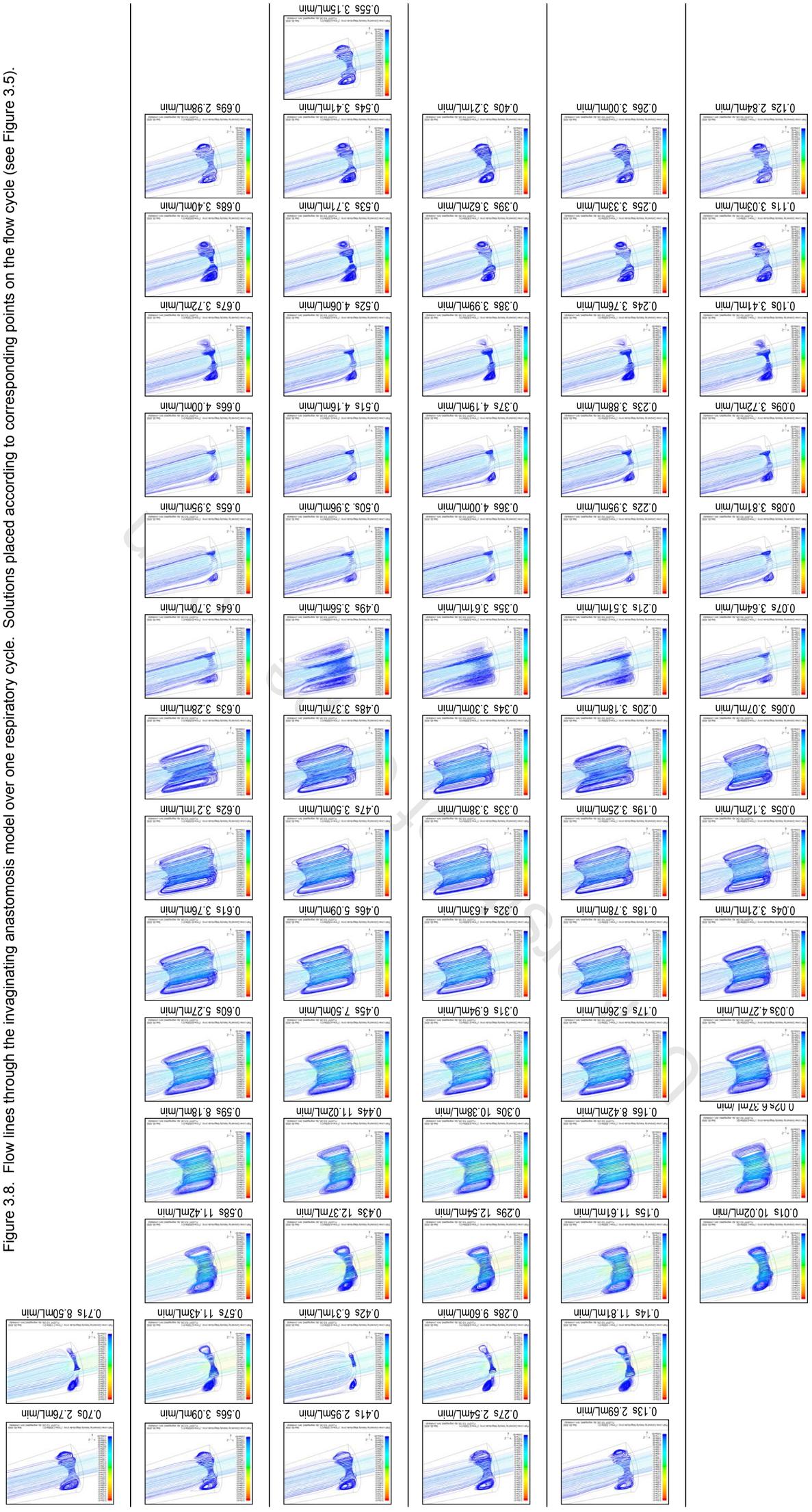

Figure 3.8. Flow lines through the invaginating anastomosis model over one respiratory cycle. Solutions placed according to corresponding points on the flow cycle (see Figure 3.5).

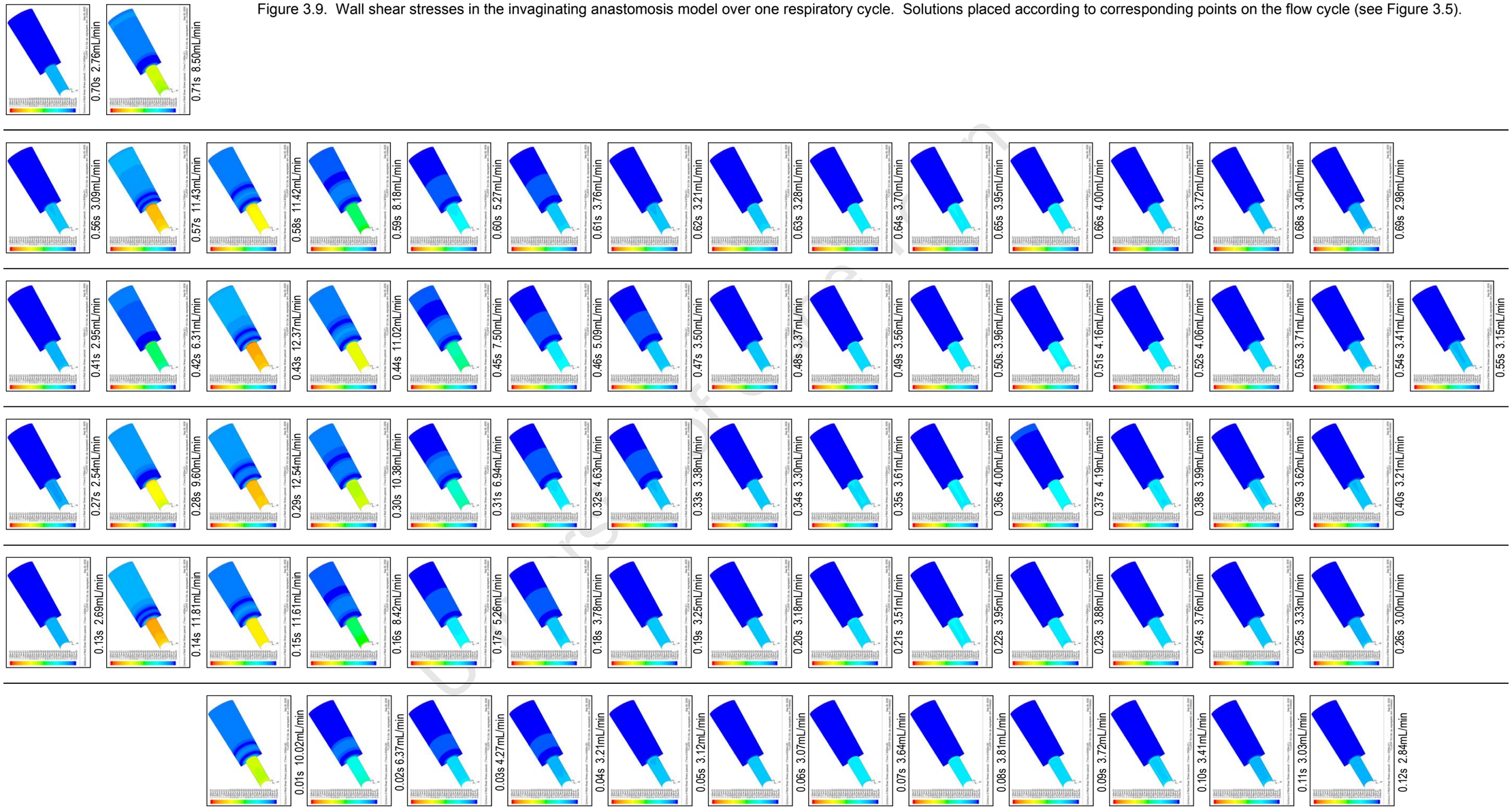

Figure 3.9. Wall shear stresses in the invaginating anastomosis model over one respiratory cycle. Solutions placed according to corresponding points on the flow cycle (see Figure 3.5).

3.3.2. Oblique End-to-End Anastomosis

Flow line and shear stress results for the entire respiratory cycle are at Figures 3.13 and 3.14. Animations of flow lines and of shear stress contours are on the accompanying CD (files ObliqueETE_Flow_Lines.mov and ObliqueETE_Shear_Stresses.mov).

Figure 3.10 shows flow lines at time points t = 0.27, 0.28, 0.29, 0.31, 0.33, 0.35, 0.36, 0.37, 0.39, and 0.40 s in greater detail. Flow through the oblique geometry is more complex than through the invagination model. High-velocity flow from the small diameter artery impinges at a 45-degree angle onto the upper surface of the large diameter artery. The upper surface deflects the flow downward as well as downstream, creating a pair of counter-rotating spiral vortices aligned along the length of the large diameter artery. These vortices dissipate in the downstream direction. Figure 3.11 shows flow vectors in a cross-sectional plane at the inlet side of, and perpendicular to the axis of the larger diameter artery, at t = 0.29s. One of the centres of the counter-rotating vortex pairs can be seen in the figure. The use of a symmetry plane means that only one half of the geometry was modelled and therefore only one of the vortices is seen in Figure 3.11.

Although the vortex formation in the case of the oblique geometry is more complex, the general trends are similar to the invagination model: The vortex appears during decelerating flow from t = 0.29 to 0.34s, corresponding to maximum vortex size in the invagination model, and it disappears again as the flow starts to accelerate at t=0.35s.

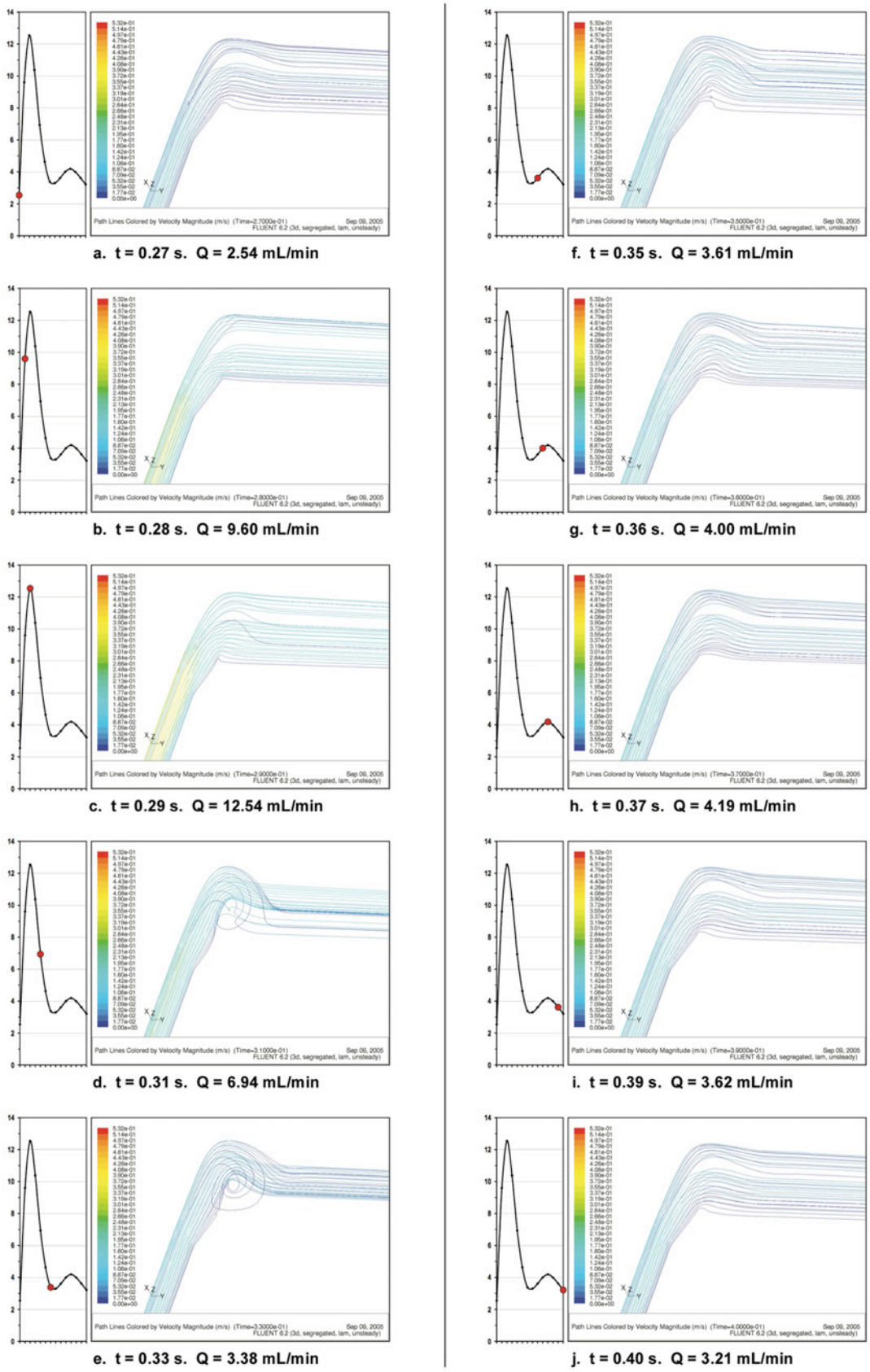

Figure 3.10. Flow lines through the oblique end-to-end anastomosis model at t = 0.27, 0.28, 0.29, 0.31, 0.33, 0.35, 0.36, 0.37, 0.39, and 0.40s. Lines coloured by velocity magnitude (ms^{-1})

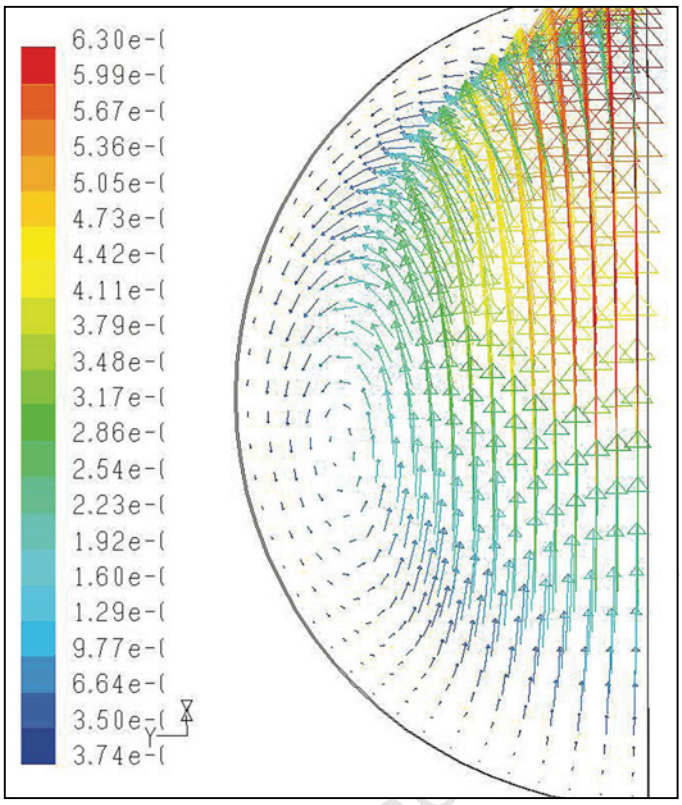

Figure 3.11. Flow lines through the oblique end-to-end anastomosis model at t = 0.27, 0.28, 0.29, 0.31, 0.33, 0.35, 0.36, 0.37, 0.39, and 0.40s. Lines coloured by velocity magnitude (ms^{-1})

Figure 3.12 shows wall shear stresses at the same time points as Figure 3.12. As with the invagination geometry, the maximum wall shear stress occurs at the point where the small diameter artery model starts to expand. Shear stresses scale with flow rate and range in the smaller vessel from 2.3 to 13.1 N/m^2, and in the larger from 0.47 to 2.8 N/m^2. Maximum shear stress occurs at the highest flow rate (t = 0.29 s), where expansion commences in the smaller vessel. Complex shear stress contours are seen in the proximal part of the larger vessel between t = 0.29 and t = 0.36s and appear to correspond to where flow impinges on the upper surface of the large diameter vessel.

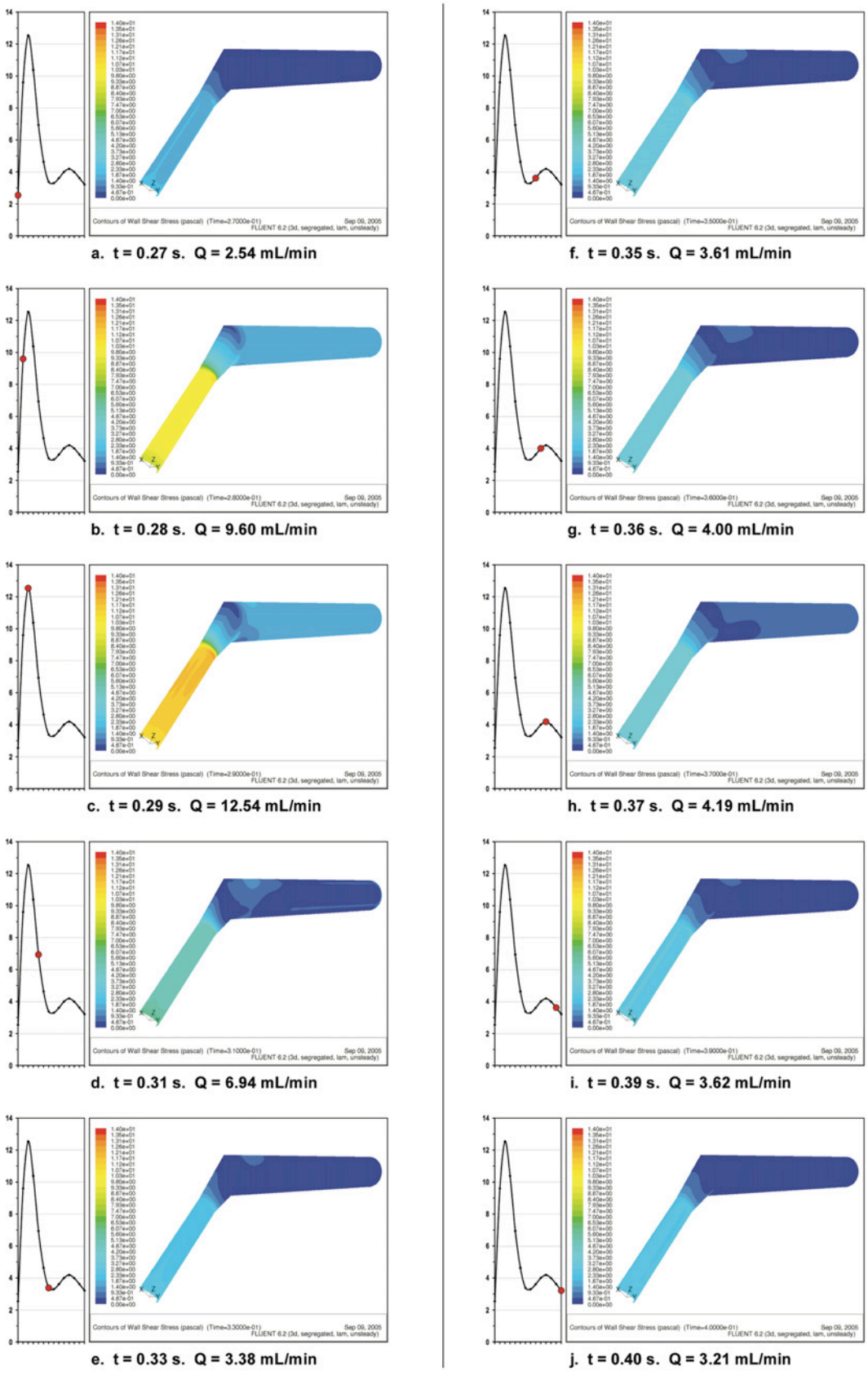

Figure 3.12. Wall shear stress contours in the oblique end-to-end anastomosis model at t = 0.27, 0.28, 0.29, 0.31, 0.33, 0.35, 0.36, 0.37, 0.39, and 0.40s. Contours coloured by shear stress magnitude (Pascal)

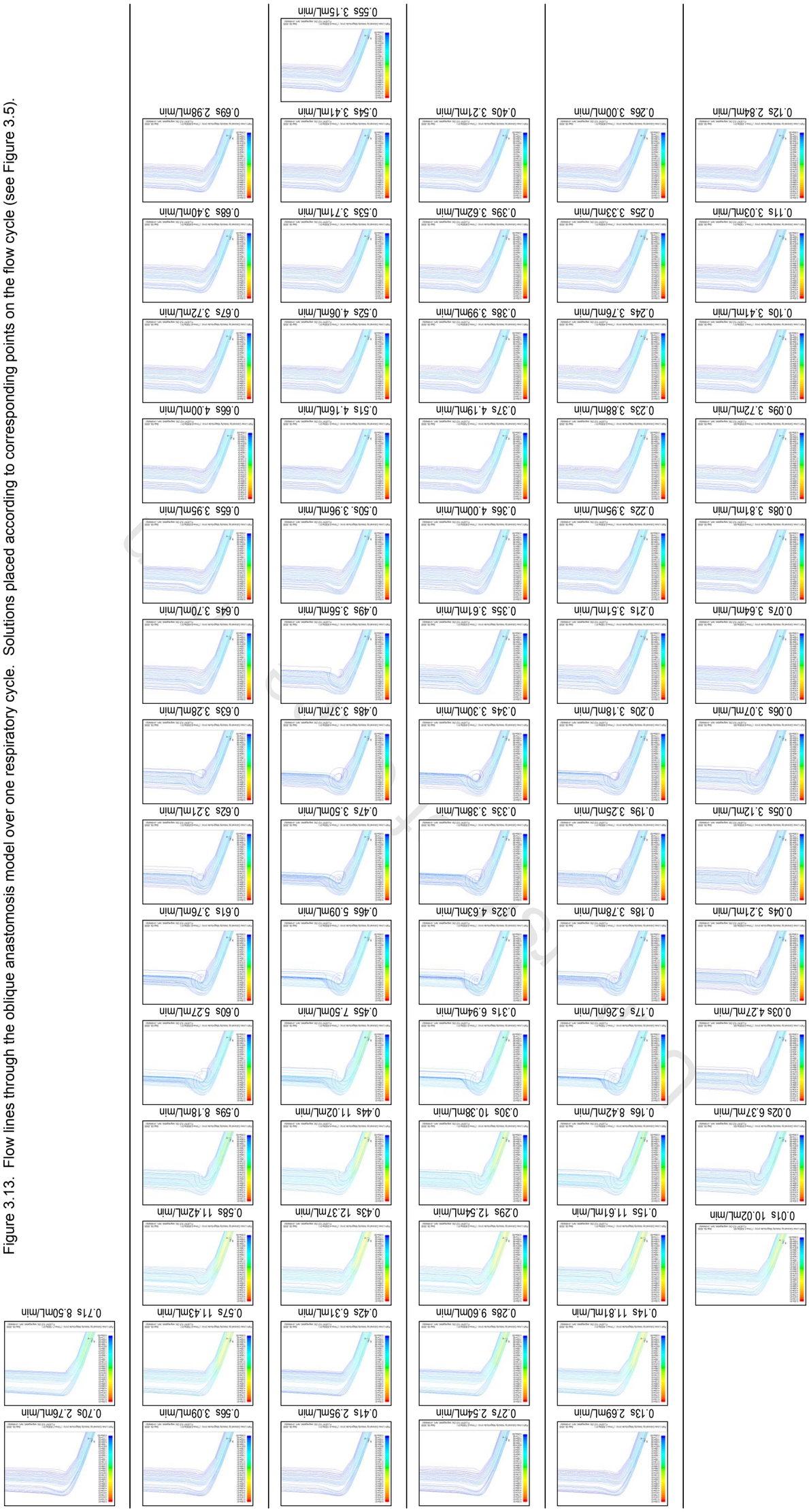

Figure 3.13. Flow lines through the oblique anastomosis model over one respiratory cycle. Solutions placed according to corresponding points on the flow cycle (see Figure 3.5).

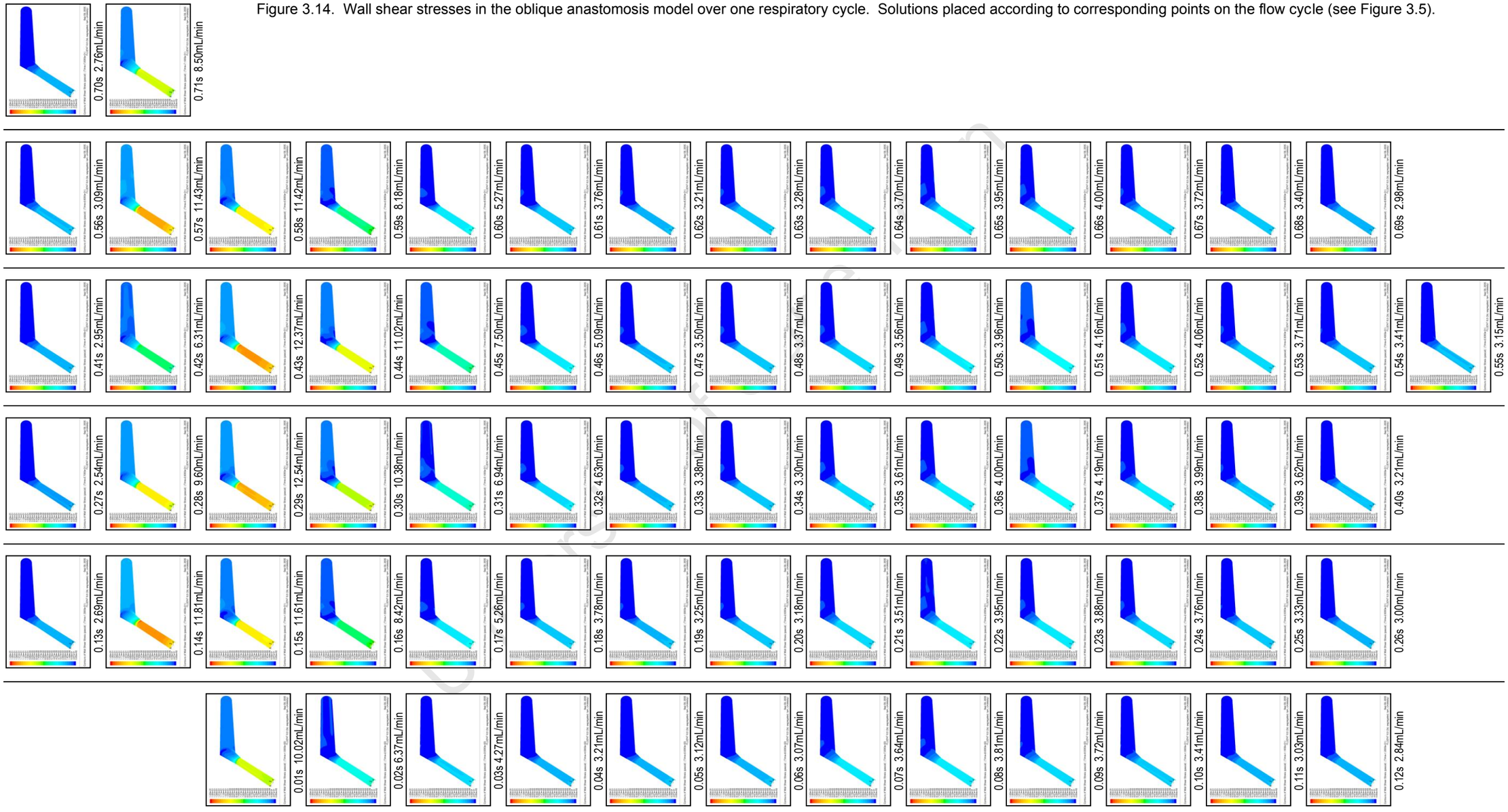

Figure 3.14. Wall shear stresses in the oblique anastomosis model over one respiratory cycle. Solutions placed according to corresponding points on the flow cycle (see Figure 3.5).

3.4. Discussion

In recent years, the *in silico* modelling of haemodynamics has found increasing application in the study of healthy, diseased, and surgically-altered cardiovascular systems (Migliavacca and Dubini, 2005). The power of these validated solutions has enabled evaluation of rheological factors such as wall shear stresses that are otherwise very difficult to study or to measure *in vivo*. To date, *ex vivo* models have used time-averaged or sinusoidal flow rates and therefore cannot generate results from unsteady flows seen in life (Sunamura *et al*., 2007). Computational studies allow modelling of these unsteady flows, but until recently have concentrated largely on coronary artery bypass graft anastomoses, peripheral arterial bypass graft geometries and materials, and in surgical procedures for the treatment of congenital cardiac abnormalities.

Rat femoral artery flow rate and flow rate variability approximated that seen in perforating arteries in man (Rubino *et al*., 2006). Regions of separated flow were observed in both constructs, occurring maximally during high but decelerating flow rate. This finding, i.e. the timing of maximum flow disturbance confirms those of other investigators who have used cinemicrographic techniques (Ishibashi *et al*., 1995). Although vortices appeared in the oblique construct during only this flow period, their geometry was more complex than in the invagination technique. The pattern of counter-rotating spiral vortices seen in the oblique construct has also been observed in 45 degree end-to-side anastomoses examined by a high-resolution photochromic tracer technique (Ojha *et al*., 1990).

Whilst the wall shear stress distribution is different for the different configurations, the minimum and maximum wall shear stress values are similar in these models.

The two primary disadvantages of these models are their idealized geometries and their non-compliant walls. The idealized geometries cannot account for any small perturbations in flow produced by sutures or by bunching up of tissues at an anastomosis. Vessel compliance (defined as change in vessel volume divided by change in blood pressure) is dynamic, and is influenced by many factors; smooth muscle tone in particular. Modelling vessels without compliant walls excludes a potential confounding factor that is difficult to quantify. However, the inclusion of compliance in *in silico* models is a subject of ongoing study in some centres. The validity of application of Newtonian fluid characteristics is more open for debate. In mammalian arteries of this magnitude, high shear rates cause blood to behave in a largely Newtonian fashion, and little difference has been demonstrated in other computational studies (Strony *et al*., 1993, Dutta and Tarbell, 1996). However, more recent work (Chen *et al*., 2006) may refute this, and the position is as yet unclear.

In conclusion, flow separation is seen in both the invagination and oblique anastomoses. A decision on the 'best' technique cannot be made on the basis of these models alone.

3.5. References

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4 ESTABLISHMENT OF AN ANIMAL MODEL

Outline

4.1. Introduction

Following the *in silico* modelling of the two anastomotic techniques, and in recognition of the limitations of this work, an animal model was sought which would allow the study of these techniques *in vivo*. The aim was to identify the least sentient species that provided mammalian cardiovascular and haematological physiology, and whose vessel anatomy provided a size discrepancy that would be clinically relevant.

In order to allow a paired study of the two techniques, a search was performed for paired, bilateral vessels that exhibited similar size and anatomical characteristics. Surgery on the hind limbs has the advantage of easy access to axial vessels and low morbidity following surgery. Branching patterns from the femoral artery were investigated. After the method of de la Peña-Salcedo *et al*. (2000), a branching pattern was sought which would allow the main, axial vessel to be tied off immediately distal to the origin of a smaller branching artery, thus diverting all blood flow into this smaller branch. The axial artery could then be divided just distal to this ligature, and the smaller branch could then be anastomosed end-to-end to the distal end of the divided axial vessel, providing a small-to-large diameter mismatch (Figure 4.1.). One technique could be performed on each side. Branching arteries were sought which would permit tension-free anastomosis, and which would offer sufficient distal axial vessel length to allow undisturbed distal run-off from the anastomosis. This last criterion was particularly important for the

invagination technique due to its requirement for longer distal vessel length.

Two animal species were initially investigated by means of small anatomical pilot studies; the New Zealand White Rabbit, and the Wistar Rat. Following this, the chosen model was anatomically and physiologically characterised in greater detail.

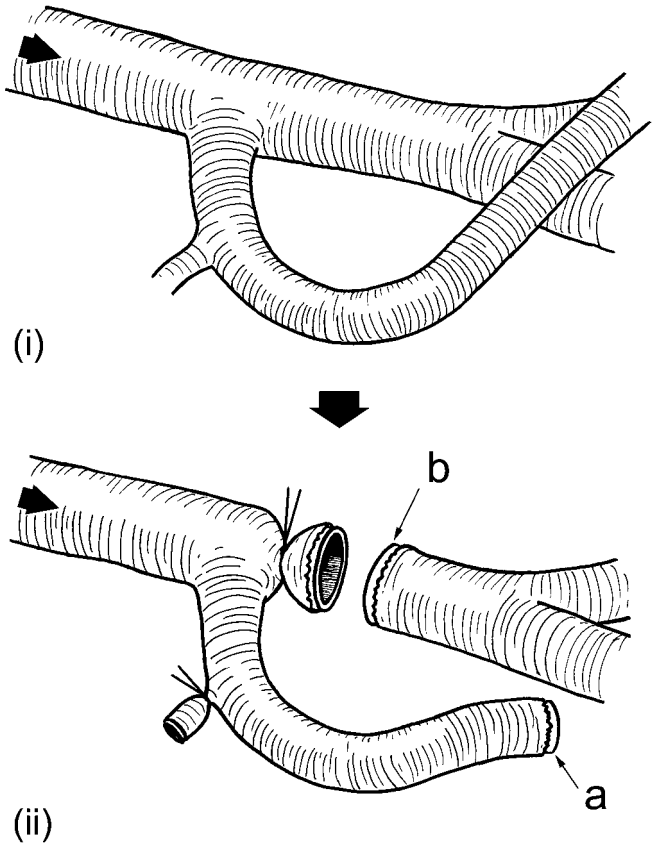

Figure 4.1. Vessel branching pattern (i) allows ligation of the main, axial vessel immediately distal to a branch, diverting all flow into a smaller side branch. (ii). The resulting small upstream vessel (a) and large downstream vessel (b) produce the small-to-large arterial diameter mismatch.

4.1.1. Ethical Approval

Ethical approval for the anatomical part of the study was granted by the University of Cape Town Animal Ethics Committee (Refs 03/04 and 03/041). Flow characteristics were studied in the UK under licence from the UK Home Office under the Terms and Conditions of the Animals (Scientific Procedures) Act 1986 (Project Licence 60/3749).

4.1.2. Animals

New Zealand White strain rabbits were obtained from an outbred breeding colony (JH Rabbitary, Kemptongate 1617, South Africa).

Wistar strain rats were obtained from two sources. Those used in the anatomical series were obtained from the breeding colony of the Central Research Facility, University of Stellenbosch. This colony was established in 2001 from HsdOla:WI rats, and maintained as an outbred colony. Those used in the flow study were HsdHan™:WIST rats from Harlan UK Ltd., Bicester, Oxfordshire, UK.

4.1.3. Husbandry

Animal care in South Africa was practised according to University of Cape Town protocols, and for the flow study, performed in the UK, the guidance given in EC Directive 91/507/EEC was followed.

Information on animal health status was obtained prior to arrival and all animals underwent an acclimatization period of at least seven days prior to

commencing the study. Rabbits were housed individually and rats were group-housed in polypropylene solid floor cages with stainless steel grid lids on racks. Wood shavings were used as bedding with nesting material and/or cardboard tubes provided as cage enrichment. The environmental temperature was maintained at 20 ± 2ºC and relative humidity at 55 ± 10%. The lighting schedule was 12h light and 12h dark and there were 15-20 air changes per hour. A non-sterile pelleted diet and domestic mains water were offered *ad libitum*.

4.2. Exploratory Studies

4.2.1. Materials and Methods

Five outbred male New Zealand White strain rabbits and five outbred male Wistar strain rats were studied. A similar dissection was carried out in both species. Animals were anaesthetised using a combination of parenteral ketamine HCl (Anaket-V®, Centaur Laboratories, Bryanston, South Africa) and xylazine HCl (Rompun®, Bayer (Pty) Ltd., Isando, South Africa. http://www.bayer.co.za).

Animals were positioned supine on a warming mat and the hind limbs were extended using loose, unconstricting limb restraints pinned into a cork board. Body temperature was maintained by the use of the warming mat, supplemented with a radiant heat source.

After shaving, bilateral caudally-concave incisions were made over the groins. The inguinal fat pad was dissected free from the underlying external oblique muscle and spermatic cord. The animal was placed under an operating microscope (SMZ-10, Nikon Corporation, Tokyo, Japan. http://www.nikon.com). With the aid of soft retractors, the Femoral Artery (FA) was found as it emerges from under the inguinal ligament and was dissected free from surrounding tissues using an atraumatic technique (Duminy, 1988). The dissection was completed from the inguinal ligament to a few millimetres distal to the bifurcation of the FA into the popliteal (PA) and saphenous arteries (SA).

Following topical application of a vessel plegic solution (2% lignocaine, B|Braun Medical (Pty) Ltd., Gauteng 2125, SA. http://www.bbraun.co.za) , digital images of these vessels were obtained using a 4.0MP consumer digital camera (Nikon Coolpix® 4500, Nikon), attached to the C-mount of a binocular operating microscope (SMZ-10, Nikon) by means of an adaptor lens (MDC-C Relay Lens, Nikon). These gave a 2272 x 1704 pixel image at a magnification of x40. Images were calibrated using a millimetre calibration slide (one pixel = 0.0045mm). Vessel measurements were performed using digital image analysis software (Carnoy v2.1, Biovolution, Leuven, Belgium http://www.biovolution.com). Three replicate measurements of vessel diameters and, in the Wistar rats, the length of FA distal to the origin of the SCEA were taken at each point and the mean values and standard deviations calculated. Results are given as means ±SD.

4.2.2. Results

4.2.2.1. New Zealand White Rabbit

After emerging from deep to the inguinal ligament, the femoral artery (FA) gives off the deep (caudal) femoral artery (DFA). Just distal to this, the lateral circumflex femoral artery (LCFA) is given off laterally. The LCFA subsequently divides into a transverse branch (LCFA-Tr) and a descending branch (LCFA-Desc) (Figure 4.2.). The DFA was unusable as a branching vessel because in some cases it could not be anastomosed to the distal FA due to the branching of the LCFA immediately distal to its origin. The LCFA itself was found to be too short to be useful. The LCFA-Tr and LCFA-Desc were of possible utility and their diameters were therefore measured, along with the diameter of the FA distal to the origin of the LCFA (Appendix A).

Mean animal body weight was 2211g. Taking results from both sides together, the mean distal FA external diameter was 1.032 ±0.146mm. Mean LCFA-Tr external diameter was 0.665 ±0.161mm. Mean LCFA-Desc external diameter was 0.579 ±0.196mm. Mean LCFA-Tr : FA ratio was therefore 1 : 1.55. Mean LCFA-Desc : FA ratio was 1 : 1.78. However, even in this small sample, the dominant vessel, i.e., either the LCFA-Tr or the LCFA-Desc, varied between animals and between sides.

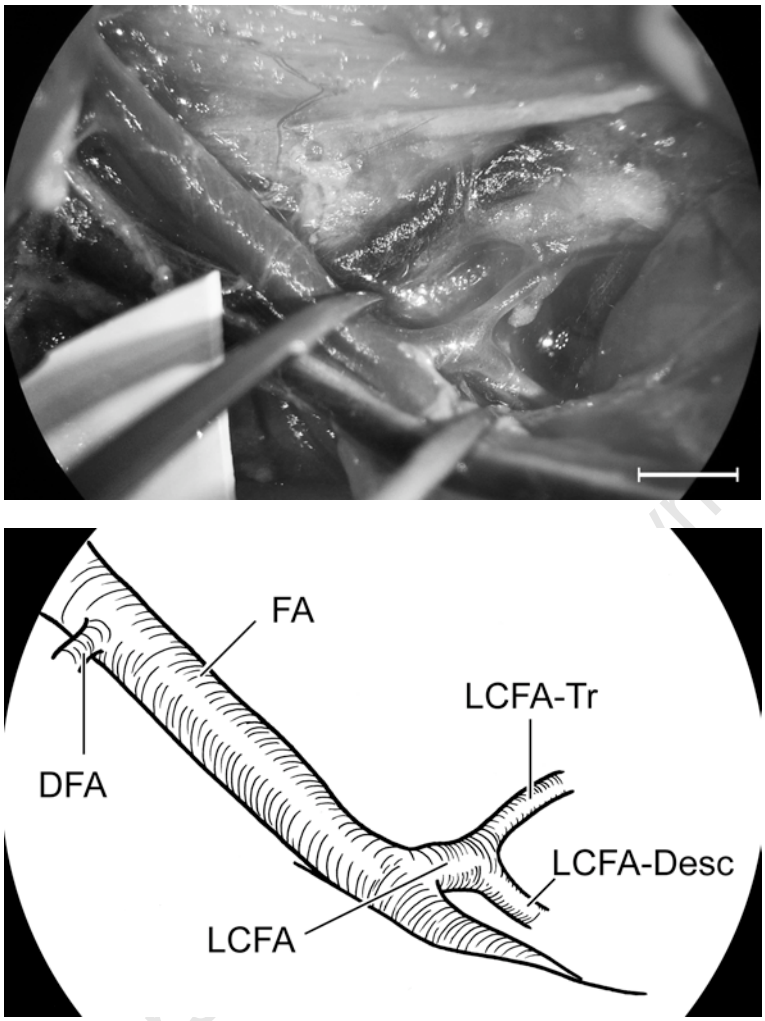

Figure 4.2. Branching pattern of the New Zealand White Rabbit femoral artery. Scale bar = 2mm. FA = Femoral Artery; DFA = Deep Femoral Artery; LCFA = Lateral Circumflex Femoral Artery; LCFA-Tr = Transverse Branch; LCFA-Desc = Descending Branch. Note that the LCFA-Desc is the dominant branch in this example.

Based on these results, attempts were made to use the LCFA-Desc as the proximal, smaller artery, anastomosing it to the distal FA. This necessitated ligating and dividing the DFA, FA and LCFA-Tr, before freeing sufficient length of LCFA-Desc to permit as tension-free an anastomosis as possible. A 10-O nylon suture swaged onto a 50µm, 3mm 3/8 circle taper point round-bodied needle (Dafilon® Black, G1118781,

B|Braun Medical (Pty) Ltd. Gauteng 2125, SA. http://www.bbraun.co.za) was used.

Atraumatic dissection of this vessel was technically demanding and time consuming. Whilst no formal biomechanical measurement was performed, the impression was gained that in all five rabbits the anastomosis was completed under unacceptably high tension (Figure 4.3).

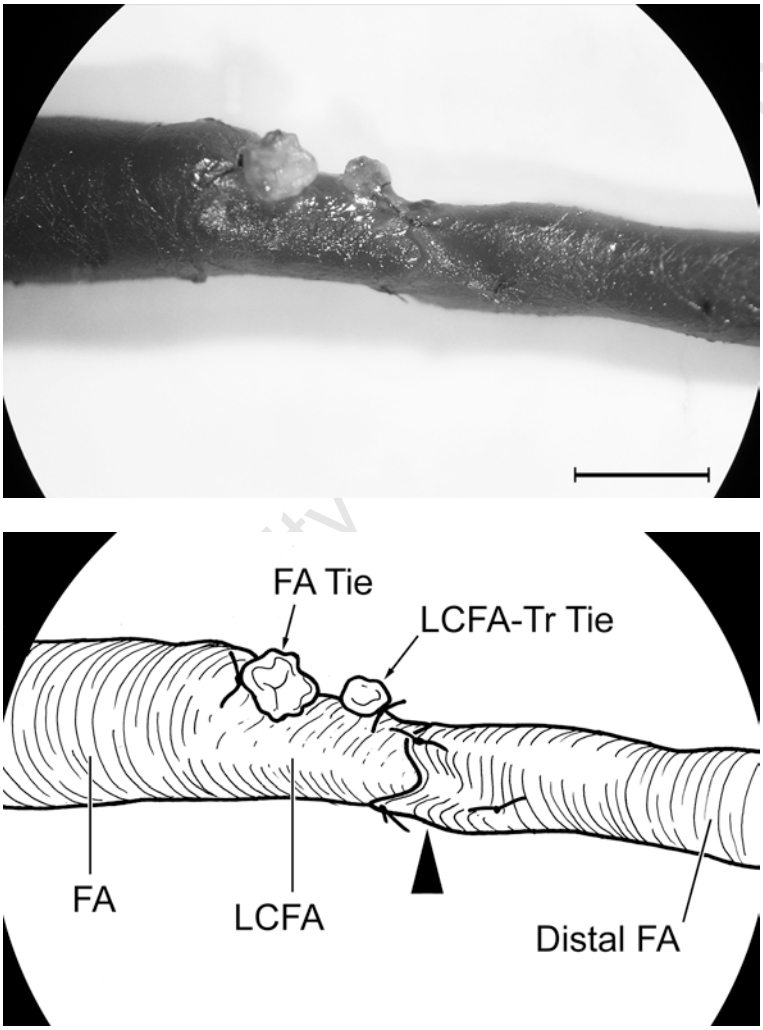

Figure 4.3. An example of the tension encountered when anastomosing the Descending Branch of the Lateral Circumflex Femoral Artery to the Distal Femoral Artery in the rabbit. Photomicrograph shows a functioning invaginating anastomosis between these vessels. Scale bar = 1mm. Arrowhead = site of anastomosis. FA = Femoral Artery; LCFA = Lateral Circumflex Femoral Artery; Distal FA = Distal Femoral Artery; FA Tie = tie around Femoral Artery immediately after origin of LCFA. LCFA-Tr Tie = Tie around Transverse Branch of LCFA.

4.2.2.2. Wistar Rat

The FA in the Wistar rat gives, as its first branch, the Deep (Caudal) Femoral Artery (DFA). The first branch given off after the DFA is the Superficial Caudal Epigastric Artery (SCEA). There is no rodent equivalent of the rabbit LCFA. A relatively short length of FA is present after the SCEA is given off, before it divides into the PA and SA. The SCEA is long and of uniform calibre.

After dissection of the FA from the inguinal ligament to this bifurcation, the SCEA was dissected free from the inguinal fat pad for a distance of eight millimetres. The utmost delicacy was necessary in dissecting free this vessel because it was very easily damaged and prone to spasm. SCEA diameter was measured 6mm from its origin and FA diameter 1mm distal to the SCEA origin.

Mean animal body weight was 415.6 ±31.9g. Taking results from both sides together, the mean FA external diameter was 0.950 ±0.092mm. Mean SCEA external diameter was 0.539 ±0.083mm, giving a mean SCEA : FA ratio of 1 : 1.76. Mean length of FA available distal to the SCEA origin was 3.534 ± 0.616mm. The plentiful length of the SCEA allowed for a tension-free anastomosis at the 6mm point (Figure 4.4.).

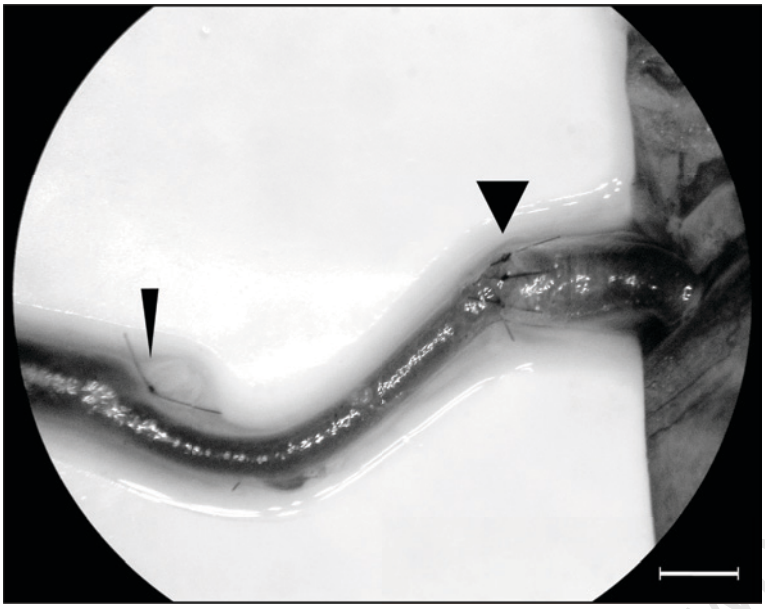

Figure 4.4. An oblique end-to-end anastomosis between the Wistar Superficial Caudal Epigastric Artery (SCEA) and distal Femoral Artery (FA). Narrow arrowhead = tie around the FA immediately distal to the origin of the SCEA. Wide arrowhead = anastomosis.

4.2.3. Discussion

Atraumatic dissection of the rabbit vessels proved technically demanding and time consuming. Furthermore, even after dissecting out this vessel to its maximum available length, there appeared to be too much tension at the anastomosis. The impression was gained that rabbit femoral arteries are more elastic than rodent arteries, and retract extensively when divided. This may have presented technical difficulties in one study of the invagination technique in equal-sized vessels (Sully *et al.*, 1982).

In contrast, atraumatic dissection of Wistar strain rat vessels was relatively straightforward, although utmost delicacy was essential when

dissecting out the SCEA. The SCEA : FA diameter ratio was within the desired range. The ample length and uniform calibre of the SCEA made it an ideal small, upstream vessel and ensured a tension-free anastomosis. In addition, sufficient length could be obtained to ensure that, at least theoretically, laminar flow would be re-established before blood passed through the anastomosis. Whilst distal FA length was variable in these five animals, sufficient length of artery was available to permit undisturbed distal run-off before this vessel bifurcated, a distance of 1-2mm being sufficient in vessels of this approximate calibre (De La Pava *et al.*, 1979).

4.3. Detailed Characterisation of the Wistar Rat Model

4.3.1. Introduction

Following these pilot studies, measurements from an additional 35 outbred male Wistar strain rats were included in a more extensive anatomical characterisation of this model. Femoral artery blood flow characteristics were determined from the study of seventeen further animals.

4.3.2. Materials and Methods

4.3.2.1. Anaesthesia

Parenteral ketamine HCl (Anaket-V®, Centaur Laboratories, Bryanston, South Africa) and xylazine HCl (Rompun®, Bayer (Pty) Ltd., Isando, South Africa. http://www.bayer.co.za) were used for the anatomical series. For flow measurement, the animals underwent gaseous induction followed by oro-endotracheal intubation with a modified 16G/45mm venous cannula (AniCath MP06216, Millpledge Veterinary, Millpledge Pharmaceuticals Ltd., Clarborough, Nottinghamshire, UK. http://www.millpledge.com) using a trans-tracheal illumination technique. Anaesthesia was maintained by an N_2O / isoflurane / O_2 gaseous mixture on a rodent ventilator (Model No. 7025, Ugo Basile, Comero, Varese, Italy. http://www.ugobasile.com) at 60 breaths per minute and a ventilator stroke volume of 4 – 5mL.

In both studies, buprenorphine HCl (Temgesic®, Schering-Plough, Johannesburg, South Africa, or Vetergesic®, Reckitt Benckiser Healthcare (UK) Ltd., Hull, UK) and either bupivacaine HCl (Marcaine®, Adcock Ingram, Bryanston, South Africa) or levobupivacaine HCl (Chirocaine®, Abbott Laboratories Ltd., Queensborough, Kent, UK) was administered during the procedure. Hydration was maintained by the administration of normal saline by either the intravenous (anatomic study) or subcutaneous routes (flow study).

4.3.2.2. Anatomic Characterisation

Forty outbred male Wistar rats (the five from the pilot study plus the additional thirty-five animals) were included in the anatomic characterisation. Animals were weighed. The FA and SCEA were dissected free as described above. Following topical application of 2% lignocaine, digital images of these vessels were obtained using the Nikon SMZ-10 microscope and Coolpix camera, and measured as described above (Section 4.2.1.).

In each animal and on each side, three replicate measurements were taken of each of the following (Figure 4.5.):

(a) External diameter of the femoral artery just distal to the SCEA origin,

(b) External diameter of the SCEA at a point 6mm from its origin from the FA

(c) Length of FA from origin of SCEA to its bifurcation into PA and SA.

SCEA / FA diameter ratio was calculated from (a) and (b).

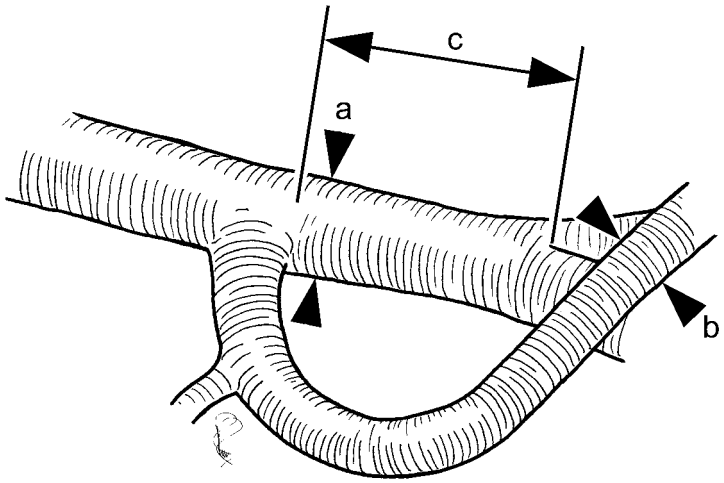

Figure 4.5. Anatomical measurements taken. (a) Femoral artery (FA) external diameter distal to the superficial caudal epigastric artery (SCEA) origin. (b) SCEA external diameter 6 mm from its origin. (c) Length of FA between the origin of the SCEA and its bifurcation into the popliteal (PA) and saphenous (SA) arteries

4.3.2.3. Flow Characterisation

Seventeen outbred male Wistar strain rats were included in the flow study. To ensure physiological stability, gaseous anaesthesia was used as described. Heart rate and arterial oxygen saturation were monitored by the use of a Nonin Model No 8500AV pulse oximeter attached to a forelimb (Nonin Medical, Inc., Plymouth, MN, USA. http://www.nonin.com). Animal core body temperature was monitored by a rectal probe, and maintained between 37.0 and 38.0 degrees centigrade by the use of a homeothermic

warming blanket (Harvard model 50-7061, Harvard Apparatus Ltd., Edenbridge, Kent, UK. http://www.harvardapparatus.co.uk), supplemented with a radiant heat source. Temperature was recorded by analogue voltage output through a calibrated analogue-to-digital interface (PowerLab® 400, AD Instruments Pty Ltd., Castle Hill, NSW, Australia. http://www.adinstruments.com) linked to an Apple® PowerBook G4® computer (Apple Computer Inc., Cupertino, CA, USA. http://www.apple.com) running Chart® v5.5.6 software (AD Instruments Pty Ltd.).

The FA and SCEA were dissected out bilaterally as described. The DFA and small muscular branches were tied off using 10-O monofilament nylon (S&T AG, Neuhausen, Switzerland. http://www.microsurgery.ch). A few drops of lignocaine 2% solution (B|Braun Medical (Pty) Ltd.) were applied. FA flow rate was recorded by placing a transit-time ultrasound flow probe (Model 1RB, Transonic Systems Inc., Ithaca, NY, USA. http://www.transonic.com) around the vessel immediately distal to the inguinal ligament. Normal saline, maintained at 40°C in a water bath, was used as the acoustic couplant. Flow rate was measured by a transit-time ultrasound flow meter (T204, Transonic Systems Inc.), digitised by the PowerLab® 400 (AD Instruments Pty Ltd.), giving a volume flow measurement every 0.0025 seconds. Flow rates on each side were measured consecutively and recorded over one minute. Rates were time-averaged. The order in which these measurements were taken was randomised by a computer-generated list.

4.3.2.4. Statistical Analysis

Statistical analysis of results was performed in Minitab® 15 for Windows® (Minitab Inc., State College, PA, USA. http://www.minitab.com). Linear regression analysis was used to test for association of animal body weight with vessel size and diameter ratio. Two-factor analysis of variance (ANOVA) was used to test for differences in vessel size by side and by measurement serial number. Three-factor ANOVA was used to test for differences in volume flow rate by side, the order in which measurements were taken, and the measurement serial number. Three-factor ANOVA was also used to test for any differences in core temperature by these factors. Linear regression analysis was used to test for an association between flow rate and core temperature. A significance level of 5% was set.

4.3.3. Results

4.3.3.1. Anatomic Characterisation

One digital image was technically inadequate for diameter measurement, leaving thirty-nine pairs available for analysis. One further image was inadequate for measurement of FA length distal to the SCEA origin, leaving thirty-eight pairs available for this analysis. Mean animal body weight was 423.1g (range 343g to 505g).

(a) *FA External Diameter* (Figure 4.6.). Mean FA external diameter (±SD) was 0.9725 ± 0.0073mm on the left and 0.9749 ± 0.0073mm on the right. Regression analysis showed a significant association between diameter and animal body weight (*p*=0.0030, R-Sq=3.8%). The regression equation was FA external diameter = 0.777 + (0.000464 x weight), predicting an FA external diameter of 0.9166 ± 0.0327mm at an animal weight of 300g, and a diameter of 1.0094 ± 0.0216mm at 500g. The predictive interval, i.e. how accurately it is possible to predict FA diameter in a given animal of known body weight, however, was wide (95%PI = 0.7514 – 1.0818mm at 300g and 0.8517 – 1.1671mm at 500g). There was no significant difference in FA external diameter between left and right-sided vessels (*p*=0.8211, ANOVA).

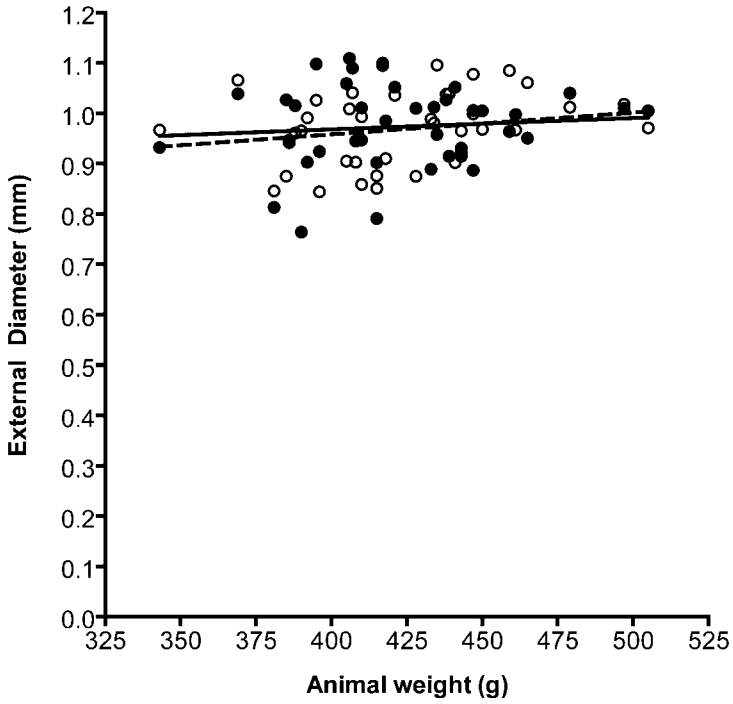

Figure 4.6. Scatterplot of the Femoral artery (FA) external diameter versus weight. Values are means of three replicate measurements. *N* = 39. Open circles and interrupted line = left FA; closed circles and solid line = right FA.

(b) *SCEA External Diameter* (Figure 4.7.). Mean SCEA external diameter (±SD) was 0.5621 ± 0.0052mm on the left side and 0.5430 ± 0.0051mm on the right. Regression analysis of both sides together showed a significant association between vessel size and animal body weight (p<0.0001, R-Sq=5.9%). The regression equation was SCEA external diameter = 0.376 + (0.000417 x weight), predicting an SCEA external diameter of 0.5012 ± 0.0226mm at an animal weight of 300g (95%PI = 0.3875 - 0.6150mm), and a diameter of 0.5846 ± 0.0148mm at 500g (95%PI = 0.4760 - 0.6932mm). There was a significant difference in SCEA external diameter between sides (p=0.0095, ANOVA). The difference in mean diameters was 0.0190mm, with the left SCEA being larger.

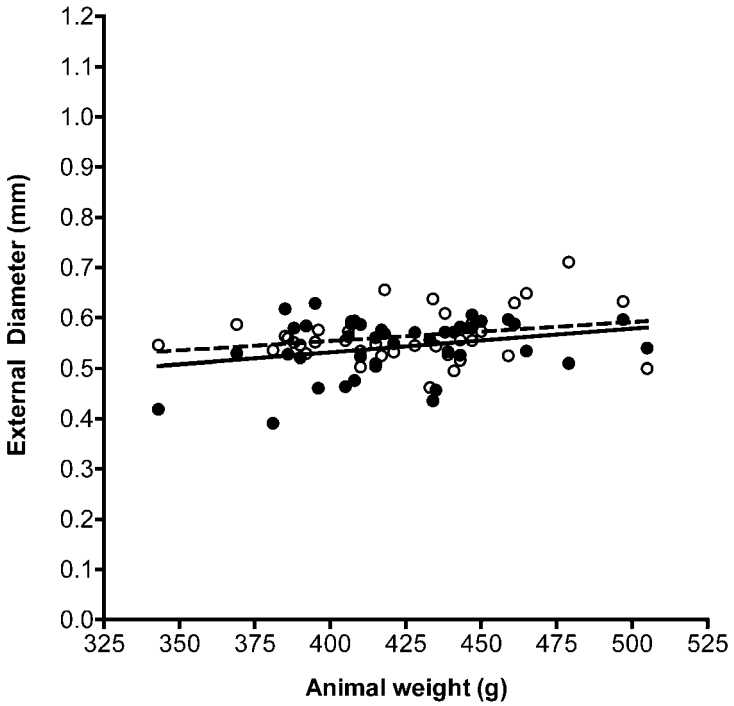

Figure 4.7. Scatterplot of the superficial caudal epigastric artery (SCEA) external diameter versus weight. Values are means of three replicate measurements. *N* = 39. Open circles and interrupted line = left SCEA; closed circles and solid line = right SCEA.

(c) *Distal FA length* (Figure 4.8.). Mean length (±SD) of FA distal to the origin of the SCEA was 3.457 ± 0.0572mm on the left side and 3.298 ± 0.0572mm on the right. Regression analysis of both sides together revealed a negative association between this length and animal body weight (p=0.0040, R-Sq=3.6%). The regression equation was Distal FA length = 4.86 – (0.00350 x weight). This predicts a length of 3.8076 ± 0.2643mm (95%PI = 2.4748 - 5.1404mm) at 300g and a length of 3.1083 ± 0.1744mm (95%PI = 1.8356 - 4.3810mm) at 500g.

There was a significant difference in distal FA length between sides (p=0.0496, ANOVA). The difference in mean lengths was 0.3189mm, with the left distal FA being longer.

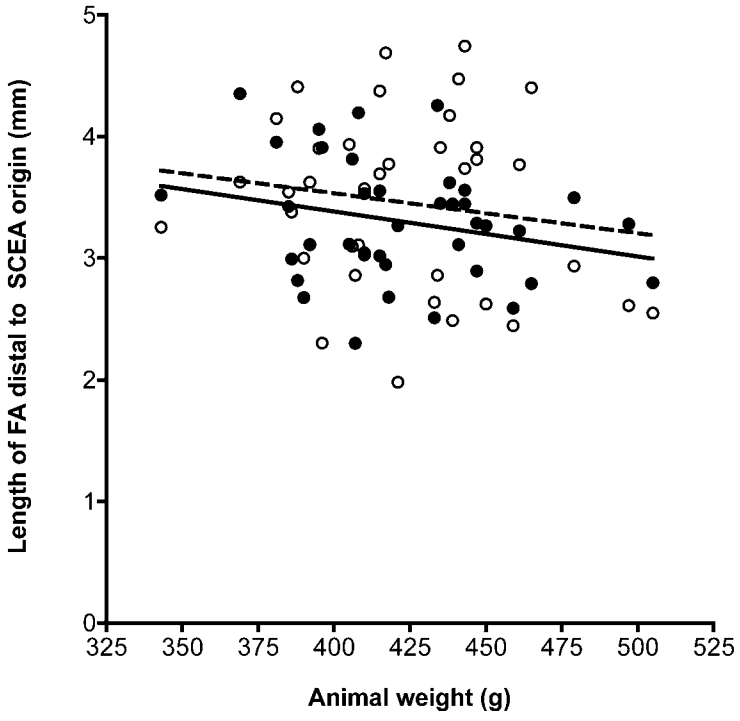

Figure 4.8. Scatterplot of the distal femoral artery (FA) length versus weight. Values are means of three replicate measurements. *N* = 38. Open circles and interrupted line = left distal FA length; closed circles and solid line = right distal FA length.

(d) *SCEA : FA Diameter Ratio* (Figure 4.9.). This ratio was calculated on each side in each animal, giving a total of thirty-nine paired calculations. Mean ratios were left side, 1:1.741 and right side, 1:1.811. There was no significant association between diameter ratio and animal body weight (p=0.4710, Linear Regression Analysis). There was no significant difference in diameter ratio between left and right sides (p=0.1580, ANOVA).

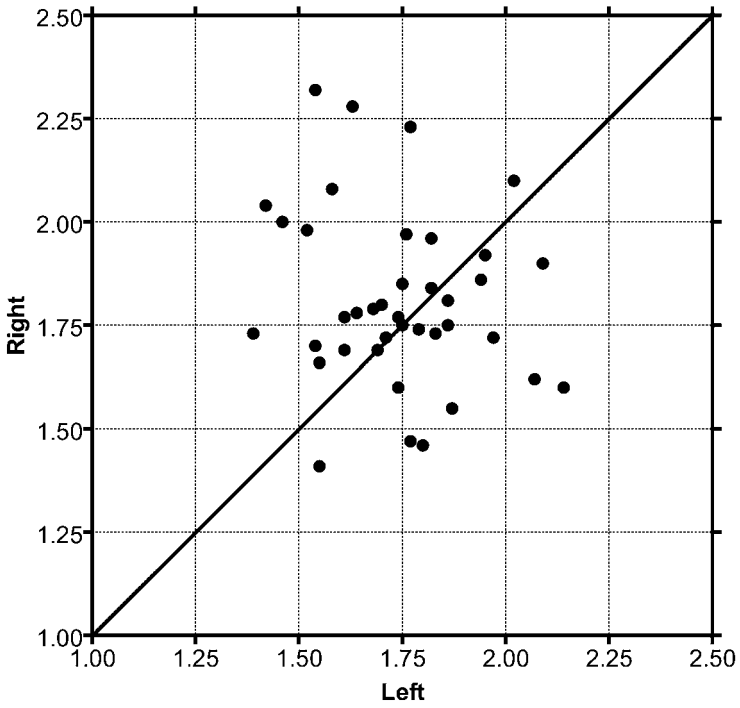

Figure 4.9. XY plot of paired superficial caudal epigastric artery : femoral artery (SCEA:FA) diameter ratios. *N* = 39. Diagonal marks equal left and right ratios.

4.3.3.2. Flow Characterisation

Paired, time-averaged flow rate results were available in all seventeen animals (Figure 4.10.). Mean animal body weight was 448.9g (range 322 – 564g). Mean core body temperature at time of measurement was 37.509 ± 0.361 degrees centigrade. There was no significant association between flow rate and core temperature of the animal at the time of measurement (p=0.079, Linear Regression Analysis). There was no association between flow rate and the side order in which measurements were taken (p=0.1801, ANOVA).

Mean flow rates were left side, 2.391mL/min, and right side, 2.882mL/min, a difference of 0.491 ± 0.1849mL/min. This difference was statistically significant (p=0.0179, ANOVA).

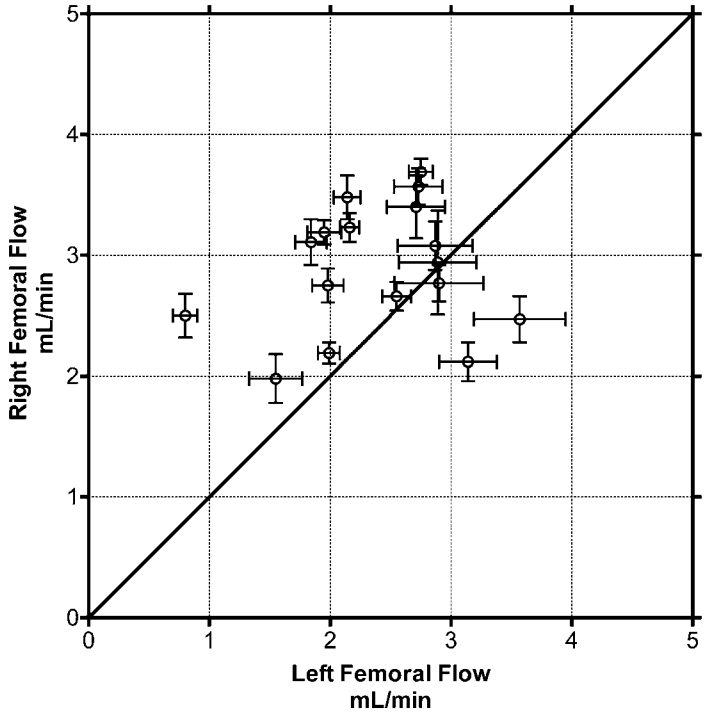

Figure 4.10. XY plot of paired femoral artery volume flow rates (temporal means ±SD). *N* = 17. Diagonal represents equal left and right volume flow rates.

4.3.4. Discussion

As detailed in chapter 1.3.3., the majority of studies that have used animals to examine the geometric design of arterial microanastomoses to overcome diameter mismatch have employed interposition vein grafts to create the diameter discrepancy (Ryan *et al.*, 1988, Gumley *et al.*, 1989, Monsivais, 1990, Ahn *et al.*, 1994). The use of vein grafts introduces two further variables into these studies: those of a significant compliance mismatch, and the requirement for a second anastomosis. In addition, the harvest of large vein grafts from the same animal is a potential source of morbidity.

The outbred male Wistar rat SCEA : FA model described here keeps any compliance mismatch within an arterial range, and avoids the need for a second anastomosis. Furthermore, it permits the paired study of the two techniques in the one animal, reducing the number of animals that need to be used to comprehensively answer this research question.

The vessel diameter mismatch provided by this model is in the range of 1:1.5 to 1:2.5. This ratio is clinically applicable - it is beyond a (small) mismatch that might be managed by judicious mechanical dilatation of the smaller vessel, but not so large as to be seldom encountered in clinical practice. There did not appear to be any association of this ratio with animal body weight, and no significant ratio difference was found between left and right sides.

The association between FA and SCEA external diameters and body weight of the outbred Wistar rat is to be expected. Whilst there was a statistically significant difference in SCEA external diameter between left and right sides, the actual difference in mean diameters was very small in this sample population and not technically significant. It is also noteworthy that the size of the vessels, as predicted by the regression equations, does not increase markedly with animal weight. Together, these observations imply that it is unnecessary to house and feed animals until they are very heavy and a younger 300g animal would suffice.

The length of FA distal to the SCEA origin is important because of the need for undisturbed distal run-off. As stated above, a length of 1-2mm would appear to be sufficient (De La Pava *et al*., 1979). This distance is particularly important in the study of the invagination technique, where the SCEA is invaginated inside the distal FA. Regression analysis showed a negative association of this length with animal weight, and with the desire for as long a distal FA as possible, a smaller animal would appear to be better in this regard. The predictive interval, however, was very wide. Whilst precise incidence figures were not recorded, very occasional anatomical anomalies have been found where the SCEA originated with the PA and SA as part of an FA trifurcation. This finding makes the animal unusable in this study.

Mean femoral artery volume flow rates in these Wistar rats were found to approximate those found in perforating arteries in man (Rubino *et al*.,

2006). In this relatively small population sample, a statistically significant difference in mean flow rate between left and right femoral arteries was found. This difference is unexplained by small variations in animal core temperature, or by the order in which consecutive measurements were taken. With a difference in mean arterial flow rates of 0.491 ± 0.185mL/min, this difference is physiologically significant. This finding has not, to our knowledge, been reported previously. In the light of mammalian anatomical asymmetry, it is perhaps logical that this should be so.

In conclusion, an animal model was found which would allow the paired study of microanastomotic techniques between small upstream and larger downstream arteries, and provides a vessel diameter mismatch of clinical relevance. Counter-intuitively, we have found that the use of larger animals is unnecessary, and may be technically disadvantageous. Of secondary, but pragmatic importance, the use of smaller animals should lead to cost savings.

In the paired study of blood flow rates through anastomoses performed using this model, the disparity in baseline FA volume flow rate between left and right sides would mandate the randomisation of anastomotic technique to one side or the other, and the comparison of flow rates following anastomosis to be made to a baseline measurement.

4.4. References

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5 PATENCY AND TIMING

Outline

5.1. Introduction

The *in silico* study has shown that complex flow separations are likely to be produced by both the oblique end-to-end and invagination techniques of anastomosing arteries with a small-to-large diameter discrepancy. However, as noted in Chapter 3, the non-compliant walls and the inorganic, geometric design of the models used in this study limit any wider conclusions that may be drawn in respect of best technique to employ clinically. An *in vivo* study can more closely match conditions in human vessels.

The principle measure of success of any anastomotic technique is patency. If patency appears equal, or nearly equal, then the ease of the procedure, and the length of time taken to perform an anastomosis, must be secondary discriminators.

An *in vivo* paired study was designed to compare the oblique end-to-end technique with the invagination technique. The Wistar rat SCEA / FA model described in the previous chapter was used.

5.2. Null Hypothesis

The following null hypothesis was formed:

"In anastomosis of arteries of unequal diameter, where a small-to-large diameter mismatch exists of between 1:1.5 and 1:2.5, there is no difference in:

a. patency rate, or

b. speed of completion,

between the invaginating anastomosis and the oblique end-to-end anastomosis in a rodent model."

5.3. Materials and Methods

5.3.1. Statistical Design

Because of the paired nature of the model, and therefore the study, the McNemar test (McNemar, 1947) for analysing matched-pair data was used to calculate the number of animals needed to test the primary outcome measure, i.e. patency (null hypothesis (a)). This test can be defined as the matched-pair version of the chi-square test. A patency rate difference of 5% was deemed clinically relevant and pragmatically possible to test. Power was set at 80% and a significance level of 5% was used.

Sample size calculation led to a total of 156 animals being required to test null hypothesis (a). An estimated 10% of animals were likely to die or require euthanasia prior to completing the study and so ethical approval was sought for a total sample size of (156+16=172) animals.

5.3.2. Study Design

The desire to facilitate extrapolation of the results beyond those of a single investigator meant that more than one investigator was necessary. The study was thus divided into two, with two investigators each putting 86 animals through the study. Order of execution in terms of which side (left or right) was operated on first and which technique (invagination or oblique end-to-end) was used on the first side were randomised by computer-generated nested randomisation. Randomisation was concealed until vessels were dissected out. The investigator was not randomised by serial number, but carried out the procedures simultaneously, allowing for other research commitments.

If flow through the anastomosis was not observed immediately following clamp release, a single revision was performed. This was to more closely replicate normal clinical practice. At one hour following completion of the anastomosis a single 'empty-and-refill' test (Hayhurst and O'Brien, 1975, Acland, 1980, 1986, Petry *et al*., 1986) was used to determine patency. At one week and at six weeks, animals were re-anaesthetised and the anastomosis was dissected free from surrounding tissues. The absence of thrombus at the anastomosis, judged by microscopic visual inspection,

and a successful 'empty-and-refill' test were used to determine patency at these time points. Unless they were designated for another study, animals were killed at this point by an intravenous injection of sodium pentobarbitone (Euthanaze®, Bayer (Pty) Ltd., Isando, South Africa. http://www.bayer.co.za).

The time taken to complete an anastomosis was measured from division of the vessels to release of the clamps (see Technical Details of Anastomoses, below).

5.3.3. Secondary Outcome Measures

Secondary outcome measures were:

a. the incidence of a revision being required,
b. the influence on patency of a revision having been performed,
c. the influence on patency of the investigator,
d. the difference in speed of completion between investigators, and;
e. the influence on patency of the side (left or right) on which the anastomoses were performed.

5.3.4. Animals

Male Wistar strain rats were obtained from the breeding colony of the Central Research Facility, University of Stellenbosch. This colony was established in 2001 from HsdOla:WI rats, and maintained as an outbred

colony. Animals were accepted if body weight was in the region of 300-450g.

5.3.5. Husbandry

Information on animal health status was obtained prior to arrival and all animals underwent an acclimatisation period of at least seven days prior to entering the study. Rats were housed two-to-a-cage in polypropylene solid floor cages with stainless steel grid lids on racks. Wood shavings were used as bedding with nesting material and cardboard tubes provided as cage enrichment. The environmental temperature was maintained at 20 ± 2°C and relative humidity at 55 ± 10%. The lighting schedule was 12 hours light and 12 hours dark and there were 15 – 20 air changes per hour. A non-sterile pelleted diet (Rat Cubes, Epol (Pty) Ltd, Rustenberg, SA) and domestic mains water were offered *ad libitum*.

5.3.6. Anaesthesia

Animals were weighed and underwent gaseous induction of anaesthesia. Anaesthesia was maintained with parenteral ketamine HCl (Anaket-V®, Centaur Laboratories, Bryanston, SA) and xylazine HCl (Rompun®, Bayer (Pty) Ltd., Isando, SA).

Bupivacaine HCl (Marcaine®, Adcock Ingram, Bryanston, SA) was infiltrated locally following wound closure and buprenorphine HCl (Temgesic®, Schering-Plough, Johannesburg, SA) was administered prior to recovery from anaesthesia. Animals were rehydrated during recovery by

the administration of 2mL of 0.9% w/v NaCl (B|Braun Medical (Pty) Ltd., Randburg, Gauteng 2125, SA. http://www.bbraun.co.za) via the dorsal penile vein.

5.3.7. Technical Details of Anastomoses

5.3.7.1. Vessel Preparation

Vessel dissection and preparation was carried out bilaterally as described in section 4.2.1. A binocular operating microscope (SMZ-10, Nikon Corporation, Tokyo, Japan. http://www.nikon.com) was used throughout. The side order and technique were then assigned from the computer-generated randomisation list.

The femoral artery (FA) was tied off immediately distal to the origin of the superficial caudal epigastric artery (SCEA) using 10-O nylon suture material (Dafilon® Black, G1118781, B|Braun Medical (Pty) Ltd.). In order to promote laminar flow in the SCEA, care was taken in the placement of this ligature, the aim being to avoid a blind-ended pouch that might cause recirculating flow at the ostium of the SCEA. The SCEA was then tied off 7mm distal to its origin using 10-O nylon suture material. A double approximating clamp (Acland Model ABB-2V, S&T AG, Neuhausen, Switzerland. http://www.microsurgery.ch) was placed on the FA proximal to the origin of the SCEA, and on the distal FA at its bifurcation. For the invagination technique, the FA was divided immediately distal to the ligature. For the oblique end-to-end technique the FA was divided 1mm further distal to the ligature. This difference was made to allow for the

extra 1mm of distal FA required to invaginate the SCEA in the invagination technique, in an attempt to remove the potential confounding factors of anastomotic tension difference and length of distal FA before bifurcation.

The SCEA was then divided 6mm distal to its origin. For the invaginating anastomosis this was performed as a 90º transverse division. For the oblique end-to-end anastomoses, the vessel was transected at a 45º angle.

The approximating clamp was then separated and the lumens of both vessels were cleared of all blood by flushing with heparinised saline (1000U heparin in 100ml 0.9% w/v NaCl). In accordance with conventional microsurgical technique, the adventitia was trimmed back far enough from the vessel ends to prevent any fronds of adventitia from entering the vessel lumen. The vessels were dilated with a 0.3mm diameter vessel dilator (Model D5aZs, S&T AG). In the side undergoing invagination, a cuff of the adventitia of the SCEA was slipped back for a distance of 1mm, in the manner of pulling back a shirt sleeve.

A 10-O nylon suture swaged onto a 50µm, 3mm 3/8 circle taper point round-bodied needle (Dafilon® Black, G1118781, B|Braun Medical (Pty) Ltd.) was used for both anastomotic techniques.

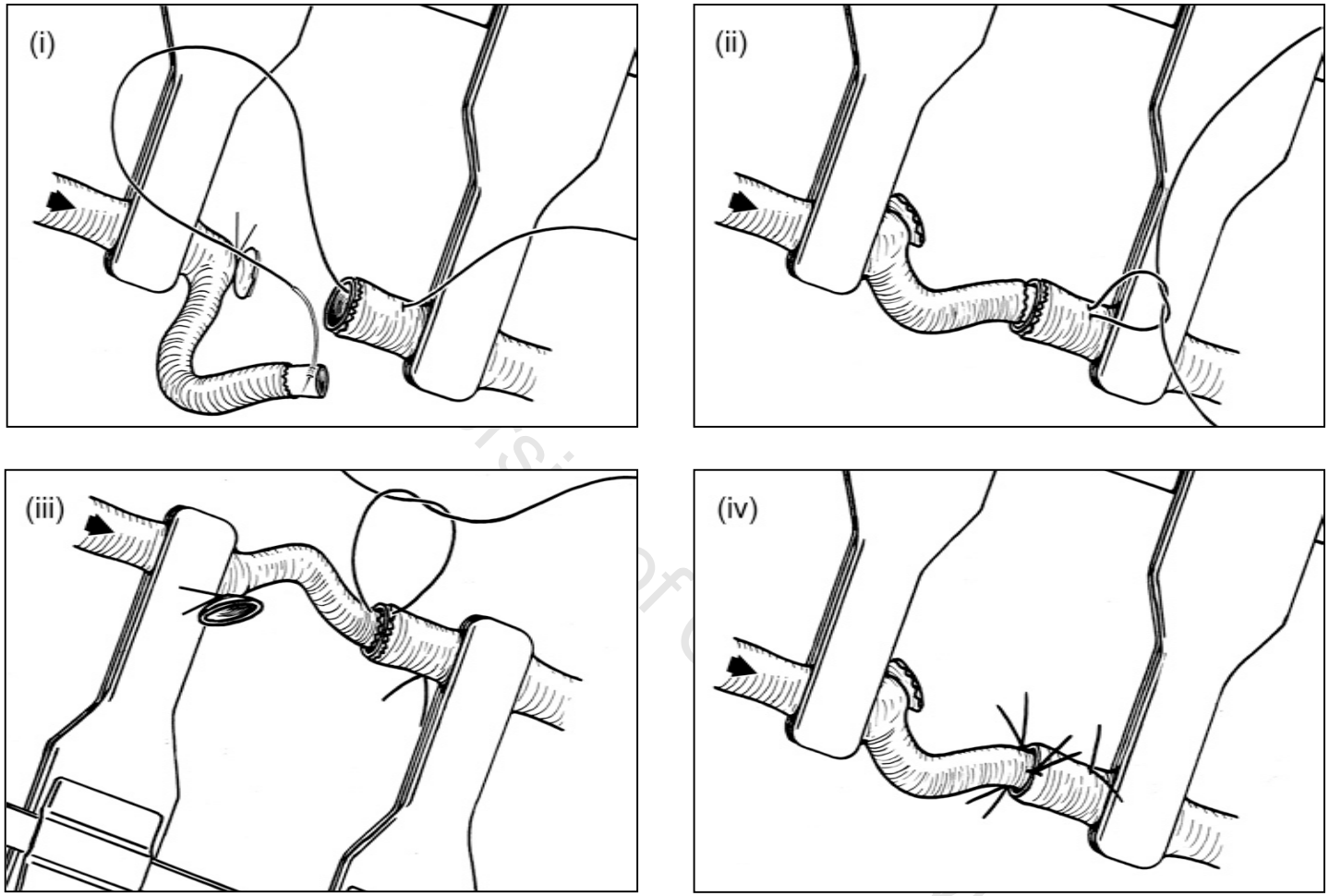

Figure 5.1. Construction of the invaginating anastomosis. The upstream vessel is on the left in all diagrams. (i). After passing through the full thickness of the larger, downstream vessel wall one larger vessel diameter from its end, a very small tangential bite is taken of the smaller, upstream vessel. (ii). The suture is passed back out of the larger vessel immediately beside itself and the smaller vessel is invaginated by drawing on this suture. (iii). A suture taking the full thickness of the larger vessel wall, and only adventitia in the smaller vessel, is placed 180º from the invaginating suture. (iv). Two further sutures are placed in a similar manner to this second suture, at 120º from it.

5.3.7.2. Invaginating Anastomosis

The invaginating anastomosis technique is based on the 'sleeve' anastomosis described by Duminy (1988, 1989). It is performed by first passing the needle through the full thickness of the wall of the FA one larger vessel diameter from its end (approximately 1mm in this model). A small, transverse, tangential bite is then taken of the end of the SCEA, without breaching the intima (Figure 5.1.(i)). The needle is then passed back through the lumen of the FA and the suture is brought out immediately beside itself (Figure 5.1.(ii)). The SCEA is then invaginated inside the FA by drawing on this suture, and this is very loosely tied by a single reef knot. It is important to keep this suture loose in order to prevent crimping of the end of the SCEA inside the FA. The approximating clamp is then turned over and the adventitia of the SCEA is pulled forward to meet the end of the FA. An interrupted suture is then placed through the end of the FA and through the adventitia and media of the SCEA, at a point 180° from the invaginating suture, in a manner similar to the 'sleeve' anastomosis of Duminy (Figure 5.1.(iii)). The clamp is then returned to its original position and two further interrupted sutures placed in a similar manner, at 120° from this second suture (Figure 5.1.(iv)). These two sutures are additional to the method of Duminy and are necessary because of the size discrepancy. Thus a total of four sutures are required to complete the invaginating anastomosis.

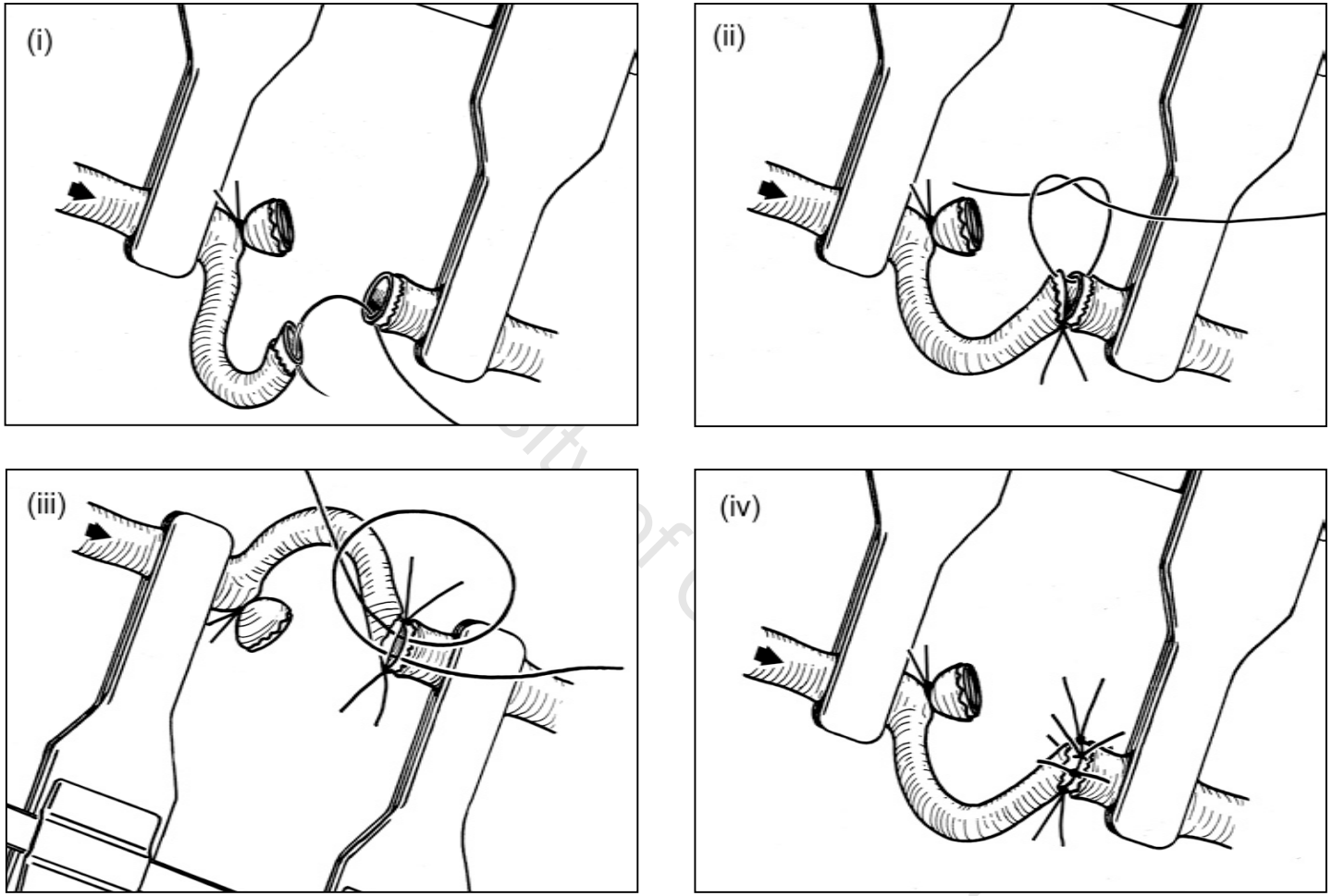

Figure 5.2. Construction of the oblique end-to-end anastomosis. The upstream vessel is on the left in all diagrams. (i). The first suture is passed through the 'heel' of the obliquely-sectioned smaller, upstream vessel. (ii). The second suture is placed in the 'toe'. (iii). Back wall sutures placed by Harashina's method to allow visualisation of the lumen at all times (Harashina, 1977). (iv). The front wall is completed in a similar manner.

5.3.7.3. Oblique End-to-End Anastomosis

The oblique end-to-end anastomosis is performed by the use of interrupted, full-thickness suture bites in the conventional manner, placing the first suture through the FA and the heel of the obliquely cut SCEA (Figure 5.2.(i)). The second suture is then placed in the toe of the SCEA (Figure 5.2.(ii)). The approximating clamp is then turned over and two interrupted full-thickness sutures are placed in the back wall, using Harashina's method to allow visualisation of the lumen at all times (Figure 5.2.(iii)) (Harashina, 1977). The clamp is then returned to its original position and two further sutures are placed in the front wall in a similar manner. Thus a total of six sutures are used to accomplish the oblique end-to-end anastomosis (Figure 5.2.(iv)).

5.3.7.4. Timing of Procedure

Anastomoses were timed from division of the SCEA until release of the clamps by the use of a digital stopwatch.

5.3.7.5. Closure and Recovery

On completion of the procedure, the inguinal fat pad was sutured back into place using interrupted 5-O polyglactin 910 sutures (Vicryl® J844G, Ethicon Ltd, Edinburgh, UK) and the skin was closed with a locked, continuous 4-O monofilament nylon suture (Ethilon® W1620, Ethicon Ltd.). The wound was dressed with an acrylic spray (Opsite®, Smith and Nephew Medical Ltd., Hull, UK. http://www.smith-nephew.com). A single

parenteral dose of enrofloxacin (Baytril®, Bayer (Pty) Ltd., Isando, South Africa. http://www.bayer.co.za) was administered during recovery.

5.3.8. Statistical Analysis

Primary statistical analysis of patency results was performed by the McNemar test online at http://www.graphpad.com/quickcalcs. Minitab® 15 for Windows® (Minitab Inc., State College, PA, USA. http://www.minitab.com) was used for all other analyses. Results are reported as means ± SD.

5.4. Results

Detailed results are at Appendix B. Mean animal body weight was 387.4 ± 53.4g. All 172 animals were available for study at one hour. At one week, 16 animals had been lost, leaving 156 available for study at this point. At six weeks, 127 animals were available for study.

5.4.1. Patency by Technique - Primary Analysis.

Of 344 anastomoses, a total of 18 failed to run at one hour, nine of each technique. At one week, 20 oblique end-to-end and 27 invagination anastomoses had failed. At six weeks, 14 oblique end-to-end anastomoses had failed, and 12 invagination anastomoses. Analysis by McNemar test of patency at one hour, one week and at six weeks showed no significant difference between oblique end-to-end and invagination

techniques. (p values = 0.8026, 0.2963 and 0.8137 respectively; Odds Ratios = 1.000, 0.650 and 1.250 respectively) (Table 5.1.).

		Oblique End-to-End		
		+	-	
Invagination	+	155	8	**163**
	-	8	1	**9**
		163	**9**	**172**

a. Patency at 1 hour. p value = 0.8026, X^2 = 0.063, DF = 1. OR = 1.000 (0.327–3.057)

		Oblique End-to-End		
		+	-	
Invagination	+	116	13	**129**
	-	20	7	**27**
		136	**20**	**156**

b. Patency at 1 week. p value = 0.2963, X^2 = 1.019, DF = 1. OR = 0.650 (0.297-1.373)

		Oblique End-to-End		
		+	-	
Invagination	+	105	10	**115**
	-	8	4	**12**
		113	**14**	**127**

c. Patency at 6 weeks. p value = 0.8137, X^2 = 0.056, DF = 1. OR = 1.250 (0.444-3.645)

Table 5.1. Primary analysis of patency, at (a) one hour, (b) one week, and; (c) six weeks (McNemar test with continuity correction). Odds ratios given with 95% confidence intervals. A positive result represents a patent anastomosis.

5.4.2. Patency by Technique - Secondary Analysis.

Patency at one hour was further analysed by Binary Logistic Regression, looking at the influence of side, technique, investigator, when in the series the animal was operated on, and whether a revision of the anastomosis had been performed.

Side did not show a significant association with one hour patency (p=0.135). Similarly, technique did not show a significant association with patency by Binary Logistic Regression (p=0.303).

The position in series was analysed by dividing the complete series approximately into thirds of 58, 57 and 57 animals. The position in series showed a significant association with patency, when the last 57 animals were compared to the first 58 (p=0.050, OR 6.3). In other words, an anastomosis was more likely to fail in the first third than in the last,

independent of all other factors. There was no significant association between first and middle thirds (p=0.228).

Whether or not a revision had been performed showed a highly significant association with an anastomosis having failed at one hour (p<0.0001, OR 33.333).

5.4.3. Timing

Timings are plotted at Figure 5.3. The time taken to complete an oblique end-to-end anastomosis varied from 11min 24sec to 42min 5sec. Mean was 19min 37sec (± 4min 26sec). Time taken to complete the invagination technique varied from 5min 9sec to 25min 52sec. Mean was 12min 44sec (± 3min 26sec).

The length of time that it took to complete the anastomoses was analysed by a four-factor ANOVA, looking at the influence of side, technique, investigator and when in the experimental series the anastomoses were done (the series again being divided approximately into thirds of 58, 57 and 57 animals each).

Side and position in series had no influence on how long it took to do an anastomosis (p = 0.402 and 0.103 respectively).

Technique showed a highly significant influence on the time taken to perform an anastomosis, independent of other factors (p<0.0001, means; Oblique end-to-end = 19.62mins, Invagination = 12.73mins, difference in means = 6.89mins).

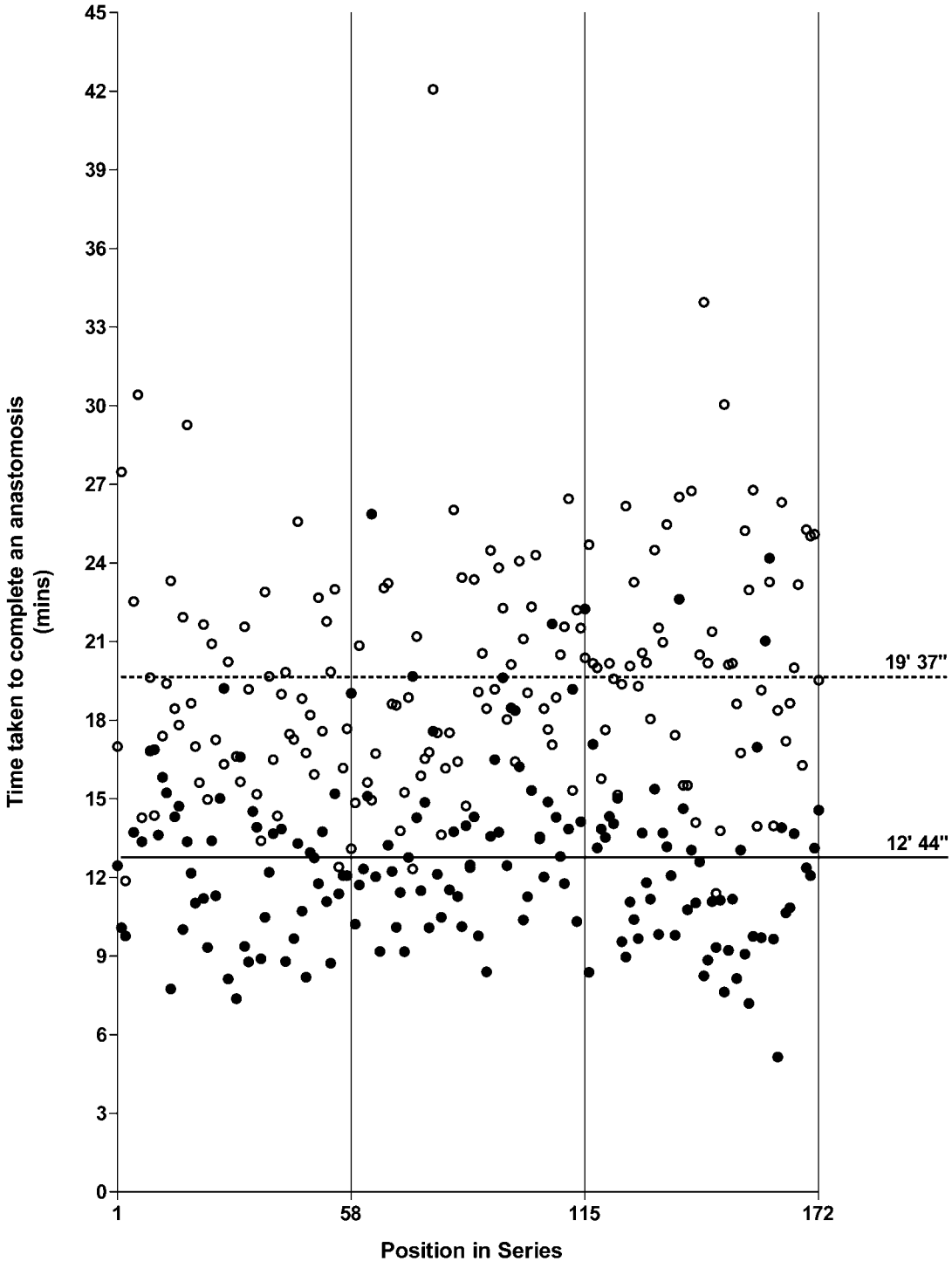

Figure 5.3. Scatterplot of time taken to complete an anastomosis by position in series. Open circles = Oblique end-to-end. Filled circles = Invagination technique. Dotted line = mean of all oblique end-to-end technique times. Solid line = mean of all invagination technique times.

5.4.4. Revision Rate

A total of 37 anastomoses failed to run immediately following clamp release and were revised. Of these, 11 occurred after invagination and 26 after an oblique end-to-end anastomosis. The number of revisions performed was analysed by Binary Logistic Regression, looking at the influence of side, technique, and investigator.

Side and investigator did not show any association with a revision having being done (p = 0.645 and 0.902 respectively).

Technique showed a significant influence on a revision having been performed (p=0.010, OR = 2.66, a revision was more likely to have been done with an oblique end-to-end technique).

The likelihood of a revision failing at one hour was analysed by technique. Of the 26 oblique end-to-end anastomoses that underwent revision, 19 were patent at one hour. Of the 11 invagination anastomoses requiring revision, six were patent at one hour. Analysis of this sample of 37 anastomoses showed no significant difference in revision outcome by technique (p = 0.4737, Chi-square) (Table 5.2.).

	Oblique ETE	Invagination	
Successful	19	6	**25**
Unsuccessful	7	5	**12**
	26	**11**	**37**

Table 5.2. Two-by-two contingency table for analysis of revision successes. X^2=0.513; df=1. P value = 0.4737. (Chi square).

If a revision had not been attempted, and if it is assumed that anastomotic failure immediately following clamp release would have remained a failure at one hour, then a further analysis is possible. An additional 19 failures can be included with the eight oblique end-to-end recorded and an additional six with the invagination technique. This analysis indicates that the difference in one-hour patency between the oblique ETE and invagination is not quite statistically significant (McNemar test, p-value = 0.061, OR = 1.929) (Table 3.).

		Oblique End-to-End		
		+	-	
Invagination	+	130	27	**157**
	-	14	1	**15**
		144	**28**	**172**

Table 5.3. Analysis of patency at one hour where successful revisions have been included in the failure category. A positive result represents a patent anastomosis. *P* value = 0.0609, X^2 = 3.512, DF = 1, OR = 1.929 (95% CI = 0.976-3.979) (McNemar test).

5.4.5. Influence of the Investigator

The influence of the investigator on one hour patency and on speed of completion was examined as part of the analysis performed in sections 5.4.2 and 5.4.3 respectively.

Who the investigator was (A or B) showed a significant association with one hour patency overall (p=0.022, OR 4.545, A more successful than B, Binary Logistic Regression).

Who the investigator was also showed a significant association with speed of completion of the anastomoses, independent of all other factors (p=0.0037, difference in means = 1.27mins, B faster than A, ANOVA).

5.5. Discussion

5.5.1. Patency

The study presented here was designed to test the null hypothesis that there is 'no' (<5%) patency difference between an invagination technique and an oblique end-to-end technique in rodent arteries with a small-to-large diameter mismatch in the region of 1:1.5 – 1:2.5. Primary analysis by McNemar test has not shown significant patency differences at one hour, one week or at six weeks in this experiment. Secondary analysis by Binary Logistic Regression of one-hour patency has agreed with this finding. Null hypothesis (a), therefore, is not rejected.

Secondary analysis of possible confounding factors or influences on patency in this study has shown that:

(1). A learning curve existed for both investigators, and;
(2). The investigator who took longer to complete an anastomosis achieved a higher overall success rate.

Although success improved overall, between the first 58 animals with the last 57, investigators neither slowed down, nor did they become faster at performing an anastomosis. Therefore they must simply have become

more dextrous. It may be concluded from finding (2) that investigator A took greater care in performing both techniques.

In analysis of the revision of unsuccessful anastomoses, the following may be concluded:

(3). A significantly higher revision rate was observed in the oblique end-to-end technique.

(4). If an anastomosis was unsuccessful at the first attempt, it was significantly less likely to be successful at a further attempt, but;

(5). No significant difference in revision success was observed between techniques, and;

(6). If in the study a revision had not been permitted, a greater number of invagination anastomoses may have been patent at one hour, although this difference was not quite statistically significant.

Findings (3), (4) and (5), taken together, suggest that the oblique end-to end technique was technically more difficult to perform in this model. This conclusion agrees with reported opinions of other authors who have examined invagination techniques in comparison to the conventional interrupted end-to-end microanastomosis of equal-sized vessels (Lauritzen, 1978, Duminy, 1989). However, technical difficulties found with the oblique end-to-end were not more difficult to remedy when compared

to the invagination technique, and the overall success rate at one hour following revision showed no significant difference between techniques.

Including successful revisions in the one-hour failure, numbers indicate that any difference in one-hour patency should be considered as not quite statistically significant. This analysis suffers the disadvantage of being based on an assumption that immediate failure would have translated into one-hour failure. Furthermore, the experiment was not designed with this analysis in mind.

5.5.2. Timing

Independent of all other factors, the invagination technique was faster to perform than the oblique end-to-end. Mean times taken were: oblique end-to-end, 19 mins 38 secs and invagination, 12 mins 46 secs. The difference in these means is 6 mins 52 secs, which is 35% of the oblique end-to-end, or 54% of the invagination duration. In other words, in this study, the oblique end-to-end took approximately one-and-a-half times longer to complete. Null hypothesis (b) is therefore rejected.

As previously mentioned, there was a significant difference in cadence between investigators, which may have influenced patency rates. There was no difference in time taken by position in series.

The finding of less time taken to complete the invagination technique is logical in that four, as opposed to six, sutures were required. Whilst a greater number of sutures are required to complete an invagination technique in this situation than in equal-sized vessels, this finding is

consistent with previous reports (Lauritzen, 1978, Lauritzen and Bagge, 1979, Hyland *et al*., 1981, Stamatopoulos *et al*., 1982, Duminy, 1988).

5.5.3. Conclusions

Within the limits of the study, it can be concluded that the invagination technique provides at least similar patency to the oblique end-to-end technique, whilst being easier and significantly faster to perform in this model.

5.6. References

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6 FLOW

Outline

6.1. Introduction

The *in silico* study has shown that both techniques exhibit regions of complex flow separation and that no clear advantage of one technique exists over the other. The *in vivo* patency and timing study has shown that no clear benefit exists in terms of gross patency. It may be said, however, that the invagination technique is easier and faster to complete in this model.

Anastomotic patency is an essential primary outcome measure. As a binary outcome measure, however, it may be regarded as a crude evaluation of a microanastomotic technique. Indeed Krag and Holck (1981) have demonstrated that the 'empty-and-refill' test (Hayhurst and O'Brien, 1975, Acland, 1980) may give a positive result for patency, even when the vessel is occluded by more than 75%. Further studies were therefore designed to examine flow through the anastomoses and to measure long term vessel remodelling. The flow experiment is reported here.

One observation made in some studies of equal-sized vessels is that flow through arteries anastomosed by an invagination technique may be reduced when compared to a conventional interrupted end-to-end anastomosis.

Lauritzen and Bagge (1979) examined flow in a paired study, cannulating the aorta and recording the time taken to pass 50mL of saline solution through invaginating and end-to-end anastomoses performed in

84 rodent femoral arteries, one side at a time. Flow was then examined at seven different time periods, with six animals in each group. These authors noted reduced flow through an invagination technique at one and three hours when compared to an end-to-end technique. There was no difference, however, at other time points up to eight weeks.

In a similar study, Duminy (1988) modified Lauritzen and Bagge's experimental technique by cannulating the rat aorta and collecting saline from both femoral vessels simultaneously (in which one technique had been performed on each side). Fluid was collected over four minutes, and the volumes collected from each side were recorded. The author conducted the experiment in 12 animals at four time points after anastomosis, with three animals in each group. Duminy found no flow difference between techniques at one, three, eight and 28 days.

However, two other studies contradict these findings. Wieslander and Åberg (1983) conducted a mixed paired / unpaired study using the central artery of the rabbit ear, measuring flow with an electromagnetic flow meter at various time points out to three days. In contrast to the studies of Lauritzen and Bagge and of Duminy, these authors noted that flow through an invagination or 'end-in-end' anastomosis was significantly reduced when compared to an end-to-end technique. This reduction occurred from clamp release up to completion of the study at three days.

Nakayama and Soeda (1984) performed a paired study also using an electromagnetic flow meter to measure flow, but in rabbit carotid arteries which had been divided and re-anastomosed by an invagination or end-to-end technique. These authors found that mean flow rate through the

invaginated anastomoses was reduced by a mean of 34% at 2-3 hours and by 29% at one week, when compared to an interrupted end-to-end technique.

No studies of blood flow through anastomoses of vessels with a small-to-large diameter mismatch have been reported. One group has suggested that in this situation, stenosis and reduced flow following invagination should, in theory, not occur (Nakayama *et al*., 1987).

An experiment was designed to test for flow differences across the two techniques under scrutiny. Transit-time ultrasound flow measurement was selected over electromagnetic flow measurement because the sensors offer the advantages of greater accuracy, smaller size, and lighter weight, and can be implanted into small animals. Nakayama and Soeda (1984), in their paired study in rabbit carotid arteries, note a volume flow measurement error of up to 20mL/min when using the electromagnetic flow meter. One comparative study noted an average flow measuring error of only 6% when using a transit-time flow probe compared to 15% error experienced with an electromagnetic flow probe (Koenig *et al*., 1996).

As well as time-averaged volume flow rates reported in these previous studies, the availability of implantable transit-time flow probes permitted the study of other indices of flow. The paired nature of the model also allowed for a resistance ratio between techniques to be calculated when flow was measured concurrently.

6.2. Null Hypothesis

The following null hypothesis was formed:

"In anastomosis of arteries of unequal diameter, where a small-to-large diameter mismatch exists of between 1:1.5 and 1:2.5, there is no difference in flow through the invaginating anastomosis and the oblique end-to-end anastomosis in a rodent model."

6.3. Materials and Methods

6.3.1. Statistical Design

Sample size calculation was carried out in StatMate™ v2.0a for Macintosh (GraphPad Software Inc., La Jolla, CA, USA. http://www.graphpad.com). Paired, time-averaged flow results from the twelve New Zealand White rabbits used in Nakayama and Soeda's study were used as the basis from which to calculate a sample size. Based on the analysis of results by a paired *t*-test, an estimate of the standard deviation of differences between pairs was taken from the 2-3 hour flow results in that paper. Power was set at 80% and significance at 5% (two-tailed). A percentage volume flow rate difference of 10% was arbitrarily selected as reasonable to test. Sample size calculation led to a total of six or seven animals being needed to answer this question at these power and significance levels. Allowing for flow measuring mishaps prompted an increase in this sample size to ten.

6.3.2. Animals

Ethical review was conducted by the University of Glasgow Ethical Review Panel. The study was licenced by the UK Government Home Office under the terms and conditions of the Animals (Scientific Procedures) Act 1986 (Project Licence 60/3749).

Outbred male Wistar (HsdHan™:WIST) strain rats were obtained from Harlan UK Ltd., Bicester, Oxfordshire, UK. Animals were accepted if body weight was in the region of 300-450g.

6.3.3. Husbandry

Animal care followed the guidance given in EC Directive 91/507/EEC. Information on animal health status was obtained prior to arrival and all animals underwent an acclimatisation period of at least seven days prior to entering the study. Following recovery, rats were housed individually in polypropylene solid floor cages with stainless steel grid lids on racks. Fleece rugs and paper towels were used in place of wood shavings as bedding in order to avoid wound contamination. The environmental temperature was maintained at 20 ± 2°C and relative humidity at 55 ± 10%. The lighting schedule was 12 hours light and 12 hours dark and there were 15 – 20 air changes per hour. A non-sterile pelleted diet (BEEKAY Rat and Mouse Standard Diet (expanded), B&K Universal Ltd., Hull, UK) and domestic mains water were offered *ad libitum*.

A prophylactic dose of ampicillin 10mg/100g s/c (Amphipen LA, Intervet/Schering Plough Animal Health, Milton Keynes, UK. http://www.intervet.co.uk) was administered at the start of surgery. A

further dose was administered the following day and on days three and five.

6.3.4. Anaesthesia

Animals were weighed and underwent gaseous induction followed by oro-endotracheal intubation with a modified 16G/45mm venous cannula (AniCath MP06216, Millpledge Veterinary, Millpledge Pharmaceuticals Ltd., Clarborough, Nottinghamshire, UK. http://www.millpledge.com) using a trans-tracheal illumination technique. Anaesthesia was maintained by an N_2O / isoflurane / O_2 gaseous mixture on a rodent ventilator (Model No. 7025, Ugo Basile, Comero, Varese, Italy. http://www.ugobasile.com) at 60 breaths per minute and a ventilator stroke volume of 4 – 5mL.

Heart rate and arterial oxygen saturation were monitored by the use of a Nonin Model No 8500AV pulse oximeter attached to a forelimb (Nonin Medical, Inc., Plymouth, MN, USA. http://www.nonin.com).

Animal core body temperature was monitored by a rectal probe, and maintained between 36.5 and 38.5 degrees centigrade by the use of a homeothermic warming blanket (Harvard model 50-7061, Harvard Apparatus Ltd., Edenbridge, Kent, UK. http://www.harvardapparatus.co.uk), supplemented with a radiant heat source. Temperature was recorded by analogue voltage output through an analogue-to-digital interface (PowerLab® 400, AD Instruments Pty Ltd., Castle Hill, NSW, Australia. http://www.adinstruments.com) linked to an Apple® PowerBook G4® computer (Apple Computer Inc., Cupertino, CA,

USA. http://www.apple.com) running Chart® v5.5.6 software (AD Instruments Pty Ltd.).

Buprenorphine HCl (Vetergesic®, Reckitt Benckiser Healthcare (UK) Ltd., Hull, UK), and levobupivacaine HCl (Chirocaine®, Abbott Laboratories Ltd., Queensborough, Kent, UK) were administered during the procedure. Hydration was maintained by the subcutaneous administration of 0.9% w/v NaCl.

6.3.5. Experimental Procedure

Nominally sterile technique was used. The Femoral Artery (FA) and Superficial Caudal Epigastric Artery (SCEA) were dissected out bilaterally as described in Chapter 4. Following this, side order and technique were assigned from a computer-generated nested randomisation list. A transit-time ultrasound flow probe (Model 1RB, Transonic Systems Inc., Ithaca, NY, USA. http://www.transonic.com) was placed around the FA immediately distal to the inguinal ligament and baseline flow measurements were taken on the first side. Normal saline, maintained at 40°C in a water bath, was used as the acoustic couplant. Flow rate was measured by a transit-time ultrasound flow meter (T204, Transonic Systems Inc.), and digitised by the PowerLab® 400 (AD Instruments Pty Ltd.), giving a discrete volume flow measurement (mL/min) every 0.0025 seconds. Flow was recorded for a period of at least one minute. The probe was then removed to allow construction of the anastomosis.

Anastomoses were created between the upstream SCEA and downstream FA as described in Chapter 5. A 10-O nylon suture swaged

onto a 50µm, 3mm 3/8 circle taper point round-bodied needle (Dafilon® Black, G1117041, B|Braun Medical Ltd, Sheffield, UK) was used. In contrast to the patency experiment, a revision was not permitted.

Following clamp release, the 1RB flow probe was replaced around the proximal FA and bathed in warmed normal saline. At ten minutes following clamp release, a further flow rate and core body temperature recording was made.

Baseline flow through the other FA was then recorded. The other anastomotic technique was then executed, and flow measurements were taken at ten minutes after clamp release on that side. On each side, flow was then measured at one hour following clamp release.

Following this, a perivascular transit-time ultrasound flow probe (1RS-JS-WC13-CM4S-chronic, Transonic Systems Inc.) was implanted in each side thus: Two dorsal incisions were made between the animal's scapulae using a 4mm biopsy punch (Stiefel® Laboratories (UK) Ltd., Maidenhead, UK. http://www.stiefel.com). Subcutaneous tunnels were created between the groins and these incisions. The connectors and wires were passed through these tunnels and secured onto the dorsal skin by suturing in place over rigid polypropylene collars (AAPC104, Transonic Systems Inc.) using 4-O braided silk sutures (Sofsilk™ SS629, Syneture, Covidien plc., Loughlinstown, Co. Dublin, Ireland. http://www.syneture.com). The perivascular flow probes were carefully placed around the proximal FA and secured to perivascular tissues by 4-O silk mattress sutures (Sofsilk™ SS629, Covidien plc.). A loop of cable was placed in a posterior inguinal pocket to allow for animal limb movement without impinging on the femoral

vessels or probe measuring heads. Warmed normal saline was instilled around the probes and the signal was tested by connection to the flow meter.

Once a reliable signal had been secured, the groins were closed by suturing the inguinal fat pad back into place using interrupted 5-O polyglactin 910 (Vicryl® J844G, Ethicon Ltd, Edinburgh, UK). The skin was closed with a locked, continuous 4-O monofilament nylon suture (Ethilon W1620, Ethicon Ltd.). The wound was dressed with an acrylic spray (Opsite®, Smith and Nephew Medical Ltd., Hull, UK. http://www.smith-nephew.com).

A paired volume flow rate measurement was taken at a mean of 3h following clamp release. Because anastomoses were effected consecutively, approximately one hour apart, the first side recording was approximately 3h 30min after clamp release, the second at 2h 30 min.

The animal was recovered from anaesthesia. Flow was measured at 24hrs, 72 hrs and at 7 days in the conscious animal by connecting the dorsal connector buttons to the flow meter. No anaesthesia, sedation or restraint was necessary, and flow measurements were taken with the animal resting. Flow from both sides was recorded simultaneously over one minute.

Animal core body temperature was recorded for all time points where consecutive (rather than simultaneous) flow measurements were taken, i.e. at baseline, ten minutes and at one hour. This was to address the

potential confounding factor of core temperature and its possible influence on flow rate at disparate time points. Where simultaneous measurements were taken, the paired nature of the model allowed this factor to be discounted, and reduced the complexity of the procedure required to record flow rates in the conscious animal.

Limb ischaemia time was recorded from last baseline flow measurement to clamp release. This time includes some time spent setting up and ligating the vessels prior to placing the vessel clamp, and hence records a longer period than reported in Chapter 5.

6.3.6. Mean Volume Flow Rate

Time-averaged flow rates (±SD) were calculated from each one-minute recording of 24,000 individual sampling points using Chart® v5.5.6 software (AD Instruments Pty Ltd.).

6.3.7. Pulsatility Index

Time-averaged Pulsatility Index (PI. Q_{MAX}-Q_{MIN} /Q_{MEAN}) was calculated using Chart® v5.5.6 software at each flow measuring point.

6.3.8. Relative Resistance

It can reasonably be assumed that in this paired model, the arteriovenous pressure differences in each limb at a single time point will

be similar. If this assumption is made, from Ohm's Law it is possible to calculate relative resistance across the two techniques thus:

1. $$Q = \frac{\Delta P}{R}$$

   where Q is flow, ΔP is the pressure gradient across the system being measured and R is resistance.

2. $\therefore$ $$\Delta P = R \cdot Q$$

3. $$\Delta P_{INV} = R_{INV} \cdot Q_{INV} \quad \text{and} \quad \Delta P_{OBQ} = R_{OBQ} \cdot Q_{OBQ},$$

   where R_{INV} is resistance across the invagination technique and F_{OBQ} is flow across the oblique end-to-end technique, *et cetera*.

4. The assumption is made that $\Delta P_{INV} = \Delta P_{OBQ}$. Therefore:

5. $$R_{INV} \cdot Q_{INV} = R_{OBQ} \cdot Q_{OBQ},$$

6. $\therefore$ $$R_{INV} = \frac{\left(R_{OBQ} \cdot Q_{OBQ}\right)}{Q_{INV}},$$

   or

7. $$R_{INV} = R_{OBQ} \cdot \frac{Q_{OBQ}}{Q_{INV}}$$

8. Assume $R_{OBQ} = 1$, then:

9. $$\textit{(relative)}\ R_{INV} = \frac{Q_{OBQ}}{Q_{INV}}$$

This resistance ratio was calculated for each paired time-averaged volume flow measurement available at time points 3h, 24h, 3d and 1wk.

6.3.9. Statistical Analysis

Statistical analysis of results was carried out in Minitab® 15 for Windows® (Minitab Inc., State College, PA, USA. http://www.minitab.com). Values are given as mean ±SD unless otherwise stated.

6.4. Results

Detailed results are at Appendix C. Mean animal body weight was 443.0 ±39.2g. Mean animal core body temperature at the measurement points was 37.58 ±0.47ºC.

Probe signal was lost from an implanted probe on 13 occasions (out of a total of 140 measuring points). Volume flow rates were available bilaterally for all ten animals at the baseline, ten minute, and one hour measuring points. At three hours, paired results from nine animals were available for analysis. Paired results were available from eight animals at the 24 hour and three day time points. At one week, paired results were available from six animals.

Means of actual time measuring start points were: 10m 26s (±1m 02s), 1h 02m (±5m), 3h 10m (±22m), 24h 54m (±1h 16m), 72h 10m (±21m) and 168h 22m (±1h 12m).

6.4.1. Ischaemia Time

Ischaemia time was analysed by two factor ANOVA, the factors being experiment serial number and technique. No statistically significant difference was noted by serial number (p = 0.842). Mean ischaemia time was 32m 00s (± 5m 13s) for the invagination technique and 42m 28s (±

5m 06s) for the oblique end-to-end technique (Figure 6.1.). This difference was statistically significant (p = 0.003, ANOVA. Difference in means 10m 28s).

A regression analysis of mean volume flow at ten minutes following clamp release versus ischaemia time was also carried out to check for any association between these two factors. No significant association was found (p = 0.089).

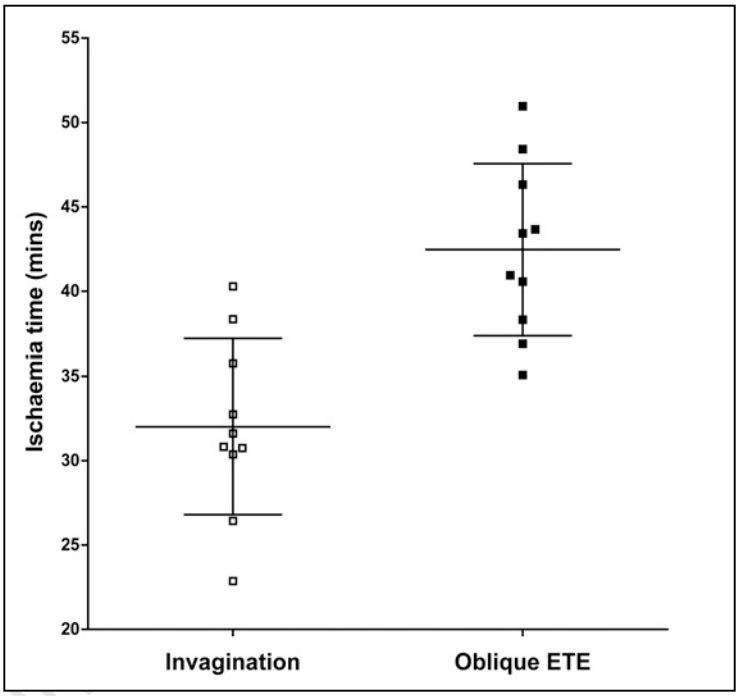

Figure 6.1. Ischaemia time by technique. Bars represent means ±SD. p = 0.003 (ANOVA, difference in means 10m 28s).

6.4.2. Mean Volume Flow Rate

Time-averaged flow rates from each animal at each time point are at Appendix C. Flow, as a mean of these individual results, is plotted at Figure 6.2. Mean flow as a percentage of baseline flow is plotted at Figure 6.3.

Flow rate was analysed by three factor ANOVA, looking for a difference in means by experiment serial number, technique and measurement time point. There was no statistically significant difference between techniques (p = 0.087). Animal serial number showed no association with flow rate (p = 0.115).

There was a significant difference in flow rate by the time point at which it was measured (p<0.0001), the latter three time points (24h, 3 days and 1 week) showing increased flow when compared to the first four (Tukey pairwise comparisons – see Appendix C). No significant difference was noted between the baseline flow measurements and flow at ten minutes or one hour (p = 0.3290 and p = 0.1459 respectively, Tukey).

6.4.3. Pulsatility Index

Time-averaged Pulsatility Index (PI) calculations from each animal at each time point are at Appendix C. PI, as a mean of these individual temporal means, is plotted at Figure 6.4. Mean PI as a percentage of baseline PI is plotted at Figure 6.5.

Mean PI was analysed by three factor ANOVA, looking for a difference in means by animal serial number, technique and measurement time point. There was no statistically significant difference between techniques (p = 0.084). PI showed a difference by animal serial number (p = 0.010).

There was a significant association between PI and the time point at which it was measured (p<0.0001), the latter three time points (24 hours, 3 days and 1 week) showing a lower PI when compared to the first four (Tukey pairwise comparisons – see Appendix C).

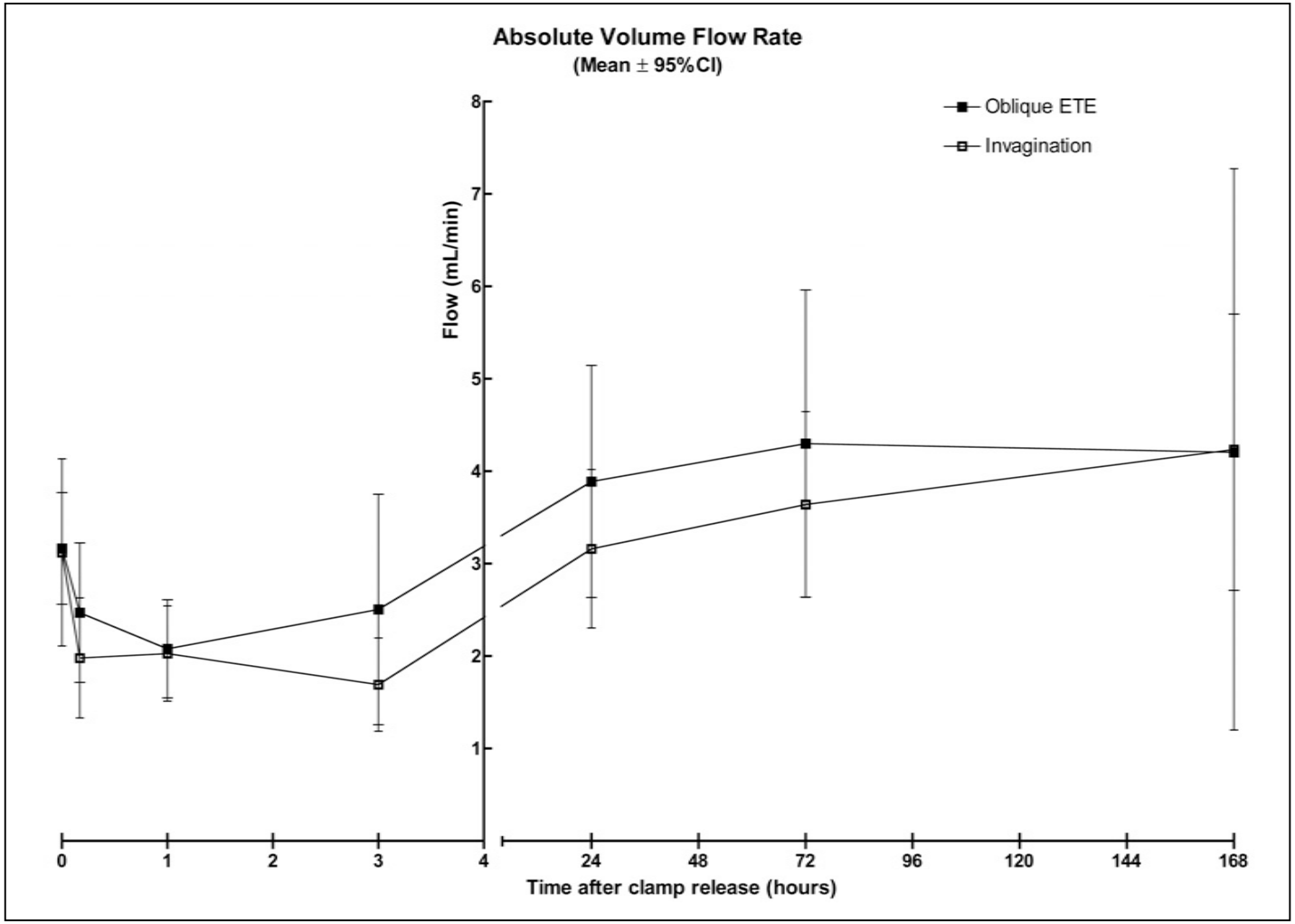

Figure 6.2. Mean volume flow rate ±95%CI measured at time points: Baseline (zero), ten minutes, one hour, three hours, 24 hours, 72 hours and 168 hours (7 days). Mean values are means of time-averaged volume flow rates taken at each time point.

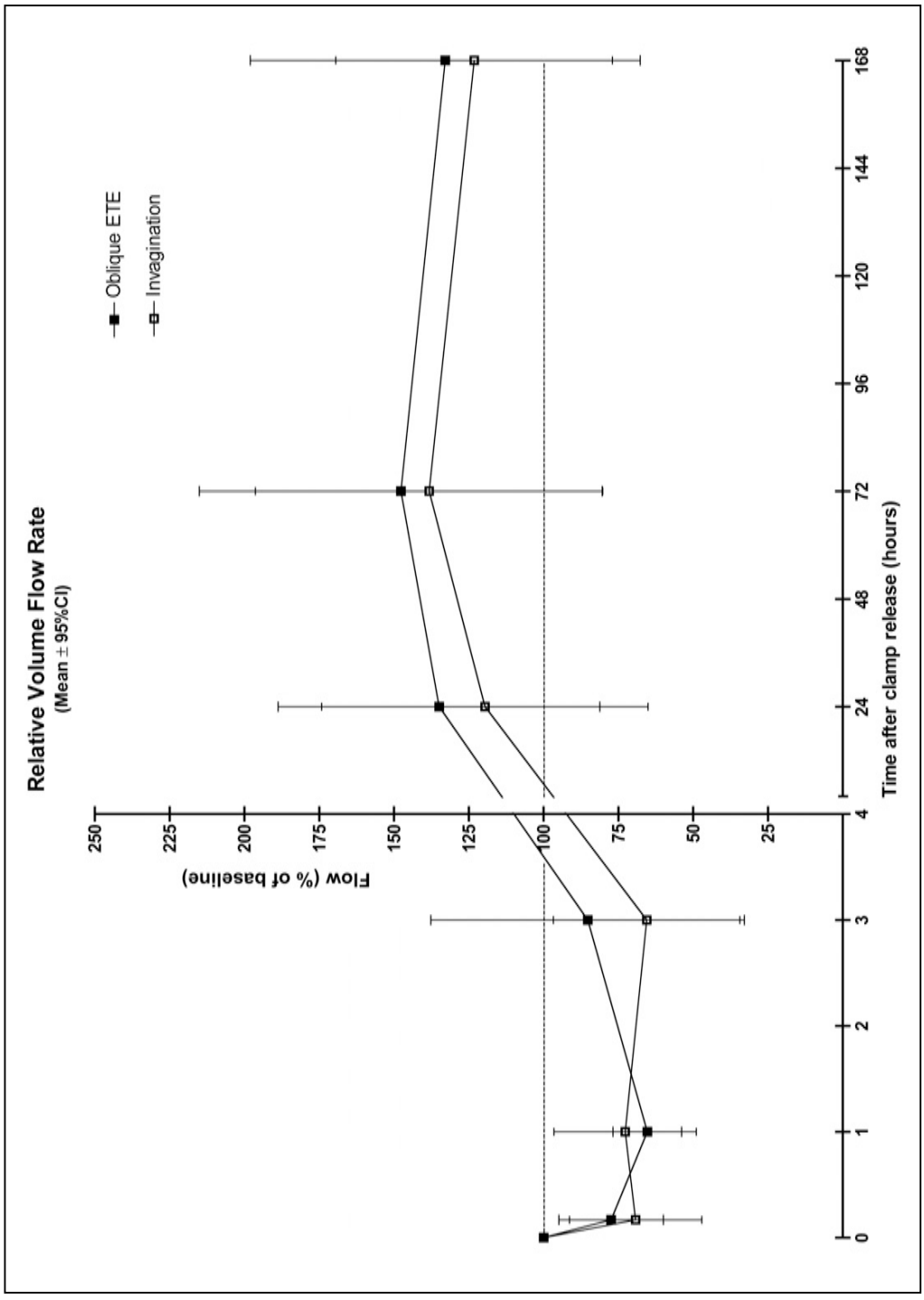

Figure 6.3. Relative mean volume flow rate ±95%CI at time points: ten minutes, one hour, three hours, 24 hours, 72 hours and 168 hours (7 days). Values are percentages of baseline measurements in each animal.

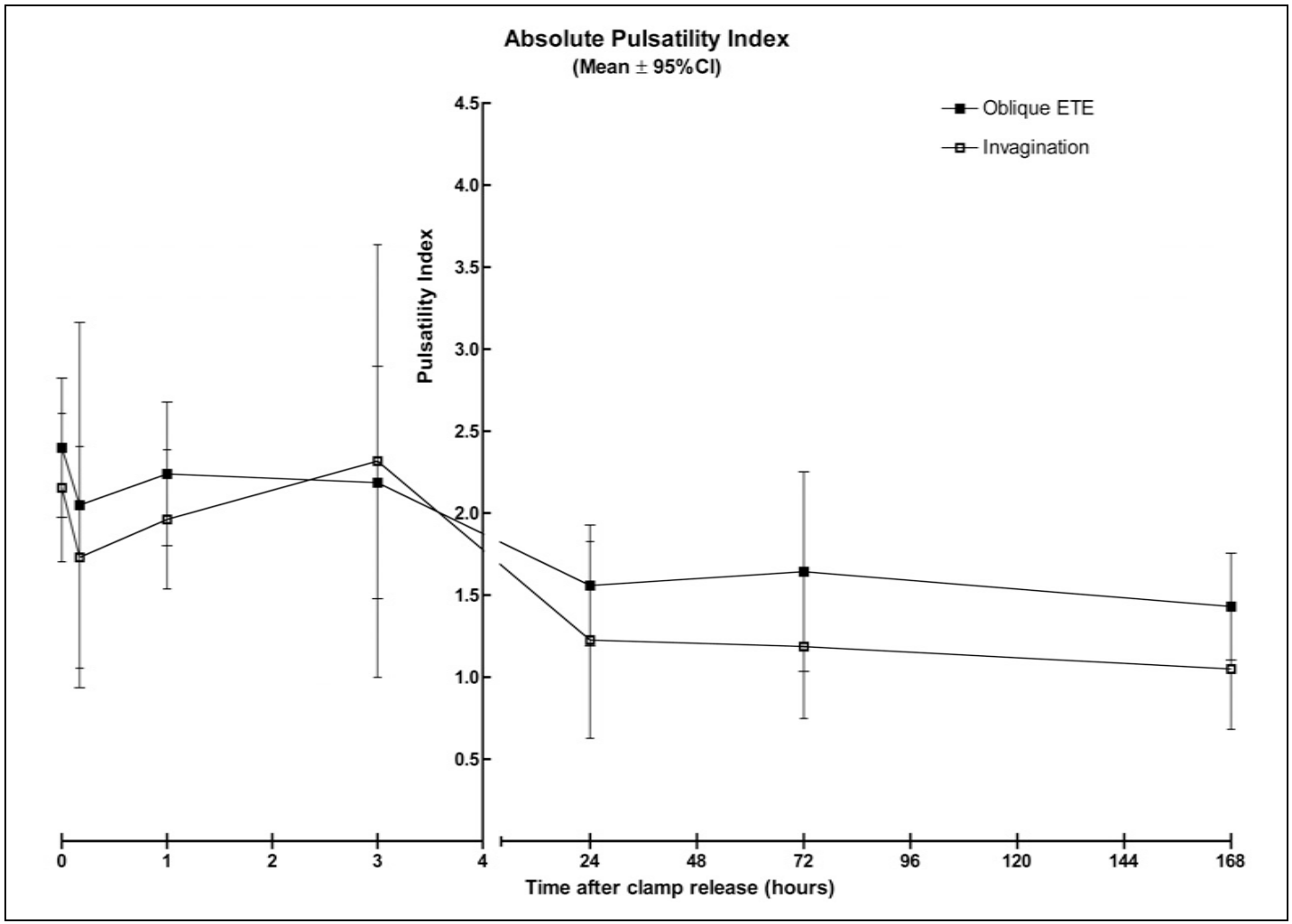

Figure 6.4. Mean pulsatility index ±95%CI measured at time points: Baseline (zero), ten minutes, one hour, three hours, 24 hours, 72 hours and 168 hours (7 days). Mean values are means of time-averaged pulsatility index values taken at each time point.

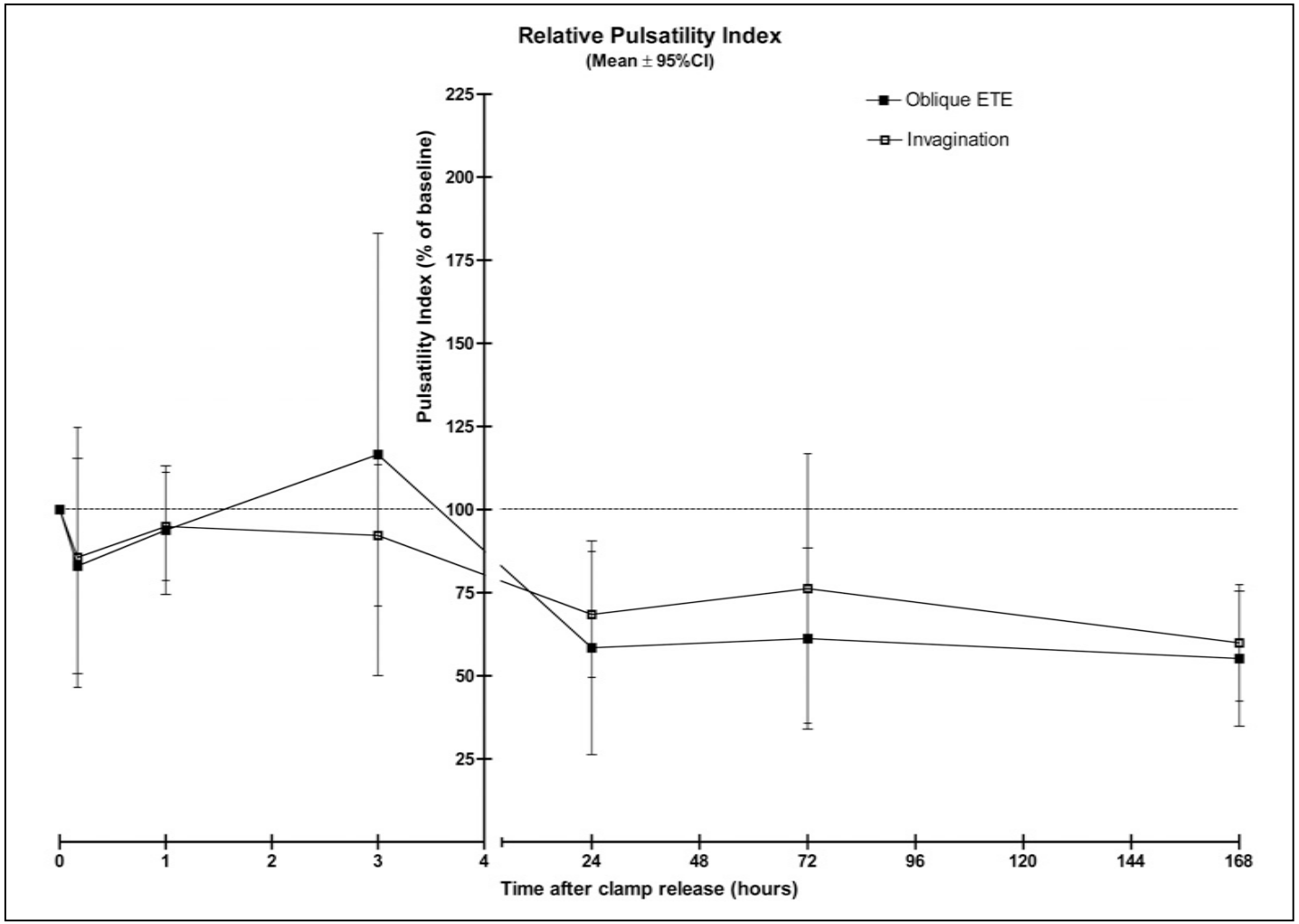

Figure 6.5. Relative mean pulsatility index ±95%CI at time points: ten minutes, one hour, three hours, 24 hours, 72 hours and 168 hours (7 days). Values are percentages of baseline measurements in each animal.

6.4.4. Relative Resistance

Resistance ratios for each of the four time points are plotted at Figure 6.6. Pooled ratios were analysed by *t*-test to look for differences between techniques. No significant difference was found ($p = 0.368$).

Data was also analysed by two factor ANOVA, looking at measurement time point and position in series. No statistically significant difference in mean resistance ratios was found between time points ($p = 0.634$, ANOVA) or animal serial numbers ($p = 0.144$, ANOVA).

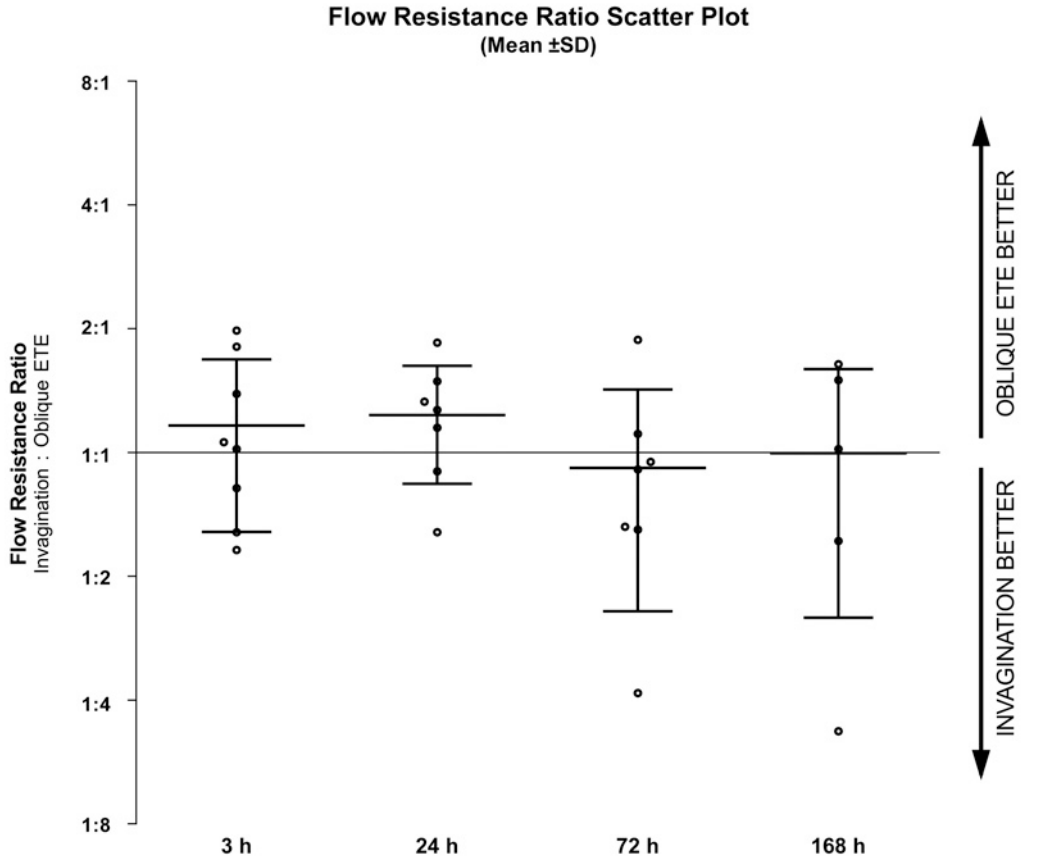

Figure 6.6. Relative flow resistance through the anastomoses at each of the four time points 3h, 24h, 72h and 168h (1wk).

6.5. Discussion

Three indices of flow across the two anastomoses have been examined. No evidence of any difference in time-averaged volume flow rate, PI or relative resistance between techniques has been found. Because of missing values, a repeated-measures ANOVA was not found possible with this model. The repeated measures used in this study may have introduced serial correlation, although using a repeated measures test is highly unlikely to have increased the chance of a significant result. The non-significant results arising from the three factor ANOVA can therefore be judged to be robust.

The difference in ischaemia time and its potential influence on flow between techniques is worthy of further scrutiny. As shown in Chapter 5, this difference is related to the differing complexities associated with performing the two techniques.

Lindbjerg (1966) studied ^{133}Xe clearance from the tibialis anterior muscles of healthy human subjects following periods of pneumatic tourniquet-induced ischaemia of three, five and ten minutes. The magnitude of the hyperaemia following tourniquet release increased between the three and five minute groups, but Lindbjerg found no difference in maximal flow rate between the five and ten minute groups.

Santavirta *et al* (1978) examined hyperaemia following pneumatic tourniquet-induced ischaemia in rabbit hind limbs of varying durations of one, two and three hours. These authors also calculated flow in the tibialis

anterior muscle using ^{133}Xe clearance. They found that the peak of the ischaemia-induced hyperaemic reaction occurred one minute after tourniquet release in each of the three groups, and that flow had returned to normal or less than normal in each group at five minutes. The magnitude of the reaction was independent of the duration of ischaemia. Vita *et al* (2008) in a human model conclude that the relative increase in hyperaemic flow is independent of the baseline flow rate.

Myhre (1975) found that the duration of hyperaemia was unaffected by the presence of a downstream stenosis, although the extent of the hyperaemic reaction was reduced in the presence of a stenosis when compared to controls. This latter conclusion concurs with some of the findings of Lindbjerg (1966).

Thus it may be concluded that the difference in ischaemia times between the two techniques found in this study is unlikely to have influenced flow following release of the clamps. Any difference that may have occurred due to stenosis at the anastomoses is likely to have recovered before the initial time measuring point at a mean of 10m 26s (±1m 02s).

Differences in mean time-averaged volume flow rates were not affected by position in series. This indicates that, in this measure at least, there was no significant inter-animal variability. Whilst mean flow rates following anastomosis dropped when compared to baseline, this difference was not statistically significant. This is in contrast to the findings of Notodihardjo et al., (1998), who attributed their finding of a drop in flow to post-

anastomotic spasm. The highly significant differences in flow rate found between the first four and the latter three time points in the study presented here may be attributed to reductions in flow seen in supine, anaesthetised animals (Roer and Dillaman, 1994).

Some researchers suggest that mean flow rate is a poor discriminator of anastomotic stenosis, this being more directly dependent on the resistance produced by the tissues perfused by that vessel, and by the overall vessel diameter. (D'Ancona *et al*., 2000, Hirotani *et al*., 2001, D'Ancona *et al*., 2001). D'Ancona and others (Aleksic *et al*., 2004, Leong *et al*., 2005) postulate that waveform analysis is a more sensitive indicator of anastomotic stenosis.

In the present study, if the assumption is made that vascular resistance produced by the hind limbs of the animals is similar on both sides, then these factors have been controlled, and any differences in mean flow rate will be created by the anastomosis. This assumption also allows for the calculation of a resistance ratio. However, the quality of data available from the transit-time sensors permits a more detailed analysis of the flow waveform. Of indices available from waveform analysis, Pulsatility Index (PI) has found greatest utility in the experimental and clinical assessment of anastomotic flow.

Pulsatility is defined as the difference between the peak (systolic) flow rate and the trough (end-diastolic) flow rate. The PI, as defined by Gosling (Gosling *et al*., 1971, Gosling and King, 1974), is the ratio of pulsatility to time-averaged flow rate. Downstream luminal obstruction causes reflected

flows and an increase in systolic flow acceleration, together with a relative reduction in mean flow rate. Together, these produce a high PI value. High PI values have been correlated with flow resistance produced by coronary artery stenosis (Aleksic *et al*., 2004), and some authors consider that the use of PI, amongst other transit-time derived flow indices, is essential in the intra-operative assessment of coronary artery bypass grafts (Leong *et al*., 2005). A recently-reported prospective trial of 1000 coronary artery bypass grafts has shown that PI alone is a useful predictor of graft failure (Kieser *et al*., 2010). Mean flow rate was not predictive of outcome in this study.

Others have used PI as an experimental measurement of microanastomotic quality (Jones and Greenhalgh, 1983) and of resistance within transplanted autologous tissue composites (Lloyd and Niranjan, 2009, Scholz *et al*., 2009).

In this study, there was no evidence that differences in mean PI values were due to anastomotic technique. There was a statistically significant difference in PI with position in series, indicating significant inter-animal variability. It is also interesting to note that, as with mean flow rates, PI was affected by the conscious state of the animal.

6.5.1. Conclusion

In conclusion, no evidence of differences in flow greater than 10% across the two techniques under study has been found. The Null Hypothesis, therefore, is not rejected.

6.6. References

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7 VESSEL REMODELLING

Outline

7.1. Introduction

The *in silico* study has shown that the two techniques under study exhibit regions of complex flow separation and that no clear advantage of one technique exists over the other. The *in vivo* patency and timing study has shown that no clear benefit exists in terms of gross patency. It may be said, however, that the invagination technique is easier and quicker to complete in this model.

The paired *in vivo* study of flow using the two techniques has not shown any statistically significant differences in mean volume flow rate, pulsatility index or relative resistance up to a maximum of one week.

A longer-term measure of flow was sought. Vessels are known to remodel their architecture in response to shear stresses at the vessel wall, endotheliocytes acting as shear stress transducers (Langille and O'Donnell, 1986). As demonstrated in the *in silico* study, shear stresses are proportional to volume flow rates. Vessels dilate with increased flow rate, and constrict with decreased flow, both responses bringing shear stresses back to within normal limits. This adaptation is a sustained response due to structural remodelling of the vessel wall.

A study was designed using lumenal cross-sectional area as a proxy for long term flow rate. A secondary outcome measure was stenosis at the anastomosis in both techniques. Animals from the patency study were studied at ten months following entry into that study.

7.2. Null Hypothesis

The following null hypothesis was formed:

"At ten months following the anastomosis of arteries of unequal diameter, where a small-to-large diameter mismatch exists of between 1:1.5 and 1:2.5, there is no difference in vessel internal diameter between the invaginating anastomosis and the oblique end-to-end anastomosis in a rodent model."

7.3. Materials and Methods

7.3.1. Study Design

Using the statistical design from the short-term flow study, paired results from six or seven animals were deemed necessary to test for vessel remodelling differences between the invagination and oblique end-to-end techniques. In order to allow for loss of anastomotic patency, loss of animals over ten months, or technical problems with casting, a total of twenty consecutive animals from the patency study were designated for long-term study. Ethical approval was gained from the Animal Ethics Committee of the University of Cape Town. Animal husbandry and anaesthesia details were the same as those used in the patency study (sections 5.3.4 to 5.3.7.).

7.3.2. Experimental Procedure

Corrosion casting of vessels was used to measure vessel internal diameter. Casting was performed according to the methods of Levesque (Levesque *et al*., 1979) and Langille (Langille *et al*., 1986, Langille and O'Donnell, 1986).

At ten months following surgery, animals were killed by an intravenous injection of sodium pentobarbitone (Euthanaze®, Bayer (Pty) Ltd., Isando, South Africa. http://www.bayer.co.za). The abdominal aorta was cannulated in an prograde direction. Two silk ligatures were placed around the cannulated aorta and one around the proximal aorta. The groins were opened and the femoral vessels exposed. A venotomy was made in the femoral vein and the limbs were perfused with warmed (37°C) 0.9% w/v NaCl via the aorta until clear solution was seen emanating from the femoral veins bilaterally. A methyl methacrylate casting medium (Batson's No. 17, Polysciences, Inc., Warrington, PA, USA. http://www.polysciences.com) was then infused manually by syringe until it was seen passing into the popliteal and saphenous arteries. An additional 2mL was infused and a constant pressure of 100mmHg was applied by the attachment of a water trap placed 136cm above the animal's aorta (1mmHg = 1.36cm H_20).

After 24hours, the casts were cleared by removing the hind limbs and lower torso together and immersing them in 25% NaOH at 50°C for 24 hours. On both sides, the portion of these casts comprised of the femoral artery (FA), superficial caudal epigastric artery (SCEA) and distal FA, including its bifurcation into the popliteal and saphenous arteries, was

removed as a single unit, cleaned with distilled water and air-dried. The casts were mounted on 32mm aluminium pin stubs using adhesive carbon tabs (Agar Scientific Ltd., Stansted, Essex, UK. http://www.agarscientific.com). Specimens were sputter-coated in gold by a Polaron E5000 low vacuum sputter coater (Quorum Technologies Ltd., Ashford, Kent, UK. http://www.quorumtech.com) and examined at 10kV in a Cambridge Stereoscan 120 scanning electron microscope (SEM) (Leica Microsystems (Cambridge) Ltd., Cambridge, UK. http://www.leica-microsystems.com). Digital images were obtained with I-SCAN 2000 image acquisition software (ISS Group Services Ltd., Manchester, UK. http://www.iss-group.co.uk).

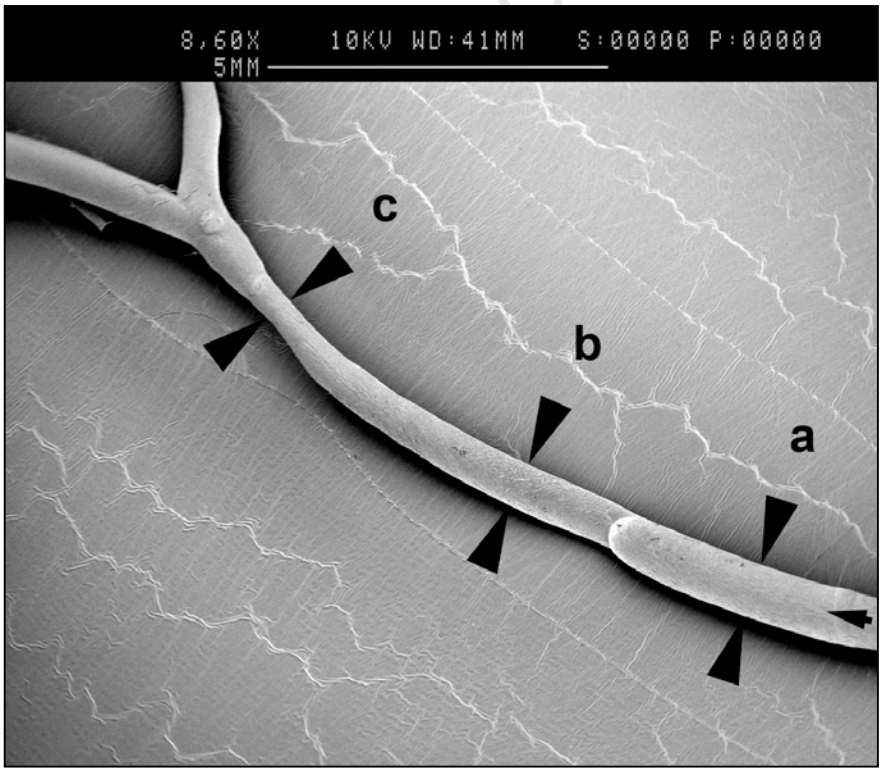

Figure 7.1. Overview SEM image of a corrosion cast. Small arrow = direction of flow (proximal vessel on the right). Cast measurement points: (a) femoral artery, (b) widest point of the superficial caudal epigastric artery ($SCEA_W$), and; (c) narrowest point of the SCEA ($SCEA_N$).

An overview of the specimen was taken and three measuring points were selected from this image. These three points were the widest point of the FA, and the widest and the narrowest points of the SCEA (Figure 7.1.). At each of these points, two images at 90º to each other were taken at 90x to 100x magnification.

Cast diameters were measured using digital image analysis software (Carnoy v2.1, Biovolution, Leuven, Belgium http://www.biovolution.com). Technique was blinded until after measurements were taken. Three replicate measurements were performed in each image and the mean value calculated. These results were divided by two to give the mean radii. Vessel cross-sectional area was calculated using the formula for the area of an ellipse:

$$A = \pi \cdot a \cdot b$$

where A is the cross-sectional area, and a and b are the radii of the long and short axes.

7.3.3. Statistical Analysis

Statistical analysis of results was carried out in Minitab® 15 for Windows® (Minitab Inc., State College, PA, USA. http://www.minitab.com). All values are given as mean ±SD.

7.4. Results

Of the twenty consecutive animals designated for long-term study, technically adequate casts of both sides were available from seven. Five animals had died or had been euthanised during the ten month period for reasons not directly related to surgery. In five additional animals at least one anastomosis had failed. Of the remaining ten animals, three casts were technically inadequate on at least one side. The remaining seven casts were from animals 27, 31, 32, 34, 38, 42 and 45, these numbers indicating their position in series in the patency experiment. Animals were sacrificed at a mean of ten months and seven days (range 10m 3d – 10m 11d). Cast measurements are at Appendix D. XY plots of each of the three measuring points are at Figures 7.2., 7.3., and 7.4..

7.4.1. Cross-sectional Area

In all specimens, the femoral artery was of uniform calibre throughout its length. Cross-sectional areas are plotted at Figure 7.2. Mean FA cross-sectional area was 0.5092 ±0.1299 mm^2 on the side anastomosed by invagination and 0.3932 ±0.1437 mm^2 on the side anastomosed by the oblique end-to-end technique.

Cross-sectional areas of the SCEA at its widest point ($SCEA_W$) are plotted at Figure 7.3. Mean $SCEA_W$ cross-sectional area was 0.2677 ±0.1049 mm^2 on the side anastomosed by invagination and 0.2230

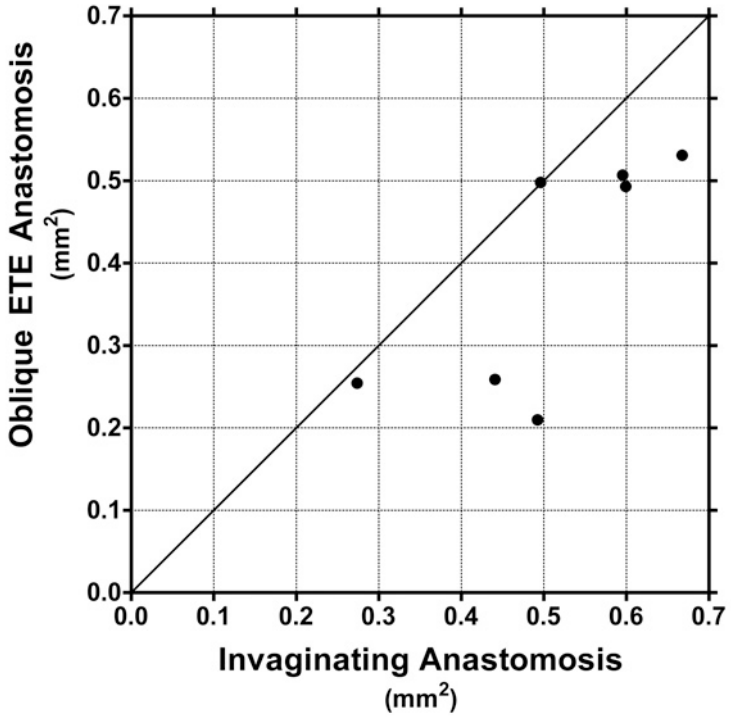

Figure 7.2. Femoral Artery. *XY* plot of paired femoral artery (FA) cross-sectional area (mm^2). Diagonal represents equal FA cross-sectional areas through both anastomoses.

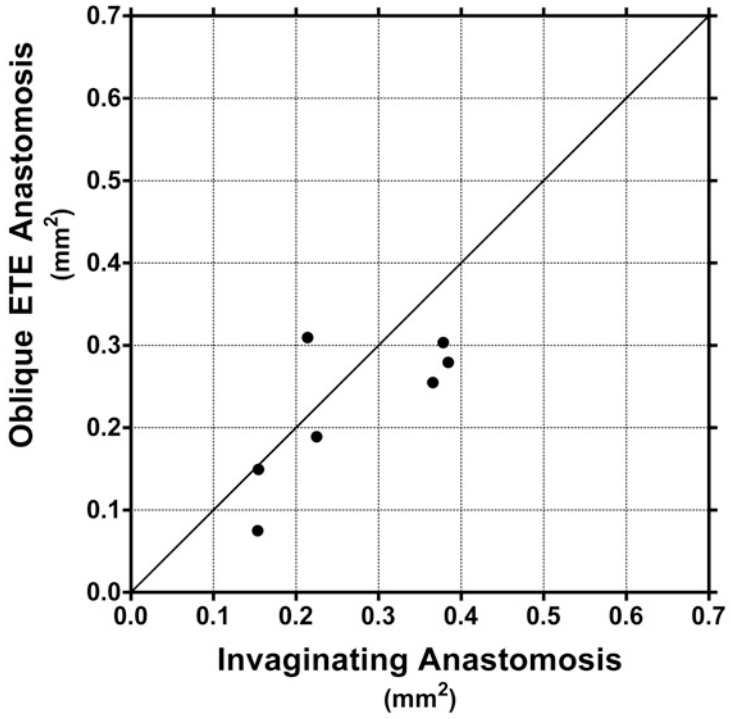

Figure 7.3. Superficial Caudal Epigastric Artery. *XY* plot of paired superficial caudal epigastric artery cross-sectional area at its widest part ($SCEA_W$) (mm^2). Diagonal represents equal $SCEA_W$ cross-sectional areas through both anastomoses.

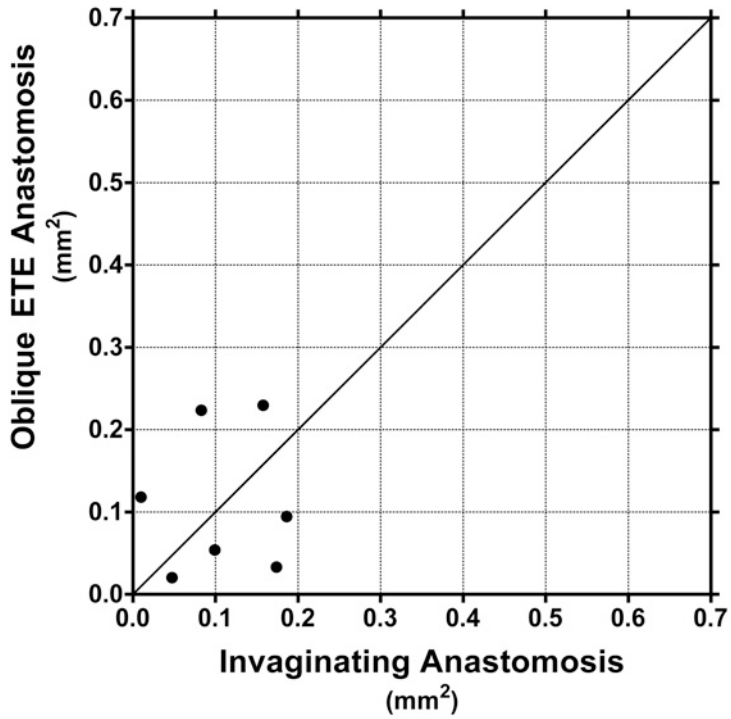

Figure 7.4. Superficial Caudal Epigastric Artery. *XY* plot of paired superficial caudal epigastric artery cross-sectional area at its narrowest part ($SCEA_N$) (mm^2). Diagonal represents equal $SCEA_N$ cross-sectional areas through both anastomoses.

±0.0881 mm^2 on the side anastomosed by the oblique end-to-end technique.

Cross-sectional areas of the SCEA at its narrowest point ($SCEA_N$) are plotted at Figure 7.4. Mean $SCEA_N$ cross-sectional area was 0.1081 ±0.0671 mm^2 on the side anastomosed by invagination and 0.1106 ±0.0862 mm^2 on the side anastomosed by the oblique end-to-end technique.

Cross-sectional area was analysed by four-factor ANOVA, looking at side, position in series, vessel measurement point and technique. Side did not show a significant influence on mean cross-sectional area ($p = 0.560$). Animal serial number showed a significant influence on cross-sectional

area (p <0.0001), indicating significant inter-animal variability in cast measurements. Mean cross-sectional area was also significantly different by measuring point overall (p <0.0001), and specifically (FA *vs* $SCEA_W$ - p <0.0001, difference in means 0.206 mm^2; FA *vs* $SCEA_N$ – p <0.0001, difference in means 0.342 mm^2; $SCEA_W$ *vs* $SCEA_N$ – p =0.0001, difference in means 0.136 mm^2).

Cross-sectional area was statistically significantly different by technique (p = 0.029, difference in means 0.026 mm^2, (invagination technique larger)). This difference in cross-sectional area is 10.73% of the mean of that of the oblique end-to-end technique.

7.4.2. Anastomotic Stenosis

The exact site of the anastomosis was not possible to identify in all specimens, and so the narrowest point of the SCEA was chosen as worst-case measuring point. The degree of stenosis through each technique was calculated by dividing the cross-sectional area of this point by that of the widest part of the SCEA:

$$\%\ stenosis = \frac{SCEA_N}{SCEA_W} \times 100$$

Cross-sectional area of the narrowest part, as a percentage of the widest part is plotted at Figure 7.5.

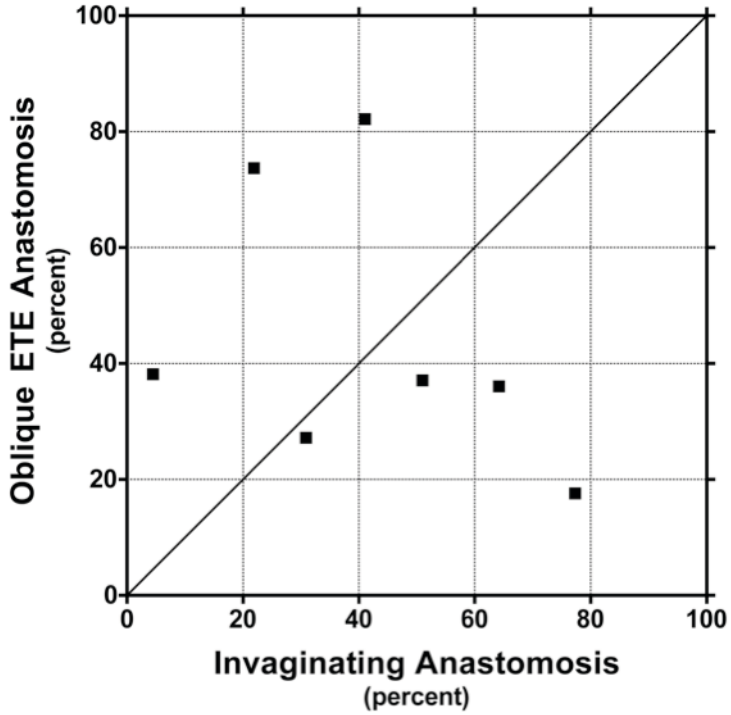

Figure 7.5. XY plot of paired SCEA cross-sectional area at its narrowest point as a percentage of the widest point. Diagonal represents equal percentages through both anastomoses. *P* = 0.819 (ANOVA).

The mean area of the stenosis was 41.53 ±25.01% of the widest part of the SCEA on the side anastomosed by invagination and 44.59 ±24.02% on the side anastomosed by the oblique end-to-end technique. Stenosis was analysed by three-factor ANOVA for side, position in series and technique.

Side did not show an influence on stenosis (p =0.693). Position in series did not show a statistically significant influence on stenosis (p =0.864). Percentage stenosis did not differ by technique (p = 0.819).

In an attempt to explain the heterogeneity in anastomotic stenosis, a further analysis was conducted to see if the operator had an influence. The model used was again a three-factor ANOVA, but using operator, side and technique as the factors. Because the operator and the animal serial number are confounded, it was not possible to simply extend the previous analysis to include both of these factors in a four factor model. This second analysis did not produce any evidence that the operator (A or B) had an influence on percentage stenosis (p = 0.890). Side and technique again were not statistically significant influences using this model (p values = 0.634 and 0.783 respectively).

7.5. Discussion

Under physiological conditions, changes in blood flow and wall shear stress stimulate compensatory changes in arterial size. Arterial remodelling occurs both during development (Chapman, 1918) and as an adaptation to repetitive exercise and some disease processes (Silver and Vita, 2006). Increases in flow lead to sustained compensatory increases in lumen diameter, both experimentally (Kamiya and Togawa, 1980) and clinically (Kojda and Hambrecht, 2005, Vita *et al*., 2008). Conversely, reductions in flow lead to a compensatory decrease in lumen diameter (Langille and O'Donnell, 1986, Olive *et al*., 2003).

Wall shear stress, the frictional force acting on the endothelial cell surface as a result of blood flow, is directly proportional to blood flow rate and viscosity, and inversely proportional to the third power of vessel

radius. This scaling is illustrated in our *in silico* study (Chapter 3). The primary transducer or mechanosensor of shear stress is the endotheliocyte, which acts both as sensor and coordinator of the remodelling response (Langille and O'Donnell, 1986, Vita *et al*., 2008). Adaptive remodelling of the arterial wall keeps shear stresses within a physiological range and maintains endotheliocyte function in a quiescent and athero-protective state (Malek *et al*., 1999). Failure of this normal mechanism has been implicated in hypertension and peripheral vascular disease (Korshunov *et al*., 2007).

The technique of vascular corrosion casting was introduced in 1935 (Schummer, 1935, Cotrufo *et al*., 2010). Batson introduced his methyl methacrylate formulation in 1955 (Batson, 1955). A study comparing the geometry of corrosion casts of the aorto-iliac bifurcation in New Zealand White rabbits with *in vivo* MRI has demonstrated excellent geometric fidelity of the casting technique used here (Moore *et al*., 1997), and its use in studies of remodelling of vessels of this size is commonplace (Levesque *et al*., 1979, Langille and O'Donnell, 1986, Calvo *et al*., 1999). It has also been used by a number of authors to assess the morphology of microvascular anastomoses (Nakayama and Soeda, 1981, Duminy, 1988, Kanaujia et al., 1988, Duminy, 1989). In combination with SEM, complex studies of three-dimensional microvascular architecture are made possible, demonstrating high fidelity in much smaller vessels (Lametschwandtner *et al*., 1990, Cotrufo *et al*., 2010).

A statistically significantly higher cross-sectional area on the side anastomosed by the invagination technique has been found. This may indicate higher flow through this anastomosis. The difference in means was 10.73% of the oblique ETE mean cross-sectional area. From Poisseuille's law, assuming that all other factors remain equal, this translates into a 50.35% increase in flow through this technique (1.1073 ^ 4 = 1.5035). However, the exact relationship between vessel remodelling and flow remains unclear, and it is almost certainly not a linear relationship. Secondly, it can be seen from the XY plots at Figures 7.2 to 7.4 that the differences in cross-sectional areas between techniques were not consistent at all vessel measuring points.

The anastomotic site was not possible to identify with precision in these casts and so the narrowest part of the SCEA was measured on the assumption that this was caused by, although not necessarily located precisely at the anastomosis. The extent of this narrowing was not significantly different in the invagination and oblique end-to-end techniques.

Anastomotic stenosis has been found by many authors who have examined the invagination technique in vessels of an equal external diameter (Lauritzen, 1978, Nakayama and Soeda, 1981, Wieslander and Aberg, 1982, 1983, Gulyas *et al*., 1984, Duminy, 1988, 1989, Siemionow, 1990). Whilst quite a marked a degree of stenosis was seen in many of the anastomoses studied here, no difference in mean stenosis as a percentage of vessel size was observed between the two techniques. This

finding is in contrast to those studies cited above; studies of larger vessels of an equal diameter.

Although there was no evidence of a difference in mean stenosis that could be attributed to the investigator, the heterogeneity of percentage stenosis seen in both techniques may still be explained by human factors, the anastomosis of vessels of this size being technically demanding.

7.5.1. Conclusion

It can be concluded that, within the limits of the study, evidence of significant remodelling differences attributable to technique was found. The null hypothesis is therefore rejected.

In addition, whilst there was marked heterogeneity, anastomotic stenosis in this model was no more pronounced in one technique when compared to the other.

7.6. References

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8 VESSEL HEALING

Outline

8.1. Introduction

In comparison with the end-to-end anastomosis of arteries of an equal diameter, temporal and spatial shear stress gradients encountered when anastomosing vessels of unequal diameter predispose them to alterations in healing, and in particular to the formation of intimal hyperplasia (Ojha, 1994, Ishibashi *et al*., 1995, Sunamura *et al*., 2007). Anastomotic intimal hyperplasia arising from shear stress abnormalities and from compliance mismatch has been implicated in late prosthetic and autogenous vein graft failure (LoGerfo *et al*., 1983, Sunamura *et al*., 2007).

No experimental studies have been found that examine healing or quantify the presence of intimal hyperplasia at microarterial anastomoses with dissimilar diameters. A histological study of a single clinical microvascular case is available which might support this theory in vessels of this size. Karl *et al.* (1980) studied the end-to-end anastomosis between a facial artery and a superficial circumflex iliac artery (SCIA) which had been harvested when thinning a groin flap four months following transplantation. Given the relative average diameters of these vessels (facial artery = 2.7mm (Magden *et al*., 2004); SCIA = 1.2mm (Smith *et al*., 1972)), it is almost certain that this would have produced a large-to-small size discrepancy at the anastomosis, although the authors do not detail this. Karl *et al*. found that the (smaller) flap vessel showed extensive narrowing of the lumen caused by intimal hyperplasia. The authors attribute this finding to the trauma of dissection. However, shear stress

gradients produced by the dissimilar vessel diameters provides an alternative explanation.

In equal-sized vessels, the healing of conventional end-to-end microanastomoses has been widely studied in animal models (Khodadad, 1970, Baxter et al., 1972, Servant et al., 1976, Acland and Trachtenberg, 1977b, Maxwell et al., 1979, Minderjahn and Dahm, 1979, Nightingale et al., 1980, Lidman and Daniel, 1981, Chow, 1983, Lidman et al., 1984). Histological examination of a single conventional end-to-end microanastomosis of equal-sized vessels from a clinical case has been reported (Lendvay and Owen, 1970).

Similarly, numerous studies have examined the healing of invaginating techniques in equal-sized vessels in animal models (Lauritzen, 1978, Lauritzen and Hansson, 1980, Lauritzen *et al*., 1980, Meyer *et al*., 1980, Siemionow, 1990, Zhang *et al*., 1991, Saitoh *et al*., 1992, Gahankari *et al*., 1995).

Three groups have performed paired animal studies directly comparing healing in conventional end-to-end anastomoses with invaginating anastomoses in equal-sized vessels.

Krag and Holck (1980), in a paired study in rodent femoral arteries at one week (*n* = 50 animals) found that transmural necrosis of end-to-end anastomoses was '*the rule*', and that intimal hyperplasia was regularly seen. In comparison, wall necrosis and intimal hyperplasia were seen less frequently in the invagination technique.

Sully *et al*. (1982) studied healing in paired rabbit femoral arteries (*n* = 50 animals) at various time periods up to six weeks. These authors observed that thrombotic deposition and endothelial necrosis were less pronounced in the invaginating technique, and that re-endothelialisation occurred more rapidly than in conventional end-to-end anastomoses. No comment is made about intimal hyperplasia.

Wieslander *et al*. (1982) studied ^{32}P-labelled platelet deposition at the two anastomotic techniques in the central artery of the rabbit ear (*n* = 8 rabbits), concluding that significantly greater platelet deposition occurred at the conventional end-to-end anastomosis. In a mixed light and scanning electron microscope study, Wieslander *et al*. (1984) observed intimal hyperplasia in both techniques. Re-endothelialisation was complete in both by 7-14 days. In a further histological comparison of techniques by light microscopy at various time points to 90 days (*n* = 21), Wieslander and Rausing (1984) conclude that intimal hyperplasia was a constant finding in both end-to-end and invaginating anastomoses, but that the process was progressive only up to 30 days, seeming to plateau or regress thereafter.

The similar wall shear stress ranges found in the *in silico* study of the two techniques under scrutiny might predict similar degrees of intimal hyperplasia where a size discrepancy exists. A study was designed to quantify intimal hyperplasia and to examine healing of the two techniques in the small-to-large vessel model described.

8.2. Null Hypothesis

The following null hypothesis was formed:

"In the anastomosis of arteries of unequal diameter, where a small-to-large diameter mismatch exists of between 1:1.5 and 1:2.5, there is no difference in:

a. healing or

b. intimal hyperplasia,

between the invaginating anastomosis and the oblique end-to-end anastomosis in a rodent model."

8.3. Materials and Methods

8.3.1. Study Design

Four time points were arbitrarily selected for study; 24 hours, one week, six weeks and eight months. There was no information on which to base a power calculation and so eight animals per group was arbitrarily deemed suitable. Those at the six week and eight month time points were consecutive animals from the patency study. Animals included in the 24h and one week groups were in addition to these animals. Ethical approval for the study was gained from the Animal Ethics Committee of the University of Cape Town.

Three further specimens from the flow study became available at one day and an additional eight paired specimens were harvested at one

week. Ethical approval and licensing were not required for these specimens because the tissues were cadaveric in origin. The inclusion of these specimens gave paired specimens from a potential total of 43 animals.

One anastomotic technique was performed on each side. Technique and side order were not formally randomised but animals were selected consecutively from their position in the patency and flow experiments. Two investigators were again employed in performing the anastomoses.

8.3.2. Experimental Procedure

The animals, their husbandry and anaesthetic details were the same as in the patency and flow studies respectively (sections 5.3.4 to 5.3.6 and 6.3.2. to 6.3.4.). Techniques were as detailed at section 5.3.7.

At the respective time points, animals underwent gaseous induction and were killed by an intravenous injection of sodium pentobarbitone (Euthanaze®, Bayer (Pty) Ltd., Isando, South Africa. http://www.bayer.co.za (patency study and additional animals)), or pentobarbital sodium (Euthatal®, Merial Animal Health, Harlow, Essex. http://uk.merial.com (flow study)).

The abdomen and groins were opened and the aorta was cannulated in a prograde direction. A ligature was placed around the cannula and proximal aorta. Bilateral femoral venotomies were made and the hind limb vessels were cleared of blood with warmed (37º) 0.9% w/v NaCl. The veins were then sealed with electrocautery. Vessels were perfusion-fixed in situ at 100mmHg by the infusion of 10% buffered formalin, followed by

the application of constant pressure by raising the formalin reservoir to 1.36m above the animal. After one hour, vessels were dissected out using an operating microscope (SMZ-10, Nikon Corporation, Tokyo, Japan. http://www.nikon.com). Prior to harvesting, the arteries were mounted *in situ* in a double approximating clamp (Acland® Model ABB-3V, S&T® AG, Neuhausen, Switzerland. http://www.microsurgery.ch) after the method of Acland and Trachtenberg (1977a). Specimens were harvested bilaterally as single units comprising the proximal femoral artery (FA), superficial caudal epigastric artery (SCEA), the distal FA and its bifurcation into the popliteal and saphenous arteries.

Specimens were immersed in 10% buffered formalin overnight and processed the following day. Processing was carried out in a Leica TP1020 tissue processor (Leica Microsystems GmbH, Wezlar, Germany. http://www.leica-microsystems.com), through graded alcohols, xylol and then paraffin wax under pressure. Unlike the method of Acland and Trachtenberg, the clamp was removed at this point. The artery was then orientated in wax in an embedding mould and embedded by a Leica EG1140 embedding centre (Leica Microsystems GmbH). Serial longitudinal sections were cut at 2µm on a sliding microtome (Leica SM2000R, Leica Microsystems GmbH) and stained with haematoxylin and eosin.

Semi-quantitative analysis of healing and intimal hyperplasia was performed using the method of Miller *et al*. (2001). The scoring system used a scale of 0 – 3, where 0 = nothing identified; 1 = mild; 2 = moderate, and; 3 = severe. In all cases an individual blinded to the technique of anastomosis performed the scoring. Each layer of the vessel was

examined and the following nine parameters were scored at each of the four time points: (i) thrombosis, (ii) endothelial necrosis, (iii) intimal hyperplasia, (iv) medial necrosis, (v) medial inflammation, (vi) medial fibrosis, (vii) adventitial inflammation, (viii) adventitial fibrosis; and, (ix) adventitial foreign body giant cell reaction or chronic inflammation. In performing the assessment, an initial overview was taken, followed by a detailed assessment of the anastomotic site, both upstream and downstream. After the initial scoring, a selection of cases was re-scored to ensure that the assessments were reproducible, including some early cases to ensure that the scores had not drifted.

8.3.3. Statistical Analysis

Statistical analysis of results was carried out in Minitab® 15 for Windows® (Minitab Inc., State College, PA, USA. http://www.minitab.com). Because of the large number of outcome measures, two analytical models were used. A univariate analysis was carried out on each of the nine outcomes by a three-way ANOVA with Tukey pairwise comparisons. In interpreting the results, a Bonferroni correction has been considered to control for the increased risk of a Type 1 error (alpha error) that arises when performing multiple comparisons on a dataset where a risk of serial correlation exists.

The second model used was a multivariate analysis by principal components analysis. The principal components analysis performs a linear transformation of a large set of potentially correlated variables to a new set of uncorrelated variables. The transformation produces a set of principal

component scores. Scores for the first three components were then analysed by three factor ANOVA, the factors being time point, side and technique.

All values are given as mean ± SE.

8.4. Results

One six-week specimen and one eight-month specimen were lost during processing. Paired results were therefore available from 40 animals (24h, *n* = 11; 1 wk, *n* = 15; 6 wks, *n* = 7, 8 months, *n* = 7).

Detailed results are at Appendix E. In the univariate analysis, each of the nine parameters is reported separately in chronological order of events.

8.4.1. Univariate Analysis

8.4.1.1. Thrombus Deposition.

Results are plotted at figure 8.1. Thrombus scores at one day were 0.73±0.23 for the oblique end-to-end and 0.73±0.27 for the invagination technique. At one week, scores were 0.73±.0.20 and 0.53±0.22 respectively. No thrombus was seen in the six week or eight month specimens. There was a significant difference in mean scores by time point (p = 0.001, ANOVA). No significant differences in the severity of thrombus were found between sides or between techniques (p = 0.180 and p = 0.410 respectively (ANOVA)).

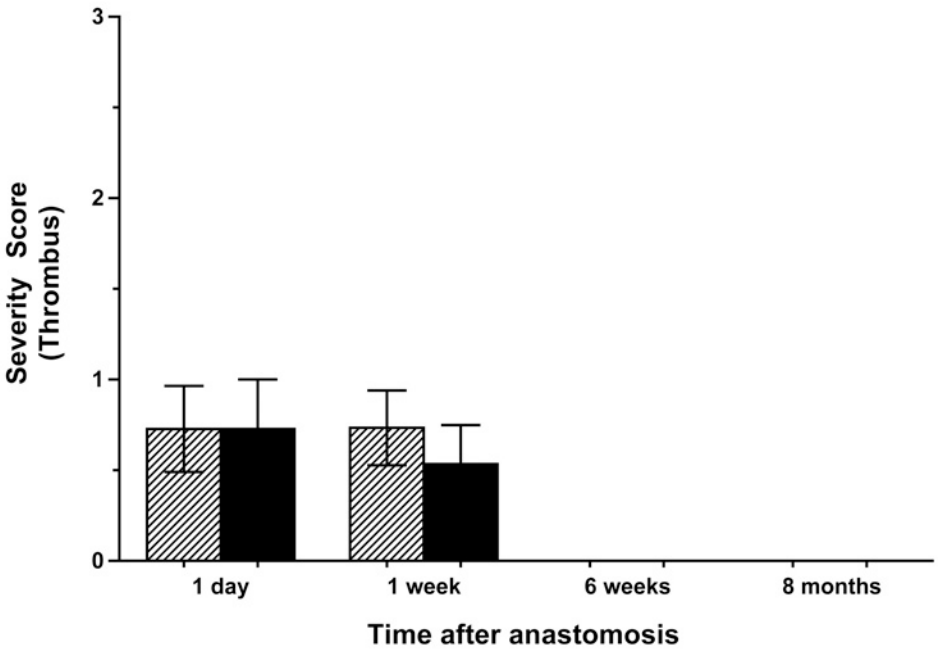

Figure 8.1. Semi-quantitative scores of thrombus formation at the oblique end-to-end anastomosis (left, hatched bars) and the invaginating anastomosis (right, solid bars). Number of vessel pairs analysed: 1d (n=11), 1wk (n=15), 6wks (n=7). 8mo (n=7). Values are expressed as means ± SE.

8.4.1.2. Endothelial Necrosis.

Results are plotted at figure 8.2. At one day, scores of endothelial necrosis were 1.91±0.09 for the oblique end-to-end and 1.55±0.16 for the invagination technique. Scores at one week were 0.87±0.13 and 0.53±0.17 respectively. No endothelial necrosis was seen at six weeks or at eight months. These differences in scores by time point were statistically significant (p <0.0001). Side had no influence on mean scores (p = 0.738). The difference between techniques was statistically significant at the p = 0.05 level (p = 0.021, ANOVA. Difference in mean scores = 0.225).

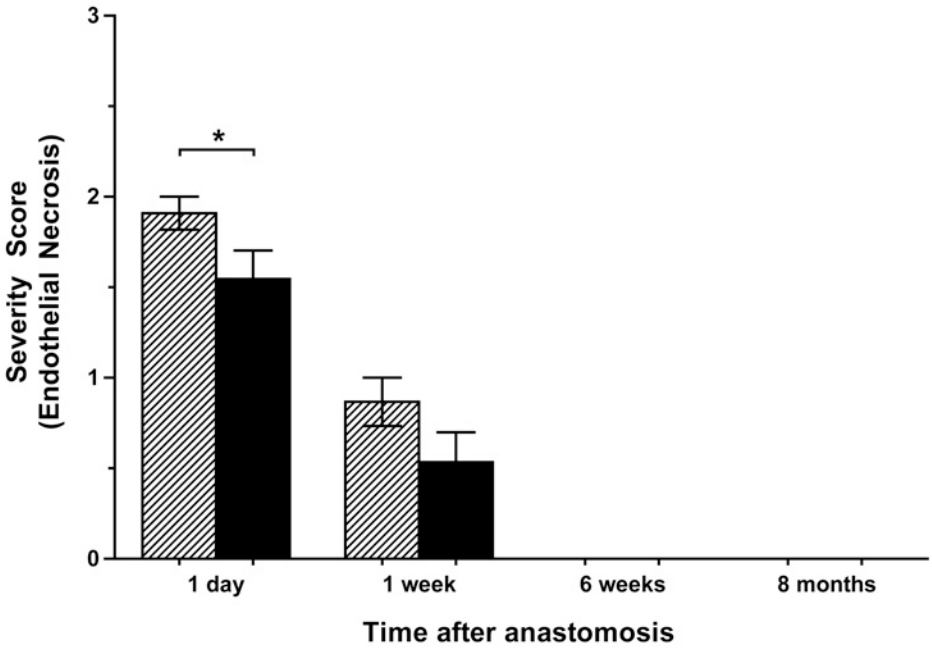

Figure 8.2. Semi-quantitative scores of endothelial necrosis at the oblique end-to-end anastomosis (left, hatched bars) and the invaginating anastomosis (right, solid bars). Number of vessels analysed: 1 day (n=11), 1 week (n=15), 6 weeks (n=7). 8 months (n=7). Values are expressed as means ± SE. *P<0.05.

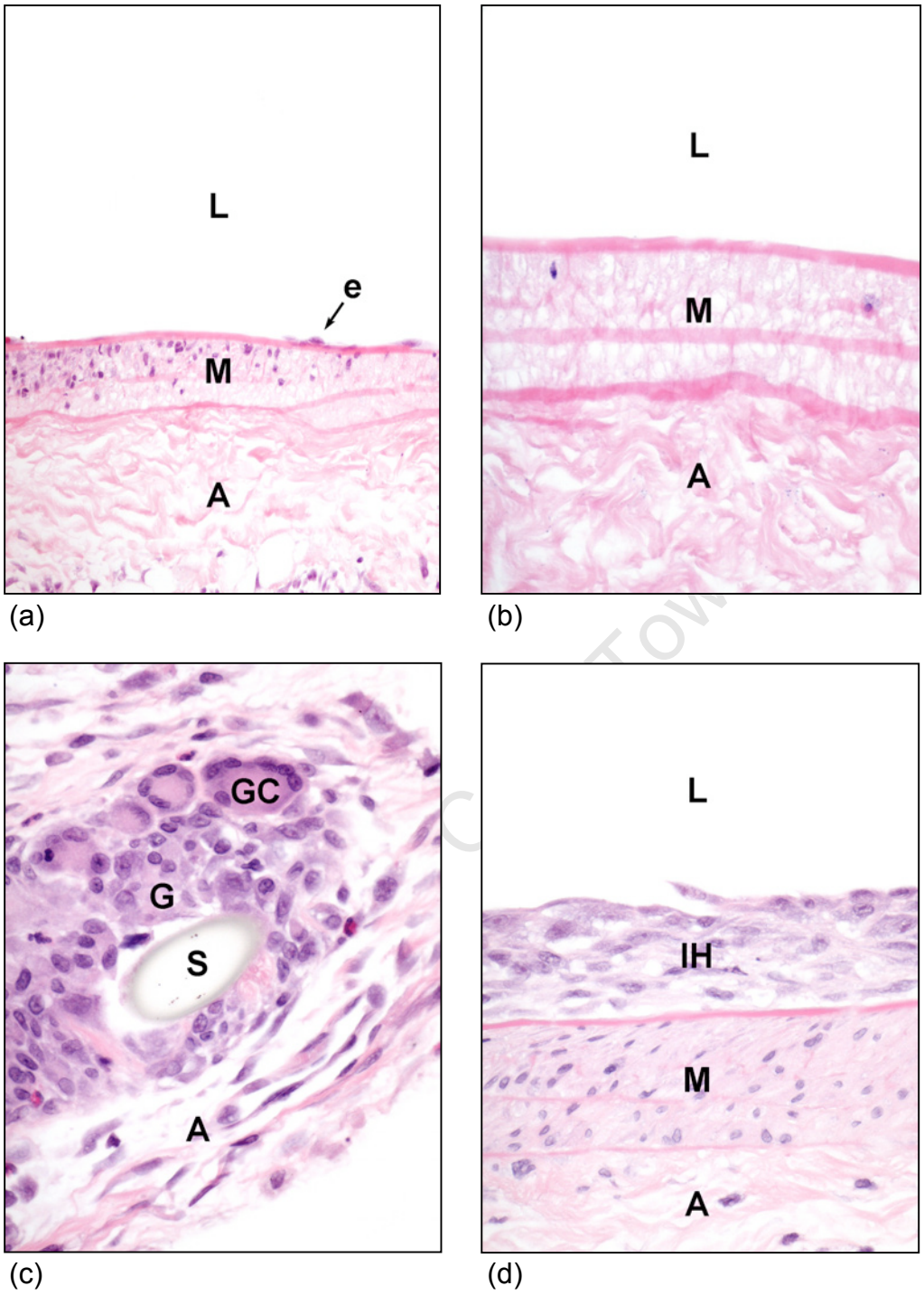

Figure 8.3. Longitudinal sections of vessel wall. Key: L = lumen, M = tunica media, A = tunica adventitia, e = endotheliocyte, GC = giant cell; G = granuloma; S = suture; IH = intimal hyperplasia. (a) Viable medial smooth muscle cells are seen on the left of the image, running into necrotic tunica media on the right, with loss of staining of cell nuclei. Little intact endothelium is seen on the luminal aspect of the internal elastic lamina. Magnification x250. (b) The tunica media in this specimen is almost completely necrotic, with only one definite nucleus visible. No intact endothelium is seen. Magnification x400. (c) Suture material present in fibrosing tunica adventitia. The suture is embedded in a foreign body giant cell granulomatous chronic inflammatory response. Magnification x600. (d) Markedly cellular intimal hyperplasia is seen above the internal elastic lamina. The tunica media is completely viable. Magnification x500. All sections stained with H&E.

8.4.1.3. Medial Necrosis.

Results are plotted at figure 8.4. Scores of medial necrosis at one day were 1.64±0.15 for the oblique end-to-end and 1.09±0.09 for the invagination technique. Scores at one week remained moderately high at 1.46±0.19 and 1.07±0.18 respectively. Medial necrosis was not seen at six weeks or at eight months. The differences in mean scores between time points were statistically significant (p <0.0001). Mean scores did not differ by side (p = 0.821). The difference between techniques was significant at the 0.05 level (p = 0.015, ANOVA. Difference in mean scores = 0.300).

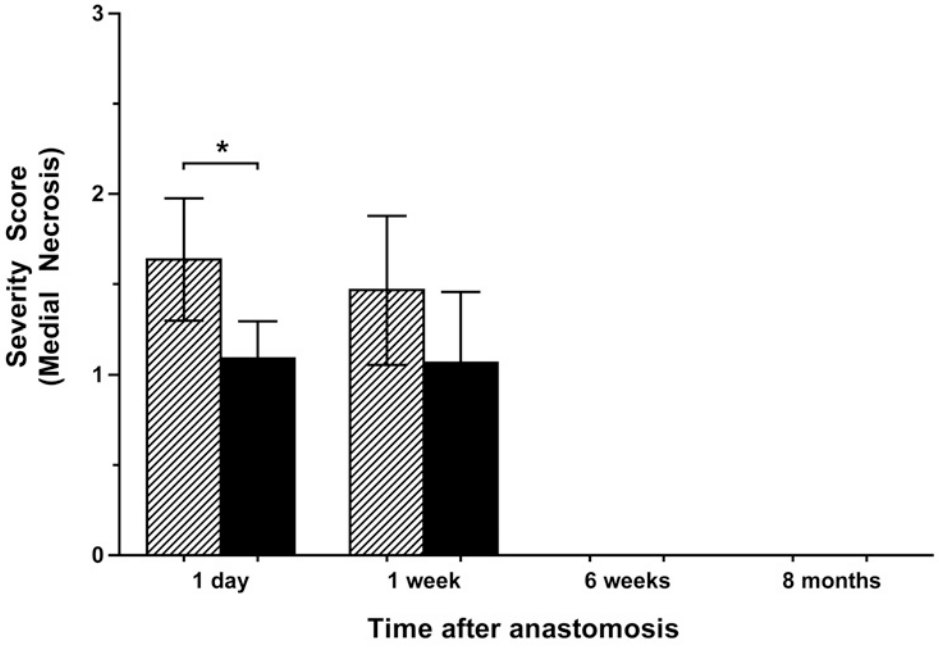

Figure 8.4. Semi-quantitative scores of medial necrosis at the oblique end-to-end anastomosis (left, hatched bars) and the invaginating anastomosis (right, solid bars). Number of vessels analysed: 1 day (n=11), 1 week (n=15), 6 weeks (n=7). 8 months (n=7). Values are expressed as means ± SE. *P<0.05.

8.4.1.4. Medial Acute Inflammation.

Results are plotted at figure 8.5. Scores of acute inflammation in the tunica media at one day were 1.18±0.12 for the oblique end-to-end and 1.00±0.14 for the invagination technique. Scores at one week had reduced to 0.27±0.12 and 0.07±0.07 respectively. Acute inflammation of the tunica media was not seen at six weeks or at eight months. These differences by time point were statistically significant (p <0.0001, ANOVA). Differences by side were not statistically significant (p = 0.531, ANOVA). Differences by technique were not statistically significant (p = 0.131, ANOVA).

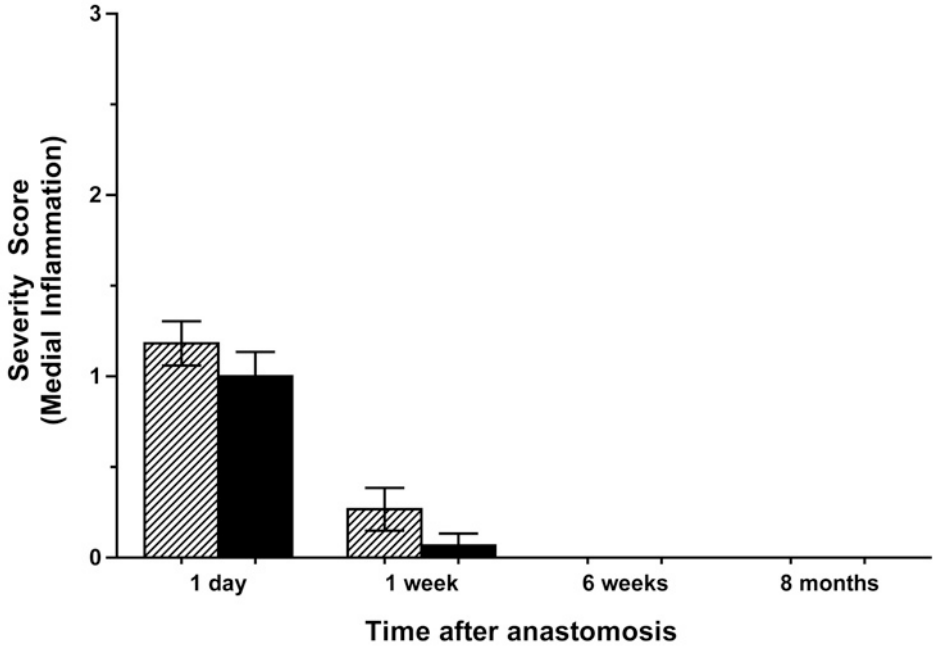

Figure 8.5. Semi-quantitative scores of medial inflammation at the oblique end-to-end anastomosis (left, hatched bars) and the invaginating anastomosis (right, solid bars). Number of vessels analysed: 1 day (n=11), 1 week (n=15), 6 weeks (n=7). 8 months (n=7). Values are expressed as means ± SE.

8.4.1.5. Adventitial Acute Inflammation.

Results are plotted at figure 8.6. Scores of acute inflammation in the adventitia at one day were 1.18±0.18 for the oblique end-to-end and 0.73±0.14 for the invagination technique. Scores at one week had reduced to 0.20±0.11 and 0.13±0.09 respectively. Acute inflammation of the adventitia was not seen at six weeks or at eight months. Differences by time point were statistically significant (p <0.0001, ANOVA). Side had no influence (p = 0.287). The differences between techniques were not quite statistically significant at the p = 0.05 level (p = 0.050, ANOVA. Differences in mean scores = 0.150).

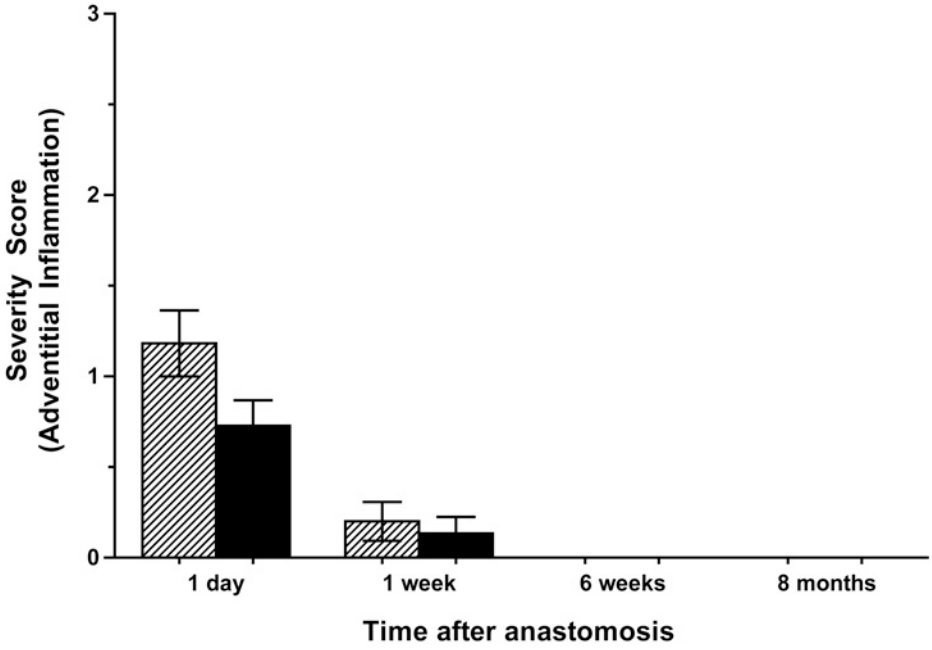

Figure 8.6. Semi-quantitative scores of adventitial inflammation at the oblique end-to-end anastomosis (left, hatched bars) and the invaginating anastomosis (right, solid bars). Number of vessels analysed: 1 day (n=11), 1 week (n=15), 6 weeks (n=7). 8 months (n=7). Values are expressed as means ± SE.

8.4.1.6. Medial Fibrosis.

Results are plotted at figure 8.7. Medial fibrosis scores at one day were zero for both techniques. At one week, scores were 0.40±0.13 for the oblique end-to-end and 0.27±0.12 for the invagination technique. Scores at six weeks were 0.857±0.14 for both techniques and 1.00±0.00 for both at eight months. The difference between scores by time point was statistically highly significant (p <0.0001, ANOVA). There were no differences by side or by technique (p = 0.607 each, ANOVA).

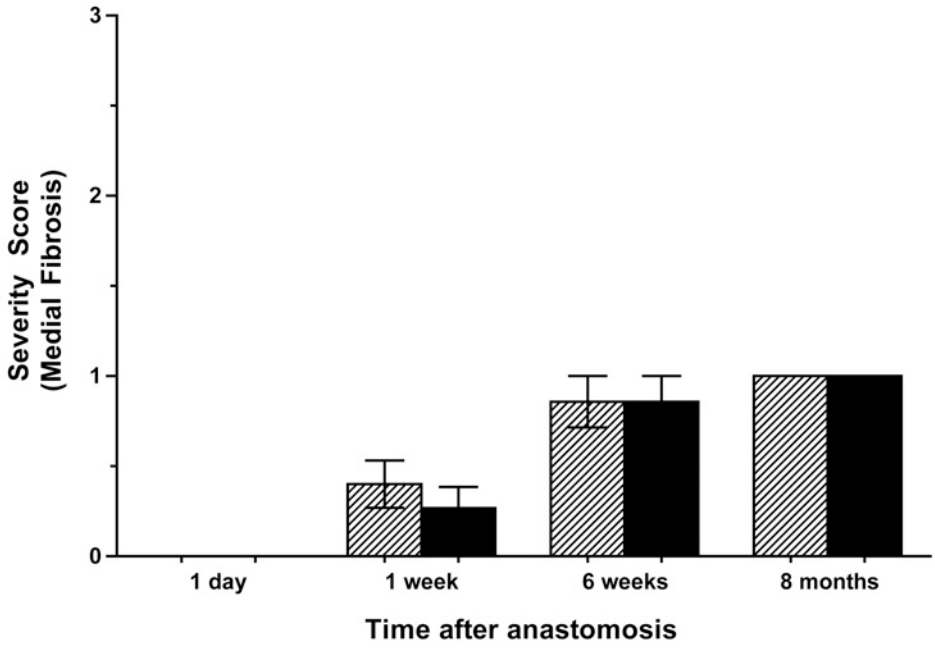

Figure 8.7. Semi-quantitative scores of medial fibrosis at the oblique end-to-end anastomosis (left, hatched bars) and the invaginating anastomosis (right, solid bars). Number of vessels analysed: 1 day (n=11), 1 week (n=15), 6 weeks (n=7). 8 months (n=7). Values are expressed as means ± SE.

8.4.1.7. Adventitial Fibrosis.

Results are plotted at figure 8.8. No adventitial fibrosis was seen at one day in either technique. At one week, scores for both techniques were 1.00±0.00. Scores at six weeks were 2.00±0.00 for the oblique end-to-end technique and 1.86±0.14 for the invagination. At eight months, scores for both techniques were 1.00±0.00. The difference between scores by time point was again statistically highly significant (p <0.0001, ANOVA). There were no differences by side or technique (p = 0.437 each, ANOVA).

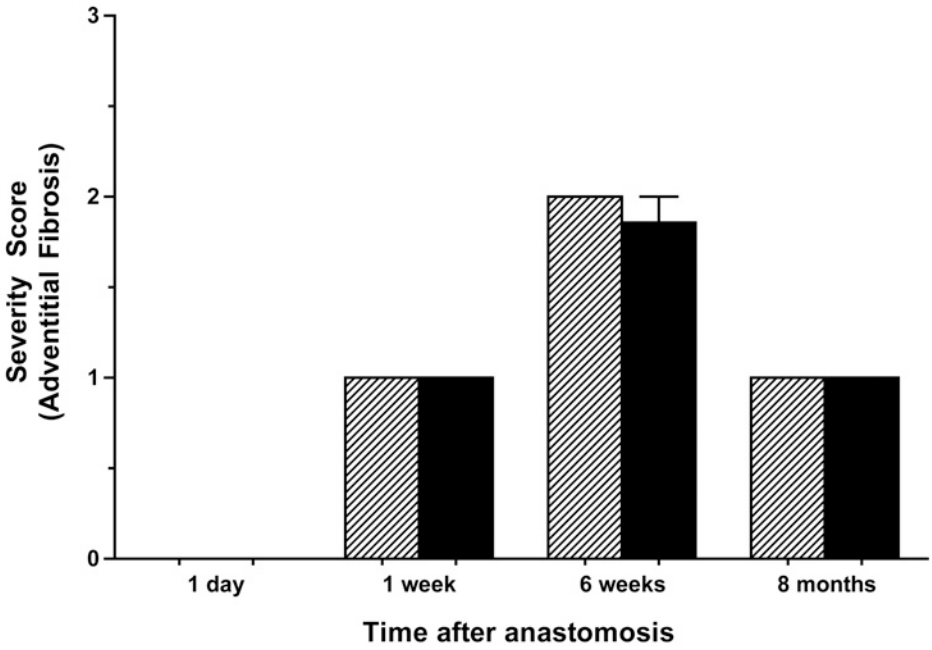

Figure 8.8. Semi-quantitative scores of adventitial fibrosis at the oblique end-to-end anastomosis (left, hatched bars) and the invaginating anastomosis (right, solid bars). Number of vessels analysed: 1 day (n=11), 1 week (n=15), 6 weeks (n=7). 8 months (n=7). Values are expressed as means ± SE.

8.4.1.8. Adventitial Chronic Inflammation

Results are plotted at figure 8.9. No chronic inflammation or signs of a foreign body giant cell reaction were seen at one day. At one week, scores for the oblique end-to-end technique were 0.93±0.07 and 0.87±0.09 for the invagination. At six weeks, scores were 1.57±0.20 and 1.14±0.14 respectively. At eight months scores were 1.00±0.00 and 1.14±0.14 respectively. The differences by time point were again statistically highly significant (p <0.0001, ANOVA). There were no statistically significant differences by side (p = 0.296) or by technique (p = 0.924).

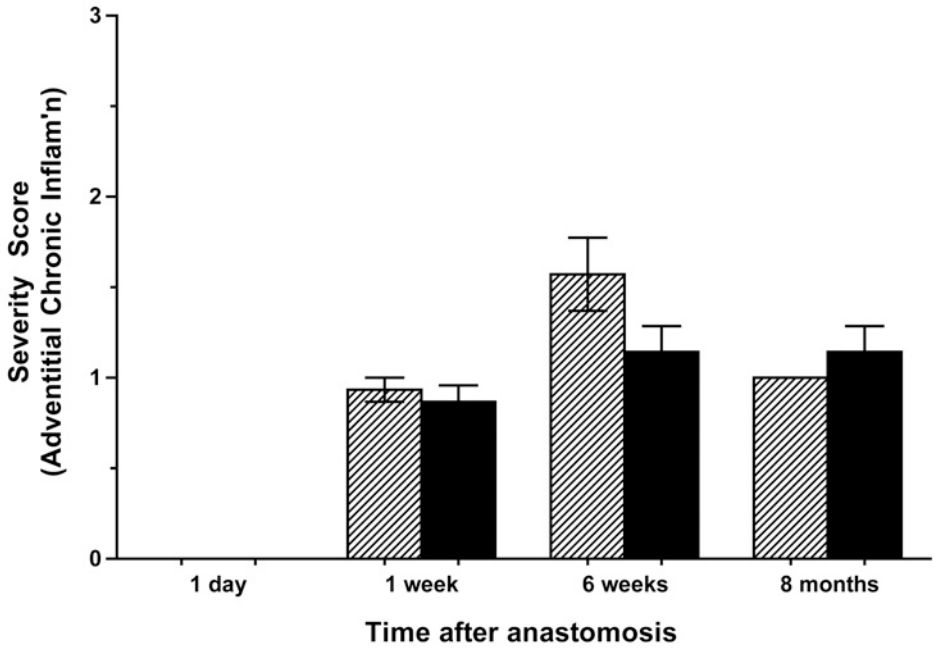

Figure 8.9. Semi-quantitative scores of adventitial chronic inflammation at the oblique end-to-end anastomosis (left, hatched bars) and the invaginating anastomosis (right, solid bars). Number of vessels analysed: 1 day (n=11), 1 week (n=15), 6 weeks (n=7). 8 months (n=7). Values are expressed as means ± SE.

8.4.1.9. Intimal Hyperplasia.

Results are plotted at figure 8.10. Intimal hyperplasia was not seen at one day in either technique. At one week, scores for the oblique end-to-end technique were 0.33±0.13 and 0.60±0.16 for the invagination. At six weeks, scores were 0.57±0.20 and 1.00±0.22 respectively. At eight months scores had reduced to 0.14±0.14 and 0.29±0.18 respectively. The differences in mean scores by time point were statistically highly significant (p <0.0001, ANOVA). Differences by side were not statistically significant (p = 0.311, ANOVA). The differences between techniques were not statistically significant (p = 0.102, ANOVA).

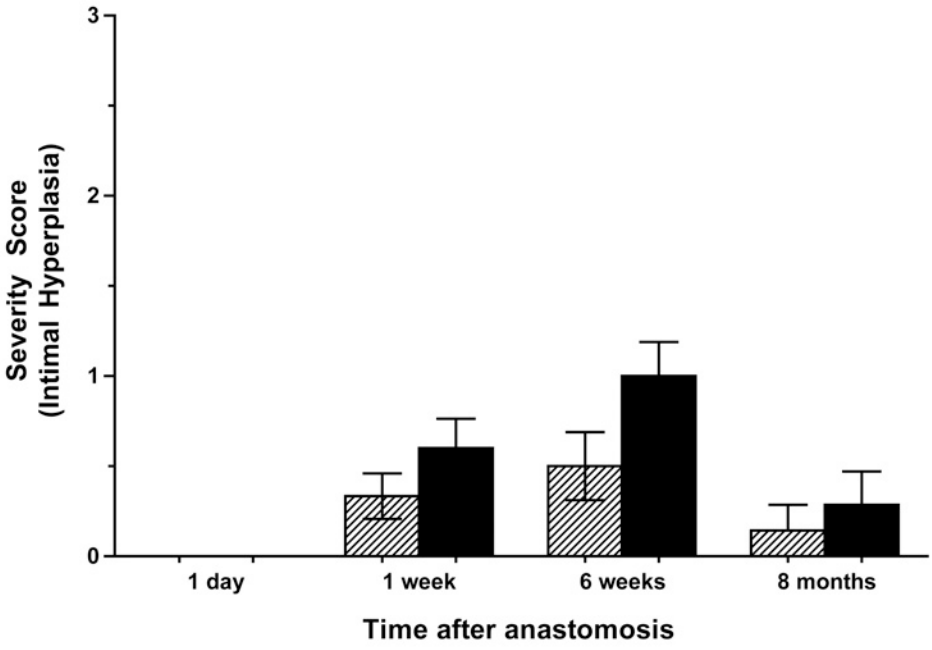

Figure 8.10. Semi-quantitative scores of intimal hyperplasia at the oblique end-to-end anastomosis (left, hatched bars) and the invaginating anastomosis (right, solid bars). Number of vessels analysed: 1 day (n=11), 1 week (n=15), 6 weeks (n=7). 8 months (n=7). Values are expressed as means ± SE.

8.4.2. Multivariate Analysis

Results of the multivariate analysis by principal component (PC) analysis are at Appendix E. Principal component analysis of the nine variables in the correlation matrix revealed a high eigenvalue for the first component, which was comprised almost exclusively of endothelial necrosis, medial necrosis, medial acute inflammation and adventitial acute inflammation. This means that these variables were highly correlated. Thrombus deposition was less strongly correlated with these other factors (Appendix E, figure E.1). The second principal component was comprised mainly of thrombosis (Appendix E, figure E.2), and the third, mainly intimal hyperplasia (Appendix E, figure E.3). Together, these accounted for 79.4% of the analysis (PC proportions 58.7%, 10.7% and 10.0%).

Scores for these first three PCs were each analysed by a three-factor ANOVA for time point, side and technique. The first component (endothelial and medial necrosis, medial and adventitial acute inflammation) showed a highly significant difference by the time point at which it was studied (p <0.0001, ANOVA), and a significant difference by technique (p = 0.025, ANOVA) (figure 8.11). Side did not influence scores from the first principal component (p = 0.657, ANOVA).

Analysis of the second component (thrombosis) (figure 8.12) showed a highly significant difference by time point (p <0.0001, ANOVA) but no difference by technique (p = 0.996, ANOVA) or by side (p=0.173, ANOVA).

Analysis of the third component (intimal hyperplasia) (figure 8.13) again showed a significant difference by time point (p = 0.006, ANOVA), but not by technique (p = 0.454, ANOVA) or by side (p = 0.693, ANOVA).

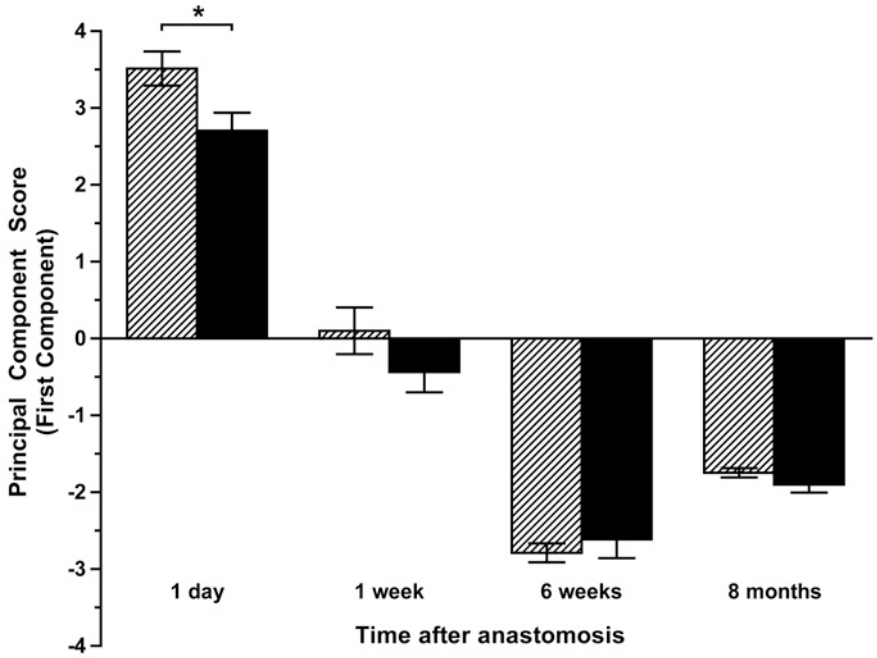

Figure 8.11. Principal component scores by time point and technique. First Component. Principal component proportion = 58.7%. Oblique end-to-end anastomosis = left, hatched bars; invaginating anastomosis = right, solid bars. Number of vessels analysed: 1 day (n=11), 1 week (n=15), 6 weeks (n=7). 8 months (n=7). Values are expressed as means ± SE.

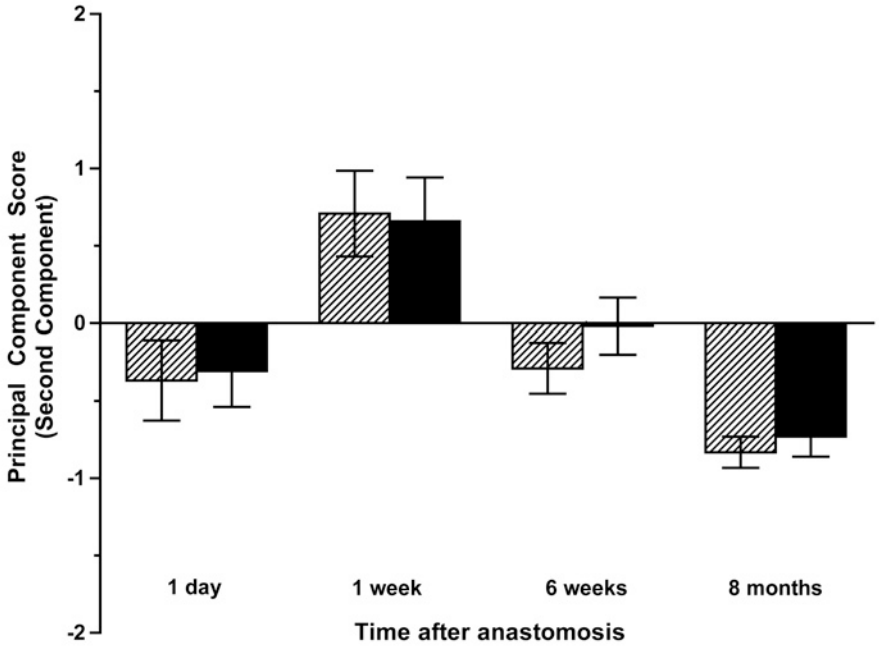

Figure 8.12. Principal component scores by time point and technique. Second Component. Principal component proportion = 10.7%. Oblique end-to-end anastomosis = left, hatched bars; invaginating anastomosis = right, solid bars. Number of vessels analysed: 1 day (*n*=11), 1 week (*n*=15), 6 weeks (*n*=7). 8 months (*n*=7). Values are expressed as means ± SE.

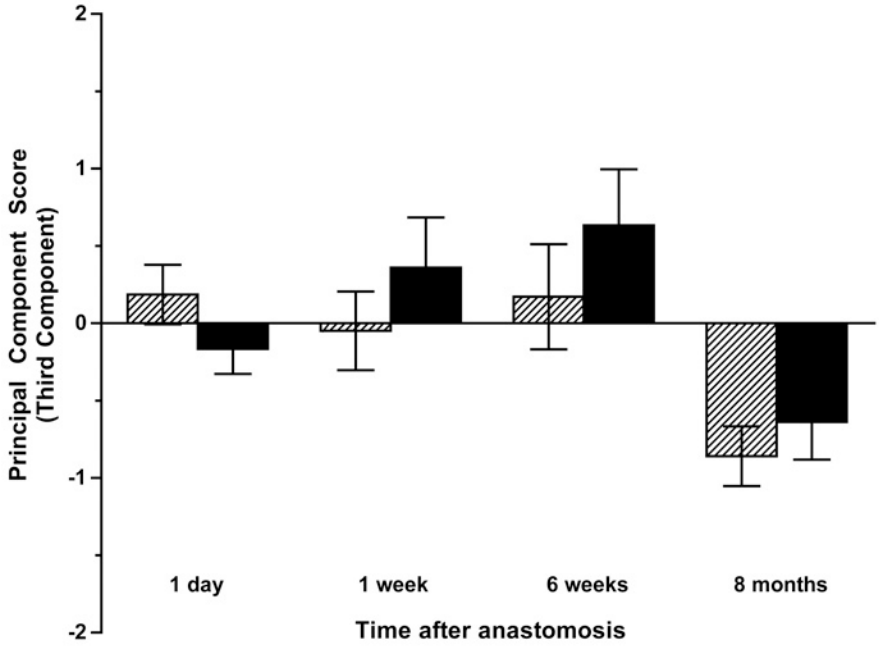

Figure 8.13. Principal component scores by time point and technique. Third Component. Principal component proportion = 10.0%. Oblique end-to-end anastomosis = left, hatched bars; invaginating anastomosis = right, solid bars. Number of vessels analysed: 1 day (*n*=11), 1 week (*n*=15), 6 weeks (*n*=7). 8 months (*n*=7). Values are expressed as means ± SE.

8.5. Discussion

8.5.1. Univariate Analysis with Bonferroni Correction

Strict application of the Bonferroni correction would lead to a division of the normally accepted significance level of five percent by the number of factors examined (nine in this study). *P* values < 0.0056 (0.05 / 9) would therefore be necessary to meet this criterion for significance. However, the Bonferroni correction produces an extreme level of significance necessary to suggest a difference and therefore increases the probability of a Type 2 error (beta error). The real risk almost certainly lies somewhere between the two.

Statistically highly significant differences in scores (*p* values <0.0001) by scoring time point were found for all nine parameters except thrombus deposition. Analysis of thrombus deposition by time point produced a *p* value of 0.001. Therefore this analysis has confirmed that all parameters differ significantly over time.

Differences in scores between techniques, significant at the 0.05 level, were found when examining endothelial and medial necrosis (*p* values = 0.021 and 0.015 respectively, ANOVA. Differences in means = 0.225 and 0.300, oblique end-to-end technique more severe necrosis). Application of the Bonferroni correction means that these differences may not be statistically significant in this analysis model.

8.5.2. Multivariate Analysis

Transformation of the results matrix into three principal components has revealed that endothelial and medial necrosis, medial and adventitial acute inflammation are all correlated in these data sets. The second principal component consisted mainly of thrombus deposition, and the third mainly of intimal hyperplasia. Analysis of the first component has shown a difference in scores by time point and by technique. Differences in the second and third components were found by time point but not by technique. Therefore it can be concluded that endothelial and medial necrosis plus medial and adventitial acute inflammation are statistically significantly greater in the oblique end-to-end technique than in the invagination.

8.5.3. Interpretation

The finding of greater necrosis in the oblique end-to-end technique concurs with that of Krag and Holck in equal-sized vessels (Krag and Holck, 1980). Most authors attribute this necrosis to the ischaemia produced by full-thickness suture bites (Baxter *et al*., 1972, Acland and Trachtenberg, 1977b, Maxwell *et al*., 1979). Maxwell *et al* (1979) found an aneurysm rate of 48% in a study of 25 arterial repairs in rats. The authors concluded that medial necrosis alone was insufficient to cause aneurysm formation, and that loss of the elastic lamellae was necessary as well. Aneurysms were not seen in either technique performed in this study. Baxter *et al*. state that '*minimal medial damage is the most important*

single entity concerned in successful vessel repair.'. Hence as few sutures as possible consistent with a successful repair would appear to be the ideal, and in this respect, the invagination technique was superior in this model.

In contrast to the work of Sully *et al*. (1982) and Wieslander *et al*. (1982), no greater thrombus deposition was found in this model of vessel size discrepancy in one technique when compared to the other.

The method of scoring used in this study did not allow the study of time to completion of re-endothelialisation, and so direct comparisons with this aspect of the work of Sully *et al*. (1982) are not possible. However, in both techniques examined here, endothelial necrosis was not seen beyond one week in either technique, although the gap of five weeks to the next observation point is wide.

In this study, no evidence of differences in intimal hyperplasia was found between techniques. This is in contrast to some findings in equal-sized vessels (Krag and Holck, 1980, Zhang *et al*., 1991, Gahankari *et al*., 1995). However, it would appear to concur with the equal or near-equal shear stresses demonstrated in the models of each technique in the *in silico* study.

Intimal hyperplasia was noted by one week. Severity scores increased to six weeks, although scores remained low throughout and the difference between these two time points was not statistically significant (p = 0.1392, Tukey; p = 0.8287, Tukey of third Principal Component). By eight months intimal hyperplasia had reduced in severity, and this difference was

statistically significant (p = 0.0071, Tukey; p = 0.0058, Tukey of third Principal Component).

8.5.4. Conclusions

Evidence for differences in healing between techniques has been found. Null Hypothesis (a) is therefore rejected.

No evidence of differences in intimal hyperplasia between techniques has been found. Null hypothesis (b), therefore, is not rejected.

8.6. References

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9 CONCLUSIONS AND FURTHER WORK

Outline

9.1. Introduction

In the last decade, the introduction of perforating arteries as recipient vessels for microvascular breast reconstruction, and more latterly, in limb reconstruction, has led to the re-emergence of arterial size discrepancy as a finding in clinical microvascular composite tissue transplantation. This increasingly common scenario prompted this investigation into which of two microarterial anastomotic constructs provided the best solution in the situation of a small-to-large anastomotic discrepancy in the region of 1:1.5 to 1:2.5, and in vessels of the approximate diameter of perforating arteries. The two techniques that have been studied are a 45º oblique section of the smaller vessel, and a technique where the smaller vessel is invaginated inside the larger.

A study programme has been carried out, which has encompassed *in silico* modelling of haemodynamics, followed by characterization of a rodent model and *in vivo* experiments into patency and timing. *In vivo* and *ex vivo* indices of blood flow through the anastomoses have been studied, and a semi-quantitative study of healing of the two techniques has been completed.

9.2. Study Results

Results from these studies are summarised below:

1. *In silico* modelling of the two techniques has shown that complex flow separations are likely to occur downstream from both

anastomoses, with maximum laminar flow disturbance seen during high, but decelerating flow rates.

2. Wall shear stress ranges across geometric models of both anastomotic techniques are similar.
3. An animal model of clinically-applicable small-to-large size mismatch has been characterized, which avoids the use of interposition vein grafts, and which approximates the size of, and mean flow rate through, small perforating arteries in humans.
4. Using this rodent Superficial Caudal Epigastric Artery / Femoral Artery model, patency rates at one hour, one week and at six weeks were found to be similar through both techniques.
5. Secondary analysis of data from this experiment shows that:
    a. A learning curve existed for the two investigators who conducted the experimental series. Across the series, neither investigator became faster at completing the techniques, but patency improved, implying that they became more dextrous.
    b. The investigator who took slightly longer to complete an anastomosis achieved a higher patency rate overall.
    c. A higher number of immediate revisions were necessary with the oblique cut end-to-end technique.
    d. An anastomosis was more likely to fail if it had undergone revision.
    e. If these revisions had not been permitted, greater patency would have been found with the invaginating technique, although this finding was not quite statistically significant.

6. The invagination technique is significantly faster to perform, by one third.
7. Time-averaged volume flow rates through both techniques, measured by transit time ultrasound, are similar at ten minutes, one hour, three hours, one day, three days and one week. Flow waveform analysis by pulsatility index did not show any evidence of a significant difference in flow across the two anastomotic techniques. Relative resistance also appeared to be similar.
8. Some proxy evidence, in the form of cross-sectional area of corrosion casts of femoral and superficial caudal epigastric arteries at eight months, was found to suggest that flow is significantly greater through the invagination technique in the longer term.
9. No evidence was found of any difference in the degree of anastomotic stenosis between the techniques.
10. The oblique cut end-to-end technique leads to greater medial and intimal necrosis, and greater inflammation than the invagination technique. Intimal hyperplasia appears to increase to six weeks, and reduces again at eight months. No evidence of a difference in intimal hyperplasia between the two techniques was found.

9.3. Discussion

9.3.1. Computational Modelling

From modelling of flow through short stenoses (Calvo *et al*., 1999) and theoretical modelling of sudden expansions (Gumley, 1987), the ring

vortex or torus found in the computational models of the larger vessel immediately downstream from the invaginating anastomosis is to be expected. The use of unsteady flow rates measured *in vivo* has revealed that maximum flow disturbance is found in high, but decelerating flow. This finding is similar to that of Ishibashi *et al*. (1995), who, using a cinemicrographic technique, found maximum flow disturbance immediately following the point of maximum flow rate.

What has not been previously reported, however, is that this torus was seen to be corrugated rather than smooth, and that it disappeared only in parts of the circumference when flow accelerated again at the start of systole. This finding is unexplained. The phenomenon warrants further work into determining whether it is an artefact of the computational modelling technique, the fluid characteristics, or the non-compliant walls.

The complex, counter-rotating spiral vortices observed in the oblique-cut technique have not been previously observed in end-to-end models. The pattern seen bears some resemblance to that observed by Ojha *et al*. (1990), who used a high resolution photochromic tracer technique to model oblique, 45º end-to-side anastomoses without flow in the recipient, axial vessel.

Wall shear stresses ranged with flow rate, as would be expected, and areas of low shear stress were observed next to the anastomoses in both geometries. Areas of low shear stress correspond to sites of intimal hyperplasia (Sunamura *et al*., 2007) and so it was of interest to note that low shear stresses of similar magnitude were present in both techniques.

Conclusions from this study, however, are limited by the computer-generated, geometric three-dimensional models, and by their non-compliant walls. The use of Newtonian fluid dynamics may also constrain conclusions and so their direct applicability to decision-making in clinical microvascular surgery is limited. However, some broader conclusions may be drawn. These are that complex flow separations are likely to occur through both anastomotic techniques, and shear stresses, whilst distributed differently, showed similar ranges. In terms of the overall study question, therefore, no evidence was found of significant physiological differences between the two techniques. Additional, *in vivo* studies were required to complement these findings and could be ethically justified.

9.3.2. Characterization of an Animal Model

The choice of animal model was reached following examination of rabbit and rodent models in a pilot study. These animals were chosen as the least sentient species that could provide mammalian cardiovascular physiology and whose vessels were likely to be of a size suitable for study. The principle of de la Peña Salcedo *et al.* (2000) was used as the basis of a technique which used a side branch to produce the diameter discrepancy. A rabbit model was explored because use of it would have allowed us to address some criticisms raised about rodent femoral artery models in examination of the invagination technique in equal-sized arteries (Meyer *et al*., 1980, Acland, 1989, Duminy, 1989). These criticisms include the fact that arterial walls are relatively thin (in relation to lumen diameter) in rodent arteries when compared to rabbit and human arteries.

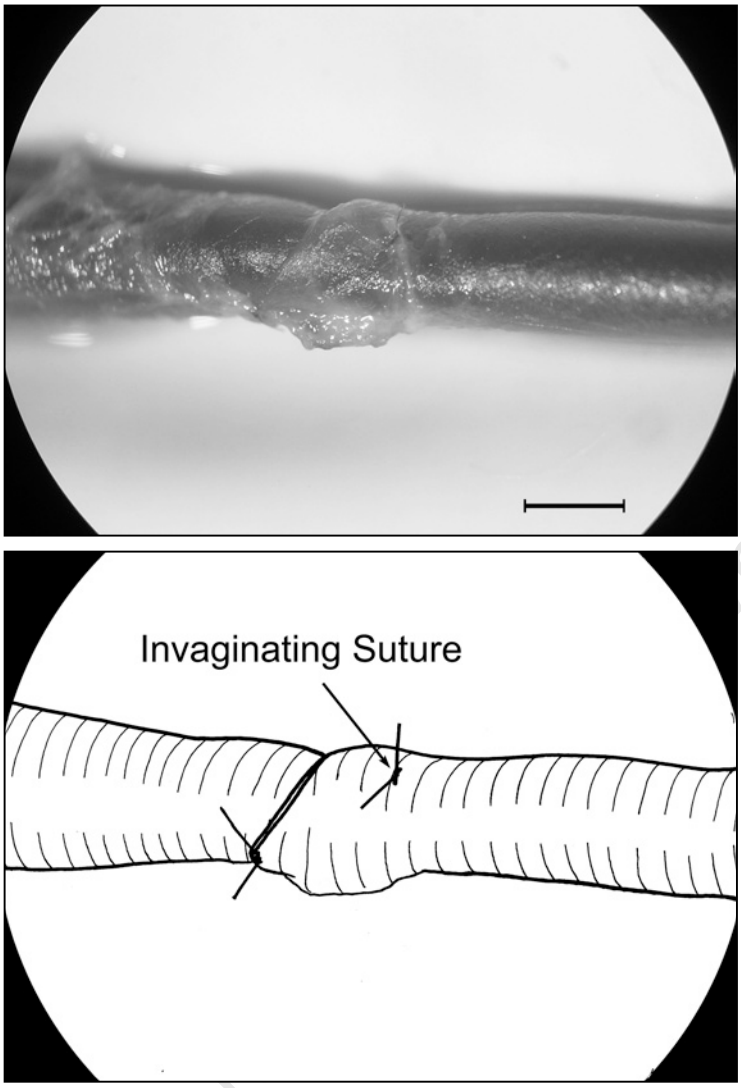

Figure 9.1 Replication of the method of Sully *et al*. (1982). Tension at an invaginating anastomosis in the femoral artery of the rabbit. Scale bar = 1mm. Flow is from left to right.

Sully *et al.*'s study into invagination in equal-sized vessels (Sully *et al*., 1982) was conducted using a rabbit femoral artery model, and these authors describe a lower patency rate with an invagination technique. In my own pilot study, a clinically-applicable size discrepancy could be found by the use of branches of the lateral circumflex femoral artery. However, tension at the anastomosis was subjectively too great for the model to be

of use. A single attempt to replicate Sully *et al.*'s model in equal-sized rabbit femoral arteries showed high tension in an invaginating anastomosis (Figure 9.1), and this was almost certainly a contributing factor to the lower patency observed with their invagination technique. Indeed these authors comment on this, although they did not deem it significant.

The rodent model allowed a tension-free anastomosis 6mm from the origin of the SCEA. The ratio produced by this model was in the clinically-applicable range of 1:1.5 - 1:2.5. There did not appear to be any significant difference in SCEA:FA ratios between sides. The paired nature of the model allowed a scientifically robust comparison of techniques to be made, and negated confounding factors such as different cardiovascular physiological parameters and larger collateral flow channels affecting anastomotic flow in individual animals. Whilst a free flap model would have been ideal, it would have been technically difficult to do this in a paired manner, whilst keeping operative morbidity to a minimum. A bilateral hind limb replantation model was also considered, but this was discounted because the theoretical advantages of using such a model did not justify the severity of the procedure if the animals were to be recovered from anaesthesia.

Femoral artery volume flow rate in the Wistar rat was statistically and physiologically higher in the right artery when compared to the left. This finding sits well with anatomical asymmetry and of dominance of one side over the other, but it has not, to my knowledge, been reported previously.

The implications for this thesis were that in any study of flow, side needed to be randomised, and that flow comparisons were most meaningful when expressed as a percentage of a baseline measurement. This was also the conclusion of Nakayama and Soeda (1984), although their reasoning was different in that widely differing baseline flow measurements were obtained because of their use of an electromagnetic flow meter. In summary, a model has been characterized that allows the paired comparison of small-to-large anastomotic techniques, whilst avoiding the use of interposition vein grafts.

9.3.3. Patency and Timing

The design of the patency experiment, i.e. using two investigators, and allowing a single revision of the anastomosis if it did not run immediately, was decided upon in order to avoid the possibility that results would be pertinent only to a single investigator, and to more closely replicate clinical practice.

Primary analysis of results at one hour, one week, and at six weeks did not reveal any evidence of a difference in patency rates between the two techniques of more than 5%. Sufficient numbers (i.e. ≥156 animals) were available at one hour and at one week to draw this conclusion with confidence. At six weeks, numbers had dropped to 127 animals, reducing the power of the study at this time point, and slightly increasing the potential for a type 2 error. However, this risk remains very much less than that pertaining to previous experimental series examining technique in size discrepancy. Of those published, Ahn *et al.* (1994) used the largest

number of 16 animals in each group; *post-hoc* calculations show a power of only 10% in that study.

Secondary analysis of the experiment presented in this dissertation revealed a patency difference between the first and last thirds of the experimental series, implying a learning curve for both investigators. It was also noteworthy that the investigator who took slightly longer to complete the anastomoses achieved a higher patency rate overall. This confirms that particularly patient and meticulous microvascular technique is necessary when anastomosing vessels of this calibre.

A significantly higher revision rate was found with the oblique cut end-to-end technique. However, the higher patency rate of the invaginating technique, which was found when revised anastomoses were included in the one hour failure figures, did not reach statistical significance.

Anastomoses that were unsuccessful at the first attempt were statistically less likely to be successful at a second attempt. This is logical, in that any intimal damage caused by performing an anastomosis, and subsequent platelet activation, will predispose a revised anastomosis to thrombus formation, no matter how meticulously the vessels are prepared again. However, no significant difference in revision success rates was found between the two techniques.

These findings imply that the invagination technique, in vessels of this size and in this model, is technically simpler than the oblique cut end-to-end technique.

The relative speed of an invagination technique is a consistent finding in many studies in equal-sized vessels, where two or three sutures are used to complete the invagination, as opposed to six or more for a conventional end-to-end technique (Lauritzen, 1978, Lauritzen and Bagge, 1979, Hyland *et al*., 1981, Stamatopoulos *et al*., 1982, Duminy, 1989).

The anastomosis of arteries of an unequal diameter is technically more complex than in equal-sized vessels. In addition, a greater number of sutures were required in the invagination technique when compared with equal-sized vessels. These two differences from previous studies in equal-sized vessels led to the desire to see if a reduction in time taken remained the case where a small-to-large discrepancy existed.

Anastomoses were timed from division of the SCEA until release of the vessel clamps. Mean timing results showed that the oblique end-to-end technique took approximately one-and-a-half times as long to complete as the invagination, a difference of just less than seven minutes in this model. This was independent of all other factors - side, position in series and investigator.

In summary, conclusions from the patency and timing study are that no clear patency advantage exists between the two techniques. However, it may be said that the invagination technique is simpler and faster to perform in the Wistar rat model used.

9.3.4. Flow Studies

Patency, judged by the 'empty-and-refill' test (Hayhurst and O'Brien, 1975, Acland, 1980) produces a binary outcome. Krag and Holck (1981) have shown that this test may give a positive result even when the lumen is occluded by more than 75%. In the absence of a significant patency advantage of one technique over the other, further study of the anastomoses was conducted by quantitative evaluation of blood flow. This was facilitated by the development of transit-time ultrasound technology and the availability of small, implantable transit-time ultrasound flow probes. Flow measurement errors from incorrect insonation angles were therefore removed, and the technology had a demonstrated measurement error in the region of only 6%. This avoided the technical difficulties described by Nakayama and Soeda, who used an electromagnetic (EM) flow meter to measure flow in their rabbit model. Correct sizing and positioning of an EM flow probe is vital in avoiding flow measurement errors, and indeed these authors experienced inter-animal flow measurement deviations of up to 20mL/min. This is over seven times the mean flow rate found in the Wistar rat model used here. Wieslander and Åberg (1983) also used an EM flow meter, but these authors do not describe the technical detail of their experiments. It is likely, however, that the EM flow meter also led to difficulties in that experiment. The methods of Lauritzen and Bagge (1979) and of Duminy (1988); using flow rates measured by the volume of saline passing through the anastomoses *ex vivo*, does not resemble the situation *in vivo*, where flow is affected by cardiovascular physiology. Furthermore, saline cannot replicate blood as a

living tissue, and so conclusions from these experiments are necessarily limited.

The paired nature of the model was very important in this experiment, because use of it controlled potential confounding factors at the flow measurement points. In microvascular tissue transplantation, flow through anastomoses is controlled largely by the resistance bed created by the transplanted tissue (Rao *et al*., 1983). Resistance has been demonstrated to be high in low volume fascial flaps, and low in high volume muscle flaps (Sasmor *et al*., 1992, Mahabir *et al*., 2001). Therefore the use of the paired hind limb model controlled for this potential factor because peripheral vascular resistance in the animals' limbs could be assumed to equate at any given time point. Paired results were available at three hours, one day, three days and one week. Where consecutive, rather than simultaneous flow measurements were taken, i.e. at ten minutes and at one hour, there was no significant association of flow rates with animal core temperature, but it is conceded that controlling peripheral vascular resistance at these points was not possible beyond ensuring that the animal's physiological parameters of temperature, heart rate and oxygenation remained stable throughout the procedure.

Some difficulties were experienced with acoustic coupling in the conscious animals. This precluded flow measurement on at least one side at approximately 10% of measurement points. Despite this, paired results were available from six animals or more at all time points, fulfilling the sample size requirement of six animals for a time-averaged flow rate difference of 10%.

Flow was assessed by time-averaged flow rates, by waveform analysis by pulsatility index, and by calculating relative resistance from time-averaged flow rates. No statistically significant differences in flow were found between the two techniques at ten minutes, one hour, three hours, one day, three days or one week.

It has long been known that arterial remodelling occurs as a response to chronic increases or decreases in wall shear stress produced by changes in flow rate (Kamiya and Togawa, 1980, Langille and O'Donnell, 1986).

Modelling of the vessels by the use of corrosion casts has been shown to produce high fidelity (Moore *et al*., 1997). Application of physiological pressure at the aorta will have applied equal pressure to both sides and both anastomotic techniques.

Mean cross-sectional area was 10.73% greater in the vessels anastomosed by the invagination technique, implying greater flow through this anastomosis. The side, left or right, did not explain this finding. From Poisseuille's law, a difference in radius of 10.73% leads to an increase in volume flow rate of 50.34%. However, the exact relationship of a change in arterial diameter to the change in flow rate has not been characterised. Caution must therefore be exercised in interpreting results from the use of vessel internal diameter as a proxy for flow rate. It may be concluded, however, that the technique influenced the vessel morphology in this study, implying that greater flow occurred through the invagination

technique than through the oblique cut end-to-end technique at the time point of ten months following anastomosis.

9.3.5. Anastomotic Stenosis

Previous studies of anastomotic morphology by casting (Nakayama and Soeda, 1981, Duminy, 1988, Kanaujia *et al*., 1988) and by angiography (Wieslander and Aberg, 1982) have demonstrated stenosis when an invagination technique has been used in equal-sized vessels. It is logical that this should be the case.

Results from the casting study presented in this dissertation, however, where the vessels have been of an unequal diameter, show that quite marked stenosis occurred in both techniques, but that there was no significant difference by technique.

An explanation for the heterogeneity of stenosis, and for the severe stenosis found in some specimens, is difficult to find. Operator technique seems an attractive option, and indeed these specimens were taken from animals that had been included in the patency study at an early stage in the learning curve. However, there did not appear to be any statistically significant difference in stenosis by investigator, and it is not possible to draw this conclusion with any certainty.

9.3.6. Anastomotic Healing and Intimal Hyperplasia

Histopathological examination of the anastomotic healing process was carried out by semi-quantitative scoring of thrombosis, and of necrosis, inflammation, and fibrosis of the three layers of the vessel wall. The degree of intimal hyperplasia was also scored. Each parameter was examined at the four time points of one day, one week, six weeks and eight months.

The study has provided some reasonably strong evidence that the oblique cut end-to-end technique causes significantly greater endothelial and medial necrosis than the invagination technique. There is also some evidence that there is greater medial and adventitial inflammation in the oblique cut end-to-end. These findings concur with some conclusions from descriptive studies in equal-sized vessels (Krag and Holck, 1980, Sully *et al*., 1982), but they provide stronger, semi-quantitative evidence that this is the case in unequal-sized vessels.

The finding of a lack of evidence of a difference in the severity of intimal hyperplasia between the two techniques is in contrast to that of Krag and Holck (1980), who examined intimal hyperplasia in equal-sized vessels. These authors report that intimal hyperplasia was seen less frequently in an invaginating anastomosis. This contrast can be explained by the diameter discrepancy of the techniques under scrutiny here, and concurs with the observation from the *in silico* modelling that areas of low shear stress of equal magnitude were found in both techniques.

9.4. Conclusions

In conclusion, it may be said that there was no patency advantage of one technique over the other. However, in the experimental model used, it was found that the invagination technique was faster and technically simpler to perform. Flow through the invagination was at least equal to that through the oblique cut end-to-end, with some experimental evidence suggesting that flow may be greater through the invagination in the long term. The invagination technique is also less traumatic, inducing less vessel wall necrosis.

9.5. Further Work

9.5.1. Clinical Trial of Technique

Microvascular composite tissue transplantations are lengthy and technically demanding procedures. Any manoeuvres that reduce morbidity, and the length of time taken must be welcomed. One of these manoeuvres is the use of perforating arteries as recipients. This, however, may lead to a small-to-large diameter mismatch in the arterial anastomosis: this situation provided the stimulus for the work presented here. The evidence gleaned from this study suggests that an invagination technique could be the method of choice in this situation. In combination with other manoeuvres, such as microvenous coupling devices (Yap *et al*., 2006) the use of these techniques could lead to significant time savings.

Furthermore, the relative technical simplicity of the invagination technique should encourage the use of perforators as recipients where a small-to-large mismatch exists. This might lead to more widespread adoption, potentially reducing morbidity for many patients.

These potential advantages encourage the further examination of these techniques in a clinical study. Sample size calculation is possible from the data available here. The large numbers involved, and the relatively low volume of free tissue transfers carried out in individual units dictate that the study should be conducted as a multicentre trial. The primary inclusion criterion would be a small-to-large arterial size discrepancy in the region of 1:1.5 to 1:2.5. Technique would be randomised by centralised computer-

generated randomisation. Allocation should remain concealed until vessels are chosen intraoperatively. An intra-operative image would be taken to record the vessel size and the size discrepancy. The primary outcome measure would be flap survival at one week. Secondary outcome measures would be anastomotic revision rate and partial flap loss.

Through this method, a robust clinical study could answer the question of best method, and would address criticisms of microvascular studies in rodents not being translatable into clinical practice.

9.5.2. Additional Avenues of Study

Further avenues of study are stimulated by the work conducted in this thesis. The computational modelling of microvascular anastomoses is potentially very interesting, and we have examined other techniques by this method (Rickard *et al*., 2009). These results are worthy of further examination in the animal model described in this dissertation. Additional work investigating varying angles of an oblique-cut end-to-end anastomotic technique would be of interest.

Computational modelling may also be applied to the study of large-to-small arterial anastomotic techniques, and to venous anastomoses. The limitations produced by the inorganic, geometric models may be addressed by combining corrosion casts of techniques with computational modelling. In converting the casts into three-dimensional computer models, laser micrometry and photogrammetric techniques have been examined, but the method most likely to be successful is micro-xray computed tomography (micro-CT).

The restrictions of non-compliant walls and Newtonian fluid dynamics may be addressed in combination with bioengineers, and this is presently a subject of study in some centres, driven by the desire to produce better intravascular stents and grafts for larger vessels. When the addition of compliance modelling becomes possible, it will also be useful to examine techniques, including the invagination, used in constructing arteriovenous shunts.

The finding of different baseline flow rates by side in the rat is of interest, and warrants further investigation to see if this is also the case in humans.

Additional work into transit-time ultrasound-derived flow waveform analysis may be of interest. In particular, it would be useful to know the precise relationship between Pulsatility and Resistance Indices and experimentally-induced stenosis of a known degree, downstream from the flow measuring probe. This would be a relatively simple project: different degrees of stenosis could be produced by a ligature placed around the artery and a cylinder of known diameter (e.g. a venous cannula or a length of wire of known gauge) before removing that cylinder to leave only the ligature around the vessel (Fig 9.2). Corrosion casts performed *post-mortem* could validate the degree of stenosis, and would be necessary because there would be variability in the tightness of a ligature placed by hand.

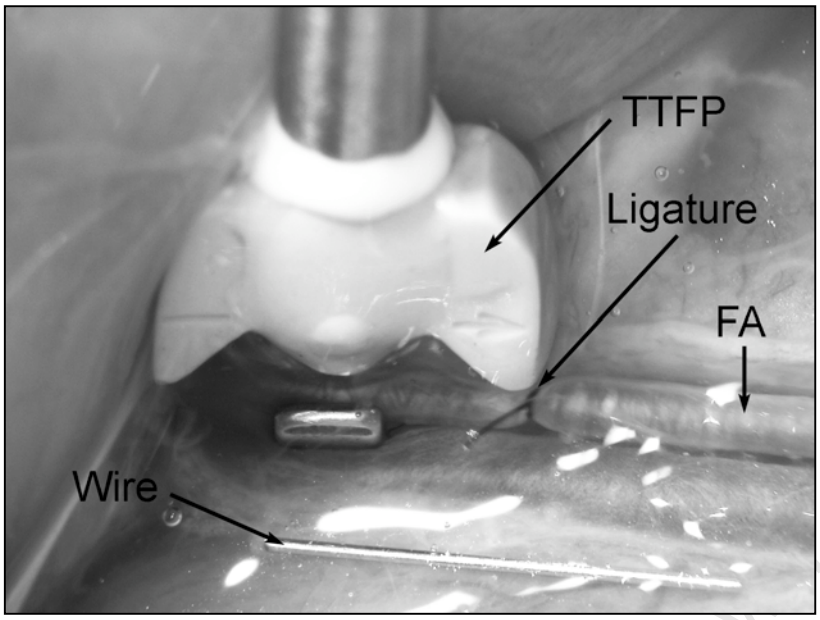

Figure 9.2 Set-up of an experiment examining the relationship between the degree of downstream stenosis and upstream Pulsatility and Resistance Indices, measured by transit-time ultrasound. TTFP = transit time flow probe, FA = Femoral Artery. Flow is from left to right, as indicated on the flow probe head.

9.6. Summary

In summary, some experimental evidence has been found to direct the choice of technique used to anastomose small arteries where a small-to-large diameter mismatch exists. The evidence suggests that of two techniques – an oblique cut of the smaller vessel, and an invagination technique, the invagination technique is the better method. This warrants further, clinical investigation.

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Appendix A. Experimental results and analysis from Animal Model Establishment

A1. Pilot Studies

A1.1. New Zealand White Rabbits

Animal No	Weight (g)	Side	FA Ext Diameter (mm) 1	2	3	Mean (a)	SD	LCFA-Tr Ext Diameter (mm) 1	2	3	Mean (b)	SD	Ratio (a)/(b)	LCFA-Desc Ext Diameter (mm) 1	2	3	Mean (c)	SD	Ratio (a)/(c)
1	2178	L	0.839	0.840	0.851	0.844	0.007	0.692	0.664	0.714	0.691	0.025	1.22	0.414	0.421	0.425	0.421	0.005	2.01
		R	1.042	1.079	1.052	1.058	0.019	0.557	0.541	0.561	0.554	0.011	1.91	0.473	0.481	0.477	0.477	0.004	2.22
2	2447	L	1.027	0.989	1.007	1.008	0.019	0.523	0.512	0.493	0.510	0.015	1.98	0.701	0.665	0.660	0.676	0.023	1.49
		R	0.983	1.017	1.053	1.018	0.035	0.468	0.489	0.440	0.466	0.024	2.18	0.619	0.584	0.592	0.599	0.018	1.70
3	1865	L	1.118	1.122	1.127	1.123	0.004	0.682	0.733	0.649	0.689	0.042	1.63	0.567	0.479	0.502	0.517	0.045	2.17
		R	0.925	0.976	0.998	0.967	0.037	0.698	0.748	0.753	0.734	0.031	1.32	0.474	0.489	0.489	0.485	0.009	2.00
4	2280	L	1.350	1.379	1.379	1.370	0.017	1.079	1.063	1.054	1.065	0.013	1.29	0.662	0.669	0.644	0.659	0.013	2.08
		R	1.094	1.130	1.131	1.119	0.021	0.662	0.694	0.710	0.689	0.024	1.62	1.086	1.147	1.035	1.090	0.056	1.03
5	2285	L	0.945	0.929	0.924	0.934	0.011	0.591	0.636	0.606	0.612	0.023	1.53	0.394	0.412	0.401	0.403	0.009	2.32
		R	0.886	0.877	0.886	0.884	0.005	0.673	0.642	0.608	0.641	0.033	1.38	0.472	0.464	0.465	0.468	0.004	1.89
Mean:	2211				L	1.056	0.011			L	0.713	0.024	1.528			L	0.535	0.019	2.014
					R	1.009	0.024			R	0.617	0.025	1.683			R	0.624	0.018	1.766
					Both	1.032	0.146			Both	0.665	0.161	1.55			Both	0.579	0.196	1.78

Table A.1. Measurements from New Zealand White Rabbit vessels. Key: FA = Femoral Artery; SD = Standard Deviation; LCFA-Tr = Transverse branch of Lateral Circumflex Femoral Artery; LCFA-Desc = Descending branch of Lateral Circumflex Femoral Artery.

A1.2. Wistar Rats

Animal No	Weight (g)	Side	FA Ext Diameter (mm)			Mean (a)	SD	SCEA Ext Diameter (mm)			Mean (b)	SD	Ratio (a)/(b)	Distal FA Length (mm)			Mean	SD
			1	2	3			1	2	3				1	2	3		
1	396	L	0.822	0.822	0.887	0.844	0.038	0.554	0.598	0.576	0.576	0.022	1.46	2.289	2.362	2.251	2.301	0.057
		R	0.949	0.916	0.905	0.924	0.023	0.468	0.456	0.456	0.461	0.007	2.00	3.942	3.919	3.871	3.911	0.036
2	381	L	0.846	0.814	0.876	0.846	0.031	0.544	0.533	0.529	0.536	0.008	1.58	4.085	4.139	4.222	4.149	0.069
		R	0.796	0.799	0.842	0.813	0.026	0.377	0.417	0.379	0.391	0.023	2.08	3.998	4.004	3.861	3.954	0.081
3	406	L	0.995	1.005	1.024	1.009	0.015	0.546	0.553	0.618	0.573	0.040	1.76	2.926	3.171	3.192	3.097	0.148
		R	1.102	1.102	1.120	1.109	0.010	0.556	0.562	0.569	0.563	0.006	1.97	3.882	3.719	3.844	3.816	0.086
4	461	L	0.957	0.970	0.971	0.967	0.008	0.641	0.631	0.615	0.630	0.013	1.54	3.862	3.749	3.695	3.769	0.085
		R	1.000	0.978	1.014	0.998	0.018	0.564	0.605	0.593	0.588	0.021	1.70	3.332	3.147	3.192	3.224	0.097
5	434	L	0.967	0.995	0.981	0.981	0.014	0.644	0.573	0.695	0.638	0.061	1.54	2.815	2.887	2.873	2.859	0.038
		R	1.064	1.006	0.963	1.012	0.051	0.426	0.466	0.415	0.436	0.027	2.32	4.351	4.315	4.105	4.257	0.133
Mean:	416				L	0.929	0.075			L	0.590	0.049	1.576			L	3.235	0.683
					R	0.971	0.105			R	0.488	0.079	2.013			R	3.832	0.359
					Both	0.950	0.092			Both	0.539	0.083	1.795			Both	3.534	0.616

Table A.2. Measurements from Wistar Rats (HsdOla-WI). FA=Femoral Artery; SD = Standard Deviation; SCEA = Superficial Caudal Epigastric Artery.

A2. Detailed Characterization of the Wistar Rat Model

Animal No	Weight (g)	Side	FA Ext Diameter (mm)			Mean (a)	SD	SCEA Ext Diameter (mm)			Mean (b)	SD	Ratio (a)/(b)	Distal FA Length (mm)			Mean	SD
			1	2	3			1	2	3				1	2	3		
6	505	L	0.992	0.939	0.979	0.971	0.028	0.445	0.525	0.529	0.500	0.047	1.94	2.589	2.459	2.598	2.549	0.078
		R	0.994	0.992	1.026	1.005	0.019	0.541	0.519	0.558	0.540	0.019	1.86	2.891	2.687	2.815	2.798	0.103
7	407	L	1.050	1.034	1.039	1.041	0.008	0.590	0.583	0.605	0.593	0.011	1.75	2.861	2.927	2.786	2.859	0.071
		R	1.113	1.102	1.053	1.090	0.032	0.551	0.593	0.618	0.588	0.034	1.85	2.400	2.312	2.185	2.299	0.108
8	441	L	0.911	0.897	0.895	0.902	0.009	0.487	0.502	0.493	0.495	0.008	1.82	4.509	4.413	4.501	4.475	0.053
		R	1.024	1.063	1.067	1.052	0.024	0.557	0.582	0.577	0.572	0.013	1.84	2.905	3.191	3.237	3.112	0.180
9	392	L	0.981	1.002	0.989	0.991	0.010	0.478	0.522	0.584	0.529	0.053	1.87	3.602	3.605	3.667	3.625	0.036
		R	0.944	0.892	0.871	0.903	0.038	0.540	0.599	0.611	0.584	0.038	1.55	2.905	3.191	3.237	3.112	0.180
10	415	L	0.828	0.865	0.858	0.851	0.020	0.546	0.568	0.530	0.548	0.020	1.55	3.728	3.636	3.715	3.694	0.050
		R	0.807	0.780	0.786	0.791	0.014	0.555	0.564	0.564	0.561	0.005	1.41	3.251	2.957	2.849	3.019	0.208
11	417	L	1.116	1.100	1.082	1.100	0.017	0.554	0.493	0.527	0.525	0.031	2.09	4.732	4.693	4.641	4.689	0.046
		R	1.111	1.088	1.085	1.095	0.014	0.558	0.587	0.583	0.576	0.016	1.90	2.871	3.030	2.940	2.947	0.080
12	447	L	1.105	1.045	1.082	1.078	0.030	0.591	0.584	0.591	0.589	0.004	1.83	3.891	3.796	3.744	3.811	0.075
		R	0.976	1.027	1.012	1.006	0.026	0.587	0.586	0.570	0.581	0.010	1.73	2.982	2.797	2.902	2.894	0.093
13	395	L	1.031	1.035	1.012	1.026	0.012	0.539	0.558	0.558	0.552	0.011	1.86	3.978	4.039	3.691	3.903	0.186
		R	1.105	1.108	1.080	1.098	0.015	0.603	0.649	0.633	0.629	0.023	1.75	4.217	3.965	4.001	4.061	0.136
14	465	L	1.070	1.065	1.046	1.061	0.013	0.660	0.646	0.639	0.649	0.011	1.64	4.479	4.499	4.230	4.403	0.150
		R	0.993	0.963	0.897	0.951	0.049	0.531	0.533	0.539	0.534	0.004	1.78	2.921	2.747	2.700	2.790	0.117
15	447	L	0.995	1.012	0.989	0.999	0.012	0.537	0.582	0.545	0.555	0.024	1.80	3.7976	4.0202	3.9147	3.911	0.111
		R	0.902	0.867	0.891	0.887	0.018	0.608	0.609	0.601	0.606	0.004	1.46	3.486	3.191	3.189	3.288	0.171
16	343	L	0.969	0.960	0.970	0.967	0.006	0.537	0.546	0.554	0.546	0.009	1.77	3.254	3.239	3.269	3.254	0.015
		R	0.907	0.965	0.923	0.932	0.030	0.413	0.399	0.442	0.419	0.022	2.23	3.431	3.502	3.624	3.519	0.098
17	408	L	0.901	0.883	0.925	0.903	0.021	0.592	0.617	0.572	0.594	0.023	1.52	3.317	3.053	2.958	3.109	0.186
		R	0.937	0.952	0.944	0.945	0.007	0.488	0.485	0.455	0.476	0.018	1.98	4.249	4.169	4.167	4.196	0.047

Animal No	Weight (g)	Side	FA Ext Diameter (mm)			Mean (a)	SD	SCEA Ext Diameter (mm)			Mean (b)	SD	Ratio (a)/(b)	Distal FA Length (mm)			Mean	SD
			1	2	3			1	2	3				1	2	3		
18	418	L	0.895	0.927	0.907	0.910	0.016	0.654	0.676	0.637	0.656	0.019	1.39	3.825	3.741	3.757	3.775	0.045
		R	0.999	0.963	0.992	0.985	0.019	0.499	0.608	0.598	0.569	0.060	1.73	2.654	2.671	2.709	2.679	0.028
19	479	L	1.019	1.004	1.013	1.012	0.008	0.697	0.697	0.736	0.711	0.023	1.42	3.058	2.885	2.862	2.935	0.107
		R	1.046	1.037	1.036	1.040	0.006	0.545	0.454	0.529	0.510	0.049	2.04	3.546	3.433	3.511	3.497	0.058
20	369	L	1.071	1.062	1.064	1.066	0.005	0.580	0.596	0.584	0.587	0.009	1.82	3.717	3.694	3.465	3.626	0.139
		R	1.030	1.024	1.063	1.039	0.021	0.515	0.548	0.526	0.530	0.017	1.96	4.386	4.243	4.433	4.354	0.099
21	428	L	0.912	0.908	0.803	0.875	0.062	0.538	0.541	0.554	0.545	0.009	1.61	Image technically inadequate for measurement				
		R	1.016	1.024	0.987	1.010	0.019	0.543	0.605	0.564	0.571	0.031	1.77					
22	415	L	0.875	0.905	0.846	0.876	0.029	0.477	0.509	0.525	0.504	0.025	1.74	4.345	4.445	4.341	4.377	0.059
		R	0.870	0.897	0.937	0.902	0.034	0.550	0.449	0.525	0.509	0.053	1.77	3.561	3.634	3.468	3.555	0.083
23	459	L	1.085	1.099	1.069	1.085	0.015	0.474	0.585	0.516	0.525	0.056	2.07	2.402	2.459	2.470	2.444	0.036
		R	0.965	0.953	0.973	0.964	0.010	0.611	0.589	0.589	0.597	0.013	1.62	2.652	2.647	2.469	2.589	0.104
24	443	L	0.950	0.893	0.921	0.922	0.029	0.516	0.522	0.509	0.516	0.007	1.79	3.710	3.751	3.752	3.738	0.024
		R	0.911	0.913	0.919	0.915	0.004	0.502	0.524	0.551	0.526	0.024	1.74	3.379	3.532	3.427	3.447	0.078
25	405	L	1.006	0.858	0.849	0.905	0.088	0.562	0.540	0.562	0.555	0.013	1.63	4.059	3.891	3.853	3.935	0.110
		R	1.098	1.067	1.009	1.059	0.045	0.452	0.482	0.457	0.464	0.016	2.28	3.105	3.067	3.168	3.114	0.051
26	410	L	0.957	0.781	0.837	0.859	0.090	0.489	0.493	0.525	0.503	0.020	1.71	3.643	3.493	3.576	3.571	0.075
		R	1.013	0.986	1.032	1.011	0.023	0.597	0.606	0.556	0.587	0.027	1.72	3.459	3.606	3.525	3.531	0.074
27	443	L	0.980	0.954	0.958	0.965	0.014	0.563	0.551	0.552	0.556	0.007	1.74	4.912	4.610	4.711	4.745	0.153
		R	0.959	0.924	0.908	0.931	0.026	0.578	0.568	0.598	0.582	0.015	1.60	3.586	3.583	3.509	3.560	0.044
28	438	L	1.028	1.014	1.069	1.038	0.029	0.625	0.615	0.584	0.609	0.021	1.70	4.304	4.183	4.031	4.173	0.137
		R	1.041	0.991	1.048	1.027	0.031	0.581	0.580	0.552	0.572	0.016	1.80	3.653	3.637	3.571	3.621	0.044
29	433	L	1.028	0.960	0.977	0.989	0.036	0.472	0.452	0.461	0.462	0.010	2.14	2.681	2.580	2.649	2.637	0.052
		R	0.855	0.914	0.897	0.889	0.031	0.548	0.567	0.552	0.556	0.010	1.60	2.621	2.500	2.405	2.509	0.108
30	497	L	0.992	1.033	1.029	1.018	0.023	0.650	0.605	0.642	0.633	0.024	1.61	2.660	2.644	2.525	2.610	0.074
		R	1.039	1.037	0.952	1.010	0.050	0.631	0.563	0.597	0.597	0.034	1.69	3.358	3.236	3.246	3.280	0.068

Animal No	Weight (g)	Side	FA Ext Diameter (mm)			Mean (a)	SD	SCEA Ext Diameter (mm)			Mean (b)	SD	Ratio (a)/(b)	Distal FA Length (mm)			Mean	SD
			1	2	3			1	2	3				1	2	3		
31	386	L	0.946	0.951	0.927	0.942	0.013	0.583	0.528	0.568	0.560	0.028	1.68	3.356	3.365	3.416	3.380	0.033
		R	0.946	0.970	0.924	0.947	0.023	0.527	0.526	0.529	0.528	0.002	1.79	3.192	2.937	2.853	2.994	0.176
32	388	L	0.934	0.963	0.986	0.961	0.026	0.547	0.551	0.554	0.551	0.004	1.75	4.316	4.463	4.450	4.410	0.082
		R	1.008	1.007	1.027	1.015	0.011	0.593	0.593	0.551	0.580	0.024	1.75	2.820	2.766	2.860	2.816	0.047
33	435	L	1.123	1.103	1.061	1.096	0.032	0.578	0.522	0.530	0.544	0.030	2.02	3.961	3.819	3.950	3.911	0.079
		R	0.962	0.950	0.961	0.958	0.007	0.385	0.505	0.480	0.457	0.063	2.10	3.507	3.393	3.449	3.450	0.057
34	385	L	0.865	0.864	0.896	0.875	0.018	0.576	0.568	0.546	0.564	0.015	1.55	3.594	3.502	3.532	3.543	0.047
		R	1.016	1.017	1.048	1.027	0.018	0.653	0.619	0.581	0.618	0.036	1.66	3.417	3.433	3.425	3.426	0.008
35	390	L	0.974	0.967	0.952	0.965	0.011	0.577	0.525	0.536	0.546	0.027	1.77	2.981	2.977	3.042	3.001	0.037
		R	0.776	0.731	0.783	0.764	0.028	0.518	0.524	0.519	0.521	0.003	1.47	2.734	2.497	2.793	2.675	0.157
36	410	L	0.980	1.018	0.980	0.993	0.022	0.513	0.533	0.555	0.534	0.021	1.86	3.174	2.952	2.952	3.026	0.128
		R	0.960	0.958	0.921	0.947	0.021	0.563	0.514	0.490	0.523	0.037	1.81	3.089	2.986	3.055	3.044	0.052
37	450	L	0.951	0.976	0.975	0.968	0.014	0.564	0.575	0.578	0.573	0.007	1.69	2.589	2.568	2.711	2.623	0.077
		R	1.009	1.016	0.988	1.005	0.015	0.587	0.589	0.603	0.594	0.009	1.69	3.316	3.244	3.238	3.267	0.043
38	421	L	1.031	1.046	1.031	1.036	0.009	0.555	0.548	0.493	0.532	0.034	1.95	2.053	1.888	2.004	1.982	0.085
		R	1.110	1.032	1.013	1.052	0.052	0.575	0.571	0.498	0.549	0.043	1.92	3.308	3.321	3.168	3.266	0.085
39	439	L	1.048	1.052	1.016	1.039	0.019	0.552	0.503	0.523	0.527	0.024	1.97	2.506	2.531	2.418	2.486	0.060
		R	0.913	0.933	0.897	0.915	0.018	0.525	0.529	0.543	0.533	0.010	1.72	3.494	3.376	3.471	3.447	0.062
Mean:	423				L	0.973	0.007			L	0.563	0.005	1.741			L	3.457	0.057
					R	0.975	0.007			R	0.543	0.005	1.811			R	3.298	0.057
					Both	0.974	0.007			Both	0.553	0.005	1.776			Both	3.378	0.057

Table A.3. Measurements from additional Wistar Rats (HsdOla-WI) used in detailed characterisation. FA=Femoral Artery; SD = Standard Deviation; SCEA = Superficial Caudal Epigastric Artery. Mean results include the five animals from the pilot study.

A3. Details of Analysis of Wistar Rat Vessel Characteristics

A3.1. Regression Analysis: FA External Diameter versus Weight

The regression equation is
FA = 0.777 + 0.000464 Wt

Predictor	Coef	SE Coef	T	P
Constant	0.77743	0.06528	11.91	0.000
Wt	0.0004639	0.0001538	3.02	0.003

S = 0.0790472 R-Sq = 3.8% R-Sq(adj) = 3.4%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.056853	0.056853	9.10	0.003
Residual Error	232	1.449641	0.006248		
Total	233	1.506494			

Unusual Observations

Obs	Wt	FA	Fit	SE Fit	Residual	St Resid
4	381	0.79690	0.95419	0.00828	-0.15729	-2.00R
11	505	0.99230	1.01172	0.01362	-0.01941	-0.25 X
12	505	0.99432	1.01172	0.01362	-0.01740	-0.22 X
20	415	0.80739	0.96996	0.00532	-0.16257	-2.06R
31	343	0.96981	0.93656	0.01336	0.03325	0.43 X
32	343	0.90771	0.93656	0.01336	-0.02885	-0.37 X
70	390	0.77686	0.95836	0.00725	-0.18151	-2.31R
89	505	0.93959	1.01172	0.01362	-0.07212	-0.93 X
90	505	0.99281	1.01172	0.01362	-0.01891	-0.24 X
98	415	0.78009	0.96996	0.00532	-0.18987	-2.41R
109	343	0.96006	0.93656	0.01336	0.02350	0.30 X
110	343	0.96568	0.93656	0.01336	0.02912	0.37 X
129	410	0.78114	0.96764	0.00555	-0.18650	-2.37R
148	390	0.73187	0.95836	0.00725	-0.22650	-2.88R
167	505	0.97961	1.01172	0.01362	-0.03211	-0.41 X
168	505	1.02692	1.01172	0.01362	0.01520	0.20 X
176	415	0.78664	0.96996	0.00532	-0.18332	-2.32R
187	343	0.97011	0.93656	0.01336	0.03355	0.43 X
188	343	0.92387	0.93656	0.01336	-0.01269	-0.16 X
197	428	0.80371	0.97599	0.00522	-0.17229	-2.18R
226	390	0.78391	0.95836	0.00725	-0.17445	-2.22R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

A3.2. Regression Analysis: SCEA External Diameter versus Weight

The regression equation is
SCEA = 0.376 + 0.000417 Wt

Predictor	Coef	SE Coef	T	P
Constant	0.37617	0.04656	8.08	0.000
Wt	0.0004168	0.0001097	3.80	0.000

S = 0.0563769 R-Sq = 5.9% R-Sq(adj) = 5.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.045894	0.045894	14.44	0.000
Residual Error	232	0.737380	0.003178		
Total	233	0.783274			

Unusual Observations

Obs	Wt	SCEA	Fit	SE Fit	Residual	St Resid
4	381	0.37719	0.53499	0.00591	-0.15779	-2.81R
10	434	0.42684	0.55708	0.00387	-0.13024	-2.32R
11	505	0.44530	0.58668	0.00971	-0.14138	-2.55RX
12	505	0.54165	0.58668	0.00971	-0.04503	-0.81 X
31	343	0.53712	0.51915	0.00953	0.01797	0.32 X
32	343	0.41364	0.51915	0.00953	-0.10551	-1.90 X
37	479	0.69774	0.57584	0.00715	0.12190	2.18R
66	435	0.38547	0.55750	0.00391	-0.17203	-3.06R
68	385	0.65319	0.53665	0.00557	0.11654	2.08R
82	381	0.41763	0.53499	0.00591	-0.11736	-2.09R
89	505	0.52558	0.58668	0.00971	-0.06109	-1.10 X
90	505	0.51995	0.58668	0.00971	-0.06673	-1.20 X
109	343	0.54605	0.51915	0.00953	0.02690	0.48 X
110	343	0.39947	0.51915	0.00953	-0.11967	-2.15RX
113	418	0.67618	0.55041	0.00373	0.12577	2.24R
115	479	0.69774	0.57584	0.00715	0.12190	2.18R
116	479	0.45430	0.57584	0.00715	-0.12154	-2.17R
160	381	0.37963	0.53499	0.00591	-0.15536	-2.77R
165	434	0.69540	0.55708	0.00387	0.13832	2.46R
166	434	0.41535	0.55708	0.00387	-0.14173	-2.52R
167	505	0.52903	0.58668	0.00971	-0.05765	-1.04 X
168	505	0.55862	0.58668	0.00971	-0.02806	-0.51 X
187	343	0.55470	0.51915	0.00953	0.03555	0.64 X
188	343	0.44293	0.51915	0.00953	-0.07622	-1.37 X
193	479	0.73678	0.57584	0.00715	0.16095	2.88R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

A3.3. Regression Analysis: Distal FA Length versus Weight

The regression equation is
DISTAL = 4.86 - 0.00350 Wt

228 cases used, 6 cases contain missing values

Predictor	Coef	SE Coef	T	P
Constant	4.8565	0.5073	9.57	0.000
Wt	-0.003496	0.001196	-2.92	0.004

S = 0.614260 R-Sq = 3.6% R-Sq(adj) = 3.2%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	3.2271	3.2271	8.55	0.004
Residual Error	226	85.2733	0.3773		
Total	227	88.5003			

Unusual Observations

Obs	Wt	DISTAL	Fit	SE Fit	Residual	St Resid
11	505	2.5899	3.0908	0.1062	-0.5010	-0.83 X
12	505	2.8913	3.0908	0.1062	-0.1996	-0.33 X
21	417	4.7328	3.3985	0.0413	1.3342	2.18R
27	465	4.4797	3.2307	0.0646	1.2490	2.04R
31	343	3.2536	3.6573	0.1039	-0.4037	-0.67 X
32	343	3.4309	3.6573	0.1039	-0.2263	-0.37 X
53	443	4.9123	3.3076	0.0472	1.6047	2.62R
75	421	2.0536	3.3845	0.0407	-1.3309	-2.17R
89	505	2.4590	3.0908	0.1062	-0.6318	-1.04 X
90	505	2.6872	3.0908	0.1062	-0.4037	-0.67 X
99	417	4.6935	3.3985	0.0413	1.2950	2.11R
105	465	4.4996	3.2307	0.0646	1.2689	2.08R
109	343	3.2387	3.6573	0.1039	-0.4186	-0.69 X
110	343	3.5021	3.6573	0.1039	-0.1551	-0.26 X
131	443	4.6109	3.3076	0.0472	1.3033	2.13R
153	421	1.8884	3.3845	0.0407	-1.4961	-2.44R
167	505	2.5984	3.0908	0.1062	-0.4925	-0.81 X
168	505	2.8158	3.0908	0.1062	-0.2751	-0.45 X
170	407	2.1854	3.4335	0.0449	-1.2481	-2.04R
177	417	4.6410	3.3985	0.0413	1.2424	2.03R
187	343	3.2694	3.6573	0.1039	-0.3879	-0.64 X
188	343	3.6243	3.6573	0.1039	-0.0330	-0.05 X
209	443	4.7111	3.3076	0.0472	1.4035	2.29R
231	421	2.0044	3.3845	0.0407	-1.3801	-2.25R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

A3.4. General Linear Model: FA External Diameter versus Side

Factor	Type	Levels	Values
Side	fixed	2	L, R

Analysis of Variance for FA, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Wt	1	0.056853	0.056853	0.056853	9.06	0.003
Side	1	0.000322	0.000322	0.000322	0.05	0.821
Error	231	1.449320	1.449320	0.006274		
Total	233	1.506494				

S = 0.0792093 R-Sq = 3.80% R-Sq(adj) = 2.96%

Term	Coef	SE Coef	T	P
Constant	0.77743	0.06542	11.88	0.000
Wt	0.000464	0.000154	3.01	0.003

Unusual Observations for FA

Obs	FA	Fit	SE Fit	Residual	St Resid
4	0.79690	0.95536	0.00978	-0.15846	-2.02 R
20	0.80739	0.97113	0.00743	-0.16374	-2.08 R
70	0.77686	0.95954	0.00892	-0.18268	-2.32 R
98	0.78009	0.97113	0.00743	-0.19104	-2.42 R
129	0.78114	0.96647	0.00760	-0.18533	-2.35 R
148	0.73187	0.95954	0.00892	-0.22767	-2.89 R
176	0.78664	0.97113	0.00743	-0.18450	-2.34 R

```
197  0.80371  0.97482  0.00736  -0.17112     -2.17 R
226  0.78391  0.95954  0.00892  -0.17562     -2.23 R

R denotes an observation with a large standardized residual.


Means for Covariates

Covariate   Mean  StDev
Wt         423.1  33.67


Least Squares Means for FA1

Side   Mean   SE Mean
L    0.9725  0.007323
R    0.9749  0.007323


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable FA1
All Pairwise Comparisons among Levels of L/R
Side = L  subtracted from:

Side    Lower    Center    Upper  -----+---------+---------+---------+-
R    -0.01806  0.002345  0.02275  (-----------------*----------------)
                                  -----+---------+---------+---------+-
                                    -0.012     0.000     0.012     0.024


Tukey Simultaneous Tests
Response Variable FA1
All Pairwise Comparisons among Levels of L/R
Side = L  subtracted from:

     Difference       SE of          Adjusted
Side   of Means  Difference  T-Value   P-Value
R      0.002345     0.01036   0.2264    0.8211
```

A3.5. General Linear Model: SCEA External Diameter versus Side

```
Factor  Type   Levels  Values
Side    fixed       2  L, R


Analysis of Variance for SCEA, using Adjusted SS for Tests

Source   DF    Seq SS    Adj SS    Adj MS      F      P
Wt        1  0.045894  0.045894  0.045894  14.80  0.000
Side      1  0.021195  0.021195  0.021195   6.84  0.010
Error   231  0.716185  0.716185  0.003100
Total   233  0.783274


S = 0.0556809   R-Sq = 8.57%   R-Sq(adj) = 7.77%


Term          Coef   SE Coef     T      P
Constant   0.37617   0.04598  8.18  0.000
Wt        0.000417  0.000108  3.85  0.000
```

Unusual Observations for SCEA

Obs	SCEA	Fit	SE Fit	Residual	St Resid
4	0.377193	0.525470	0.006878	-0.148277	-2.68 R
10	0.426844	0.547563	0.005281	-0.120719	-2.18 R
11	0.445298	0.596193	0.010258	-0.150895	-2.76 R
37	0.697737	0.585355	0.007948	0.112382	2.04 R
66	0.385469	0.547980	0.005307	-0.162511	-2.93 R
68	0.653194	0.527138	0.006599	0.126057	2.28 R
104	0.649522	0.531306	0.005981	0.118216	2.14 R
110	0.399473	0.509630	0.010090	-0.110157	-2.01 R
113	0.676184	0.559928	0.005177	0.116256	2.10 R
115	0.697737	0.585355	0.007948	0.112382	2.04 R
116	0.454302	0.566321	0.007948	-0.112019	-2.03 R
135	0.452504	0.566180	0.005258	-0.113677	-2.05 R
160	0.379625	0.525470	0.006878	-0.145845	-2.64 R
165	0.695396	0.566597	0.005281	0.128799	2.32 R
166	0.415352	0.547563	0.005281	-0.132210	-2.39 R
193	0.736785	0.585355	0.007948	0.151430	2.75 R

R denotes an observation with a large standardized residual.

Means for Covariates

Covariate	Mean	StDev
Wt	423.1	33.67

Least Squares Means for SCEA

Side	Mean	SE Mean
L	0.5621	0.005148
R	0.5430	0.005148

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable SCEA
All Pairwise Comparisons among Levels of L/R
Side = L subtracted from:

Side	Lower	Center	Upper	---+---------+---------+---------+---
R	-0.03338	-0.01903	-0.004691	(-------------*-------------)
				---+---------+---------+---------+---
				-0.030 -0.020 -0.010 0.000

Tukey Simultaneous Tests
Response Variable SCEA
All Pairwise Comparisons among Levels of L/R
Side = L subtracted from:

Side	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
R	-0.01903	0.007280	-2.615	0.0095

A3.6. General Linear Model: Distal FA Length versus Side

```
Factor  Type   Levels  Values
Side    fixed       2  L, R


Analysis of Variance for DISTAL, using Adjusted SS for Tests

Source   DF   Seq SS   Adj SS  Adj MS     F      P
Wt        1   3.2271   3.2271  3.2271  8.66  0.004
Side      1   1.4521   1.4521  1.4521  3.90  0.050
Error   225  83.8211  83.8211  0.3725
Total   227  88.5003


S = 0.610359   R-Sq = 5.29%   R-Sq(adj) = 4.45%


Term           Coef   SE Coef      T      P
Constant     4.8565    0.5041   9.63  0.000
Wt        -0.003496  0.001188  -2.94  0.004


Unusual Observations for DISTAL

Obs  DISTAL   Fit      SE Fit   Residual     St Resid
  1  2.28937  3.55176  0.06553  -1.26239     -2.08 R
 21  4.73275  3.47834  0.05760   1.25442      2.06 R
 53  4.91228  3.38743  0.06192   1.52485      2.51 R
 75  2.05359  3.46435  0.05721  -1.41076     -2.32 R
131  4.61088  3.38743  0.06192   1.22345      2.01 R
153  1.88842  3.46435  0.05721  -1.57593     -2.59 R
157  2.25131  3.55176  0.06553  -1.30045     -2.14 R
209  4.71114  3.38743  0.06192   1.32371      2.18 R
231  2.00443  3.46435  0.05721  -1.45992     -2.40 R

R denotes an observation with a large standardized residual.


Means for Covariates

Covariate   Mean  StDev
Wt         423.0  34.10


Least Squares Means for DISTAL

Side  Mean  SE Mean
L    3.457  0.05717
R    3.298  0.05717


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable DISTAL
All Pairwise Comparisons among Levels of L/R
Side = L  subtracted from:

Side   Lower   Center      Upper   --+---------+---------+---------+----
R    -0.3189  -0.1596  -0.000304   (---------------*---------------)
                                   --+---------+---------+---------+----
                                  -0.30     -0.20     -0.10     -0.00
```

Tukey Simultaneous Tests
Response Variable DISTAL
All Pairwise Comparisons among Levels of L/R
Side = L subtracted from:

Side	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
R	-0.1596	0.08084	-1.974	0.0496

A4. Wistar Rat Femoral Artery Flow Rates

Animal No	Weight (g)	Side	Order	Animal Core Temp (ºC)	FA Mean Flow (mL/min)	SD
1	453	L	2	37.99	2.16	0.08
		R	1	37.61	3.23	0.12
2	564	L	1	37.79	1.95	0.14
		R	2	37.88	3.19	0.10
3	504	L	1	37.56	2.73	0.20
		R	2	37.47	3.57	0.15
4	440	L	2	37.34	1.84	0.13
		R	1	37.44	3.11	0.19
5	485	L	2	36.98	2.75	0.10
		R	1	37.24	3.69	0.11
6	463	L	2	36.89	1.98	0.13
		R	1	37.11	2.75	0.14
7	322	L	2	37.37	1.55	0.22
		R	1	37.52	1.98	0.20
8	449	L	1	37.42	3.14	0.24
		R	2	37.12	2.12	0.16
9	430	L	1	37.03	2.55	0.12
		R	2	37.32	2.66	0.12
10	412	L	1	37.83	2.87	0.31
		R	2	37.59	3.08	0.20
11	453	L	1	37.26	0.80	0.10
		R	2	36.80	2.50	0.18
12	426	L	1	38.17	3.57	0.38
		R	2	37.39	2.47	0.19
13	384	L	1	37.43	2.90	0.37
		R	2	37.54	2.77	0.15
14	483	L	2	37.51	1.99	0.09
		R	1	37.49	2.19	0.09
15	470	L	2	37.79	2.89	0.32
		R	1	38.15	2.94	0.43
16	451	L	2	37.54	2.14	0.11
		R	1	37.43	3.48	0.18
17	442	L	2	38.05	2.71	0.24
		R	1	38.24	3.40	0.26

Table A.4. Time-averaged mean femoral artery flow rates of Wistar Rats (HsdHan™:WIST).

A5. Details of Analysis of Femoral Artery Flow Rates

A5.1. General Linear Model: Mean FA Flow versus Animal Serial Number, Order, Side

```
Factor  Type    Levels  Values
Ser No  random      17  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                        15, 16, 17
Order   fixed        2  1, 2
Side    fixed        2  L, R


Analysis of Variance for Mean FA Flow, using Adjusted SS for Tests

Source  DF   Seq SS  Adj SS  Adj MS     F      P
Ser No  16   6.6561  6.6561  0.4160  1.44  0.244
Order    1   0.7091  0.5723  0.5723  1.98  0.180
Side     1   2.0436  2.0436  2.0436  7.06  0.018
Error   15   4.3424  4.3424  0.2895
Total   33  13.7511


S = 0.538045   R-Sq = 68.42%   R-Sq(adj) = 30.53%


Unusual Observations for Mean FA Flow

     Mean FA
Obs     Flow      Fit   SE Fit  Residual  St Resid
 21  0.80000  1.53437  0.40353  -0.73437     -2.06 R
 22  2.50000  1.76562  0.40353   0.73438      2.06 R

R denotes an observation with a large standardized residual.


Least Squares Means for Mean FA Flow

Ser No   Mean
 1      2.695
 2      2.570
 3      3.150
 4      2.475
 5      3.220
 6      2.365
 7      1.765
 8      2.630
 9      2.605
10      2.975
11      1.650
12      3.020
13      2.835
14      2.090
15      2.915
16      2.810
17      3.055

Order
1       2.767
2       2.507

Side
L       2.391
R       2.882
```

```
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Mean FA Flow
All Pairwise Comparisons among Levels of Order
Order = 1  subtracted from:

Order    Lower   Center   Upper  ------+---------+---------+---------+
2      -0.6540  -0.2599  0.1341  (---------------*--------------)
                                 ------+---------+---------+---------+
                                    -0.50     -0.25      0.00      0.25


Tukey Simultaneous Tests
Response Variable Mean FA Flow
All Pairwise Comparisons among Levels of Order
Order = 1  subtracted from:

       Difference       SE of           Adjusted
Order    of Means  Difference  T-Value   P-Value
2         -0.2599      0.1849   -1.406    0.1801


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Mean FA Flow
All Pairwise Comparisons among Levels of L/R
Side = L  subtracted from:

Side   Lower  Center   Upper  ------+---------+---------+---------+
R    0.09714  0.4912  0.8852  (---------------*--------------)
                              ------+---------+---------+---------+
                                  0.25      0.50      0.75      1.00


Tukey Simultaneous Tests
Response Variable Mean FA Flow
All Pairwise Comparisons among Levels of L/R
Side = L  subtracted from:

     Difference       SE of           Adjusted
Side   of Means  Difference  T-Value   P-Value
R        0.4912      0.1849    2.657    0.0179
```

A5.2. General Linear Model: Temp versus Ser No, Side, Order

```
Factor  Type    Levels  Values
Ser No  random      17  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                        15, 16, 17
Side    fixed        2  L, R
Order   fixed        2  1, 2


Analysis of Variance for Temp, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS   Adj MS     F      P
Ser No  16  3.51548  3.51548  0.21972  5.29  0.001
Side     1  0.01094  0.01601  0.01601  0.39  0.544
Order    1  0.14102  0.14102  0.14102  3.39  0.085
Error   15  0.62359  0.62359  0.04157
Total   33  4.29103


S = 0.203893   R-Sq = 85.47%   R-Sq(adj) = 68.03%
```

Unusual Observations for Temp

Obs	Temp	Fit	SE Fit	Residual	St Resid
23	38.1700	37.8663	0.1529	0.3038	2.25 R
24	37.3900	37.6938	0.1529	-0.3038	-2.25 R

R denotes an observation with a large standardized residual.

Least Squares Means for Temp

Ser No	Mean
1	37.80
2	37.84
3	37.52
4	37.39
5	37.11
6	37.00
7	37.45
8	37.27
9	37.17
10	37.71
11	37.03
12	37.78
13	37.48
14	37.50
15	37.97
16	37.48
17	38.15
L/R	
L	37.53
R	37.49
Order	
1	37.57
2	37.44

* WARNING * No multiple comparisons were calculated for the following terms
which contain or interact with random factors.

Ser No

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Temp
All Pairwise Comparisons among Levels of L/R
Side = L subtracted from:

Side	Lower	Center	Upper	---------+---------+---------+-------
R	-0.1928	-0.04347	0.1058	(--------------*--------------)
				---------+---------+---------+-------
				-0.10 0.00 0.10

Tukey Simultaneous Tests
Response Variable Temp
All Pairwise Comparisons among Levels of L/R
Side = L subtracted from:

Side	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
R	-0.04347	0.07006	-0.6205	0.5442

```
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Temp
All Pairwise Comparisons among Levels of Order
Order = 1  subtracted from:

Order    Lower   Center    Upper  --------+---------+---------+--------
2      -0.2783  -0.1290  0.02029  (--------------*--------------)
                                 --------+---------+---------+--------
                                      -0.20     -0.10     -0.00


Tukey Simultaneous Tests
Response Variable Temp
All Pairwise Comparisons among Levels of Order
Order = 1  subtracted from:

       Difference       SE of           Adjusted
Order    of Means  Difference  T-Value   P-Value
2         -0.1290     0.07006   -1.842    0.0854
```

A5.3. Regression Analysis: Mean FA Flow versus Temp

```
The regression equation is
Mean FA Flow = - 17.8 + 0.546 Temp


Predictor    Coef  SE Coef      T      P
Constant   -17.84    11.30  -1.58  0.124
Temp       0.5459   0.3014   1.81  0.079


S = 0.624305   R-Sq = 9.3%   R-Sq(adj) = 6.5%


Analysis of Variance

Source          DF       SS      MS     F      P
Regression       1   1.2789  1.2789  3.28  0.079
Residual Error  32  12.4722  0.3898
Total           33  13.7511



Unusual Observations

           Mean FA
Obs  Temp     Flow    Fit  SE Fit  Residual  St Resid
 21  37.3    0.800  2.501   0.131    -1.701     -2.79R

R denotes an observation with a large standardized residual.
```

Appendix B. Experimental results and analysis from Patency and Timing Experiments

B1. Results

Position in Series	Side	Technique	Investigator	Time (mm:ss)	1 hour patency	1 week patency	6 week patency	Revised ?
1	R	Oblq ETE	A	27:29	Y	Y	-	N
	L	Invagination		13:43	Y	Y	-	N
2	R	Oblq ETE	B	11:52	Y	-	-	N
	L	Invagination		05:09	N	-	-	Y
3	R	Oblq ETE	A	-	Y	Y	-	N
	L	Invagination		-	Y	Y	-	N
4	R	Oblq ETE	A	22:32	Y	Y	-	N
	L	Invagination		13:54	N	N	-	Y
5	L	Oblq ETE	B	30:25	Y	Y	Y	N
	R	Invagination		13:22	Y	Y	Y	N
6	L	Oblq ETE	B	14:17	Y	-	-	N
	R	Invagination		-	Y	-	-	N
7	L	Oblq ETE	A	-	Y	Y	-	N
	R	Invagination		16:50	Y	Y	-	N
8	R	Oblq ETE	A	19:38	Y	Y	-	N
	L	Invagination		16:53	Y	Y	-	N
9	L	Oblq ETE	B	14:22	Y	Y	Y	N
	R	Invagination		13:37	Y	Y	Y	N
10	L	Oblq ETE	A	-	Y	Y	-	N
	R	Invagination		15:49	Y	Y	-	N
11	R	Oblq ETE	B	17:24	Y	N	-	N
	L	Invagination		15:14	Y	Y	-	N
12	L	Oblq ETE	B	19:24	Y	Y	Y	N
	R	Invagination		07:45	Y	Y	Y	N
13	R	Oblq ETE	A	23:19	Y	Y	-	N
	L	Invagination		14:19	Y	Y	-	N
14	R	Oblq ETE	B	18:27	Y	-	-	N
	L	Invagination		10:39	N	-	-	Y
15	R	Oblq ETE	A	16:45	Y	Y	Y	Y
	L	Invagination		14:43	Y	Y	Y	N
16	R	Oblq ETE	B	17:49	Y	Y	Y	N
	L	Invagination		10:01	Y	Y	Y	N
17	R	Oblq ETE	A	21:56	Y	-	-	N
	L	Invagination		13:22	Y	-	-	N
18	L	Oblq ETE	A	29:16	Y	Y	Y	N
	R	Invagination		12:10	Y	Y	Y	N
19	R	Oblq ETE	B	18:39	Y	Y	Y	N
	L	Invagination		11:01	Y	Y	Y	N
20	L	Oblq ETE	A	17:00	Y	Y	Y	N
	R	Invagination		-	Y	Y	Y	N

Position in Series	Side	Technique	Investigator	Time (mm:ss)	1 hour patency	1 week patency	6 week patency	Revised ?
21	L	Oblq ETE	B	15:37	Y	Y	Y	N
	R	Invagination		11:12	Y	Y	Y	N
22	R	Oblq ETE	A	21:39	Y	Y	-	N
	L	Invagination		10:51	N	N	-	Y
23	L	Oblq ETE	B	14:59	Y	Y	Y	N
	R	Invagination		09:20	Y	Y	Y	N
24	R	Oblq ETE	A	20:55	Y	Y	Y	N
	L	Invagination		13:24	Y	Y	Y	N
25	R	Oblq ETE	B	17:15	Y	Y	Y	N
	L	Invagination		11:18	Y	Y	Y	N
26	R	Oblq ETE	A	-	Y	Y	Y	N
	L	Invagination		-	Y	N	N	Y
27	L	Oblq ETE	B	16:19	Y	Y	Y	N
	R	Invagination		15:01	Y	Y	Y	N
28	L	Oblq ETE	A	20:14	Y	Y	Y	N
	R	Invagination		19:13	Y	Y	Y	N
29	L	Oblq ETE	B	25:14	Y	N	N	Y
	R	Invagination		08:08	Y	Y	N	N
30	R	Oblq ETE	A	-	Y	Y	Y	N
	L	Invagination		-	Y	Y	Y	N
31	L	Oblq ETE	B	16:37	Y	Y	Y	N
	R	Invagination		07:23	Y	Y	Y	N
32	R	Oblq ETE	A	15:39	Y	Y	Y	N
	L	Invagination		16:36	Y	Y	Y	N
33	R	Oblq ETE	B	21:34	Y	-	-	N
	L	Invagination		09:22	Y	-	-	N
34	L	Oblq ETE	B	19:11	Y	Y	Y	N
	R	Invagination		08:47	Y	Y	Y	N
35	R	Oblq ETE	A	-	Y	N	-	N
	L	Invagination		14:31	Y	N	-	N
36	R	Oblq ETE	A	15:11	Y	Y	-	N
	L	Invagination		13:55	Y	N	-	N
37	R	Oblq ETE	B	13:24	Y	Y	Y	N
	L	Invagination		08:54	Y	Y	N	N
38	R	Oblq ETE	B	22:54	Y	Y	Y	N
	L	Invagination		10:29	Y	N	Y	N
39	R	Oblq ETE	A	19:40	Y	Y	-	N
	L	Invagination		12:12	Y	Y	-	N
40	L	Oblq ETE	A	16:30	Y	Y	N	N
	R	Invagination		13:40	Y	Y	Y	N
41	R	Oblq ETE	B	14:21	Y	Y	Y	N
	L	Invagination		-	Y	Y	Y	N
42	L	Oblq ETE	A	19:00	Y	Y	Y	N
	R	Invagination		13:51	Y	Y	Y	N
43	L	Oblq ETE	B	19:50	Y	-	-	N
	R	Invagination		08:48	Y	-	-	N

Position in Series	Side	Technique	Investigator	Time (mm:ss)	1 hour patency	1 week patency	6 week patency	Revised ?
44	L	Oblq ETE	A	17:28	Y	Y	Y	N
	R	Invagination		-	Y	Y	Y	N
45	R	Oblq ETE	B	17:16	Y	Y	Y	N
	L	Invagination		09:40	Y	Y	Y	N
46	L	Oblq ETE	B	25:35	Y	Y	Y	N
	R	Invagination		13:18	Y	N	N	N
47	R	Oblq ETE	A	18:50	Y	Y	Y	N
	L	Invagination		10:43	Y	Y	Y	N
48	L	Oblq ETE	A	16:45	Y	Y	Y	N
	R	Invagination		08:12	Y	Y	Y	N
49	L	Oblq ETE	B	18:12	Y	Y	Y	N
	R	Invagination		12:57	Y	Y	Y	N
50	L	Oblq ETE	B	-	N	N	-	N
	R	Invagination		12:27	N	N	-	N
51	L	Oblq ETE	A	15:56	Y	Y	Y	N
	R	Invagination		12:45	Y	Y	Y	N
52	L	Oblq ETE	A	22:41	Y	N	N	N
	R	Invagination		11:46	Y	Y	Y	N
53	L	Oblq ETE	B	17:00	N	N	-	N
	R	Invagination		13:45	Y	N	-	N
54	R	Oblq ETE	A	17:35	Y	Y	Y	N
	L	Invagination		11:05	Y	Y	Y	N
55	L	Oblq ETE	B	21:46	Y	Y	Y	N
	R	Invagination		08:44	Y	Y	Y	N
56	L	Oblq ETE	A	19:51	Y	Y	Y	N
	R	Invagination		15:12	Y	Y	Y	N
57	R	Oblq ETE	B	23:00	Y	Y	N	N
	L	Invagination		10:05	N	N	N	N
58	R	Oblq ETE	A	12:24	Y	Y	Y	N
	L	Invagination		11:23	Y	Y	Y	N
59	L	Oblq ETE	A	16:11	Y	Y	Y	N
	R	Invagination		12:04	Y	Y	Y	N
60	R	Oblq ETE	A	22:58	Y	Y	-	Y
	L	Invagination		12:04	Y	Y	-	N
61	R	Oblq ETE	A	17:41	Y	-	-	N
	L	Invagination		19:02	Y	-	-	N
62	L	Oblq ETE	A	13:06	Y	Y	Y	N
	R	Invagination		-	Y	Y	Y	Y
63	L	Oblq ETE	B	14:51	Y	Y	-	N
	R	Invagination		10:13	Y	N	-	N
64	L	Oblq ETE	A	20:51	Y	Y	Y	N
	R	Invagination		11:43	Y	Y	Y	N
65	L	Oblq ETE	B	21:23	N	N	N	Y
	R	Invagination		12:20	Y	Y	Y	N
66	R	Oblq ETE	A	-	Y	Y	Y	N
	L	Invagination		15:06	Y	Y	Y	N

Position in Series	Side	Technique	Investigator	Time (mm:ss)	1 hour patency	1 week patency	6 week patency	Revised ?
67	L	Oblq ETE	B	15:38	Y	Y	Y	N
	R	Invagination		25:52	Y	N	N	N
68	R	Oblq ETE	A	14:57	Y	Y	Y	N
	L	Invagination		12:02	Y	Y	Y	N
69	R	Oblq ETE	B	26:47	Y	N	N	Y
	L	Invagination		09:11	Y	Y	Y	N
70	L	Oblq ETE	A	16:44	Y	Y	Y	N
	R	Invagination		-	Y	Y	Y	N
71	L	Oblq ETE	A	-	Y	Y	Y	N
	R	Invagination		13:14	Y	Y	Y	N
72	L	Oblq ETE	B	23:03	Y	Y	Y	N
	R	Invagination		12:14	Y	Y	Y	N
73	L	Oblq ETE	B	23:14	Y	Y	Y	N
	R	Invagination		10:06	Y	Y	Y	N
74	R	Oblq ETE	A	11:24	N	N	N	Y
	L	Invagination		12:22	Y	N	N	Y
75	R	Oblq ETE	B	18:37	Y	Y	Y	N
	L	Invagination		11:26	Y	Y	Y	N
76	L	Oblq ETE	B	18:34	Y	N	-	N
	R	Invagination		09:10	Y	N	-	N
77	L	Oblq ETE	B	13:47	Y	Y	-	N
	R	Invagination		12:46	Y	N	-	N
78	L	Oblq ETE	A	15:15	Y	Y	Y	N
	R	Invagination		19:40	Y	Y	Y	N
79	R	Oblq ETE	B	13:47	N	N	N	Y
	L	Invagination		14:17	Y	Y	Y	N
80	L	Oblq ETE	A	18:52	Y	Y	Y	N
	R	Invagination		11:30	Y	Y	Y	N
81	R	Oblq ETE	B	12:20	Y	N	N	N
	L	Invagination		14:52	Y	Y	Y	N
82	L	Oblq ETE	B	21:12	Y	Y	Y	N
	R	Invagination		10:05	Y	Y	Y	N
83	R	Oblq ETE	A	15:53	Y	-	-	N
	L	Invagination		17:35	Y	-	-	N
84	R	Oblq ETE	B	16:32	Y	N	Y	N
	L	Invagination		12:07	Y	Y	Y	N
85	L	Oblq ETE	A	16:47	Y	Y	Y	N
	R	Invagination		10:29	Y	Y	Y	N
86	R	Oblq ETE	B	42:05	Y	Y	Y	N
	L	Invagination		12:04	Y	N	Y	Y
87	R	Oblq ETE	A	17:31	Y	Y	Y	N
	L	Invagination		-	Y	Y	Y	N
88	L	Oblq ETE	A	13:38	Y	Y	Y	N
	R	Invagination		11:32	Y	Y	Y	N
89	R	Oblq ETE	A	13:57	Y	Y	-	Y
	L	Invagination		13:45	Y	Y	-	N

Position in Series	Side	Technique	Investigator	Time (mm:ss)	1 hour patency	1 week patency	6 week patency	Revised ?
90	R	Oblq ETE	A	16:10	Y	-	-	N
	L	Invagination		11:17	Y	-	-	N
91	R	Oblq ETE	A	17:31	Y	Y	Y	N
	L	Invagination		10:08	Y	N	N	N
92	R	Oblq ETE	B	26:02	Y	Y	-	N
	L	Invagination		13:59	Y	N	-	N
93	R	Oblq ETE	A	19:09	Y	Y	Y	Y
	L	Invagination		13:07	Y	Y	Y	Y
94	R	Oblq ETE	B	-	Y	N	-	Y
	L	Invagination		12:23	Y	Y	-	N
95	R	Oblq ETE	A	16:25	Y	Y	Y	N
	L	Invagination		14:19	Y	Y	Y	N
96	R	Oblq ETE	B	23:17	Y	Y	Y	Y
	L	Invagination		09:46	Y	Y	Y	N
97	R	Oblq ETE	A	13:58	Y	Y	Y	Y
	L	Invagination		-	Y	Y	Y	N
98	R	Oblq ETE	B	18:23	Y	Y	Y	Y
	L	Invagination		08:24	Y	Y	Y	N
99	L	Oblq ETE	A	23:27	Y	Y	Y	N
	R	Invagination		13:34	Y	Y	Y	N
100	L	Oblq ETE	A	14:44	Y	Y	Y	N
	R	Invagination		16:30	Y	Y	Y	N
101	L	Oblq ETE	B	30:03	N	N	N	Y
	R	Invagination		13:44	Y	Y	Y	N
102	R	Oblq ETE	A	26:19	Y	Y	Y	Y
	L	Invagination		19:37	Y	Y	Y	N
103	R	Oblq ETE	B	12:29	Y	Y	Y	N
	L	Invagination		09:46	N	N	N	N
104	L	Oblq ETE	B	23:22	Y	N	-	N
	R	Invagination		12:27	Y	N	-	N
105	L	Oblq ETE	A	19:05	Y	Y	Y	N
	R	Invagination		18:28	Y	Y	Y	N
106	L	Oblq ETE	A	17:12	Y	Y	Y	Y
	R	Invagination		18:22	Y	Y	Y	N
107	L	Oblq ETE	B	20:33	Y	Y	Y	N
	R	Invagination		16:14	Y	Y	Y	N
108	R	Oblq ETE	A	18:27	Y	-	-	N
	L	Invagination		10:23	Y	-	-	N
109	L	Oblq ETE	B	24:29	Y	Y	-	N
	R	Invagination		13:40	N	N	-	Y
110	L	Oblq ETE	A	18:39	Y	Y	Y	Y
	R	Invagination		11:16	Y	Y	Y	N
111	L	Oblq ETE	B	19:11	Y	Y	Y	N
	R	Invagination		15:19	Y	Y	Y	N
112	L	Oblq ETE	A	23:49	Y	Y	Y	N
	R	Invagination		-	Y	Y	Y	N

Position in Series	Side	Technique	Investigator	Time (mm:ss)	1 hour patency	1 week patency	6 week patency	Revised ?
113	R	Oblq ETE	B	22:17	Y	-	-	N
	L	Invagination		13:29	Y	-	-	N
114	L	Oblq ETE	B	20:07	N	N	-	Y
	R	Invagination		12:01	Y	Y	-	N
115	R	Oblq ETE	A	18:02	Y	Y	Y	N
	L	Invagination		14:53	Y	Y	Y	N
116	L	Oblq ETE	A	20:08	Y	-	-	N
	R	Invagination		21:41	Y	-	-	N
117	L	Oblq ETE	B	16:25	Y	-	-	N
	R	Invagination		14:18	Y	-	-	N
118	L	Oblq ETE	A	24:04	Y	Y	Y	N
	R	Invagination		12:48	Y	Y	Y	N
119	L	Oblq ETE	B	20:10	N	N	N	Y
	R	Invagination		11:46	Y	N	N	N
120	L	Oblq ETE	B	21:06	Y	Y	Y	N
	R	Invagination		13:51	Y	Y	Y	N
121	R	Oblq ETE	A	19:03	Y	Y	Y	N
	L	Invagination		19:11	Y	Y	Y	N
122	L	Oblq ETE	B	20:00	Y	Y	Y	Y
	R	Invagination		10:19	Y	Y	Y	N
123	L	Oblq ETE	A	22:20	Y	Y	Y	N
	R	Invagination		14:08	Y	Y	Y	N
124	R	Oblq ETE	A	24:18	Y	Y	Y	N
	L	Invagination		22:15	Y	Y	Y	N
125	R	Oblq ETE	B	13:33	Y	Y	Y	N
	L	Invagination		08:23	Y	Y	Y	N
126	R	Oblq ETE	A	18:27	Y	Y	Y	N
	L	Invagination		17:05	Y	Y	Y	N
127	L	Oblq ETE	A	17:39	Y	Y	Y	N
	R	Invagination		13:08	Y	Y	Y	N
128	R	Oblq ETE	A	17:04	Y	Y	Y	N
	L	Invagination		13:51	Y	Y	Y	N
129	L	Oblq ETE	A	18:52	Y	Y	Y	N
	R	Invagination		13:32	Y	N	N	N
130	R	Oblq ETE	A	20:30	Y	Y	Y	N
	L	Invagination		14:20	Y	Y	Y	N
131	L	Oblq ETE	B	23:11	Y	-	-	Y
	R	Invagination		14:03	Y	-	-	N
132	L	Oblq ETE	A	21:34	Y	Y	Y	N
	R	Invagination		15:02	Y	Y	Y	N
133	L	Oblq ETE	B	26:27	Y	Y	-	N
	R	Invagination		09:33	Y	Y	-	N
134	R	Oblq ETE	B	15:19	Y	Y	Y	N
	L	Invagination		08:58	Y	Y	Y	N
135	L	Oblq ETE	A	22:12	Y	Y	Y	N
	R	Invagination		11:04	Y	Y	Y	N

Position in Series	Side	Technique	Investigator	Time (mm:ss)	1 hour patency	1 week patency	6 week patency	Revised ?
136	L	Oblq ETE	B	21:31	Y	Y	Y	N
	R	Invagination		10:24	Y	Y	Y	N
137	R	Oblq ETE	B	20:23	Y	Y	Y	N
	L	Invagination		09:40	Y	Y	Y	N
138	L	Oblq ETE	B	24:42	Y	Y	Y	N
	R	Invagination		13:42	Y	Y	Y	N
139	L	Oblq ETE	A	20:10	Y	Y	Y	N
	R	Invagination		11:48	Y	Y	Y	N
140	R	Oblq ETE	A	20:00	Y	Y	Y	N
	L	Invagination		11:11	Y	Y	Y	N
141	R	Oblq ETE	A	15:46	Y	Y	Y	N
	L	Invagination		-	N	N	N	N
142	R	Oblq ETE	A	17:38	Y	Y	Y	N
	L	Invagination		15:22	Y	N	Y	N
143	R	Oblq ETE	B	20:10	Y	Y	Y	N
	L	Invagination		09:50	Y	Y	Y	N
144	R	Oblq ETE	B	19:35	Y	Y	Y	N
	L	Invagination		13:42	Y	Y	Y	N
145	R	Oblq ETE	A	16:17	Y	Y	Y	Y
	L	Invagination		13:10	Y	Y	Y	N
146	L	Oblq ETE	B	15:09	Y	Y	-	N
	R	Invagination		12:04	Y	Y	-	N
147	R	Oblq ETE	B	19:23	Y	Y	Y	N
	L	Invagination		09:48	Y	Y	Y	N
148	L	Oblq ETE	A	26:10	Y	Y	Y	N
	R	Invagination		22:37	Y	Y	Y	N
149	R	Oblq ETE	A	20:04	Y	Y	Y	N
	L	Invagination		14:38	Y	Y	Y	N
150	L	Oblq ETE	B	25:17	Y	Y	-	Y
	R	Invagination		10:46	Y	N	-	N
151	R	Oblq ETE	A	18:37	N	N	N	Y
	L	Invagination		13:03	Y	Y	Y	N
152	R	Oblq ETE	B	23:16	Y	N	N	N
	L	Invagination		11:02	Y	Y	Y	N
153	L	Oblq ETE	B	19:18	Y	Y	Y	N
	R	Invagination		12:36	Y	N	Y	N
154	R	Oblq ETE	B	20:34	Y	Y	N	N
	L	Invagination		08:15	Y	Y	Y	N
155	R	Oblq ETE	B	20:12	Y	Y	Y	N
	L	Invagination		08:51	Y	Y	Y	N
156	R	Oblq ETE	B	18:03	Y	Y	Y	N
	L	Invagination		11:05	Y	Y	Y	N
157	L	Oblq ETE	B	24:30	Y	Y	Y	N
	R	Invagination		09:20	Y	Y	Y	N
158	L	Oblq ETE	B	25:02	Y	Y	Y	Y
	R	Invagination		11:08	Y	Y	Y	N

Position in Series	Side	Technique	Investigator	Time (mm:ss)	1 hour patency	1 week patency	6 week patency	Revised ?
159	R	Oblq ETE	B	21:32	Y	Y	Y	N
	L	Invagination		07:38	Y	Y	Y	N
160	R	Oblq ETE	B	20:59	Y	Y	Y	N
	L	Invagination		09:13	Y	Y	Y	N
161	L	Oblq ETE	B	25:28	Y	Y	Y	N
	R	Invagination		11:11	Y	Y	Y	N
162	R	Oblq ETE	B	19:32	Y	Y	Y	N
	L	Invagination		08:09	Y	Y	Y	N
163	R	Oblq ETE	B	17:26	Y	-	-	N
	L	Invagination		13:03	Y	-	-	N
164	L	Oblq ETE	B	26:31	Y	Y	Y	N
	R	Invagination		09:05	Y	Y	Y	N
165	R	Oblq ETE	B	15:31	Y	-	-	N
	L	Invagination		07:12	Y	-	-	N
166	L	Oblq ETE	B	15:31	Y	Y	Y	N
	R	Invagination		09:45	Y	Y	Y	N
167	L	Oblq ETE	A	26:45	Y	Y	-	N
	R	Invagination		16:58	Y	Y	-	N
168	L	Oblq ETE	B	14:06	Y	Y	Y	N
	R	Invagination		09:42	Y	Y	Y	N
169	R	Oblq ETE	A	20:30	Y	Y	Y	N
	L	Invagination		21:02	Y	Y	Y	N
170	L	Oblq ETE	A	25:06	Y	Y	Y	Y
	R	Invagination		14:34	Y	Y	Y	Y
171	L	Oblq ETE	A	33:57	Y	Y	-	N
	R	Invagination		24:11	Y	Y	-	N
172	R	Oblq ETE	A	20:11	Y	Y	Y	N
	L	Invagination		09:39	Y	Y	Y	N

Table B.1. Patency and Timing Results.

B2. Analysis

B2.1. Binary Logistic Regression: 1 hour patency versus Side, Technique, Investigator, Series Position, Revision

```
Link Function: Logit


Response Information

Variable        Value  Count
1 hour patency  1        326  (Event)
                0         18
                Total    344
```

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P	Odds Ratio	95% CI Lower	95% CI Upper
Constant	3.63190	0.695266	5.22	0.000			
Side							
R	0.924073	0.617940	1.50	0.135	2.52	0.75	8.46
Technique							
Oblq ETE	0.644394	0.625944	1.03	0.303	1.90	0.56	6.50
Investigator							
B	-1.51460	0.659476	-2.30	0.022	0.22	0.06	0.80
When							
2	0.826852	0.685728	1.21	0.228	2.29	0.60	8.77
3	1.83977	0.943418	1.95	0.050	6.30	0.99	40.00
Revision							
1	-3.68703	0.669048	-5.51	0.000	0.03	0.01	0.09

Log-Likelihood = -45.550
Test that all slopes are zero: G = 50.150, DF = 6, P-Value = 0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	49.6075	33	0.032
Deviance	35.2073	33	0.364
Hosmer-Lemeshow	7.4675	6	0.280

Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

	Group								
Value	1	2	3	4	5	6	7	8	Total
1									
Obs	26	44	43	44	43	45	42	39	326
Exp	24.7	46.2	42.9	43.2	42.5	44.7	42.8	38.9	
0									
Obs	11	5	1	0	0	0	1	0	18
Exp	12.3	2.8	1.1	0.8	0.5	0.3	0.2	0.1	
Total	37	49	44	44	43	45	43	39	344

Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs	Number	Percent	Summary Measures	
Concordant	5213	88.8	Somers' D	0.80
Discordant	526	9.0	Goodman-Kruskal Gamma	0.82
Ties	129	2.2	Kendall's Tau-a	0.08
Total	5868	100.0		

B2.2. Correlations: 1 hour patency, revision

Pearson correlation of 1 hour patency and revision = -0.424
P-Value = 0.000

B2.3. Tabulated statistics: 1 hour patency, revision

```
Rows: 1 hour patency   Columns: revision

          0   1  All

0         6  12   18
1       301  25  326
All     307  37  344

Cell Contents:      Count


Fisher's exact test: P-Value =  0.0000000
```

B2.4 General Linear Model: Time versus Side, Technique, Investigator, Position in Series

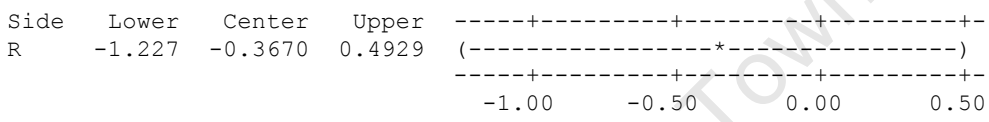
```
Factor     Type   Levels  Values
Side       fixed       2  L, R
Technique  fixed       2  Oblq ETE, Invag
Investig.. fixed       2  A, B
Position   fixed       3  1, 2, 3


Analysis of Variance for Time, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS   Adj MS      F      P
Side          1    15.66    10.81    10.81   0.71  0.402
Technique     1  3808.24  3797.87  3797.87 247.82  0.000
Investig..    1   119.33   128.91   128.91   8.41  0.004
Position      2    70.24    70.24    35.12   2.29  0.103
Error       315  4827.43  4827.43    15.33
Total       320  8840.90


S = 3.91474   R-Sq = 45.40%   R-Sq(adj) = 44.53%


Unusual Observations for Time

Obs     Time      Fit  SE Fit  Residual  St Resid
  2  27.4833  19.4620  0.5364    8.0214      2.07 R
  9  30.4167  18.5581  0.5388   11.8586      3.06 R
 35  29.2667  19.8290  0.5331    9.4377      2.43 R
134  25.8667  12.0778  0.5291   13.7889      3.55 R
138  26.7833  18.9580  0.5282    7.8254      2.02 R
148  11.4000  20.2288  0.5325   -8.8288     -2.28 R
172  42.0833  18.9580  0.5282   23.1254      5.96 R
201  30.0500  19.3250  0.5250   10.7250      2.76 R
232  21.6833  13.3487  0.5347    8.3347      2.15 R
247  22.2500  14.0839  0.5544    8.1661      2.11 R
296  22.6167  13.7169  0.5529    8.8998      2.30 R
341  33.9500  20.9641  0.5504   12.9859      3.35 R
342  24.1833  13.7169  0.5529   10.4664      2.70 R

R denotes an observation with a large standardized residual.
```

Least Squares Means for Time

Side	Mean	SE Mean
L	16.39	0.3088
R	16.02	0.3097
Technique		
Oblq ETE	19.64	0.3078
Invag	12.76	0.3107
Investigator		
A	16.84	0.3161
B	15.57	0.3031
When		
1	15.57	0.3803
2	16.34	0.3700
3	16.71	0.3867

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Time
All Pairwise Comparisons among Levels of Side
Side = L subtracted from:

Side	Lower	Center	Upper
R	-1.227	-0.3670	0.4929

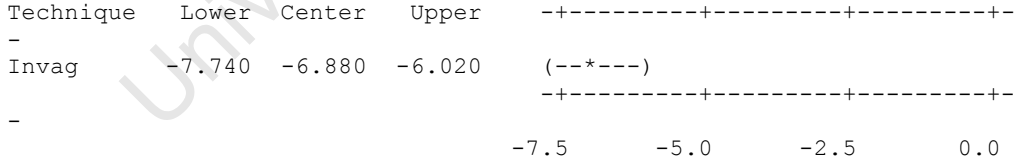


Tukey Simultaneous Tests
Response Variable Time
All Pairwise Comparisons among Levels of Side
Side = L subtracted from:

Side	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
R	-0.3670	0.4371	-0.8397	0.4011

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Time
All Pairwise Comparisons among Levels of Technique
Technique = Oblq ETE subtracted from:

Technique	Lower	Center	Upper
Invag	-7.740	-6.880	-6.020



Tukey Simultaneous Tests
Response Variable Time
All Pairwise Comparisons among Levels of Technique
Technique = Oblq ETE subtracted from:

Technique	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Invag	-6.880	0.4371	-15.74	0.0000

```
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Time
All Pairwise Comparisons among Levels of Investigator
Investigator = A  subtracted from:

Investig.. Lower  Center    Upper  ------+---------+---------+---------+
B         -2.133  -1.271  -0.4087  (--------------*-------------)
                                   ------+---------+---------+---------+
                                      -1.80     -1.20     -0.60      0.00


Tukey Simultaneous Tests
Response Variable Time
All Pairwise Comparisons among Levels of Investigator
Investigator = A  subtracted from:

          Difference       SE of           Adjusted
Investig..  of Means  Difference  T-Value   P-Value
B            -1.271      0.4382   -2.900    0.0037


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Time
All Pairwise Comparisons among Levels of Pos'n
Pos'n = 1  subtracted from:

Pos'n   Lower  Center  Upper  ---------+---------+---------+-------
2     -0.4749  0.7669  2.009      (------------*-----------)
3     -0.1348  1.1351  2.405          (-----------*------------)
                             ---------+---------+---------+-------
                                    0.0       1.0       2.0


Pos'n = 2  subtracted from:

Pos'n   Lower  Center  Upper  ---------+---------+---------+-------
3     -0.8839  0.3682  1.620  (------------*-----------)
                             ---------+---------+---------+-------
                                    0.0       1.0       2.0


Tukey Simultaneous Tests
Response Variable Time
All Pairwise Comparisons among Levels of When
Pos'n = 1  subtracted from:

      Difference       SE of           Adjusted
Pos'n   of Means  Difference  T-Value   P-Value
2         0.7669      0.5306    1.445    0.3176
3         1.1351      0.5426    2.092    0.0914


Pos'n = 2  subtracted from:

      Difference       SE of           Adjusted
Pos'n   of Means  Difference  T-Value   P-Value
3         0.3682      0.5350   0.6883    0.7703
```

B2.5 Binary Logistic Regression: Revision versus Side, Technique, Investigator

```
Link Function: Logit


Response Information

Variable  Value  Count
Redo?     1         37  (Event)
          0        307
          Total    344


Logistic Regression Table

                                                        Odds      95% CI
Predictor         Coef   SE Coef      Z      P  Ratio  Lower  Upper
Constant     -3.12892  0.514720  -6.08  0.000
Side
 R          -0.165000  0.358066  -0.46  0.645   0.85   0.42   1.71
Technique
 Oblq ETE    0.979096  0.382282   2.56  0.010   2.66   1.26   5.63
Investigator
 B          -0.0441682  0.358168  -0.12  0.902   0.96   0.47   1.93


Log-Likelihood = -109.542
Test that all slopes are zero: G = 15.785, DF = 5, P-Value = 0.007


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             30.1205  18  0.036
Deviance            30.8045  18  0.030
Hosmer-Lemeshow     22.2000   7  0.002


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value     1     2     3     4     5     6     7     8     9  Total
1
  Obs     0     6     0     3     2     3     7    14     2     37
  Exp   1.6   1.7   2.4   3.4   4.7   4.1   4.6  10.4   4.2
0
  Obs    43    35    40    33    45    35    32    30    14    307
  Exp  41.4  39.3  37.6  32.6  42.3  33.9  34.4  33.6  11.8
Total    43    41    40    36    47    38    39    44    16    344


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant    7470     65.8  Somers' D              0.39
Discordant    3084     27.2  Goodman-Kruskal Gamma  0.42
Ties           805      7.1  Kendall's Tau-a        0.07
Total        11359    100.0
```

Appendix C. Experimental Results and Analysis from Flow Experiment

C1. Results

Ser No	Wt	Sched Meas Pt	Invagination										Oblique End-to-End										Timing Means
			Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	
1	402	Femoral Baseline	13:34:48		38.45		6.79	0.15	12.37	3.92	1.2449	100.00%	12:05:35		37.07		5.27	0.16	11.6	2.51	1.7243	100.00%	
		Recorded Finish	14:14:00			32.75							13:04:00			50.97							
		10 mins	14:25:13	00:11:13	37.60		3.86	0.16	5.93	2.17	1.0513	56.85%	13:15:52	00:11:52	38.96		4.49	0.53	9.01	2.83	1.3925	85.20%	00:11:32
		1 hour	15:19:50	01:05:50	39.02		2.93	0.07	4.93	1.72	1.1176	43.15%	14:29:38	01:25:38	37.62		3.47	0.76	7.39	2.11	1.5907	65.84%	01:15:44
		3 hours	17:14:08	03:00:08	39.46		1.38	0.04	2.82	0.67	1.5553	20.32%	16:12:48	03:08:48	38.31		2.73	0.08	5.63	1.57	1.4740	51.80%	03:04:28
		24 hours	13:20:21	23:06:21			2.03	0.09	2.73	1.47	0.6247	29.90%	13:20:19	24:16:19			2.69	0.09	5.20	1.59	1.3431	51.04%	23:41:20
		3 days	13:24:02	71:10:02			4.24	0.34	6.81	2.19	1.0935	62.44%	13:24:02	72:20:02			3.87	0.28	8.58	1.73	1.7816	73.43%	71:45:02
		1 week	13:53:45	167:39:45			9.74	0.58	14.04	5.84	0.8511	143.45%	13:53:43	168:49:43			2.08	0.14	3.28	1.17	1.0213	39.47%	168:14:44

Ser No	Wt	Sched Meas Pt	Invagination										Oblique End-to-End										Timing Means
			Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	
2	453	Femoral Baseline	12:57:36		37.99		2.16	0.08	5.09	0.77	1.9990	100.00%	11:43:16		37.61		3.22	0.12	11.89	0.67	3.4736	100.00%	
		Recorded Finish Time	13:31:00			26.43							12:39:00			48.43							
		10 mins	13:42:39	00:11:39	37.83		2.11	0.07	3.54	1.24	1.0532	97.69%	12:50:01	00:11:01	37.83		3.71	0.16	6.89	2.29	1.1789	115.22%	00:11:20
		1 hour	14:34:03	01:03:03	36.88		1.59	0.07	3.25	0.58	1.6772	73.61%	13:38:44	00:59:44	37.49		3.20	0.10	7.92	1.29	2.0712	99.38%	01:01:23
		3 hours	17:01:52	03:30:52			2.61	0.09	3.95	1.79	0.8266	120.83%	17:01:52	04:22:52			2.13	0.13	5.07	0.95	1.9721	66.15%	03:56:52
		24 hours	13:08:11	23:37:11			4.60	0.19	5.98	3.36	0.5765	212.96%	13:08:11	24:29:11			5.31	0.29	9.15	3.12	1.1375	164.91%	24:03:11
		3 days	13:07:52	71:36:52			4.28	0.39	5.47	3.27	0.5204	198.15%	13:07:52	72:28:52			4.77	0.37	6.91	3.15	0.7979	148.14%	72:02:52
		1 week	13:05:08	167:34:08			3.37	0.13	4.48	2.48	0.5945	156.02%	13:05:08	168:26:08			5.52	0.19	8.04	3.58	0.8095	171.43%	168:00:08

Ser No	Wt	Sched Meas Pt	Invagination										Oblique End-to-End										Timing Means
			Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	
3	504	Femoral Baseline	12:03:46		37.51		3.57	0.15	8.60	1.31	1.9644	100.00%	10:55:58		37.56		2.73	0.20	5.74	0.85	1.8028	100.00%	
		Recorded Finish Time	12:41:00			30.82							11:48:00			46.33							
		10 mins	12:51:31	00:10:31	37.56		0.54	0.04	2.27	0.11	3.9165	15.13%	11:58:43	00:10:43	37.54		1.65	0.06	2.60	1.28	0.7319	60.44%	00:10:37
		1 hour	13:41:48	01:00:48	37.59		0.65	0.03	2.18	0.17	3.0843	18.21%	12:48:05	01:00:05	37.56		1.15	0.03	1.89	0.65	1.0803	42.12%	01:00:27
		3 hours	15:52:43	03:11:43	37.30		0.54	0.05	3.42	-0.13	6.5672	15.13%	15:52:43	04:04:43	37.30		6.01	0.18	10.38	2.93	1.2374	220.15%	03:38:13
		24 hours	16:00:03	27:19:03			1.72	0.23	5.51	0.87	2.7774	48.18%	16:00:03	28:12:03			7.28	1.32	14.86	4.42	1.4752	266.67%	27:45:33
		3 days	13:08:34	72:27:34			1.70	0.36	4.95	0.95	2.3194	47.62%	13:11:38	73:23:38			8.33	1.57	17.58	4.86	1.5502	305.13%	72:55:36
		1 week	15:11:11	170:30:11			1.42	0.10	3.03	0.74	1.6199	39.78%	15:11:11	171:23:11			4.64	0.41	9.94	2.26	1.6666	169.96%	170:56:41

Ser No	Wt	Sched Meas Pt	Invagination										Oblique End-to-End										Timing Means
			Time at Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	Time at Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	
4	485	Femoral Baseline	10:59:59		37.24		3.69	0.11	11.66	0.73	2.9647	100.00%	11:53:39		36.98		2.75	0.10	8.69	0.51	2.9793	100.00%	
		Recorded Finish Time	11:37:00			31.62							12:35:00			38.32							
		10 mins	11:48:31	00:11:31	37.25		1.64	0.16	3.33	0.93	1.4702	44.44%	12:47:53	00:12:53	37.16		3.26	0.12	7.00	1.62	1.6516	118.55%	00:12:12
		1 hour	12:38:50	01:01:50	37.36		1.73	0.08	4.07	0.61	1.9981	46.88%	13:35:01	01:00:01	37.24		1.60	0.09	4.98	0.25	2.9603	58.18%	01:00:56
		3 hours	14:58:49	03:21:49	37.07		0.98	0.17	3.40	0.45	3.0887	26.56%	14:58:49	02:23:49	37.07		1.36	0.19	5.36	0.13	3.8856	49.45%	02:52:49
		24 hours	13:26:39	25:49:39			3.77	0.11	6.14	2.20	1.0452	102.17%	13:26:39	24:51:39			4.77	0.14	9.43	2.48	1.4546	173.45%	25:20:39
		3 days	-				-		-	-	-		12:56:09	72:21:09			5.68		10.43	3.20	1.2762	206.55%	72:21:09
		1 week	-				-		-	-	-		12:04:07	167:29:07			3.88		9.09	1.85	1.9355	141.09%	167:29:07

Ser No	Wt	Sched Meas Pt	Invagination										Oblique End-to-End										Timing Means
			Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	
5	430	Femoral Baseline	11:16:21		37.03		2.55	0.12	8.22	0.59	2.9986	100.00%	12:27:45		37.32		2.66	0.12	8.09	0.72	2.7734	100.00%	
		Recorded Finish Time	11:59:35			38.35							13:13:19			43.43							
		10 mins	12:08:36	00:09:01	37.49		2.23	0.09	4.74	1.02	1.6747	87.45%	13:22:08	00:08:49	37.34		1.17	0.08	7.28	0.01	6.2482	43.98%	00:08:55
		1 hour	12:52:47	00:53:12	37.47		2.10	0.07	5.40	0.66	2.2590	82.35%	14:12:44	00:59:25	37.77		1.97	0.05	6.69	0.60	3.0958	74.06%	00:56:18
		3 hours																					
		24 hours	13:45:05	25:45:30			3.95	0.17	6.40	2.61	0.9611	154.90%	13:45:05	24:31:46			3.55	0.16	6.36	2.03	1.2218	133.46%	25:08:38
		3 days	13:03:36	73:04:01			5.45	0.59	8.71	3.44	0.9756	213.73%	13:03:36	71:50:17			3.62	0.47	6.94	2.13	1.3540	136.09%	72:27:09
		1 week	12:38:04	168:38:29			2.78	0.18	5.14	1.74	1.2427	109.02%	12:38:04	167:24:45			2.84	0.45	4.94	1.77	1.1538	106.77%	168:01:37

Ser No	Wt	Sched Meas Pt	Invagination										Oblique End-to-End										Timing Means
			Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	
6	412	Femoral Baseline	11:49:58		37.59		3.08	0.20	6.57	1.41	1.6791	100.00%	10:40:30		37.83		2.87	0.31	8.56	0.64	2.7837	100.00%	
		Recorded Finish Time	12:21:26			22.87							11:32:18	00:40:35		40.58							
		10 mins	12:30:48	00:09:22	37.70		1.36	0.10	3.10	0.75	1.7428	44.16%	11:42:22	00:10:04	37.94		1.97	0.16	4.04	1.22	1.4379	68.64%	00:09:43
		1 hour	13:21:09	00:59:43	37.73		2.78	0.15	5.79	1.37	1.5893	90.26%	12:36:48	01:04:30	37.55		1.44	0.13	3.51	0.35	2.2154	50.17%	01:02:06
		3 hours	14:56:24	02:34:58	37.24		1.69	0.09	3.89	0.73	1.8709	54.87%	14:56:24	03:24:06	37.24		1.72	0.13	5.72	0.21	3.2029	59.93%	02:59:32
		24 hours	13:35:55	25:14:29			2.33	0.09	4.67	1.21	1.4876	75.65%	13:35:55	26:03:37			4.31	0.20	10.57	1.79	2.0424	150.17%	25:39:03
		3 days	12:06:27	71:45:01			2.80	0.15	5.14	1.48	1.3088	90.91%	12:06:27	72:34:09			5.26	0.33	11.96	2.42	1.8220	183.28%	72:09:35
		1 week	12:29:06	168:07:40			4.63	0.21	7.65	2.86	1.0374	150.32%	12:29:06	168:56:48			6.96	0.35	14.37	3.59	1.5540	242.51%	168:32:14

Ser No	Wt	Sched Meas Pt	Invagination										Oblique End-to-End										Timing Means
			Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	
7	426	Femoral Baseline	12:07:43		37.39		2.47	0.19	6.17	1.18	2.0306	100.00%	11:01:45		38.17		3.57	0.38	9.53	1.57	2.2425	100.00%	
		Recorded Finish Time	12:48:21			30.75							11:48:31			43.67							
		10 mins	12:58:29	00:10:08	37.79		2.95	0.18	4.77	1.89	0.9790	119.43%	11:59:54	00:11:23	37.49		2.01	0.20	4.55	0.76	1.8945	56.30%	00:10:45
		1 hour	13:48:24	01:00:03	37.96		2.12	0.16	4.65	1.13	1.6675	85.83%	12:53:15	01:04:44	38.03		1.91	0.18	5.24	0.63	2.4171	53.50%	01:02:24
		3 hours	15:21:47	02:33:26	37.71		1.64	0.11	4.15	0.64	2.1429	66.40%	15:21:47	03:33:16	37.71		1.05	0.11	3.04	0.27	2.6543	29.41%	03:03:21
		24 hours											13:07:15	25:18:44			1.40	0.13	3.95	0.23	2.6695	39.22%	25:18:44
		3 days	12:19:28	71:31:07			3.21	0.16	6.22	1.85	1.3633	129.96%	12:19:28	72:30:57			2.10	0.09	4.83	1.01	1.8229	58.82%	72:01:02
		1 week	13:07:24	168:19:03			3.48	0.15	5.64	2.32	0.9560	140.89%	13:07:24	169:18:53			2.11	0.27	4.54	1.01	1.7099	59.10%	168:48:58

Ser No	Wt	Sched Meas Pt	Invagination										Oblique End-to-End										Timing Means
			Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	
8	384	Femoral Baseline	12:01:47		37.54		2.77	0.15	6.13	1.65	1.6213	100.00%	10:50:03		37.43		2.90	0.33	7.31	1.48	2.0279	100.00%	
		Recorded Finish Time	12:35:45			30.38							11:33:36			40.95							
		10 mins	12:45:12	00:09:27	37.21		1.60	0.10	2.98	0.95	1.2680	57.76%	11:42:58	00:09:22	37.37		1.83	0.10	4.24	1.01	1.7679	63.10%	00:09:25
		1 hour	13:36:33	01:00:48	37.67		1.62	0.10	4.04	0.80	2.0054	58.48%	12:33:44	01:00:08	37.60		1.98	0.18	4.88	0.99	1.9798	68.28%	01:00:28
		3 hours	15:04:59	02:29:14	36.09		1.86	0.11	4.27	1.03	1.7446	67.15%	15:04:59	03:31:23	36.09		1.07	0.07	2.94	0.55	2.3538	36.90%	03:00:19
		24 hours											12:16:05	24:42:29			2.39	0.17	5.65	0.98	1.9709	82.41%	24:42:29
		3 days																					
		1 week																					

Ser No	Wt	Sched Meas Pt	Invagination										Oblique End-to-End										Timing Means
			Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	
9	483	Femoral Baseline	13:02:52		37.51		1.99	0.09	6.47	0.43	3.0442	100.00%	11:56:24		37.49		2.19	0.09	6.25	0.95	2.4189	100.00%	
		Recorded Finish Time	13:46:04			35.77							12:39:01			35.08							
		10 mins	13:55:49	00:09:45	37.60		1.64	0.07	5.05	0.37	2.8599	82.41%	12:49:02	00:10:01	37.47		1.82	0.08	5.34	0.52	2.6492	83.11%	00:09:53
		1 hour	14:46:04	01:00:00	37.64		1.79	0.10	5.37	0.51	2.7245	89.95%	13:38:32	00:59:31	37.50		1.65	0.07	5.00	0.52	2.7138	75.34%	00:59:46
		3 hours	16:12:01	02:25:57	37.77		2.23	0.18	5.34	1.08	1.9185	112.06%	16:12:01	03:33:00	37.77		4.04	0.36	8.87	2.22	1.6539	184.47%	02:59:28
		24 hours	12:35:30	22:49:26			3.24	0.10	6.37	1.47	1.5151	162.81%	12:35:30	23:56:29			4.84	0.24	8.58	2.45	1.2688	221.00%	23:22:58
		3 days	13:01:04	71:15:00			4.55	0.16	6.81	2.72	0.8986	228.64%	13:01:04	72:22:03			4.31	0.23	6.37	2.54	0.8887	196.80%	71:48:32
		1 week											11:32:02	166:53:01			5.61	0.14	11.14	2.23	1.5906	256.16%	166:53:01

Ser No	Wt	Sched Meas Pt	Invagination										Oblique End-to-End										Timing Means
			Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	Time Measuring Point hh:mm:ss	Actual Study Time Point hh:mm:ss	Temp ºC	Isch Time mm.00	Mean Flow mL/min	SD	Max	Min	PI	% of baseline	
10	451	Femoral Baseline	12:10:17		37.54		2.14	0.11	5.29	1.01	2.0073	100.00%	11:12:59		37.43		3.48	0.18	7.98	1.88	1.7567	100.00%	
		Recorded Finish Time	13:00:39			40.30							11:53:50			36.90							
		10 mins	13:10:39	00:10:00	37.30		1.87	0.09	3.38	0.98	1.2879	87.38%	12:03:50	00:10:00	37.51		2.79	0.12	5.81	1.54	1.5343	80.17%	00:10:00
		1 hour	14:04:28	01:03:49	37.57		2.97	0.20	6.07	1.63	1.4975	138.79%	12:53:50	01:00:00	37.72		2.32	0.27	6.18	0.97	2.2722	66.67%	01:01:54
		3 hours	15:27:19	02:26:40	38.24		2.29	0.16	3.99	1.38	1.1435	107.01%	15:27:19	03:33:29	38.24		2.42	0.16	4.47	1.47	1.2444	69.54%	03:00:05
		24 hours	12:28:13	23:27:34			3.65	0.17	5.51	2.51	0.8228	170.56%	12:28:13	24:34:23			2.35	0.11	3.87	1.52	1.0028	67.53%	24:00:58
		3 days	12:28:38	71:27:59			2.89	0.13	4.71	1.79	1.0164	135.05%	12:28:38	72:34:48			0.75	0.10	2.39	-0.18	3.4921	21.55%	72:01:23
		1 week																					

Table C.1. Observations from each animal in the flow experiment. Key: Wt = Animal body weight (grams); Isch = Ischaemia; PI = Pulsatility Index.

C2. Analysis

C2.1. General Linear Model: Ischaemia Time versus Ser No, Technique

```
Factor     Type   Levels  Values
Ser No     fixed      10  1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Technique  fixed       2  ETE, Sleeve


Analysis of Variance for Ischaemia Time, using Adjusted SS for Tests

Source       DF   Seq SS  Adj SS  Adj MS      F      P
Ser No        9   159.36  159.36   17.71   0.50  0.842
Technique     1   547.27  547.27  547.27  15.46  0.003
Error         9   318.51  318.51   35.39
Total        19  1025.14


S = 5.94896   R-Sq = 68.93%   R-Sq(adj) = 34.41%
```

C2.2. Regression Analysis: Ischaemia Time versus 10 min flow

```
The regression equation is
Ischaemia Time = 30.8 + 2.90 10 min flow


Predictor      Coef  SE Coef     T      P
Constant     30.793    3.907  7.88  0.000
10 min flow   2.895    1.611  1.80  0.089


S = 6.94900   R-Sq = 15.2%   R-Sq(adj) = 10.5%


Analysis of Variance

Source          DF      SS      MS     F      P
Regression       1  155.94  155.94  3.23  0.089
Residual Error  18  869.20   48.29
Total           19 1025.14


Unusual Observations

     10 min  Ischaemia
Obs    flow       Time    Fit  SE Fit  Residual  St Resid
  2    4.49      50.97  43.79    3.97      7.18      1.26 X

X denotes an observation whose X value gives it large leverage.
```

C2.3. General Linear Model: FA versus Technique, Ser No, Time

```
Factor     Type   Levels  Values
Technique  fixed       2  ObqETE, Invag
Ser No     fixed      10  1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Time       fixed       7  1 Baseline, 2 10 mins, 3 1 hour, 4 3 hours,
                       5 24 hours, 6 3 days, 7 1 week


Analysis of Variance for FA, using Adjusted SS for Tests

Source      DF   Seq SS   Adj SS  Adj MS     F      P
Technique    1    6.906    5.404   5.404  2.98  0.087
Ser No       9   35.508   26.629   2.959  1.63  0.115
Time         6   77.358   77.358  12.893  7.11  0.000
Error      110  199.507  199.507   1.814
Total      126  319.279


S = 1.34674   R-Sq = 37.51%   R-Sq(adj) = 28.42%


Unusual Observations for FA

Obs       FA      Fit   SE Fit  Residual  St Resid
  1  6.79000  3.90932  0.47130   2.88068      2.28 R
 66  6.01000  2.27699  0.48383   3.73301      2.97 R
 86  7.28000  3.68382  0.47857   3.59618      2.86 R
106  8.33000  4.12662  0.48501   4.20338      3.35 R
120  0.75000  3.80645  0.50326  -3.05645     -2.45 R
121  9.74000  4.85110  0.50792   4.88890      3.92 R
122  2.08000  5.26541  0.50144  -3.18541     -2.55 R
132  6.96000  4.38184  0.50144   2.57816      2.06 R

R denotes an observation with a large standardized residual.


Least Squares Means for FA

Ser No       Mean  SE Mean
 1          3.969   0.3599
 2          3.470   0.3599
 3          2.995   0.3599
 4          3.059   0.3908
 5          2.752   0.3919
 6          3.086   0.3599
 7          2.330   0.3743
 8          2.480   0.4577
 9          3.137   0.3745
10          2.675   0.3927
Technique
ObqETE      3.202   0.1667
Invag       2.788   0.1752
Time
1 Baseline  3.143   0.3011
2 10 mins   2.225   0.3011
3 1 hour    2.048   0.3011
4 3 hours   2.070   0.3201
5 24 hours  3.477   0.3190
6 3 days    3.920   0.3309
7 1 week    4.084   0.3680
```

```
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable FA
All Pairwise Comparisons among Levels of Technique
Technique = ObqETE  subtracted from:

Technique    Lower  Center   Upper  +---------+---------+---------+------
Invag      -0.8900 -0.4143 0.06135  (---------------*---------------)
                                    +---------+---------+---------+------
                                  -0.90     -0.60     -0.30      0.00


Tukey Simultaneous Tests
Response Variable FA
All Pairwise Comparisons among Levels of Technique
Technique = ObqETE  subtracted from:

           Difference       SE of           Adjusted
Technique    of Means  Difference  T-Value   P-Value
Invag         -0.4143      0.2400   -1.726    0.0871


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable FA
All Pairwise Comparisons among Levels of Time
Time = 1 Baseline  subtracted from:

Time         Lower  Center   Upper  -----+---------+---------+---------+-
2 10 mins   -2.197  -0.917  0.3623   (-------*-------)
3 1 hour    -2.374  -1.094  0.1858  (-------*-------)
4 3 hours   -2.393  -1.072  0.2484  (-------*--------)
5 24 hours  -0.984   0.334  1.6529          (-------*-------)
6 3 days    -0.567   0.777  2.1218            (--------*-------)
7 1 week    -0.487   0.942  2.3708             (--------*--------)
                                  -----+---------+---------+---------+-
                                    -1.6       0.0       1.6       3.2


Time = 2 10 mins  subtracted from:

Time         Lower   Center  Upper  -----+---------+---------+---------+-
3 1 hour    -1.456  -0.1765  1.103        (-------*-------)
4 3 hours   -1.476  -0.1549  1.166        (-------*-------)
5 24 hours  -0.066   1.2520  2.570                 (-------*-------)
6 3 days     0.350   1.6948  3.039                   (--------*-------)
7 1 week     0.430   1.8593  3.288                    (--------*--------)
                                  -----+---------+---------+---------+-
                                    -1.6       0.0       1.6       3.2


Time = 3 1 hour  subtracted from:

Time         Lower   Center  Upper  -----+---------+---------+---------+-
4 3 hours   -1.299  0.02165  1.342         (-------*-------)
5 24 hours   0.110  1.42848  2.747                 (-------*-------)
6 3 days     0.527  1.87128  3.216                   (--------*-------)
7 1 week     0.607  2.03578  3.465                    (--------*--------
                                  -----+---------+---------+---------+-
                                    -1.6       0.0       1.6       3.2
```

```
Time = 4 3 hours  subtracted from:

Time          Lower  Center  Upper  -----+---------+---------+---------+-
5 24 hours  0.04701   1.407  2.767                (--------*-------)
6 3 days    0.46336   1.850  3.236                   (--------*-------)
7 1 week    0.54284   2.014  3.485                   (---------*--------
)
                                     -----+---------+---------+---------+-
                                       -1.6       0.0       1.6       3.2


Time = 5 24 hours  subtracted from:

Time        Lower  Center  Upper  -----+---------+---------+---------+-
6 3 days  -0.9315  0.4428  1.817          (--------*-------)
7 1 week  -0.8501  0.6073  2.065           (--------*--------)
                                  -----+---------+---------+---------+-
                                    -1.6       0.0       1.6       3.2


Time = 6 3 days  subtracted from:

Time       Lower  Center  Upper  -----+---------+---------+---------+-
7 1 week  -1.306  0.1645  1.635         (--------*--------)
                                 -----+---------+---------+---------+-
                                   -1.6       0.0       1.6       3.2


Tukey Simultaneous Tests
Response Variable FA
All Pairwise Comparisons among Levels of Time
Time = 1 Baseline  subtracted from:

            Difference       SE of             Adjusted
Time          of Means  Difference  T-Value   P-Value
2 10 mins       -0.917      0.4259   -2.154    0.3290
3 1 hour        -1.094      0.4259   -2.569    0.1459
4 3 hours       -1.072      0.4395   -2.440    0.1923
5 24 hours       0.334      0.4387    0.762    0.9880
6 3 days         0.777      0.4474    1.737    0.5927
7 1 week         0.942      0.4755    1.981    0.4329


Time = 2 10 mins  subtracted from:

            Difference       SE of             Adjusted
Time          of Means  Difference  T-Value   P-Value
3 1 hour       -0.1765      0.4259  -0.4144    0.9996
4 3 hours      -0.1549      0.4395  -0.3523    0.9998
5 24 hours      1.2520      0.4387   2.8537    0.0741
6 3 days        1.6948      0.4474   3.7881    0.0045
7 1 week        1.8593      0.4755   3.9101    0.0030


Time = 3 1 hour  subtracted from:

            Difference       SE of             Adjusted
Time          of Means  Difference  T-Value   P-Value
4 3 hours      0.02165      0.4395  0.04926    1.0000
5 24 hours     1.42848      0.4387  3.25604    0.0245
6 3 days       1.87128      0.4474  4.18259    0.0011
7 1 week       2.03578      0.4755  4.28126    0.0008
```

Time = 4 3 hours subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
5 24 hours	1.407	0.4525	3.109	0.0374
6 3 days	1.850	0.4613	4.010	0.0021
7 1 week	2.014	0.4896	4.114	0.0014

Time = 5 24 hours subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
6 3 days	0.4428	0.4573	0.9683	0.9597
7 1 week	0.6073	0.4850	1.2523	0.8717

Time = 6 3 days subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7 1 week	0.1645	0.4892	0.3362	0.9999

C2.4. General Linear Model: PI versus Ser No, Technique, Time

```
Factor     Type   Levels  Values
Ser No     fixed      10  1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Technique  fixed       2  ETE, Sleeve
Time       fixed       7  1 Baseline, 2 10 mins, 3 1 hour, 4 3 hours, 5
24
                          hours, 6 3 days, 7 1 week


Analysis of Variance for PI, using Adjusted SS for Tests

Source      DF    Seq SS   Adj SS  Adj MS     F      P
Ser No       9   14.8286  15.4889  1.7210  2.57  0.010
Technique    1    1.3791   2.0379  2.0379  3.05  0.084
Time         6   20.5357  20.5357  3.4226  5.12  0.000
Error      110   73.5582  73.5582  0.6687
Total      126  110.3016


S = 0.817748   R-Sq = 33.31%   R-Sq(adj) = 23.61%


Unusual Observations for PI

Obs       PI      Fit   SE Fit  Residual  St Resid
  4  3.47360  1.94153  0.28805   1.53207      2.00 R
 25  3.91650  2.23587  0.28618   1.68063      2.19 R
 26  0.73190  2.49029  0.28805  -1.75839     -2.30 R
 30  6.24820  2.46665  0.30067   3.78155      4.97 R
 46  1.08030  2.70162  0.28805  -1.62132     -2.12 R
 65  6.56720  2.64837  0.29171   3.91883      5.13 R
 66  1.23740  2.90279  0.29379  -1.66539     -2.18 R
120  3.49210  1.23672  0.30558   2.25538      2.97 R

R denotes an observation with a large standardized residual.


Least Squares Means for PI

Ser No       Mean  SE Mean
 1          1.276   0.2186
 2          1.335   0.2186
 3          2.271   0.2186
 4          2.124   0.2373
 5          2.247   0.2380
 6          1.841   0.2186
 7          1.847   0.2273
 8          1.585   0.2779
 9          1.956   0.2274
10          1.492   0.2385
Technique
ObqETE      1.925   0.1012
Invag       1.670   0.1064
Time
1 Baseline  2.277   0.1829
2 10 mins   1.890   0.1829
3 1 hour    2.101   0.1829
4 3 hours   2.302   0.1944
5 24 hours  1.388   0.1937
6 3 days    1.415   0.2009
7 1 week    1.210   0.2235
```

```
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable PI
All Pairwise Comparisons among Levels of Technique
Technique = ObqETE  subtracted from:

Technique   Lower  Center   Upper  ----+---------+---------+---------+--
Invag    -0.5432  -0.2544 0.03440  (-----------------*-----------------)
                                   ----+---------+---------+---------+--
                                    -0.48     -0.32     -0.16      0.00


Tukey Simultaneous Tests
Response Variable PI
All Pairwise Comparisons among Levels of Technique
Technique = ObqETE  subtracted from:

           Difference       SE of           Adjusted
Technique    of Means  Difference  T-Value   P-Value
Invag         -0.2544      0.1457   -1.746    0.0837


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable PI
All Pairwise Comparisons among Levels of Time
Time = 1 Baseline  subtracted from:

Time         Lower  Center    Upper +---------+---------+---------+------
2 10 mins   -1.164  -0.387   0.3898         (-------*-------)
3 1 hour    -0.953  -0.176   0.6011          (-------*-------)
4 3 hours   -0.777   0.025   0.8271            (-------*-------)
5 24 hours  -1.690  -0.889  -0.0885    (-------*-------)
6 3 days    -1.678  -0.862  -0.0454    (-------*--------)
7 1 week    -1.935  -1.067  -0.1994  (-------*--------)
                             +---------+---------+---------+------
                           -2.0      -1.0       0.0       1.0


Time = 2 10 mins  subtracted from:

Time         Lower   Center   Upper +---------+---------+---------+------
3 1 hour    -0.566   0.2113  0.9885              (-------*-------)
4 3 hours   -0.389   0.4125  1.2145                (-------*-------)
5 24 hours  -1.302  -0.5018  0.2988        (-------*-------)
6 3 days    -1.291  -0.4745  0.3419        (-------*-------)
7 1 week    -1.547  -0.6797  0.1880      (-------*--------)
                           +---------+---------+---------+------
                         -2.0      -1.0       0.0       1.0


Time = 3 1 hour  subtracted from:

Time         Lower   Center     Upper +---------+---------+---------+----
4 3 hours   -0.601   0.2012   1.00315                  (-------*-------)
5 24 hours  -1.514  -0.7131   0.08747          (-------*-------)
6 3 days    -1.502  -0.6858   0.13058          (-------*-------)
7 1 week    -1.759  -0.8911  -0.02335       (--------*--------)
                             +---------+---------+---------+----
                           -2.0      -1.0       0.0       1.0


Time = 4 3 hours  subtracted from:

Time         Lower  Center    Upper +---------+---------+---------+------
5 24 hours  -1.740  -0.914  -0.0886    (-------*-------)
6 3 days    -1.729  -0.887  -0.0452    (-------*--------)
7 1 week    -1.986  -1.092  -0.1988 (--------*--------)
                             +---------+---------+---------+------
                           -2.0      -1.0       0.0       1.0
```

Time = 5 24 hours subtracted from:

```
Time       Lower   Center   Upper   +---------+---------+---------+------
6 3 days  -0.807   0.0273  0.8618              (-------*--------)
7 1 week  -1.063  -0.1780  0.7070           (--------*--------)
                                   +---------+---------+---------+------
                                  -2.0      -1.0       0.0       1.0
```

Time = 6 3 days subtracted from:

```
Time       Lower   Center   Upper   +---------+---------+---------+------
7 1 week  -1.098  -0.2052  0.6875           (--------*--------)
                                   +---------+---------+---------+------
                                  -2.0      -1.0       0.0       1.0
```

Tukey Simultaneous Tests
Response Variable PI
All Pairwise Comparisons among Levels of Time
Time = 1 Baseline subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 10 mins	-0.387	0.2586	-1.498	0.7455
3 1 hour	-0.176	0.2586	-0.681	0.9934
4 3 hours	0.025	0.2669	0.094	1.0000
5 24 hours	-0.889	0.2664	-3.338	0.0192
6 3 days	-0.862	0.2717	-3.172	0.0313
7 1 week	-1.067	0.2887	-3.696	0.0062

Time = 2 10 mins subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3 1 hour	0.2113	0.2586	0.817	0.9827
4 3 hours	0.4125	0.2669	1.546	0.7166
5 24 hours	-0.5018	0.2664	-1.884	0.4956
6 3 days	-0.4745	0.2717	-1.747	0.5866
7 1 week	-0.6797	0.2887	-2.354	0.2284

Time = 3 1 hour subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4 3 hours	0.2012	0.2669	0.754	0.9887
5 24 hours	-0.7131	0.2664	-2.677	0.1141
6 3 days	-0.6858	0.2717	-2.525	0.1608
7 1 week	-0.8911	0.2887	-3.086	0.0399

Time = 4 3 hours subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
5 24 hours	-0.914	0.2748	-3.328	0.0198
6 3 days	-0.887	0.2801	-3.167	0.0318
7 1 week	-1.092	0.2973	-3.674	0.0066

Time = 5 24 hours subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
6 3 days	0.0273	0.2777	0.0982	1.0000
7 1 week	-0.1780	0.2945	-0.6043	0.9966

Time = 6 3 days subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7 1 week	-0.2052	0.2971	-0.6909	0.9929

C2.5. Descriptive Statistics: Rel Resistance

```
Variable  Time         Mean  SE Mean  StDev  Minimum  Median  Maximum
Rel Res   1 3 hours   1.162    0.184  0.521    0.580   1.040    1.980
          2 24 hours  1.233    0.149  0.393    0.640   1.270    1.850
          3 3 days    0.917    0.191  0.506    0.260   0.910    1.880
          4 1 week    0.996    0.268  0.599    0.210   1.020    1.640
```

C2.6. Wilcoxon Signed Rank Test: Rel Resistance

```
Test of median = 1.000 versus median not = 1.000

                      N for   Wilcoxon         Estimated
                 N  N*   Test  Statistic      P     Median
Rel Resistance  27   9     27      221.5  0.442      1.070
```

C2.7. One-Sample T: Rel Resistance

```
Test of mu = 1 vs not = 1


Variable        N    Mean   StDev  SE Mean       95% CI          T      P
Rel Resistance 27  1.0863  0.4897   0.0942  (0.8926, 1.2800)  0.92  0.368
```

C2.8. General Linear Model: Resistance versus Time, Ser No

```
Factor    Type   Levels  Values
Time      fixed       4  1 3 hours, 2 24 hours, 3 3 days, 4 1 week
Ser No    fixed       9  1, 2, 4, 5, 6, 7, 8, 9, 10


Analysis of Variance for Resistance, using Adjusted SS for Tests

Source    DF  Seq SS  Adj SS  Adj MS     F      P
Time       3  0.4379  0.3411  0.1137  0.58  0.634
Ser No     8  2.8813  2.8813  0.3602  1.85  0.144
Error     15  2.9163  2.9163  0.1944
Total     26  6.2354


S = 0.440928   R-Sq = 53.23%   R-Sq(adj) = 18.93%


Unusual Observations for Resistance

Obs  Resistance      Fit   SE Fit  Residual  St Resid
  1     1.98000  1.24764  0.26834   0.73236      2.09 R
  7     0.58000  0.58000  0.44093   0.00000         * X
 28     0.21000  1.01931  0.28146  -0.80931     -2.38 R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.
```

Least Squares Means for Resistance

Time	Mean	SE Mean
1 3 hours	1.1497	0.1591
2 24 hours	1.1022	0.1783
3 3 days	0.8649	0.1800
4 1 week	0.9213	0.2185
Ser No		
1	1.1075	0.2205
2	1.1800	0.2205
4	1.2136	0.3256
5	0.9067	0.2596
6	1.5625	0.2205
7	0.6642	0.2596
8	0.4399	0.4667
9	1.3873	0.2612
10	0.6239	0.2612

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Resistance
All Pairwise Comparisons among Levels of Time
Time = 1 3 hours subtracted from:

Time	Lower	Center	Upper	+---------+---------+---------+-----+
2 24 hours	-0.744	-0.0475	0.6492	(----------*----------)
3 3 days	-0.987	-0.2847	0.4173	(----------*----------)
4 1 week	-1.023	-0.2283	0.5660	(----------*----------)
				+---------+---------+---------+-----+
				-1.00 -0.50 0.00 0.50

Time = 2 24 hours subtracted from:

Time	Lower	Center	Upper	+---------+---------+---------+-----+
3 3 days	-0.9392	-0.2372	0.4648	(----------*----------)
4 1 week	-0.9752	-0.1808	0.6135	(----------*----------)
				+---------+---------+---------+-----+
				-1.00 -0.50 0.00 0.50

Time = 3 3 days subtracted from:

Time	Lower	Center	Upper	+---------+---------+---------+-----+
4 1 week	-0.7100	0.05641	0.8228	(----------*----------)
				+---------+---------+---------+-----+
				-1.00 -0.50 0.00 0.50

Tukey Simultaneous Tests
Response Variable Resistance
All Pairwise Comparisons among Levels of Time
Time = 1 3 hours subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 24 hours	-0.0475	0.2415	-0.197	0.9972
3 3 days	-0.2847	0.2433	-1.170	0.6538
4 1 week	-0.2283	0.2753	-0.829	0.8398

Time = 2 24 hours subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3 3 days	-0.2372	0.2433	-0.9749	0.7655
4 1 week	-0.1808	0.2753	-0.6567	0.9115

Time = 3 3 days subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4 1 week	0.05641	0.2657	0.2123	0.9965

Appendix D. Experimental Results and Analysis from Casting Experiment

D1. Vessel Cross-sectional Area – Results

Animal No.	Side	Technique	Investigator	Measuring Point	Cross-Sectional Area (mm^2)
1	R	Invag	B	FA	0.4406
1	L	ObqETE	B	FA	0.2589
1	R	Invag	B	$SCEA_W$	0.1533
1	L	ObqETE	B	$SCEA_W$	0.0751
1	R	Invag	B	$SCEA_N$	0.0473
1	L	ObqETE	B	$SCEA_N$	0.0204
2	R	Invag	B	FA	0.4960
2	L	ObqETE	B	FA	0.4980
2	R	Invag	B	$SCEA_W$	0.3655
2	L	ObqETE	B	$SCEA_W$	0.2549
2	R	Invag	B	$SCEA_N$	0.1862
2	L	ObqETE	B	$SCEA_N$	0.0946
3	L	Invag	A	FA	0.5954
3	R	ObqETE	A	FA	0.5070
3	L	Invag	A	$SCEA_W$	0.3842
3	R	ObqETE	A	$SCEA_W$	0.2795
3	L	Invag	A	$SCEA_N$	0.1577
3	R	ObqETE	A	$SCEA_N$	0.2297
4	R	Invag	B	FA	0.2736
4	L	ObqETE	B	FA	0.2544
4	R	Invag	B	$SCEA_W$	0.1543
4	L	ObqETE	B	$SCEA_W$	0.1494
4	R	Invag	B	$SCEA_N$	0.0990
4	L	ObqETE	B	$SCEA_N$	0.0540
5	L	Invag	B	FA	0.5990
5	R	ObqETE	B	FA	0.4932
5	L	Invag	B	$SCEA_W$	0.3781
5	R	ObqETE	B	$SCEA_W$	0.3034
5	L	Invag	B	$SCEA_N$	0.0828
5	R	ObqETE	B	$SCEA_N$	0.2237
6	R	Invag	A	FA	0.6674
6	L	ObqETE	A	FA	0.5310
6	R	Invag	A	$SCEA_W$	0.2139
6	L	ObqETE	A	$SCEA_W$	0.3095
6	R	Invag	A	$SCEA_N$	0.0096
6	L	ObqETE	A	$SCEA_N$	0.1181
7	L	Invag	B	FA	0.4921
7	R	ObqETE	B	FA	0.2098
7	L	Invag	B	$SCEA_W$	0.2248
7	R	ObqETE	B	$SCEA_W$	0.1892
7	L	Invag	B	$SCEA_N$	0.1738
7	R	ObqETE	B	$SCEA_N$	0.0333

Table D.1. Corrosion cast cross-sectional areas (mm^2). Key: Spec = animal serial number from patency and casting experiment; FA = Femoral Artery; $SCEA_W$ = Widest point of the Superficial Caudal Epigastric Artery; $SCEA_N$ = Narrowest point of the Superficial Caudal Epigastric Artery.

D2. Vessel Cross-sectional Area – Analysis

D2.1. General Linear Model: Area versus Ser No, Side, Technique, Meas Pt

```
Factor     Type    Levels  Values
Ser No     random       7  1, 2, 3, 4, 5, 6, 7
Side       fixed        2  L, R
Technique  fixed        2  ObqETE, Invag
Meas Pt    fixed        3  FA, SCEAN, SCEAW


Analysis of Variance for Area, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS   Adj MS      F      P
Ser No        6  0.25097  0.25097  0.04183   7.16  0.000
Side          1  0.00041  0.00203  0.00203   0.35  0.560
Technique     1  0.03082  0.03082  0.03082   5.27  0.029
Meas Pt       2  0.82953  0.82953  0.41477  70.98  0.000
Error        31  0.18115  0.18115  0.00584
Total        41  1.29288


S = 0.0764437   R-Sq = 85.99%   R-Sq(adj) = 81.47%


Expected Mean Squares, using Adjusted SS

            Expected Mean Square
   Source   for Each Term
1  Ser No     (5) + 6.0000 (1)
2  Side       (5) + Q[2]
3  Technique  (5) + Q[3]
4  Meas Pt    (5) + Q[4]
5  Error      (5)


Error Terms for Tests, using Adjusted SS

                            Synthesis
   Source   Error DF  Error MS  of Error MS
1  Ser No      31.00   0.00584  (5)
2  Side        31.00   0.00584  (5)
3  Technique   31.00   0.00584  (5)
4  Meas Pt     31.00   0.00584  (5)


Variance Components, using Adjusted SS

        Estimated
Source      Value
Ser No    0.00600
Error     0.00584
```

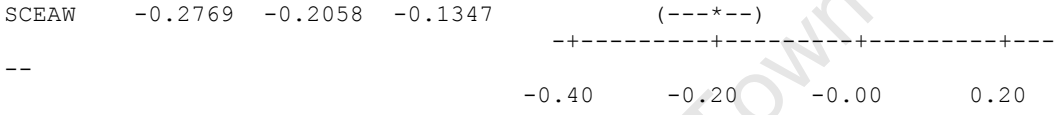
Grouping Information Using Tukey Method and 95.0% Confidence

Meas Pt	N	Mean	Grouping
FA	14	0.5	A
SCEAW	14	0.2	B
SCEAN	14	0.1	C

Means that do not share a letter are significantly different.

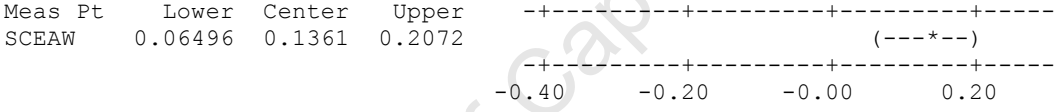
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Area
All Pairwise Comparisons among Levels of Meas Pt
Meas Pt = FA subtracted from:

Meas Pt	Lower	Center	Upper
SCEAN	-0.4130	-0.3419	-0.2708
SCEAW	-0.2769	-0.2058	-0.1347



Meas Pt = SCEAN subtracted from:

Meas Pt	Lower	Center	Upper
SCEAW	0.06496	0.1361	0.2072



Tukey Simultaneous Tests
Response Variable Area
All Pairwise Comparisons among Levels of Meas Pt
Meas Pt = FA subtracted from:

Meas Pt	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
SCEAN	-0.3419	0.02889	-11.83	0.0000
SCEAW	-0.2058	0.02889	-7.12	0.0000

Meas Pt = SCEAN subtracted from:

Meas Pt	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
SCEAW	0.1361	0.02889	4.709	0.0002

D3. Anastomotic Stenosis – Results

Animal No.	Side	Technique	Investigator	$SCEA_N$	$SCEA_W$	Percentage Stenosis ($SCEA_N$ / $SCEA_W$)
1	R	Invag	B	0.0473	0.1533	72.79
1	L	ObqETE	B	0.0204	0.0751	69.14
2	R	Invag	B	0.1862	0.3655	62.88
2	L	ObqETE	B	0.0946	0.2549	49.05
3	L	Invag	A	0.1577	0.3842	17.81
3	R	ObqETE	A	0.2297	0.2795	58.95
4	R	Invag	B	0.0990	0.1543	63.90
4	L	ObqETE	B	0.0540	0.1494	35.82
5	L	Invag	B	0.0828	0.3781	26.28
5	R	ObqETE	B	0.2237	0.3034	78.11
6	R	Invag	A	0.0096	0.2139	61.82
6	L	ObqETE	A	0.1181	0.3095	95.52
7	L	Invag	B	0.1738	0.2248	82.40
7	R	ObqETE	B	0.0333	0.1892	22.69

Table D.2. Anastomotic Stenosis. Stenosis calculated from the narrowest point of the Superficial Caudal Epigastric Artery ($SCEA_N$), and expressed as a percentage of the widest ($SCEA_W$)

D4. Anastomotic Stenosis – Analysis

D4.1. General Linear Model: Stenosis versus Ser No, Side, Technique

```
Factor      Type    Levels  Values
Ser No      random       7  1, 2, 3, 4, 5, 6, 7
Side        fixed        2  L, R
Technique   fixed        2  ETE, Invag


Analysis of Variance for Stenosis, using Adjusted SS for Tests

Source      DF  Seq SS  Adj SS  Adj MS     F      P
Ser No       6  2212.6  2212.6   368.8  0.38  0.864
Side         1   145.4   169.2   169.2  0.18  0.693
Technique    1    56.5    56.5    56.5  0.06  0.819
Error        5  4834.5  4834.5   966.9
Total       13  7249.0


S = 31.0950   R-Sq = 33.31%   R-Sq(adj) = 0.00%


Term          Coef  SE Coef      T      P
Constant    56.940    8.310   6.85  0.001
Ser No
1            14.02    20.36   0.69  0.522
2            -0.97    20.36  -0.05  0.964
3           -18.56    20.36  -0.91  0.404
4            -7.08    20.36  -0.35  0.742
```

```
5           -4.74    20.36  -0.23  0.825
6           21.73    20.36   1.07  0.335
Side
L          -3.513    8.397  -0.42  0.693
Technique
ETE         2.030    8.397   0.24  0.819


Expected Mean Squares, using Adjusted SS

              Expected Mean Square
   Source     for Each Term
1  Ser No     (4) + 2.0000 (1)
2  Side       (4) + Q[2]
3  Technique  (4) + Q[3]
4  Error      (4)


Error Terms for Tests, using Adjusted SS

                                  Synthesis
   Source     Error DF  Error MS  of Error MS
1  Ser No         5.00     966.9  (4)
2  Side           5.00     966.9  (4)
3  Technique      5.00     966.9  (4)


Variance Components, using Adjusted SS

        Estimated
Source      Value
Ser No      -299.1
Error       966.9
```

D4.2. General Linear Model: Stenosis versus Operator, Side, Technique

```
Factor      Type    Levels  Values
Operator    fixed        2  A, B
Side        fixed        2  L, R
Technique   fixed        2  ETE, Invag


Analysis of Variance for Stenosis, using Adjusted SS for Tests

Source      DF  Seq SS  Adj SS  Adj MS     F      P
Operator     1    14.1    14.1    14.1  0.02  0.890
Side         1   145.4   169.2   169.2  0.24  0.634
Technique    1    56.5    56.5    56.5  0.08  0.783
Error       10  7033.0  7033.0   703.3
Total       13  7249.0


S = 26.5198   R-Sq = 2.98%   R-Sq(adj) = 0.00%


Term          Coef  SE Coef      T      P
Constant    57.416    7.845   7.32  0.000
Operator
A            1.109    7.845   0.14  0.890
Side
L           -3.513    7.161  -0.49  0.634
Technique
ETE          2.030    7.161   0.28  0.783
```

Expected Mean Squares, using Adjusted SS

	Source	Expected Mean Square for Each Term
1	Operator	(4) + 5.7143 (1)
2	Side	(4) + Q[2]
3	Technique	(4) + Q[3]
4	Error	(4)

Error Terms for Tests, using Adjusted SS

	Source	Error DF	Error MS	Synthesis of Error MS
1	Operator	10.00	703.3	(4)
2	Side	10.00	703.3	(4)
3	Technique	10.00	703.3	(4)

Variance Components, using Adjusted SS

Source	Estimated Value
Operator	-120.6
Error	703.3

Appendix E. Experimental Results and Analysis from Histology Experiment

E1. Histology Semi-Quantitative Scoring – Results

Ser No	Day	Technique	Side	Th	EN	MN	MF	MA	AF	AA	AFB	IH
1	1	Invag	R	0	2	1	0	1	0	1	0	0
1	1	ObqETE	L	0	2	1	0	1	0	1	0	0
2	1	Invag	L	2	1	1	0	1	0	1	0	0
2	1	ObqETE	R	1	2	1	0	1	0	1	0	0
3	1	Invag	L	2	2	1	0	1	0	1	0	0
3	1	ObqETE	R	2	2	1	0	1	0	1	0	0
4	1	Invag	R	0	2	1	0	1	0	1	0	0
4	1	ObqETE	L	0	2	2	0	1	0	1	0	0
5	1	Invag	R	0	2	1	0	1	0	1	0	0
5	1	ObqETE	L	0	2	2	0	1	0	1	0	0
6	1	Invag	R	1	2	1	0	1	0	1	0	0
6	1	ObqETE	L	2	2	1	0	1	0	1	0	0
7	1	Invag	L	0	2	2	0	1	0	1	0	0
7	1	ObqETE	R	0	2	2	0	1	0	1	0	0
8	1	Invag	L	2	1	1	0	2	0	1	0	0
8	1	ObqETE	R	1	2	2	0	2	0	2	0	0
9	1	Invag	R	0	1	1	0	1	0	0	0	0
9	1	ObqETE	L	0	2	2	0	2	0	2	0	0
10	1	Invag	L	0	1	1	0	0	0	0	0	0
10	1	ObqETE	R	1	1	2	0	1	0	0	0	0
11	1	Invag	R	1	1	1	0	1	0	0	0	0
11	1	ObqETE	L	1	2	2	0	1	0	2	0	0
12	7	Invag	L	2	2	1	0	0	1	0	0	0
12	7	ObqETE	R	1	1	1	1	1	1	0	1	1
13	7	Invag	L	0	0	1	0	1	1	1	1	0
13	7	ObqETE	R	0	1	1	0	1	1	1	1	0
14	7	Invag	L	0	0	0	0	0	1	0	1	2
14	7	ObqETE	R	0	1	2	0	0	1	0	1	0
15	7	Invag	L	0	0	1	0	0	1	0	1	1
15	7	ObqETE	R	0	1	2	0	1	1	1	1	0
16	7	Invag	L	0	1	1	0	0	1	1	1	1
16	7	ObqETE	R	1	2	3	0	1	1	1	1	0
17	7	Invag	R	0	1	1	0	0	1	0	1	1
17	7	ObqETE	L	0	1	1	0	0	1	0	1	1
18	7	Invag	L	0	1	2	0	0	1	0	1	1
18	7	ObqETE	R	0	1	2	0	0	1	0	1	0
19	7	Invag	L	1	1	2	0	0	1	0	1	1
19	7	ObqETE	R	1	1	2	0	0	1	0	1	1
20	7	Invag	R	1	1	2	0	0	1	0	0	0
20	7	ObqETE	L	2	1	2	0	0	1	0	0	0
21	7	Invag	R	0	0	1	1	0	1	0	1	1
21	7	ObqETE	L	0	1	1	1	0	1	0	1	0

Ser No	Day	Technique	Side	Th	EN	MN	MF	MA	AF	AA	AFB	IH
22	7	Invag	L	2	0	0	0	0	1	0	1	1
22	7	ObqETE	R	1	0	1	1	0	1	0	1	1
23	7	Invag	L	2	1	2	1	0	1	0	1	0
23	7	ObqETE	R	2	1	2	1	0	1	0	1	0
24	7	Invag	R	0	0	1	1	0	1	0	1	0
24	7	ObqETE	L	1	0	1	1	0	1	0	1	1
25	7	Invag	R	0	0	1	1	0	1	0	1	0
25	7	ObqETE	L	2	1	1	1	0	1	0	1	0
26	7	Invag	L	0	0	0	0	0	1	0	1	0
26	7	ObqETE	R	0	0	0	0	0	1	0	1	0
27	42	Invag	L	0	0	0	0	0	2	0	1	1
27	42	ObqETE	R	0	0	0	0	0	2	0	2	1
28	42	Invag	L	0	0	0	1	0	2	0	2	2
28	42	ObqETE	R	0	0	0	1	0	2	0	2	1
29	42	Invag	L	0	0	0	1	0	1	0	1	0
29	42	ObqETE	R	0	0	0	1	0	2	0	2	0
30	42	Invag	L	0	0	0	1	0	2	0	1	1
30	42	ObqETE	R	0	0	0	1	0	2	0	1	1
31	42	Invag	R	0	0	0	1	0	2	0	1	1
31	42	ObqETE	L	0	0	0	1	0	2	0	1	0
32	42	Invag	L	0	0	0	1	0	2	0	1	1
32	42	ObqETE	R	0	0	0	1	0	2	0	1	1
33	42	Invag	R	0	0	0	1	0	2	0	1	1
33	42	ObqETE	L	0	0	0	1	0	2	0	2	0
34	238	Invag	R	0	0	0	1	0	1	0	1	0
34	238	ObqETE	L	0	0	0	1	0	1	0	1	0
35	238	Invag	R	0	0	0	1	0	1	0	1	0
35	238	ObqETE	L	0	0	0	1	0	1	0	1	1
36	238	Invag	L	0	0	0	1	0	1	0	1	0
36	238	ObqETE	R	0	0	0	1	0	1	0	1	0
37	238	Invag	L	0	0	0	1	0	1	0	2	0
37	238	ObqETE	R	0	0	0	1	0	1	0	1	0
38	238	Invag	L	0	0	0	1	0	1	0	1	1
38	238	ObqETE	R	0	0	0	1	0	1	0	1	0
39	238	Invag	L	0	0	0	1	0	1	0	1	1
39	238	ObqETE	R	0	0	0	1	0	1	0	1	0
40	238	Invag	L	0	0	0	1	0	1	0	1	0
40	238	ObqETE	R	0	0	0	1	0	1	0	1	0

Table E.1. Semi-quantitative scores of histological specimens. Scoring: 0 = Nothing identified; 1 = Mild; 2 = Moderate; 3 = Severe. Key: Th = Thrombosis; EN = Endothelial Necrosis; MN = Medial Necrosis; MF = Medial Fibrosis; MA = Medial Acute Inflammation; AF = Adventitial Fibrosis; AA = Adventitial Acute Inflammation; AFB = Adventitial Foreign Body Giant Cell Chronic Inflammation; IH = Intimal Hyperplasia.

E2. Histology Semi-Quantitative Scoring – Univariate Analysis

E2.1. General Linear Model: Th versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for Th, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS     F      P
Day      3   8.3572   8.3572  2.7857  6.36  0.001
Tech     1   0.1125   0.3008  0.3008  0.69  0.410
Side     1   0.8008   0.8008  0.8008  1.83  0.180
Error   74  32.4170  32.4170  0.4381
Total   79  41.6875


S = 0.661866   R-Sq = 22.24%   R-Sq(adj) = 16.98%


Grouping Information Using Tukey Method and 95.0% Confidence

Day      N  Mean  Grouping
  1     22   0.7  A
  7     30   0.6  A
 42     14   0.0    B
238     14  -0.0    B

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Th
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

Day      Lower   Center    Upper   --+---------+---------+---------+----
  7     -0.583  -0.0939   0.3947              (-------*--------)
 42     -1.322  -0.7273  -0.1321   (---------*---------)
238     -1.322  -0.7273  -0.1321   (---------*---------)
                                   --+---------+---------+---------+----
                                  -1.20     -0.60      0.00      0.60


Day    =   7  subtracted from:

Day      Lower   Center     Upper   --+---------+---------+---------+----
 42     -1.197  -0.6333  -0.06983     (--------*---------)
238     -1.197  -0.6333  -0.06983     (--------*---------)
                                    --+---------+---------+---------+----
                                   -1.20     -0.60      0.00      0.60


Day    =  42  subtracted from:

Day       Lower     Center   Upper   --+---------+---------+---------+---
-
238     -0.6580  -0.000000  0.6580              (----------*----------)
                                     --+---------+---------+---------+---
-
                                    -1.20     -0.60      0.00      0.60
```

```
Tukey Simultaneous Tests
Response Variable Th
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
  7        -0.0939      0.1858   -0.506    0.9575
 42        -0.7273      0.2263   -3.214    0.0103
238        -0.7273      0.2263   -3.214    0.0103


Day    =   7  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
 42        -0.6333      0.2142   -2.956    0.0213
238        -0.6333      0.2142   -2.956    0.0213


Day    =  42  subtracted from:

        Difference       SE of             Adjusted
Day       of Means  Difference    T-Value   P-Value
238      -0.000000      0.2502  -0.000000     1.000


Grouping Information Using Tukey Method and 95.0% Confidence

Tech    N  Mean  Grouping
ObqETE 40   0.4  A
Invag  40   0.3  A

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Th
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE  subtracted from:

Tech     Lower   Center   Upper   --+---------+---------+---------+----
Invag  -0.4312  -0.1267  0.1779   (---------------*--------------)
                                  --+---------+---------+---------+----
                                 -0.40     -0.20     -0.00      0.20


Tukey Simultaneous Tests
Response Variable Th
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE  subtracted from:

       Difference       SE of           Adjusted
Tech      of Means  Difference  T-Value   P-Value
Invag     -0.1267      0.1529  -0.8287    0.4100


Grouping Information Using Tukey Method and 95.0% Confidence

Side   N  Mean  Grouping
L     40   0.4  A
R     40   0.2  A

Means that do not share a letter are significantly different.
```

```
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Th
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

Side    Lower   Center    Upper  ------+---------+---------+---------+
R     -0.5112  -0.2067  0.09790  (---------------*--------------)
                                ------+---------+---------+---------+
                                   -0.40     -0.20     -0.00      0.20


Tukey Simultaneous Tests
Response Variable Th
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

      Difference       SE of           Adjusted
Side    of Means  Difference  T-Value   P-Value
R        -0.2067      0.1529   -1.352    0.1805
```

E2.2. General Linear Model: EN versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for EN, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS   Adj MS      F      P
Day      3  36.8239  36.8239  12.2746  66.64  0.000
Tech     1   1.0125   1.0208   1.0208   5.54  0.021
Side     1   0.0208   0.0208   0.0208   0.11  0.738
Error   74  13.6303  13.6303   0.1842
Total   79  51.4875


S = 0.429177   R-Sq = 73.53%   R-Sq(adj) = 71.74%


Grouping Information Using Tukey Method and 95.0% Confidence

Day      N  Mean  Grouping
  1     22   1.7  A
  7     30   0.7    B
 42     14   0.0      C
238     14  -0.0      C

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable EN
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

Day      Lower  Center   Upper     +---------+---------+---------+------
  7     -1.344  -1.027  -0.710               (---*----)
 42     -2.113  -1.727  -1.341     (----*-----)
238     -2.113  -1.727  -1.341     (----*-----)
                                   +---------+---------+---------+------
                                -2.10     -1.40     -0.70      0.00
```

```
Day    =   7  subtracted from:

Day      Lower   Center    Upper     +---------+---------+---------+-----
-
 42     -1.065  -0.7000  -0.3346                   (----*----)
238     -1.065  -0.7000  -0.3346                   (----*----)
                                   +---------+---------+---------+-----
-
                                -2.10     -1.40     -0.70      0.00


Day    =  42  subtracted from:

Day       Lower     Center   Upper     +---------+---------+---------+---
---
238     -0.4267  -0.000000  0.4267                            (-----*---
--)
                                     +---------+---------+---------+---
---
                                  -2.10     -1.40     -0.70      0.00


Tukey Simultaneous Tests
Response Variable EN
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
  7         -1.027      0.1205    -8.53    0.0000
 42         -1.727      0.1467   -11.77    0.0000
238         -1.727      0.1467   -11.77    0.0000


Day    =   7  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
 42        -0.7000      0.1389   -5.039    0.0000
238        -0.7000      0.1389   -5.039    0.0000


Day    =  42  subtracted from:

        Difference       SE of             Adjusted
Day       of Means  Difference    T-Value   P-Value
238      -0.000000      0.1622  -0.000000     1.000


Grouping Information Using Tukey Method and 95.0% Confidence

Tech    N  Mean  Grouping
ObqETE 40   0.7  A
Invag  40   0.5    B

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable EN
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE  subtracted from:

Tech     Lower   Center     Upper  ------+---------+---------+---------+
Invag  -0.4308  -0.2333  -0.03584  (----------------*---------------)
                                  ------+---------+---------+---------+
                                     -0.36     -0.24     -0.12      0.00
```

```
Tukey Simultaneous Tests
Response Variable EN
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE  subtracted from:

       Difference       SE of           Adjusted
Tech     of Means  Difference  T-Value   P-Value
Invag     -0.2333     0.09911   -2.354    0.0212


Grouping Information Using Tukey Method and 95.0% Confidence

Side   N  Mean  Grouping
L     40   0.6  A
R     40   0.6  A

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable EN
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

Side    Lower    Center   Upper  ---------+---------+---------+-------
R     -0.2308  -0.03333  0.1642  (---------------*---------------)
                                 ---------+---------+---------+-------
                                       -0.12     0.00      0.12


Tukey Simultaneous Tests
Response Variable EN
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

      Difference       SE of           Adjusted
Side    of Means  Difference  T-Value   P-Value
R       -0.03333     0.09911  -0.3363    0.7376
```

E2.3. General Linear Model: MN versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for MN, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS   Adj MS      F      P
Day      3  31.2424  31.2424  10.4141  40.25  0.000
Tech     1   1.8000   1.6133   1.6133   6.24  0.015
Side     1   0.0133   0.0133   0.0133   0.05  0.821
Error   74  19.1442  19.1442   0.2587
Total   79  52.2000


S = 0.508631   R-Sq = 63.33%   R-Sq(adj) = 60.85%
```

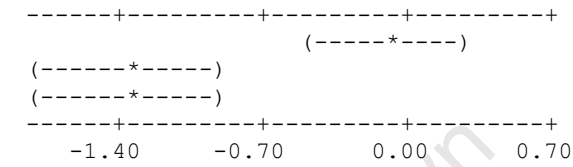
Grouping Information Using Tukey Method and 95.0% Confidence

Day	N	Mean	Grouping
1	22	1.4	A
7	30	1.3	A
42	14	0.0	B
238	14	-0.0	B

Means that do not share a letter are significantly different.

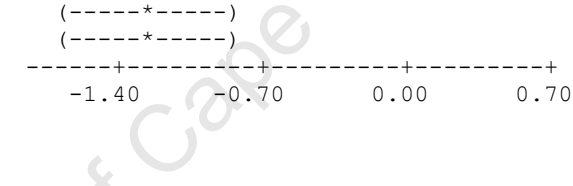
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable MN
All Pairwise Comparisons among Levels of Day
Day = 1 subtracted from:

Day	Lower	Center	Upper
7	-0.473	-0.097	0.2786
42	-1.821	-1.364	-0.9062
238	-1.821	-1.364	-0.9062



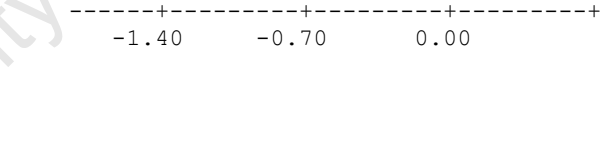
Day = 7 subtracted from:

Day	Lower	Center	Upper
42	-1.700	-1.267	-0.8336
238	-1.700	-1.267	-0.8336



Day = 42 subtracted from:

Day	Lower	Center	Upper
238	-0.5057	-0.000000	0.5057



Tukey Simultaneous Tests
Response Variable MN
All Pairwise Comparisons among Levels of Day
Day = 1 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	-0.097	0.1428	-0.679	0.9047
42	-1.364	0.1739	-7.842	0.0000
238	-1.364	0.1739	-7.842	0.0000

Day = 7 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
42	-1.267	0.1646	-7.694	0.0000
238	-1.267	0.1646	-7.694	0.0000

Day = 42 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
238	-0.000000	0.1922	-0.000000	1.000

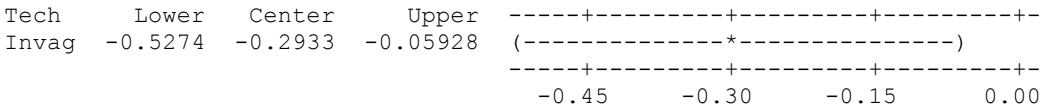
Grouping Information Using Tukey Method and 95.0% Confidence

Tech	N	Mean	Grouping
ObqETE	40	0.8	A
Invag	40	0.5	B

Means that do not share a letter are significantly different.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable MN
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE subtracted from:

Tech	Lower	Center	Upper
Invag	-0.5274	-0.2933	-0.05928



Tukey Simultaneous Tests
Response Variable MN
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE subtracted from:

Tech	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Invag	-0.2933	0.1175	-2.497	0.0148

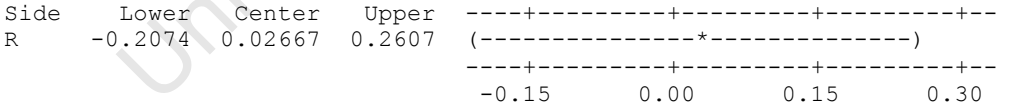
Grouping Information Using Tukey Method and 95.0% Confidence

Side	N	Mean	Grouping
R	40	0.7	A
L	40	0.6	A

Means that do not share a letter are significantly different.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable MN
All Pairwise Comparisons among Levels of Side
Side = L subtracted from:

Side	Lower	Center	Upper
R	-0.2074	0.02667	0.2607



Tukey Simultaneous Tests
Response Variable MN
All Pairwise Comparisons among Levels of Side
Side = L subtracted from:

Side	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
R	0.02667	0.1175	0.2270	0.8210

E2.4. General Linear Model: MA versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for MA, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS      F      P
Day      3  16.5027  16.5027  5.5009  53.34  0.000
Tech     1   0.3125   0.2408  0.2408   2.34  0.131
Side     1   0.0408   0.0408  0.0408   0.40  0.531
Error   74   7.6315   7.6315  0.1031
Total   79  24.4875


S = 0.321136   R-Sq = 68.84%   R-Sq(adj) = 66.73%


Grouping Information Using Tukey Method and 95.0% Confidence

Day      N  Mean  Grouping
  1     22   1.1  A
  7     30   0.2    B
 42     14   0.0    B
238     14  -0.0    B

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable MA
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

Day      Lower  Center    Upper --------+---------+---------+--------
  7     -1.161  -0.924  -0.6871       (----*---)
 42     -1.380  -1.091  -0.8021  (-----*-----)
238     -1.380  -1.091  -0.8021  (-----*-----)
                           --------+---------+---------+--------
                                -1.00     -0.50      0.00


Day    =   7  subtracted from:

Day       Lower   Center   Upper  --------+---------+---------+--------
 42     -0.4401  -0.1667  0.1067                    (-----*----)
238     -0.4401  -0.1667  0.1067                    (-----*----)
                                 --------+---------+---------+--------
                                      -1.00     -0.50      0.00


Day    =  42  subtracted from:

Day       Lower     Center   Upper  --------+---------+---------+--------
238     -0.3193  -0.000000  0.3193                       (-----*-----)
                                   --------+---------+---------+--------
                                        -1.00     -0.50      0.00
```

Tukey Simultaneous Tests
Response Variable MA
All Pairwise Comparisons among Levels of Day
Day = 1 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	-0.924	0.09014	-10.25	0.0000
42	-1.091	0.10979	-9.94	0.0000
238	-1.091	0.10979	-9.94	0.0000

Day = 7 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
42	-0.1667	0.1039	-1.603	0.3830
238	-0.1667	0.1039	-1.603	0.3830

Day = 42 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
238	-0.000000	0.1214	-0.000000	1.000

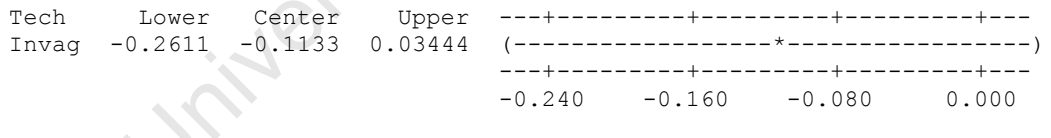
Grouping Information Using Tukey Method and 95.0% Confidence

Tech	N	Mean	Grouping
ObqETE	40	0.4	A
Invag	40	0.3	A

Means that do not share a letter are significantly different.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable MA
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE subtracted from:

Tech	Lower	Center	Upper
Invag	-0.2611	-0.1133	0.03444



Tukey Simultaneous Tests
Response Variable MA
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE subtracted from:

Tech	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Invag	-0.1133	0.07416	-1.528	0.1307

Grouping Information Using Tukey Method and 95.0% Confidence

Side	N	Mean	Grouping
R	40	0.3	A
L	40	0.3	A

Means that do not share a letter are significantly different.

```
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable MA
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

Side    Lower   Center   Upper  ---+---------+---------+---------+---
R     -0.1011  0.04667  0.1944  (-----------------*-----------------)
                                ---+---------+---------+---------+---
                                -0.080     0.000     0.080     0.160


Tukey Simultaneous Tests
Response Variable MA
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

      Difference       SE of           Adjusted
Side    of Means  Difference  T-Value   P-Value
R        0.04667     0.07416   0.6292    0.5311
```

E2.5. General Linear Model: AA versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for AA, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS     F      P
Day      3  12.4288  12.4288  4.1429  29.18  0.000
Tech     1   0.4500   0.5633  0.5633   3.97  0.050
Side     1   0.1633   0.1633  0.1633   1.15  0.287
Error   74  10.5079  10.5079  0.1420
Total   79  23.5500


S = 0.376827   R-Sq = 55.38%   R-Sq(adj) = 52.37%


Grouping Information Using Tukey Method and 95.0% Confidence

Day      N  Mean  Grouping
  1     22   1.0  A
  7     30   0.2     B
 42     14   0.0     B
238     14  -0.0     B

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable AA
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

Day      Lower   Center    Upper  ------+---------+---------+---------+
  7     -1.066  -0.7879  -0.5097       (----*-----)
 42     -1.293  -0.9545  -0.6157  (------*------)
238     -1.293  -0.9545  -0.6157  (------*------)
                                 ------+---------+---------+---------+
                                    -1.00     -0.50      0.00      0.50
```

```
Day    =   7  subtracted from:

Day       Lower   Center   Upper  ------+---------+---------+---------+
 42     -0.4875  -0.1667  0.1542                  (------*-----)
238     -0.4875  -0.1667  0.1542                  (------*-----)
                                  ------+---------+---------+---------+
                                     -1.00     -0.50      0.00      0.50


Day    =  42  subtracted from:

Day       Lower     Center   Upper  ------+---------+---------+---------+
238     -0.3746  -0.000000  0.3746                     (------*------)
                                  ------+---------+---------+---------+
                                     -1.00     -0.50      0.00
0.50


Tukey Simultaneous Tests
Response Variable AA
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
  7        -0.7879      0.1058   -7.449    0.0000
 42        -0.9545      0.1288   -7.409    0.0000
238        -0.9545      0.1288   -7.409    0.0000


Day    =   7  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
 42        -0.1667      0.1220   -1.366    0.5242
238        -0.1667      0.1220   -1.366    0.5242


Day    =  42  subtracted from:

        Difference       SE of             Adjusted
Day       of Means  Difference    T-Value   P-Value
238     -0.000000      0.1424  -0.000000     1.000


Grouping Information Using Tukey Method and 95.0% Confidence

Tech    N  Mean  Grouping
ObqETE 40   0.4  A
Invag  40   0.2  A

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable AA
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE  subtracted from:

Tech     Lower   Center     Upper  -----+---------+---------+---------+-
Invag  -0.3467  -0.1733  0.000067  (-----------------*----------------)
                                  -----+---------+---------+---------+-
                                    -0.30     -0.20     -0.10     -0.00
```

```
Tukey Simultaneous Tests
Response Variable AA
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE  subtracted from:

       Difference       SE of           Adjusted
Tech     of Means  Difference  T-Value   P-Value
Invag     -0.1733     0.08702   -1.992    0.0501


Grouping Information Using Tukey Method and 95.0% Confidence

Side   N  Mean  Grouping
L     40   0.3  A
R     40   0.2  A

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable AA
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

Side    Lower    Center    Upper  -------+---------+---------+---------
R     -0.2667  -0.09333  0.08007  (------------------*----------------)
                                  -------+---------+---------+---------
                                      -0.20     -0.10     -0.00


Tukey Simultaneous Tests
Response Variable AA
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

      Difference       SE of          Adjusted
Side    of Means  Difference  T-Value   P-Value
R       -0.09333     0.08702   -1.072    0.2870
```

E2.6. General Linear Model: MF versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for MF, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS      F      P
Day      3  11.4190  11.4190  3.8063  33.93  0.000
Tech     1   0.0500   0.0300  0.0300   0.27  0.607
Side     1   0.0300   0.0300  0.0300   0.27  0.607
Error   74   8.3010   8.3010  0.1122
Total   79  19.8000


S = 0.334925   R-Sq = 58.08%   R-Sq(adj) = 55.24%
```

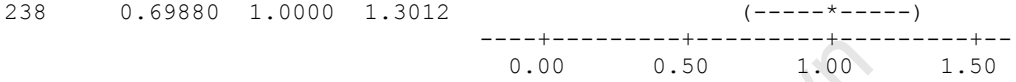
Grouping Information Using Tukey Method and 95.0% Confidence

Day	N	Mean	Grouping
238	14	1.0	A
42	14	0.9	A
7	30	0.3	B
1	22	-0.0	C

Means that do not share a letter are significantly different.

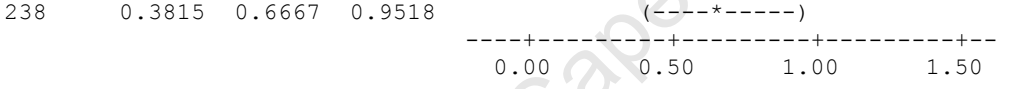
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable MF
All Pairwise Comparisons among Levels of Day
Day = 1 subtracted from:

Day	Lower	Center	Upper
7	0.08604	0.3333	0.5806
42	0.55594	0.8571	1.1583
238	0.69880	1.0000	1.3012



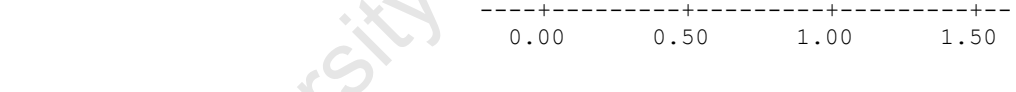
Day = 7 subtracted from:

Day	Lower	Center	Upper
42	0.2387	0.5238	0.8090
238	0.3815	0.6667	0.9518



Day = 42 subtracted from:

Day	Lower	Center	Upper
238	-0.1901	0.1429	0.4758



Tukey Simultaneous Tests
Response Variable MF
All Pairwise Comparisons among Levels of Day
Day = 1 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	0.3333	0.09401	3.546	0.0037
42	0.8571	0.11450	7.486	0.0000
238	1.0000	0.11450	8.733	0.0000

Day = 7 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
42	0.5238	0.1084	4.832	0.0001
238	0.6667	0.1084	6.150	0.0000

Day = 42 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
238	0.1429	0.1266	1.129	0.6732

Grouping Information Using Tukey Method and 95.0% Confidence

Tech	N	Mean	Grouping
ObqETE	40	0.6	A
Invag	40	0.5	A

Means that do not share a letter are significantly different.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable MF
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE subtracted from:

Tech	Lower	Center	Upper	---------+---------+---------+-------
Invag	-0.1941	-0.04000	0.1141	(--------------*--------------)
				---------+---------+---------+-------
				-0.10 0.00 0.10

Tukey Simultaneous Tests
Response Variable MF
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE subtracted from:

Tech	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Invag	-0.04000	0.07735	-0.5171	0.6066

Grouping Information Using Tukey Method and 95.0% Confidence

Side	N	Mean	Grouping
R	40	0.6	A
L	40	0.5	A

Means that do not share a letter are significantly different.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable MF
All Pairwise Comparisons among Levels of Side
Side = L subtracted from:

Side	Lower	Center	Upper	-+---------+---------+---------+-----
R	-0.1141	0.04000	0.1941	(--------------*--------------)
				-+---------+---------+---------+-----
				-0.10 0.00 0.10 0.20

Tukey Simultaneous Tests
Response Variable MF
All Pairwise Comparisons among Levels of Side
Side = L subtracted from:

Side	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
R	0.04000	0.07735	0.5171	0.6066

E2.7. General Linear Model: AF versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for AF, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS   Adj MS       F      P
Day      3  33.0589  33.0589  11.0196  897.51  0.000
Tech     1   0.0125   0.0075   0.0075    0.61  0.437
Side     1   0.0075   0.0075   0.0075    0.61  0.437
Error   74   0.9086   0.9086   0.0123
Total   79  33.9875


S = 0.110806   R-Sq = 97.33%   R-Sq(adj) = 97.15%


Grouping Information Using Tukey Method and 95.0% Confidence

Day      N  Mean  Grouping
 42     14   1.9  A
238     14   1.0    B
  7     30   1.0    B
  1     22  -0.0      C

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable AF
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

Day      Lower  Center  Upper     +---------+---------+---------+------
  7     0.9182   1.000  1.082                       (*)
 42     1.8289   1.929  2.028                                 (*)
238     0.9004   1.000  1.100                       (*)
                           +---------+---------+---------+------
                        -1.0       0.0       1.0       2.0


Day    =   7  subtracted from:

Day        Lower    Center    Upper     +---------+---------+---------+--
----
 42      0.83423  0.928571  1.02291                       (*)
238     -0.09434  0.000000  0.09434              (*)
                                      +---------+---------+---------+--
----
                                   -1.0       0.0       1.0       2.0


Day    =  42  subtracted from:

Day      Lower   Center    Upper     +---------+---------+---------+-----
-
238     -1.039  -0.9286  -0.8184     (*)
                                     +---------+---------+---------+-----
-
                                  -1.0       0.0       1.0       2.0
```

```
Tukey Simultaneous Tests
Response Variable AF
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
  7          1.000     0.03110    32.15    0.0000
 42          1.929     0.03788    50.91    0.0000
238          1.000     0.03788    26.40    0.0000


Day    =   7  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
 42       0.928571     0.03586  25.8911    0.0000
238       0.000000     0.03586   0.0000    1.0000


Day    =  42  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
238        -0.9286     0.04188   -22.17    0.0000


Grouping Information Using Tukey Method and 95.0% Confidence

Tech    N  Mean  Grouping
ObqETE 40   1.0  A
Invag  40   1.0  A

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable AF
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE  subtracted from:

Tech      Lower    Center     Upper  ----+---------+---------+---------+--
Invag  -0.07099  -0.02000   0.03099  (-----------------*----------------)
                                    ----+---------+---------+---------+--
                                     -0.060    -0.030     0.000     0.030


Tukey Simultaneous Tests
Response Variable AF
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE  subtracted from:

       Difference       SE of           Adjusted
Tech     of Means  Difference  T-Value   P-Value
Invag    -0.02000     0.02559  -0.7816    0.4370


Grouping Information Using Tukey Method and 95.0% Confidence

Side   N  Mean  Grouping
R     40   1.0  A
L     40   1.0  A

Means that do not share a letter are significantly different.
```

```
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable AF
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

Side     Lower   Center    Upper     +---------+---------+---------+-----
-
R     -0.03099  0.02000  0.07099     (-----------------*----------------)
                                     +---------+---------+---------+-----
-
                                  -0.030     0.000     0.030     0.060


Tukey Simultaneous Tests
Response Variable AF
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

      Difference       SE of           Adjusted
Side    of Means  Difference  T-Value   P-Value
R        0.02000     0.02559   0.7816    0.4370
```

E2.8. General Linear Model: AFB versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for AFB, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS      F      P
Day      3  19.6446  19.6446  6.5482  72.01  0.000
Tech     1   0.1125   0.1008  0.1008   1.11  0.296
Side     1   0.0008   0.0008  0.0008   0.01  0.924
Error   74   6.7295   6.7295  0.0909
Total   79  26.4875


S = 0.301562   R-Sq = 74.59%   R-Sq(adj) = 72.88%


Grouping Information Using Tukey Method and 95.0% Confidence

Day      N  Mean  Grouping
 42     14   1.4  A
238     14   1.1  A B
  7     30   0.9    B
  1     22  -0.0      C

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable AFB
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

Day      Lower  Center  Upper     +---------+---------+---------+------
  7     0.6773  0.9000  1.123                        (---*---)
 42     1.0859  1.3571  1.628                               (----*---)
238     0.8002  1.0714  1.343                          (----*---)
                                  +---------+---------+---------+------
                               -0.60      0.00      0.60      1.20
```

```
Day    =   7  subtracted from:

Day        Lower  Center   Upper     +---------+---------+---------+-----
-
 42      0.20040  0.4571  0.7139                (----*---)
238     -0.08532  0.1714  0.4282             (---*---)
                                     +---------+---------+---------+-----
-
                                   -0.60      0.00      0.60      1.20


Day    =  42  subtracted from:

Day        Lower   Center    Upper     +---------+---------+---------+----
--
238     -0.5855  -0.2857  0.01410      (----*----)
                                      +---------+---------+---------+----
--
                                  -0.60      0.00      0.60      1.20


Tukey Simultaneous Tests
Response Variable AFB
All Pairwise Comparisons among Levels of Day
Day    =   1  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
  7         0.9000     0.08465    10.63    0.0000
 42         1.3571     0.10310    13.16    0.0000
238         1.0714     0.10310    10.39    0.0000


Day    =   7  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
 42         0.4571     0.09761    4.684    0.0001
238         0.1714     0.09761    1.756    0.3026


Day    =  42  subtracted from:

        Difference       SE of           Adjusted
Day       of Means  Difference  T-Value   P-Value
238        -0.2857      0.1140   -2.507    0.0671


Grouping Information Using Tukey Method and 95.0% Confidence

Tech    N  Mean  Grouping
ObqETE 40   0.9  A
Invag  40   0.8  A

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable AFB
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE  subtracted from:

Tech     Lower    Center    Upper  -------+---------+---------+---------
Invag  -0.2121  -0.07333  0.06543  (-----------------*----------------)
                                  -------+---------+---------+---------
                                      -0.160    -0.080     0.000
```

```
Tukey Simultaneous Tests
Response Variable AFB
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE  subtracted from:

       Difference       SE of           Adjusted
Tech     of Means  Difference  T-Value   P-Value
Invag    -0.07333     0.06964   -1.053    0.2958


Grouping Information Using Tukey Method and 95.0% Confidence

Side   N  Mean  Grouping
R     40   0.8  A
L     40   0.8  A

Means that do not share a letter are significantly different.


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable AFB
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

Side    Lower    Center   Upper  -------+---------+---------+---------
R     -0.1321  0.006667  0.1454  (-----------------*----------------)
                                 -------+---------+---------+---------
                                     -0.080     0.000     0.080


Tukey Simultaneous Tests
Response Variable AFB
All Pairwise Comparisons among Levels of Side
Side = L  subtracted from:

      Difference       SE of           Adjusted
Side    of Means  Difference  T-Value   P-Value
R       0.006667     0.06964  0.09573    0.9240
```

E2.9. General Linear Model: IH versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for IH, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS     F      P
Day      3   6.0190   6.0190  2.0063  9.79  0.000
Tech     1   0.8000   0.5633  0.5633  2.75  0.102
Side     1   0.2133   0.2133  0.2133  1.04  0.311
Error   74  15.1676  15.1676  0.2050
Total   79  22.2000


S = 0.452734   R-Sq = 31.68%   R-Sq(adj) = 27.06%
```

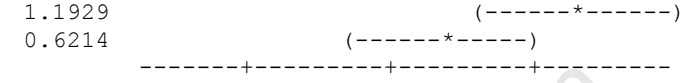
Grouping Information Using Tukey Method and 95.0% Confidence

Day	N	Mean	Grouping
42	14	0.8	A
7	30	0.5	A B
238	14	0.2	B C
1	22	-0.0	C

Means that do not share a letter are significantly different.

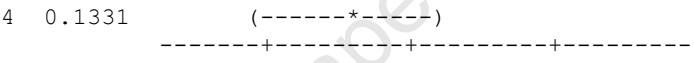
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable IH
All Pairwise Comparisons among Levels of Day
Day = 1 subtracted from:

Day	Lower	Center	Upper
7	0.1324	0.4667	0.8009
42	0.3786	0.7857	1.1929
238	-0.1929	0.2143	0.6214



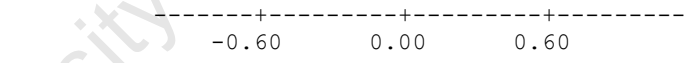
Day = 7 subtracted from:

Day	Lower	Center	Upper
42	-0.0664	0.3190	0.7045
238	-0.6378	-0.2524	0.1331



Day = 42 subtracted from:

Day	Lower	Center	Upper
238	-1.022	-0.5714	-0.1213



Tukey Simultaneous Tests
Response Variable IH
All Pairwise Comparisons among Levels of Day
Day = 1 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	0.4667	0.1271	3.672	0.0025
42	0.7857	0.1548	5.076	0.0000
238	0.2143	0.1548	1.384	0.5130

Day = 7 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
42	0.3190	0.1465	2.177	0.1392
238	-0.2524	0.1465	-1.722	0.3196

Day = 42 subtracted from:

Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
238	-0.5714	0.1711	-3.339	0.0071

Grouping Information Using Tukey Method and 95.0% Confidence

Tech	N	Mean	Grouping
Invag	40	0.5	A
ObqETE	40	0.3	A

Means that do not share a letter are significantly different.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable IH
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE subtracted from:

Tech	Lower	Center	Upper	---+---------+---------+---------+---
Invag	-0.03500	0.1733	0.3817	(----------*----------)
				---+---------+---------+---------+---
				0.00 0.12 0.24 0.36

Tukey Simultaneous Tests
Response Variable IH
All Pairwise Comparisons among Levels of Tech
Tech = ObqETE subtracted from:

Tech	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Invag	0.1733	0.1046	1.658	0.1016

Grouping Information Using Tukey Method and 95.0% Confidence

Side	N	Mean	Grouping
L	40	0.4	A
R	40	0.3	A

Means that do not share a letter are significantly different.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable IH
All Pairwise Comparisons among Levels of Side
Side = L subtracted from:

Side	Lower	Center	Upper	------+---------+---------+---------+
R	-0.3150	-0.1067	0.1017	(----------*----------)
				------+---------+---------+---------+
				-0.24 -0.12 0.00 0.12

Tukey Simultaneous Tests
Response Variable IH
All Pairwise Comparisons among Levels of Side
Side = L subtracted from:

Side	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
R	-0.1067	0.1046	-1.020	0.3110

E3. Histology Semi-Quantitative Scoring - Multivariate Analysis by Principal Component Analysis

E3.1. Eigenanalysis of the Correlation Matrix

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	5.2821	0.9588	0.8977	0.5886	0.5012	0.3258	0.1889	0.1489	0.1080
Proportion	0.587	0.107	0.100	0.065	0.056	0.036	0.021	0.017	0.012
Cumulative	0.587	0.693	0.793	0.859	0.914	0.950	0.971	0.988	1.000
Th	0.190	0.743	-0.419	-0.415	-0.094	0.217	-0.035	0.054	-0.061
EN	0.400	0.083	0.090	0.095	-0.110	-0.165	0.615	0.349	0.526
MN	0.317	0.337	0.130	0.565	-0.445	-0.334	-0.268	-0.200	-0.171
MF	-0.321	-0.156	-0.435	-0.274	-0.353	-0.692	0.063	0.017	0.016
MA	0.370	-0.249	0.116	-0.405	-0.222	0.050	-0.419	-0.365	0.512
AF	-0.382	0.125	0.139	0.014	-0.386	0.283	0.478	-0.594	0.088
AA	0.355	-0.293	0.223	-0.347	-0.395	0.110	0.225	0.153	-0.613
AFB	-0.374	0.006	0.117	0.081	-0.529	0.329	-0.295	0.565	0.211
NI	-0.226	0.375	0.716	-0.366	0.145	-0.363	-0.057	0.069	-0.005

Table E2. Eigenanalysis of the Correlation Matrix (Table E1.). Key: PC1 = First Principal Component, etc.; Th = Thrombosis; EN = Endothelial Necrosis; MN = Medial Necrosis; MF = Medial Fibrosis; MA = Medial Acute Inflammation; AF = Adventitial Fibrosis; AA = Adventitial Acute Inflammation; AFB = Adventitial Foreign Body Giant Cell Chronic Inflammation; IH = Intimal Hyperplasia.

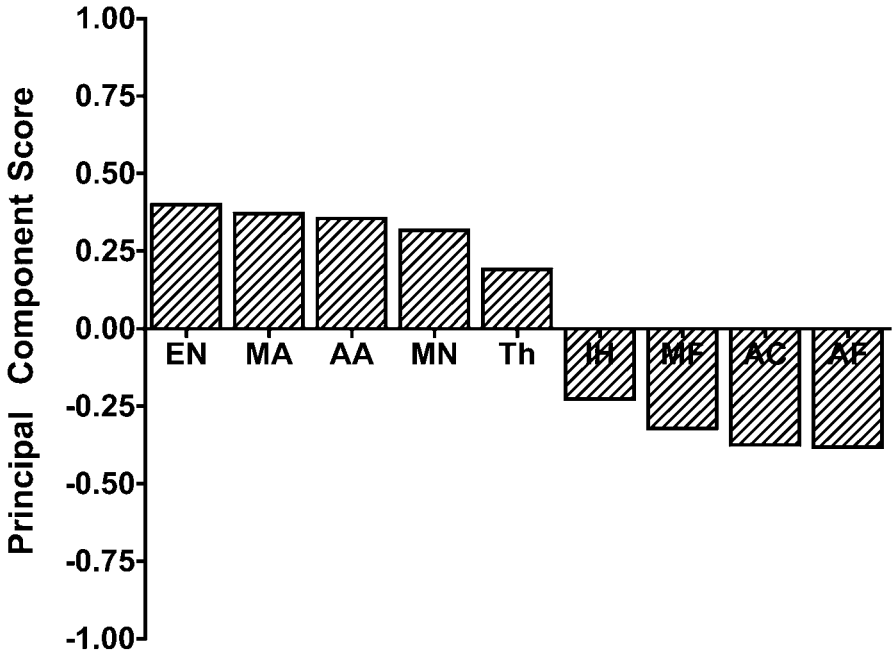


Figure E.1. Principal Component Analysis of the nine observations. First Component. Principal component proportion = 58.7%. Key: EN = Endothelial Necrosis, MA = Medial Acute Inflammation; AA = Adventitial Acute Inflammation; MN = Medial Necrosis; Th = Thrombosis; IH = Intimal Hyperplasia; MF = Medial Fibrosis; AC = Adventitial Chronic Inflammation; AF = Adventitial Fibrosis.

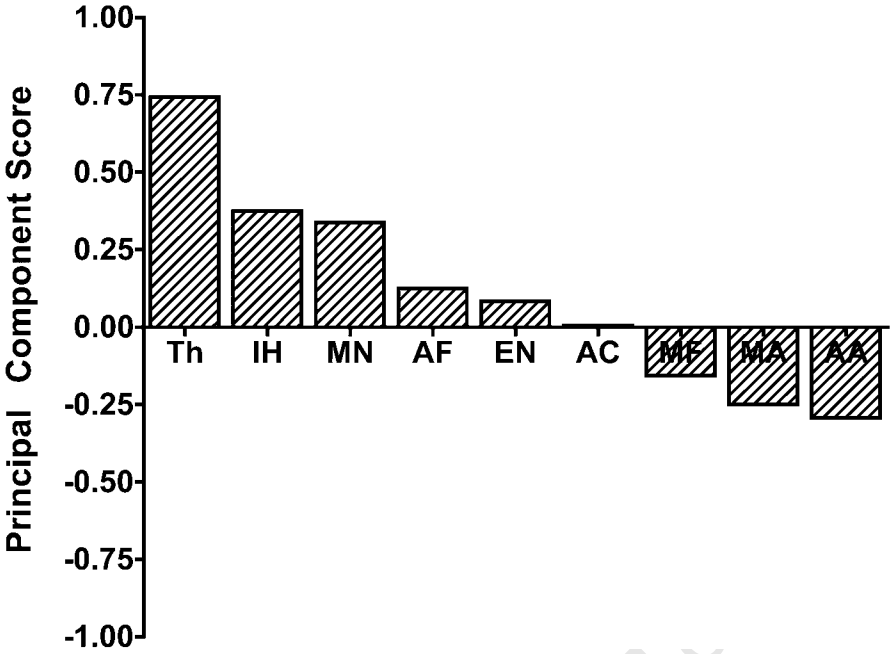


Figure E.2. Principal Component Analysis of the nine observations. Second Component. Principal component proportion = 10.7%. Th = Thrombosis; IH = Intimal Hyperplasia; MN = Medial Necrosis; AF = Adventitial Fibrosis; EN = Endothelial Necrosis; AC = Adventitial Chronic Inflammation; MF = Medial Fibrosis; MA = Medial Acute Inflammation; AA = Adventitial Acute Inflammation.

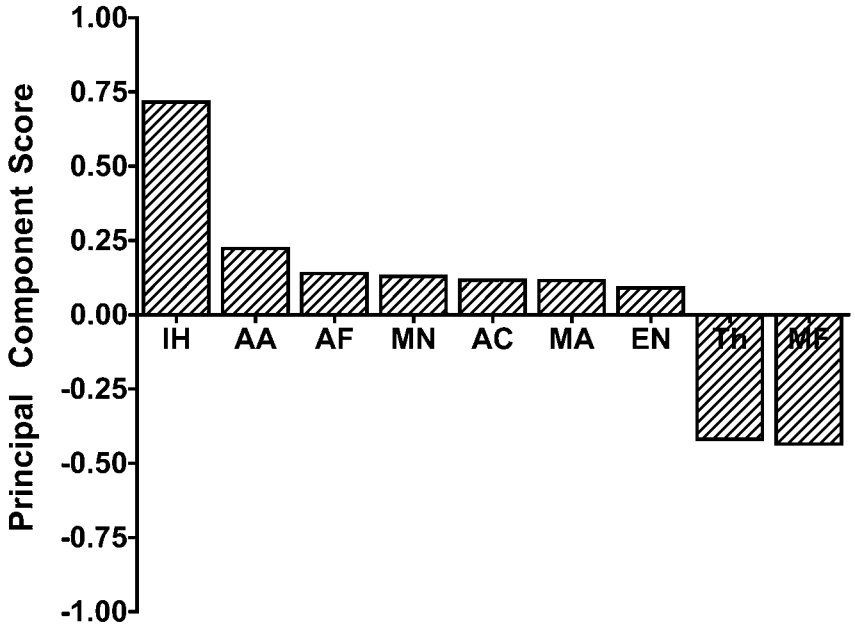


Figure E.3. Principal Component Analysis of the nine observations. Third Component. Principal component proportion = 10.0%. IH = Intimal Hyperplasia; AA = Adventitial Acute Inflammation; AF = Adventitial Fibrosis; MN = Medial Necrosis; AC = Adventitial Chronic Inflammation; MA = Medial Acute Inflammation; EN = Endothelial Necrosis; Th = Thrombosis; MF = Medial Fibrosis.

E3.2. Correlations: Th, EN, IH, MN, MF, MA, AF, AA, AFB

	Th	EN	IH	MN	MF	MA	AF	AA
EN	0.40							
IH	-0.17	-0.40						
MN	0.37	0.73	-0.29					
MF	-0.24	-0.68	0.16	-0.58				
MA	0.26	0.72	-0.39	0.48	-0.55			
AF	-0.32	-0.75	0.52	-0.53	0.58	-0.72		
AA	0.18	0.74	-0.35	0.48	-0.54	0.86	-0.64	
AFB	-0.38	-0.76	0.44	-0.51	0.59	-0.67	0.83	-0.59

Table E.3. Pearson Correlations: Th, EN, IH, MN, MF, MA, AF, AA, AFB. Key as before.

E3.3. Principal Component Scores, PCs 1-3.

Ser No	Day	Technique	Side	PC1	PC2	PC3
1	1	Invag	R	2.880	-1.187	0.401
1	1	ObqETE	L	2.880	-1.187	0.401
2	1	Invag	L	3.142	-0.165	-0.176
2	1	ObqETE	R	2.908	0.755	-0.865
3	1	Invag	L	3.404	0.858	-0.753
3	1	ObqETE	R	3.404	0.858	-0.753
4	1	Invag	R	3.270	-0.772	0.561
4	1	ObqETE	L	2.880	-1.187	0.401
5	1	Invag	R	3.270	-0.772	0.561
5	1	ObqETE	L	2.880	-1.187	0.401
6	1	Invag	R	3.404	0.858	-0.753
6	1	ObqETE	L	3.142	-0.165	-0.176
7	1	Invag	L	3.270	-0.772	0.561
7	1	ObqETE	R	3.270	-0.772	0.561
8	1	Invag	L	4.848	-0.734	0.601
8	1	ObqETE	R	3.573	0.308	-0.656
9	1	Invag	R	4.586	-1.756	1.178
9	1	ObqETE	L	1.734	-0.753	-0.118
10	1	Invag	L	2.385	0.684	-0.535
10	1	ObqETE	R	1.068	-0.305	-0.327
11	1	Invag	R	4.182	-0.286	0.392
11	1	ObqETE	L	1.996	0.269	-0.696
12	7	Invag	L	-0.300	0.865	0.201
12	7	ObqETE	R	1.506	2.032	-1.158
13	7	Invag	L	1.156	-1.089	0.705
13	7	ObqETE	R	0.660	-1.191	0.593
14	7	Invag	L	0.230	0.310	0.248
14	7	ObqETE	R	-1.900	0.791	2.519
15	7	Invag	L	1.546	-0.674	0.865
15	7	ObqETE	R	-1.083	0.499	1.328
16	7	Invag	L	2.693	0.865	0.559
16	7	ObqETE	R	0.064	0.066	1.848
17	7	Invag	R	-0.587	0.602	1.439
17	7	ObqETE	L	-0.587	0.602	1.439

Ser No	Day	Technique	Side	PC1	PC2	PC3
18	7	Invag	L	0.230	0.310	0.248
18	7	ObqETE	R	-0.197	1.016	1.600
19	7	Invag	L	0.064	2.039	1.022
19	7	ObqETE	R	0.064	2.039	1.022
20	7	Invag	R	1.399	2.344	-1.109
20	7	ObqETE	L	1.138	1.322	-0.532
21	7	Invag	R	-0.800	-0.416	-0.782
21	7	ObqETE	L	-1.723	0.188	0.458
22	7	Invag	L	-1.462	1.210	-0.119
22	7	ObqETE	R	-0.949	2.129	0.013
23	7	Invag	L	0.113	2.043	-1.776
23	7	ObqETE	R	0.113	2.043	-1.776
24	7	Invag	R	-1.462	1.210	-0.119
24	7	ObqETE	L	-1.296	-0.519	-0.893
25	7	Invag	R	-0.277	1.628	-1.936
25	7	ObqETE	L	-1.296	-0.519	-0.893
26	7	Invag	L	-1.045	-0.622	-0.184
26	7	ObqETE	R	-1.045	-0.622	-0.184
27	42	Invag	L	-2.701	0.285	1.583
27	42	ObqETE	R	-2.055	0.275	1.380
28	42	Invag	L	-3.341	-0.026	0.713
28	42	ObqETE	R	-3.768	0.680	2.064
29	42	Invag	L	-2.914	-0.733	-0.638
29	42	ObqETE	R	-1.686	-0.933	-1.053
30	42	Invag	L	-2.695	-0.037	0.510
30	42	ObqETE	R	-2.695	-0.037	0.510
31	42	Invag	R	-2.268	-0.743	-0.841
31	42	ObqETE	L	-2.695	-0.037	0.510
32	42	Invag	L	-2.695	-0.037	0.510
32	42	ObqETE	R	-2.695	-0.037	0.510
33	42	Invag	R	-2.914	-0.733	-0.638
33	42	ObqETE	L	-2.695	-0.037	0.510
34	238	Invag	R	-1.686	-0.933	-1.053
34	238	ObqETE	L	-1.686	-0.933	-1.053
35	238	Invag	R	-2.113	-0.227	0.298
35	238	ObqETE	L	-1.686	-0.933	-1.053
36	238	Invag	L	-1.686	-0.933	-1.053
36	238	ObqETE	R	-1.686	-0.933	-1.053
37	238	Invag	L	-1.686	-0.933	-1.053
37	238	ObqETE	R	-2.332	-0.923	-0.851
38	238	Invag	L	-1.686	-0.933	-1.053
38	238	ObqETE	R	-2.113	-0.227	0.298
39	238	Invag	L	-1.686	-0.933	-1.053
39	238	ObqETE	R	-2.113	-0.227	0.298
40	238	Invag	L	-1.686	-0.933	-1.053
40	238	ObqETE	R	-1.686	-0.933	-1.053

Table E.4. Principal Component scores of histological specimens. PC1 = First Principal Component, etc.

E3.4. General Linear Model: PC1 versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for PC1, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS   Adj MS       F      P
Day      3  362.067  362.067  120.689  173.19  0.000
Tech     1    3.516    3.643    3.643    5.23  0.025
Side     1    0.138    0.138    0.138    0.20  0.657
Error   74   51.569   51.569    0.697
Total   79  417.290


S = 0.834790   R-Sq = 87.64%   R-Sq(adj) = 86.81%


Unusual Observations for PC1

Obs      C15       Fit   SE Fit  Residual  St Resid
 20  1.06845   2.93062  0.21357  -1.86217     -2.31 R
 24  1.50558  -0.34519  0.19279   1.85077      2.28 R
 31  2.69327   0.00970  0.19279   2.68357      3.30 R

R denotes an observation with a large standardized residual.
```

E3.5. General Linear Model: PC2 versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for PC2, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS      F      P
Day      3  25.3788  25.3788  8.4596  12.77  0.000
Tech     1   0.0810   0.0000  0.0000   0.00  0.996
Side     1   1.2525   1.2525  1.2525   1.89  0.173
Error   74  49.0331  49.0331  0.6626
Total   79  75.7454


S = 0.814008   R-Sq = 35.27%   R-Sq(adj) = 30.89%


Unusual Observations for PC2

Obs       PC2      Fit   SE Fit  Residual  St Resid
 25  -1.08880  0.55454  0.18799  -1.64334     -2.07 R
 26  -1.19130  0.81203  0.18799  -2.00333     -2.53 R

R denotes an observation with a large standardized residual.
```

E3.6. General Linear Model: PC3 versus Day, Tech, Side

```
Factor  Type   Levels  Values
Day     fixed       4  1, 7, 42, 238
Tech    fixed       2  ObqETE, Invag
Side    fixed       2  L, R


Analysis of Variance for PC3, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS     F      P
Day      3  10.8453  10.8453  3.6151  4.51  0.006
Tech     1   0.6206   0.4545  0.4545  0.57  0.454
Side     1   0.1256   0.1256  0.1256  0.16  0.693
Error   74  59.3234  59.3234  0.8017
Total   79  70.9150


S = 0.895359   R-Sq = 16.35%   R-Sq(adj) = 10.69%


Unusual Observations for PC3

Obs       PC3      Fit   SE Fit  Residual  St Resid
 28   2.51931  0.27368  0.20677   2.24563      2.58 R
 45  -1.77614  0.03613  0.20677  -1.81227     -2.08 R
 46  -1.77614  0.27368  0.20677  -2.04982     -2.35 R
 49  -1.93630  0.11799  0.23118  -2.05429     -2.37 R

R denotes an observation with a large standardized residual.
```