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Occlusion prevents the use of vascular loops for blood sampling and dosing in minipigs

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Introduction

Due to the lack of easily accessible superficial blood vessels intravenous dosing and sampling are difficult in pigs; particularly, in Göttingen minipigs, which have small auricular veins (*Gurzwiller 1988, Morits et al 1989, Matte 1999*). Although reliable for single samples (*Matte 1999*) sampling into a vacuum blood glass from the jugular vein is less desirable for repeated samplings and dosing. Furthermore, hormone and metabolite variables may be altered by restraint or venepuncture (*Takahashi 1986*). Surgically placed heparinized catheters, commonly used for such studies (*Gutzwiller 1988*), can be functional for six months or more, but animals need to be singly housed and often they are prematurely withdrawn from experiments due to postoperative problems, such as lost or damaged catheters, loss of catheter patency and infections. (*Kemp & Kruger 1987, Gutzwiller 1988, Pijpers et al 1989*). Large external fixation is a disadvantage of some non-surgical procedures recently developed for jugular vein catheterization of unanaesthetized pigs (*Caroll et al 1998, Matte 1999, Damm et al 2000*). A skin loop containing a vein or an artery has been widely used in other species, such as goat (*Graham et al. 1937, Jha et al 1961*), sheep (*Bone et al 1962*), cow (*McClymont 1950*) and dog (*Gross & Hamlin 1973, Michie 1972*), but so far it has not been applied to pigs. The thickness of the porcine skin may prevent the use of this technique.

The survival and usefulness of the loop can also be reduced by postoperative problems, such as edema due to disruption of lymphatic flow, necrosis as the blood supply to the loop skin passes through the base of the flap, and occlusions in the loops if fibrotic tissue forms between the vessel and the skin (*Bone et al. 1962, Linzell 1963, Gross & Hamlin 1973*). The aim of this study was to test the possibility of making an extra-vascular loop in the ventral neck region of a minipig.

Materials and Methods

Animals and housing.

Six Göttingen minipigs (Ellegaard Göttingen Minipigs ApS, Denmark) housed individually on bedding with free access to water and fed restrictedly twice daily with Altromin 9023 Ekstra Minipigs (Brogaarden, Denmark) were used (Table 1). After one or two weeks of acclimatization they were deprived of feed the night before surgery.

Anesthesia and surgical preparation.

0.07 ml kg body weight^{-1} of a mixture of 12.5 mg tiletamine and 12.5 mg zolezepam (Zoletil® 50 vet., Boehringer Ingelheim, Denmark), 12.5 mg xylazin (Narcoxyl® vet. (20 mg/ml), Rosco,

Pig			Surgery		Approximate dimensions (cm)				Technique			Wound dressing		Gluco-corticoid treatment
					Incision length		Loop		Timing of blunt dissection of the loop skin					
Sex	*Age (months)*	*Weight (kg)*	*No*	*Type*	*Medial*	*Lateral*	*Circum-ference*	*Length*	*Prior to finding the vessel*	*Prior to making the second incision*	*Removal of subcutaneous fat*	*Gauze additives*	*Diaper & adhesive tape*	
F	12	32.0	**1**	Venous	12	8	5	5-6	+	+	Thoroughly	None	+	-
			3	Arterial	15	8	5-6	4-5	+	+	Thoroughly	None	+	-
F	12	26.5	**2**	Venous	12	8	4	7	-	+	Thoroughly	None	+	-
F	12	27.7	**4**	Arterial	6-7	6-7	5-6	3-4	+	-	Little	None	-	-
			5	Venous	6-7	6-7	5-6	5-6	+	-	Little	Fucidine	-	+
M	> 24	30.5	**6**	Venous	6-7	6-7	5-6	5-6	+	-	None	Fucidine	-	+
M	> 24	29.5	**7**	Arterial	6-7	6-7	5-6	3	+	-	None	Fucidine	-	+
M	3	6.6	**8**	Venous	4	4	4-5	4	+	-	None	Fucidine	-	-

Table 1: Differences in animals, dimensions, techniques, type, suture gauges and post surgical treatment of different surgical attempts to create a skin loop in six Göttingen minipigs.

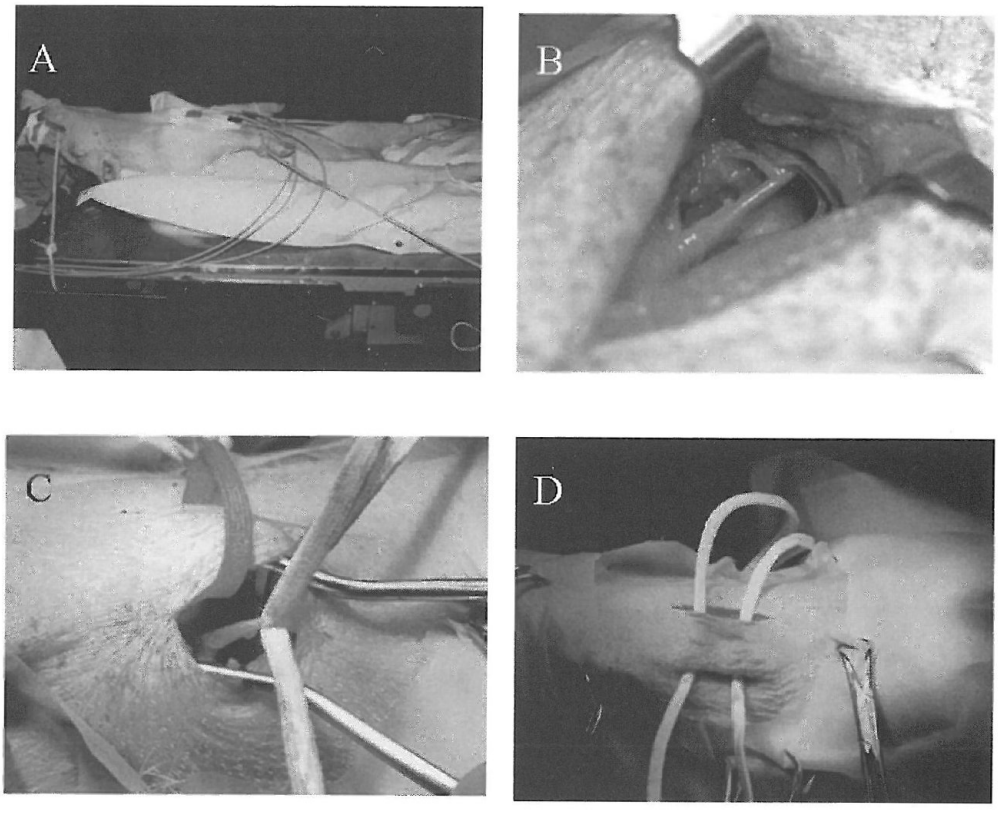
Denmark), 12.5 mg ketamine (Ketaminol® vet (100 mg/ml), Rosco) and 2.5 mg butorphanol (Torbugesic® vet. (10 mg/ml), ScanVet, Denmark) ml^{-1} was given i.m. After approximately 20 minutes 6.3 mg thiopenthone-sodium (25 mg/ml, Sygehus Apotekerne, Denmark) kg body $weight^{-1}$ was given i.v. Hereafter anesthesia was maintained on artificial respiration with 1 – 2 % isoflurane (IsoFlo™ vet., Schering-Plough Animal Helth, Denmark). 0.25 ml isotonic NaCl kg body $weight^{-1}$ min^{-1} was given i.v. throughout the surgery. The pig was placed in dorsal recumbence as shown in Figure 1A. In order to prepare the animal for surgical asepsis the neck was shaved, scrubbed with soap (Hibiscrub®, Zeneca, Denmark) and chlorhexidine (Medi-scrub®, Rovers BV, Netherlands), and disinfected with 62% surgical ethanol and 2.5% iodine ethanol and finally draped with a sterile drape (Kruuse, Denmark).

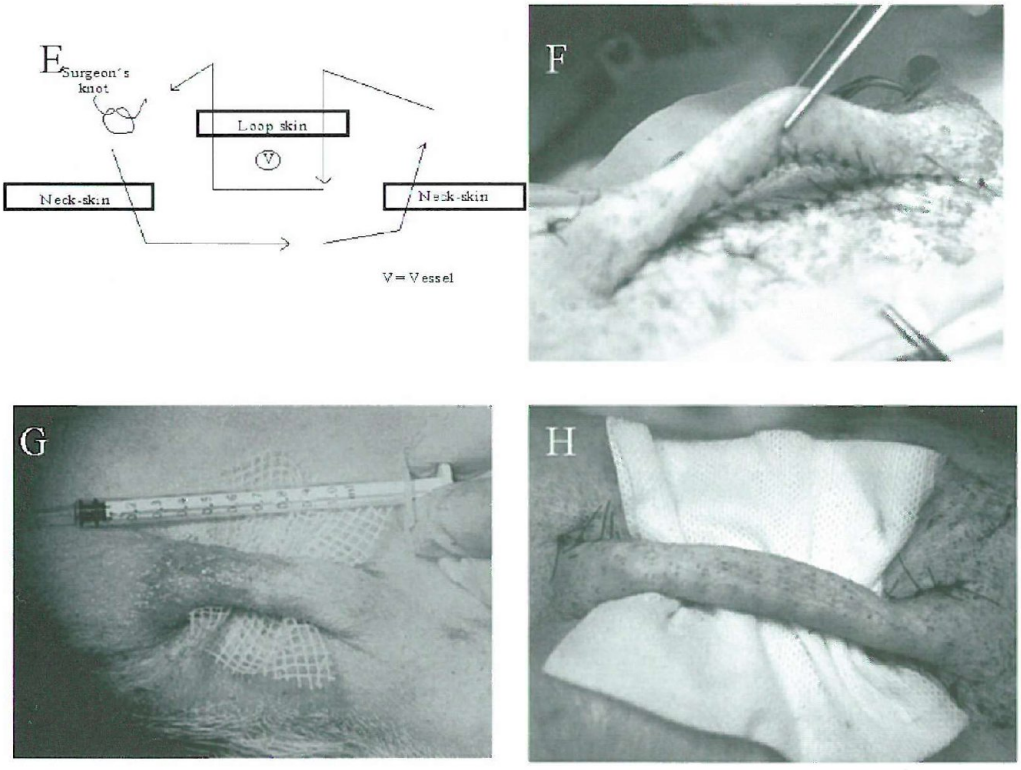
Surgery.
Eight loop surgeries were performed (Figure 1). The procedure for loop surgery and/or postoperative treatment was slightly changed for each new surgery according to observations made on the previous surgery, i.e. the technique was improved in relation to the dimensions of the loop, the timing of blunt dissection and the amount of subcutaneous fat removed (Table 1). In surgeries 3, 4 and 7 the common carotid artery was placed in the loop while the external jugular vein was placed in the other surgeries. Due to difficulties in stretching the artery the arterial loops were shorter (3 –5 cm) than the venous loops (5 – 6 cm). In the immature pig in surgery 8 the venous loop was only four cm long. The loop and the neck skin were sutured with several, closely placed, superficial, simple interrupted sutures using Dermalon (Davis & Geck, United Kingdom) 2-0 or 3-0. Two interrupted horizontal mattress sutures were placed in the underlying neck skin to reduce tension in the neck wound. Two assembling sutures determined the length of the loop (Figure 1E).

Post surgical treatment and observation.
After the first three surgeries a 10x10 cm^2 sterile gauze (Mesoft, Mölnlycke) was placed between the loop and the neck (Figure 1). The surgical wound and the loop were covered with a 10x20 cm^2 diaper (Vliwazell saugkompresse, Rauscher, Austria) secured by adhesive tape (Tensoplast, Smith + Nephew, United Kingdom). This dressing was changed every second day and removed after six days in surgery 1 and 2 and after two days in surgery 3. As long as the surgical wound exudated the sterile gauze was maintained and changed whenever needed, at least once daily. After surgery 4 the sterile gauze was the only wound dressing used. After surgeries 5 to 8, a 10x10 cm^2 Fucidine gauze (Fucidin®, Leo, Denmark) replaced the sterile gauze (Figure 1). After the first three surgeries the animal was treated with Sulfadiazine and trimethoprim (Tribrissen® vet. 48% inj., Schering-Plough Animal Health, Denmark; 0.05 ml kg body $weight^{-1}$ day^{-1}) i.m. for four days. After surgeries 4 to 8, dihydrostreptomycin/ benzylpencilline (Streptocillin® vet., Boehringer Ingelheim; 0.1 ml kg body $weight^{-1}$ day^{-1}) was given once daily i.m. for four days. Twice on any of the four days after surgery ketoprofen (Romefen® vet. (100mg/ml), Merial, France; 0.05 ml kg body $weight^{-1}$) was injected i.m. for analgesia. In surgeries 5 to 7, the animals were additionally injected with prednisolone i.m. once daily for the six days after surgery (Prednisolonacetat vet., Hoechst Roussel Vet., Denmark, 3 mg kg body $weight^{-1}$). Sutures were removed around day ten postoperatively. All pigs were examined daily after surgery. The progression of healing was protocolled, and the presence, extent, and duration of exudation, necrosis, swelling, hyperthermia, reddening and hardening of the loops was observed. Four attempts - two for each loop – were made to catheterize the two loops made in surgeries 1 and 6. Each loop was ultrasound scanned (Interspec XL, 5.0 MHz probe) to reveal the placement of the vessel in the loop. The pigs used for surgeries 1 to 5 were euthanized, and the loops were dissected to locate the placement of the vessel.

Results
It was possible to perform the operation and functional loops were produced in all eight surgeries. More tissue undermining and probably

Figure 1: Surgical creation of a vascular skin loop in the neck region of a minipig.
The animal is placed in dorsal recumbence and fastened to the operating table with its limbs stretched caudally. The head and neck are stretched cranially with the neck elevated by an object placed under it, and the nose fastened to the operating table (A). A skin incision is made into the subcutis approximately one cm lateral to the trachea. The subcutaneous fat may or may not be removed from the loop skin by blunt dissection as close to the cutis as possible. The incision is deepened by undermining through the subcutaneous fat. The brachio- and sternocephalic muscles are separated exposing the external jugular vein (B). Alternatively, the carotid artery may be exposed instead by deeper dissection. The vein is bluntly freed from the surrounding tissue and fascia both cranially and caudally. The artery must be removed from its fascia without damaging the vagal nerve. Two sterile tapes are placed under the vein to lift it above the wound surface (C). The width of the loop skin is determined by wrapping the dissected skin around the vein. Hereafter, a second incision is made lateral and parallel to the first one, and the last part of the loop skin is freed (D). After placing two stay sutures at each end of the loop (E), as well as two interrupted horizontal mattress sutures in the underlying neck skin to reduce tension, the loop and the neck skin are sutured with many, closely placed, superficial, simple interrupted sutures (F). A sterile gauze with (G) or without (H) antibiotic additives is placed between the loop and the neck for the first couple of days post surgically.





more traumatic dissection was needed when the vessel was dissected before the skin (surgery 2), as it was more difficult to find the vessel. The short incision of surgery 8 did not allow dissection of the artery from its deep position. Various degrees of necrosis, fibrosis and improper wound healing disabled intravascular access in all of the eight loops. Occlusion occurred in all of the loops five to six days post surgically. Insufficient wound healing of the surgical wounds in the loop and sometimes in the neck under the loop was evident for all the pigs except the piglet in surgery 8, but swelling, reddening and hyperthermina diminished as the loops were made shorter and wider and less subcutaneous fat was removed (Table 1). The loops in surgeries 5 to 6, which were treated post surgically with prednisolone, differed slightly, as they became edematous for some days prior to hardening, but apart from this healing in glucocorticoid treated pigs did not seem to differ from the non-treated surgeries. The surgical wound healed fully in surgery 8. In surgery 4 and 7 the improper healing made the artery appear in the wound. After removal of the artery the wound healed slowly secondarily. When removing the sutures from the loop made in surgery 3, necrosis inside the loop was observed, and as a result the pig was euthanized on the same day. The loops treated with Fucidine gauze (surgeries 5, 6, 7 and 8) showed less exudation. It was difficult to determine the absence or presence of a post surgical blood flow in the venous loops, but pulse

could be observed or palpated in the arterial loops from surgeries 3, 4 and 7 for up to six days postoperatively. None of the loops made could be catheterized. By scanning the loop from surgery 1, it was possible to localize the vein, although its lumen was very small. At necropsy (surgeries 1 to 5) the vessel could be located in all the loops, except in the artery loop from surgery 4, as the artery had been removed. All vessel lumens were less than one mm in diameter. In the loop from surgery 2 most of the tissue was necrotic. Compared to the loop from surgery 2 less necrotic tissue was found in the loop from surgery 3, while its remaining tissue was fibrotic. In the loops made in surgeries 1 and 5 the entire loop lumen was filled with fibrotic tissue with small amounts of fat enlayered.

Discussion

The thick skin of a minipig does not seem to impose an obstruction for the creation of an arterial or venous skin loop. Although the technique could be improved to reduce necrosis and improper wound healing, occlusion occurred in all of the loops five to six days post surgically. The observation of a pulse for six days post surgically in the arterial loops shows that the loops are functional until fibrosis occludes them. Such spontaneous post surgical loop occlusion has not previously been reported in other species, although it may be observed in loops frequently punctured (*Linzell 1963*). Problems of improper healing have not been reported since the goats of Graham et al (1937). In the first loops too long skin incisions and too narrow space between the two parallel skin incisions may have interfered with the blood supply to the loop skin, which might have resulted in hypoxia (*Linzell 1963*), and too much removal of subcutaneous fat from the loop skin may have compromised the dermal blood supply to the skin. This may have induced necrosis, and it may have damaged the tissue, which has been replaced by connective tissue. Improving the techniques by shortening the incisions and removing less fat reduced the severity of necrosis. E.g. in surgeries 4, 6 and 7 little or no subcutaneous fat was removed, and these loops did not become necrotic, but none of the improvements prevented occlusion. Treatment with glucocorticoids made the loops edematous prior to becoming fibrotic, but in all of these three loops fibrosis was only slightly less compared to loops from untreated surgeries, and anyway too progressive for the loops to be functional. The application of fucidine in the wound dressing reduced exudation. The loop of the piglet used in surgery 8 occluded similarly to those of the older pigs in the other surgeries, while in other aspects it showed the most complete and uncomplicated healing. Therefore, if the problem of occlusion can be solved, performing surgery in a very young pig may be the most optimal. Probably, all post-operative problems are indicative of too limited a vascularisation of the porcine skin.

In conclusion, we have been successful in reducing necrosis and improper wound healing by avoiding the removal of subcutaneous fat as well as reducing the length of the incisions and widening the distance between them. The inability to produce long loops without observing post surgical necrosis shows a difference in skin vascularisation in pigs compared to other species, in which such long loops do not render a problem. The vascularisation of the porcine skin seems to be too sparse to allow primary healing without fibrosis, which is a precondition for a continued blood flow in the vessel. Therefore, the technique needs further modification by the implantation of a more rigid, artificial vessel or the placement of an in-dwelling catheter, which is able to withstand the pressure from the connective tissue.

Summary

Continous blood sampling and intravenous dosing are difficult in pigs, especially minipigs. A skin loop containing the jugular vein or the carotid artery has been used for solving this problem in other species of animals, but it has never been attempted in the pig. In this study eight such skin loops were made on minipigs, which technically caused only a few problems. Shortening the loop and widening the distance between the two parallel incisions reduced problems of necrosis and improper wound healing. A juvenile pig did not show such problems. However, in all loops excessive formation of fibrotic tissue occluded the

vessel after approximately six days. We, therefore, conclude that although the surgical technique is possible, further modification, e.g. by insertion of a rigid artificial vessel or an in-dwelling catheter, is necessary, before vascular loops can be used as a method for continuous sampling and dosing in minipigs.

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