Supplementary Table 1. Blood gas analysis, chemistry analysis, osmolarity and oncotic pressure measured from the perfusate at the end of NEsLP (5hr).

	NEsLP Steen (5hr) (n=6)	NEsLP Gelofusine (5hr) (n=6)	NEsLP Whole blood (5hr) (n=6)	p-value
*pH	7.50±0.06	7.43±0.06	7.47±0.03	**0.01**
*Na^+ (mmol/L)	145±2.27	143±1.94	141±1.53	**<0.01**
*K^+ (mmol/L)	3.27±1.36	3.36±0.24	3.78±0.93	0.67
*Ca^{++} (mmol/L)	0.84±0.03	0.55±0.01	0.72±0.25	**0.04**
*Cl^- (mmol/L)	111±2	111±2	120±3	**<0.001**
*$HCO3^-$ (mmol/L)	16.25±2.05	14.21±2.40	15.1±0.83	0.25
*Glucose (mmol/L)	11.88±4.72	13.28±7.43	9.68±2.10	0.55
*BUN (mmol/L)	6.78±1.64	7.12±1.07	8.09±1.50	0.32
*Lactate (mmol/L)	0.78±0.36	0.68±0.69	0.69±0.17	0.95
*Osmolarity (Osm/L)	310±7	307±10	300±6	0.06
**Albumin (g/dl)	4.8±0.2	-	1±0	**<0.001**
*Oncotic pressure (mmHg)	17.85±3.66	19.52±4.07	4.86±0.58	**<0.001**

Data is presented as mean and standard deviation. P-values were determined by One-way ANOVA (*) and Student´s t test (**).

Supplementary Methods

Circuit priming

The circuit temperature was started at 25°C and progressively increased after priming with all the components until it reached 37°C prior to liver placement. HA and PV flows were assessed during perfusion. Perfusate was constituted by 300-400mls of washed red blood cells combined with 1.5L of either Gelofusine or Steen to reach a hematocrit between 15-20%. The hematocrit in the whole blood group was adjusted accordingly until it reached the same range. Heparin was added to the circuit (10, 000IU; Sandoz Canada, Quebec, QC, Canada). Caloric requirements were provided by administration of amino acids (Travasol 4.25%, 50ml bolus plus infusion of 12ml/hr, Baxter, Hamilton, ON, Canada). Insulin was used to maintain glucose levels between 4 and 10mmol/L (started at 60 IU/hr, NovoRapid, Novo Nordisk, Mississauga, ON, Canada). Cefazolin (1g, Pharmaceutical Partners of Canada, Richmond Hill, ON, Canada) and Metronidazole (500mg, Baxter, Mississauga, ON, Canada) were added during the priming. Infusion of prostacyclin I2 (Epoprostenol) at 4ml/h (8µg/h) was used as vasodilator. Two percent taurocholic acid (Sigma-Aldrich, St Louis, MO, USA) was administrated at 7ml/h to stimulate bile production. Additional electrolytes were supplemented during the circuit priming (sodium bicarbonate, 20mmol and calcium gluconate, 9.2mmol). Mixture of gases (95%O2 and 5%CO2) was added through the gas exchanger membrane.

Donor surgery

A DCD model with 30mins of WI was used to simulate the current clinical practice in most of the centers. After liver and vascular dissection pigs received 500IU/kg of heparin and blood was retrieved for NEsLP. Five minutes following heparin injection cardiac arrest was induced by intra-cardiac potassium chloride injection (20mEq). Thirty minutes after cardiac arrest livers were flushed through aorta and portal vein (PV) with 3L of cold Custodiol-HTK™ (Essential Pharmaceuticals, LCC, Ewing, NJ, USA). Back table surgery and liver preparation for perfusion were performed under cold conditions (4°C) and then transferred to ice for a total of 2hrs of SCS. In the SCS group, the livers were kept on ice following cold flush with Custodiol-HTK™ (Essential Pharmaceuticals, LCC, Ewing, NJ, USA) and until the 7hrs of preservation time was completed. Donor serum and bile samples were taken before any intervention and used as baseline samples to compare against serum and bile characteristics during NEsLP.

Normothermic Ex-Situ Liver Perfusion

NEsLP set-up was established to resemble the system used in the human clinical trials [1, 2]. Livers were taken from SCS and then placed under perfusion for five hours. As described in previous publications [3] the machine is based on a centrifugal pump that was set-up to achieve pressures of 60mmHg and 3mmHg for the hepatic artery (HA) and PV, respectively.

At the end of NEsLP the livers were again cold flushed with 2L of Custodiol-HTK™ solution and kept on static cold storage while performing the cross clamp, bypass placement and initial cava stitches. The period of time from liver out of ice until portal vein reperfusion was 25-30mins.

Post-transplant care and follow-up

Animals were extubated 3hrs after the surgery finished and brought to the recovery pens where they received the corresponding analgesic. Post-operative care and immunosuppression was established as in our prior studies[4]. The clinical evaluation was done in the morning and afternoon for 3 days, including blood sampling. If signs of distress were noted (lethargy, agitation, excessive pain, shortness of breath, severe acid-base disequilibrium) pigs were euthanized under deep anesthesia. During euthanasia liver characteristics and anastomoses patency was assessed. Animals were sacrificed on postoperative day (POD) 3 under deep anesthesia. Liver biopsies were taken and stored on 10% formalin.

Colloid solutions composition and characteristics

Composition from both solutions (Steen and Gelofusine) was determined from the different bottles prior to priming of the NEsLP circuit with RAPIDPoint® (Siemens, Munich, Germany). Additionally, albumin and BUN concentration was assessed with Piccolo Xpress®(Abbot, Princeton, NJ). Furthermore, colloid-osmotic pressure was evaluated with the Wescor 4400 Colloid Osmometer (Labequip, Markham, ON, Canada). Viscosity was measured with the cone/plate wells viscometer (AMETEK, Brookfield, Middleboro, MA, USA). Density was determined by measuring the weight of 1ml of solution.

Perfusate characteristics during NEsLP

Sodium, potassium, calcium, chloride, bicarbonate and glucose levels together with acid-base characteristics were determined by using RAPIDPoint® (Siemens, Munich, Germany) at the beginning of perfusion (5mins after the liver was placed on pump). After the baseline levels were assessed, electrolytes and pH adjustments were done with potassium bicarbonate until perfusate reached physiologic levels. Albumin and BUN concentrations were determined with Piccolo Xpress® machine. Furthermore, oncotic pressure was determined at the start of perfusion with the Wescor 4400 Colloid Osmometer.

Bile composition

Bile composition was determined by using RAPIDPoint®. Comparison of bile pH, electrolyte concentrations (Na^+, $HCO3^-$) and electrolyte bile/perfusate ratio (Bile/Perfusate Na^+, K^+, Ca^{++}) from Gelofusine and Steen groups versus pig baseline samples was performed.

The purpose of this evaluation was to determine for the first time the behavior of bile electrolyte secretion during NEsLP and its comparison with sham samples that were taken directly from the donor bile duct without any previous intervention. Since the objective of NEsLP is to simulate physiologic characteristics we consider this as a direct manner to evaluate in a quick fashion the integrity in the process of bile electrolytes secretion [5, 6.] In addition, lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT) were determined as markers of cellular injury.

Liver function assessment

International Normalized Ratio (INR) was utilized to evaluate liver function after transplantation and was measured 3hrs and on a daily basis following PV reperfusion. Lactate clearance was evaluated during perfusion and after transplantation. Liver function was also assessed by bile production and bile pH during NEsLP. Finally, oxygen consumption (OC) was calculated by subtracting the oxygen content from the vena cava to the combined oxygen content from the HA and PV with the formula described below.

Oxygen Consumption Formula

The oxygen content of the perfusate was calculated by summing the free dissolved oxygen fraction to the Hb-bound oxygen fraction using the following formula: $[O_2]= 1.39 \times [Hb] \times FO_2Hb + 0.00314 \times pO_2$, where [Hb] is the hemoglobin concentration in g/dl, FO_2Hb is the fraction oxygenated hemoglobin and the pO_2 is the partial pressure of oxygen in mmHg. OC was determined as: $OC= (([O_2]in – [O_2]out)/100) \times$ flow rate/ liver weight (kg).

Histological analysis

Biopsies were stored and maintain in 10% formalin for 72hrs and then transferred into 70%-alcohol. Biopsies were embedded in paraffin and sections of 5μm were cut and processed. Two hours post-reperfusion samples (core biopsies) were evaluated to measure the level of apoptosis by using cleaved-caspase-3 immunohistochemistry staining (Cell Signaling Technology, Danvers, MA, USA)[7]. Endothelial cell (EC) integrity was also quantified 2hrs after reperfusion by using CD31 (PECAM, Santa Cruz

Biotechnology, Dallas, TX, USA)[8]. .H&E staining was used to delineate the percentage of necrosis of samples that were taken on POD3. A blinded pathologist with the use of ImageScope software performed the analysis for cleaved-caspase-3 staining. The CD31 staining was assessed by using the endothelial integrity score as described in our prior publications[9].

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