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SYSTEMATIC REVIEWS

Small animal *ex vivo* machine perfusion of the liver: A comprehensive literature review

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Abstract

BACKGROUND

Liver transplantation is the only treatment for acute and chronic liver failure, but the global organ shortage has increased reliance on extended criteria donor livers, which are more susceptible to ischemia-reperfusion injury. While static cold storage is standard, these grafts often require improved preservation strategies.

AIM

To summarize the current state of small animal liver machine perfusion (MP), highlight variability in protocols, and emphasize the need for standardization to guide future research.

METHODS

A comprehensive literature search of PubMed was conducted to identify studies on small animal (rat and mouse) *ex vivo* liver MP. Only English-language animal studies were included, with no restrictions on publication date. Relevant full-text articles were reviewed, and reference lists were screened to ensure completeness.

RESULTS

Small animal liver MP provides a cost-effective model to explore dynamic preservation strategies. Rat perfusion studies face challenges including dual-vessel perfusion, maintaining physiological perfusate volumes, and lack of standardized protocols. Open- and closed-circuit setups have distinct advantages and limitations, and experimental designs vary widely across studies.

CONCLUSION

This review illustrates the wide variability in small animal liver MP protocols and underscores the urgent need for standardization. Addressing these inconsistencies will enhance reproducibility, facilitate comparison across studies, and support the development of optimized liver preservation strategies.

Key Words: Liver transplant; Liver transplantation; Machine perfusion; *Ex vivo* liver machine perfusion; Small animal machine perfusion

Core Tip: Small animal liver machine perfusion models are essential for studying dynamic liver preservation in transplantation research. Rat liver perfusion provides a cost-effective and accessible platform, but currently no standardized protocols exist, limiting reproducibility and progress. This review highlights variations in existing studies, technical challenges, and limitations, emphasizing the urgent need for standardization. By summarizing key developments and system differences, it offers researchers practical insights to optimize perfusion strategies, improve reproducibility, and reduce animal use in future studies.

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INTRODUCTION

Liver transplantation (LTx) is the only possible treatment option for acute and chronic liver failure. Recent advances in LTx outcomes, immunosuppressive techniques, and cancer therapies have expanded the eligibility of individuals for inclusion in transplant waiting lists[1]. The global organ shortage obligates centers to use grafts from extended criteria donors (ECDs) including grafts donated after circulatory death (DCD)[2]. ECD livers are susceptible to greater ischemia reperfusion injury, contributing to inferior graft function and outcomes[3]. While static cold storage (SCS) has long been considered the gold standard, ECD do not fare well under this traditional preservation method. Dynamic preservation methods such as normothermic (NMP), subnormothermic (SNMP), and hypothermic machine perfusion (HMP) have emerged to enhance the viability of donor livers and optimize transplantation outcomes[4,5].

In contrast to conventional SCS, *ex situ* machine perfusion (MP) enhances LTx outcomes, enabling extended preservation times and viability testing[6,7]. The prospective application of MP is anticipated to be even more extensive, with currently investigated therapeutic interventions like defatting cocktails, RNA interference, senolytics, and stem cell therapy showing promise in facilitating the repair and regeneration of injured livers before LTx[8].

Large animal studies on MP are expensive and face feasibility challenges[9]. Therefore, small animal experiments are necessary to clarify potential applications in the future. Adherence to the 3R rule (replacement, reduction, refinement) is crucial for ethical animal experimentation[10]. While the demand for animal experiments in MP is significant, a universally accepted standard protocol for its implementation is currently lacking.

This literature review outlines the steps and diverse possibilities involved in establishing MP for small animal livers, aiming to assist in considering essential elements and ultimately contributing to a reduction in the number of animals used for experiments.

MATERIALS AND METHODS

The comprehensive literature search was conducted using the PubMed database. The following combination of medical subject headings and keywords with the employment of “AND” or “OR” or “NOT” Boolean operators were used: (“HMP” OR “hope” OR “NMP” OR “SNMP” OR “machine perfusion”) AND “liver perfusion” AND (“rat” OR “mice” OR “small animal”) NOT “kidneys”. Only articles written about animals and not humans were included in the search. No restrictions on publication dates were applied, allowing for the inclusion of studies from all available years. Only abstracts written in the English language were reviewed. Full-text articles were retrieved if relevant abstracts were identified. An additional manual search of the reference lists was performed to ensure the comprehensive literature search procedure. The most recent search was performed on June 23rd, 2024. A preferred reporting items for systematic reviews and meta-analyses flow diagram of the literature search and study selection is presented in Figure 1.

RESULTS

Liver preparation for MP

Several different techniques are described in literature. Before connecting the liver to the *ex vivo* MP circuit, a standard cold flush *in situ* is performed to remove blood remnants and reduce warm ischemia (WI) time[11,12]. Clear descriptions of the flushing technique are crucial for achieving favorable outcomes in liver preservation[13]. Table 1 outlines various liver flushing solutions, routes, and volumes. The administration routes for the cold flush vary among authors. Most perform the flush through the portal vein (PV) only[14-66]. Others use a dual vessel approach, flushing through both the PV and the aorta (A)[67-74] or the PV and the hepatic artery (HA)[75-77]. Flushing *via* the A alone is another option[78-84]. The use of HA exclusively is a rare approach, employed by only a single author[85].

The solutions used for cold flushes vary significantly, with volumes ranging from 3 mL to 80 mL. Some researchers choose simple heparinized saline, which is a standard choice for removing blood remnants[80,83,85-87]. In contrast, others use more advanced preservation solutions such as University of Wisconsin (UW), Histidine Tryptophan Ketoglutarate (HTK), Krebs Henseleit, Belzer MP Solution (Belzer MPS), 3-O-methyl-D-glucose, Institute George Lopez (IGL)-1, and William's E Medium (WEM)[66-73,79,81,82,84,88,89]. These more complex solutions are often chosen because they are also used during the *ex vivo* MP process, potentially offering better preservation properties.

Additionally, the temperature at which these solutions are administered can vary. Many protocols employ solutions cooled to 0-4 °C to minimize metabolic activity. Others use solutions at higher temperatures, such as 20 °C or even 37 °C [30,33,36,65,75]. Some authors perform the flush with a solution at the same temperature that will be used for the subsequent MP[25]. While cold flush has long been standard procedure before MP, controversially, some suggest that a cold flush before NMP subjects grafts to higher WI damage[12]. Some studies also incorporate oxygenated solutions, which might affect oxygen delivery and tissue viability during the flushing process[20,23].

While authors suggest different solutions and volumes for cold flush, it is important that the pressure during the cold flush for small animal livers is not too high and that adequate blood remnant washout is achieved. Some authors described using constant flow for the cold flush[67], others performed the cold flush until the liver color changed to khaki [89], while the majority applied different volumes and performed it multiple times, both *in situ* and *ex vivo*[90-98]. However, some studies do not detail any flushing procedure before applying MP, and future studies should not omit the description of this crucial step.

Heparin administration

Heparin, an essential anticoagulant that prevents clotting, is crucial for use during rat liver explant surgery[99,100]. Administering heparin before liver perfusion in the donor and/or into the perfusion solution aids in optimizing liver harvesting[101]. Authors propose diverse routes and dosages for heparin administration, noting its non-liver-toxic effect in rats[102]. Several studies have delved into the administration of heparin *via* the vena cava (VC), with dosages spanning from 250 IU to 5000 IU[17,20,23,28,31,35,37,40,45,48,54,56]. While other suggested heparinization *via* iliac vein or abdominal A[41,43,52,78,80]. Alternative routes, including the tibial vein and dorsal penile vein, were explored in other investigations, employing heparin dosages ranging from 100 IU to 500 IU[59,68,75,76]. Moreover, some researchers chose to apply heparin *via* intraperitoneal administration, dosages ranged from 1000 IU to 1500 IU[60,79]. Various techniques have been employed for administering heparin during liver flushing *via* the PV, with some studies opting to incorporate heparin into the perfusate solutions[30,42,50,65,77]. These findings show the variability in heparin administration protocols and highlight the importance of further research to establish standardized guidelines for optimal anticoagulation during liver explant surgery and MP.

Cannulation

Anatomically, small animal livers are perfused by proportionally less arterial and more portal blood compared to large animals[103]. Thus, a significant number of authors prefer single-vessel MP *via* the PV, which necessitates simpler surgery and a more cost-effective MP circuit. Oxygenated MP through the PV provides oxygen and perfusate to the entire graft, including the extrahepatic biliary tree[104]. In contrast, other groups argue that although the PV supplies nutrients to hepatocytes and maintains a higher flow rate compared to the HA, it does not serve as the liver's primary route for oxygen delivery and does not support the vascularization of the biliary tree as effectively as the HA does[105]. In addition, when selecting a perfusion circuit among the PV, VC, and HA, it's noted that retrograde perfusion is comparable to PV perfusion, while perfusion *via* the HA is considered less advantageous[67]. Furthermore, concerns arise regarding the potential direct damage caused by arterial cannulation to the arterial intima, which could compromise vascular anastomosis[106].

However, some authors advocate for dual vessel MP, asserting that it results in superior outcomes compared to single-vessel MP[71]. Although HA cannulation is a complicated procedure, scientists often opt to cannulate *via* the celiac artery due to its larger diameter[39,84].

To establish a closed MP circuit, the VC must be cannulated, with the other VC outflow either ligated or sutured. Alternatively, the perfusate can flow freely *via* the infrahepatic and/or suprahepatic VC into the organ chamber, where it is immediately recirculated inside the system[20,23,35].

Table 1 Liver graft preparation for small animal liver *ex vivo* machine perfusion

Ref.	*Via*	Liver flush before MP
Kim *et al*[14]	PV	10 mL of cold UW-G solution
Dutkowski *et al*[15]	PV	2 mL + 3 mL + 1 mL of 4 °C UW solution (hydroxyethyl starch and glutathione free)
Compagnon *et al*[67]	A, PV	25 mL of ice-cold Celsior-HES solution with 500 IU of heparin
Lauschke *et al*[16]	PV	60 mL of HTK or Belzer MPS solutions
Lee *et al*[90]	NA	NA
Tan *et al*[91]	NA	NA
Xu *et al*[92]	NA	NA
Bessems *et al*[17]	PV	50 mL of ringer lactate
Dutkowski *et al*[18]	PV	2 mL + 3 mL + 1 mL of 4 °C UW solution (hydroxyethyl starch and glutathione free)
Tolboom *et al*[19]	PV	5 mL + 5 mL of 4 °C UW solution
Vairetti *et al*[20]	PV	Oxygenated KH medium
Manekeller *et al*[21]	PV	HTK or Belzer MPS solutions
Stegemann *et al*[22]	PV	20 mL of HTK or Custodiol-N solution at 4 °C
Ferrigno *et al*[23]	PV	Oxygenated KH medium
Lüer *et al*[24]	PV	20 mL of HTK solution
Olschewski *et al*[25]	PV	20 mL of Lifor organ preservation solution (at 4 °C, 12 °C and 21 °C)
Minor *et al*[26]	PV	20 mL of HTK solution
Tolboom *et al*[27]	PV	10 mL of saline
Giannone *et al*[28]	PV	20 mL of cold Celsior solution (*in situ*) + 30 mL of cold Celsior solution
Perk *et al*[29]	PV	10 mL of saline
Schlegel *et al*[30]	PV	6 mL of 20 °C heparinized (1 IU/mL) saline
Bruinsma *et al*[88]	NA	Cold UW solution
Liu *et al*[31]	PV	10 mL of 4 °C perfusate (control or defatting)
Carnevale *et al*[93]	NA	NA
Schlegel *et al*[32]	PV	6 mL of 20 °C heparinized (1 IU/mL) saline
Schlegel *et al*[33]	PV	6 mL of heparinized (1 IU/mL) saline at room temperature and UW solution at 4 °C
Bae *et al*[34]	PV	200 mL of 0.9% saline
Niu *et al*[68]	A, PV	50 mL of ice-cold ross perfusion fluid with 10 IU/mL heparin. 12 mL of cold supplemented UW solution. 20 mL of Hartmann's solution with heparin (5 U/mL)
Tarantola *et al*[35]	PV	KH medium
Bruinsma *et al*[36]	PV	10 mL of 21 °C 3-OMG loading solution + 10 mL of 21 °C 3-OMG loading solution
Ferrigno *et al*[37]	PV	50 mL of modified KHB
Jia *et al*[38]	PV	Cooled saline containing 25 IU/mL heparin
Westerkamp *et al*[94]	NA	NA
Carbonell *et al*[95]	NA	NA
Op den Dries *et al*[75]	PV, HA	5 mL of 37 °C 0.9% NaCl *via* PV + 5 mL of 4 °C 0.9% NaCl *via* SIVC + 20 mL of 4 °C 0.9% NaCl *via* PV + 5 mL of 4 °C 0.9% NaCl *via* HA
Okamura *et al*[39]	PV	50 mL of preservation solution
Berardo *et al*[40]	PV	Ringer lactate

Chai *et al*[89]	NA	Cold UW solution until liver color changed to khaki
Zeng *et al*[41]	PV	20 mL of 4 °C HTK solution
Beal *et al*[42]	PV	60 mL of cold 0.9% saline with 1 mL heparin (100 U)
Tabka *et al*[69]	A, PV	15 mL of 20 °C Ringer's lactate or Celsior or with Celsior supplemented with 10^{-9} M of Ang IV
Xue *et al*[43]	PV	20 mL of 4 °C HTK solution
He *et al*[78]	A	Approximately 10 mL of 0-4 °C saline
Gassner *et al*[70]	A, PV	20 mL of 4 °C HTK solution (with/without 12 mmol/L glycine)
Zeng *et al*[79]	A	2 mL of cold HTK solution with 10 IU/mL heparin
Jia *et al*[80]	A	Cold saline with 25 IU/mL of heparin
Oldani *et al*[44]	PV	20 mL of cold IGL-1 solution with 100 IU heparin
Chin *et al*[45]	PV	50 mL of 4 °C 0.9% NaCl
Gillooly *et al*[46]	PV	10 CC of cold saline with 100 IU heparin
Martins *et al*[47]	PV	4 °C Celsior solution
Scheuermann *et al*[48]	PV	40 mL of cold UW solution
Claussen *et al*[71]	A, PV	20 mL of 4 °C HTK solution supplemented with 12 mmol/L glycine
Haque *et al*[49]	PV	50 mL of ice cold Ringer's lactate with heparin
Schlegel *et al*[50]	PV	10 mL of cold heparinized IGL-1 solution
Nösser *et al*[51]	PV	20 mL of Ringer solution
Yamada *et al*[96]	NA	NA
Hu *et al*[52]	PV	20 mL of 0-4 °C HTK solution
Von Horn and Minor[53]	PV	60 mL of HTK solution
Raigani *et al*[54]	PV	50 mL of ice-cold 0.9% saline
Yang *et al*[55]	PV	2 mL of saline
Westerkamp *et al*[76]	PV, HA	10 mL of 37 °C 0.9% NaCl *via* PV. 5 mL of 4 °C HTK solution *via* PV. 20 mL of 4 °C HTK solution *via* PV. 5 mL of 4 °C HTK solution *via* HA
De Vries *et al*[86]	NA	60 mL of ice-cold saline
Liu *et al*[85]	HA	Heparin saline 40 mL (50 IU/L)
Lin *et al*[56]	PV	NA
Rigo *et al*[57]	PV	10 mL of cold Celsior solution. 30 mL of cold Celsior solution. 10 mL of WEM
Xu *et al*[97]	NA	NA
Carlson *et al*[58]	PV	20 mL of NaCl saline
Zhou *et al*[81]	A	50 mL of 4 °C HTK solution
Cao *et al*[59]	PV	10 mL of UW solution
De Stefano *et al*[60]	PV	10 mL of saline or Celsior solution
Sun *et al*[61]	PV	NA
Jennings *et al*[62]	PV	20 mL of cold 0.9% saline
Wang *et al*[72]	A, PV	3 mL of cold HTK solution
Asong-Fontem *et al*[73]	A, PV	50 mL of preservation solution. 20 mL of preservation solution at 5 °C ± 3 °C
Shi *et al*[77]	PV, HA	0-4 °C heparinized saline
Von Horn *et al*[63]	PV	60 mL of 4 °C HTK solution
Zhou *et al*[82]	A	50 mL of 4 °C HTK solution
Luo *et al*[83]	A	40 mL of heparinized saline (50 IU/mL)

Ohara *et al*[64]	PV	Ringer's solution
Chen *et al*[65]	PV	5 mL of 37 °C heparinized saline (2500 IU/mL)
Fukai *et al*[74]	A, PV	60 mL of ice-chilled saline 25 mL of ice-chilled Belzer UW or Belzer MPS solutions
Hughes *et al*[84]	A	80 mL of 4 °C HTK solution
Bai *et al*[98]	NA	NA
Von Horn *et al*[66]	PV	60 mL of 4 °C HTK solution
Li *et al*[87]	NA	NaCl solution

A: Aorta; NA: Not written or not applicable in the main article; HA: Hepatic artery; UW: University of Wisconsin; UW-G: University of Wisconsin-gluconate; HTK: Histidine Tryptophan Ketoglutarate; HES: Hydroxyethyl starch; Belzer MPS: Belzer machine perfusion solution; KH: Krebs Henseleit; KHB: Krebs Henseleit bicarbonate; 3-OMG: 3-O-methyl-D-glucose; NaCl: Sodium chloride; PV: Portal vein; SIVC: Suprahepatic inferior vena cava; Ang IV: Angiotensin IV; IGL: Institute George Lopez; WEM: William's E Medium; MP: Machine perfusion.

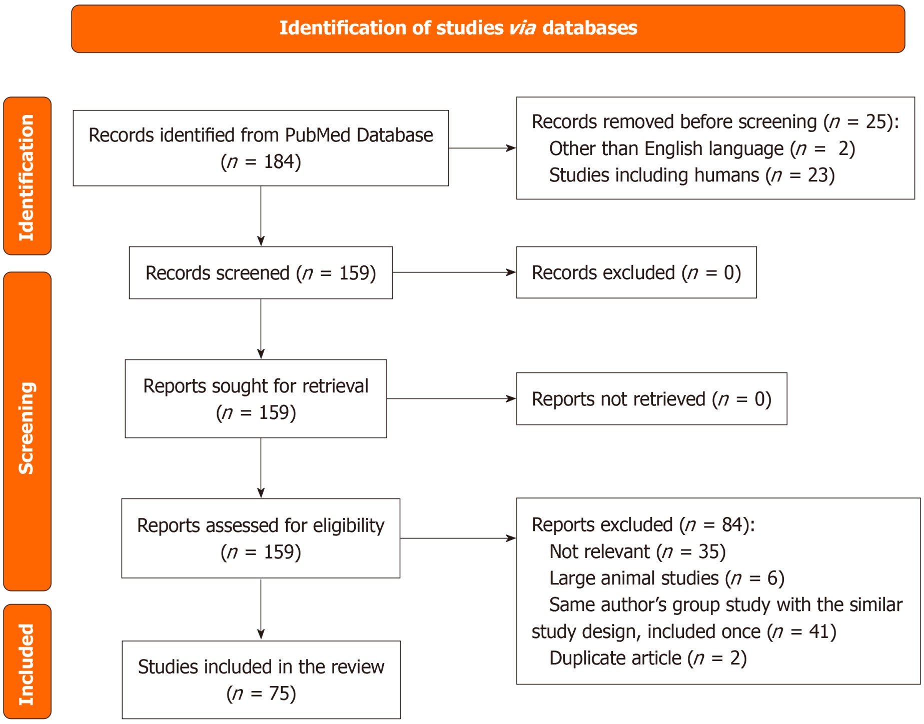

Figure 1 Preferred reporting items for systematic reviews and meta-analyses flow diagram of study selection.

Bile duct cannulation is essential for measuring bile output. Moreover, it allows to test bile composition which is a great marker of biliary viability[107]. While some authors provide detailed descriptions of the bile duct cannulation process, others may omit it entirely. Nevertheless, it is crucial not to overlook this step. Bile flow is heavily influenced by perfusion temperature and oxygen delivery rate. At 37 °C and adequate oxygenation, bile flow should be at least 1 μL/minute/g liver[108]. Table 2 summarizes which vessels (PV, HA, VC) and common bile duct cannulations were performed by different authors in their MP settings.

MP applications

Scientists have studied dynamic preservation methods under different durations and temperatures, with varying warm and cold ischemia times before applying MP. As there are still no standard times for the MP application, various studies demonstrate possible outcomes under different durations ranging from 30 minutes to 120 hours (Table 3). Some authors aim to minimize MP time which results in improved liver function[50,72,81,95,96], while others seek to extend MP time as much as possible to prolong preservation[24,26,36,49,88]. Additionally, some researchers focus on determining the optimal time for MP application[52].

Ex vivo rat liver MP studies are performed for a number of different reasons. They compare dynamic preservation techniques with SCS, especially concerning older or fatty livers[67,75,78,90,93,94]. Comparisons between dynamic preservation methods aim to identify the most effective approach[33,37,50,63,96]. Some authors study the role of oxygenation in liver preservation, providing evidence of its advantageous outcomes in improving liver function[22,24]. Researchers

Table 2 Cannulation techniques for small animal liver *ex vivo* machine perfusion

Ref.	Portal vein	Hepatic artery	Vena cava	Common bile duct
Kim *et al*[14]; Manekeller *et al*[21]; Stegemann *et al*[22]; Giannone *et al*[28]; Perk *et al*[29]; Bae *et al*[34]; Zeng *et al*[79]; Jia *et al*[80]; Gillooly *et al*[46]; Martins *et al*[47]; Haque *et al*[49]; Schlegel *et al*[50]; Cao *et al*[59]; Wang *et al*[72]; Asong-Fontem *et al*[73]; Li *et al*[87]	Yes	No or NA	No or NA	No or NA
Dutkowski *et al*[15]; Lee *et al*[90]; Bessems *et al*[17]; Olschewski *et al*[25]; Tolboom *et al*[27]; Bruinsma *et al*[88]; Carnevale *et al*[93]; Niu *et al*[68]; Bruinsma *et al*[36]; Zeng *et al*[41]; Beal *et al*[42]; Tabka *et al*[69]; Xue *et al*[43]; Gassner *et al*[70]; Oldani *et al*[44]; Nösser *et al*[51]; Hu *et al*[52]; Lin *et al*[56]; Xu *et al*[97]; Sun *et al*[61]	Yes	No or NA	Yes	Yes
Compagnon *et al*[67]; Westerkamp *et al*[94]; Op den Dries *et al*[75]; Okamura *et al*[39]; Claussen *et al*[71]	Yes	Yes	Yes	Yes
Lauschke *et al*[16]; Xu *et al*[92]; Dutkowski *et al*[18]; Tolboom *et al*[19]; Vairetti *et al*[20]; Ferrigno *et al*[23]; Lüer *et al*[24]; Minor *et al*[26]; Liu *et al*[31]; Tarantola *et al*[35]; Ferrigno *et al*[37]; Berardo *et al*[40]; He *et al*[78]; Chin *et al*[45]; Scheuermann *et al*[48]; Yamada *et al*[96]; Von Horn and Minor[53]; Raigani *et al*[54]; Yang *et al*[55]; De Vries *et al*[86]; Rigo *et al*[57]; Carlson *et al*[58]; De Stefano *et al*[60]; Jennings *et al*[62]; Von Horn *et al*[63]; Luo *et al*[83]; Chen *et al*[65]; Fukai *et al*[74]; Von Horn *et al*[66]	Yes	No or NA	No or NA	Yes
Tan *et al*[91]; Carbonell *et al*[95]; Chai *et al*[89]	No or NA	No or NA	No or NA	No or NA
Schlegel *et al*[30]; Schlegel *et al*[32]; Zhou *et al*[81]; Zhou *et al*[82]	Yes	No or NA	Yes	No or NA
Schlegel *et al*[33]	Yes	Yes	Yes	No or NA
Jia *et al*[38]; Shi *et al*[77]	Yes	Yes	No or NA	No or NA
Westerkamp *et al*[76]; Liu *et al*[85]; Ohara *et al*[64]; Hughes *et al*[84]; Bai *et al*[98]	Yes	Yes	No or NA	Yes

This table shows which vessels (vena cava, hepatic artery, portal vein) and the common bile duct were cannulated in various studies, with "Yes" denoting cannulation and "No" or "NA" indicating absence or non-application. NA: Not written or not applicable in the main article.

investigate various perfusates[17,21,92], including additives such as tacrolimus[32], α-tocopherol[34], pegylated-catalase [42], dopamine[26], angiotensin IV[69], metamizole[71], metformin[76], oxygen carriers[62], and IGL-2[73], to understand how they impact liver function. Some studies explore the introduction of stem cells to improve liver function[55,59-61], while others aim to treat fatty liver disease during MP[23,31,35,39,54,56,72,87,97]. Additionally, efforts are made to enhance donation after DCD liver function[81-85,94,96]. Moreover, there are promising initial attempts to apply gene therapy during MP[46].

MP system

A standard perfusion system is made of organ chamber, perfusate reservoir, peristaltic pump, heat exchanger, bubble trap, oxygenator and tubing[65] (Figures 2 and 3). Various MP systems have been described, ranging from simpler self-made setups to more complex designs[70-110]. The organ chamber lies at the center of the MP system, where the liver is carefully positioned hilum facing upwards. Some authors suggest using an elastic pillow to aid in positioning the liver [51]. The perfusate reservoir, connected to the chamber, holds the perfusion solution to be pumped through the organ. Alternatively, the organ chamber could also serve as perfusate reservoir[19]. The peristaltic pump regulates the flow rate of the perfusate, ensuring precise delivery to the organ. When perfusion is performed *via* both PV and HA, two peristaltic pumps are necessary[11,75]. Additionally, controlling temperature is crucial, often achieved through the heat exchanger, thermostat[76], heating water bath with temperature sensor[41,52], combined heat exchanger-oxygenator[54], and other alternatives, maintaining the perfusate at the desired temperature throughout the process. The oxygenator enriches the solution with oxygen to sustain the metabolic needs of the organ. Various types of oxygenators, including membrane[29, 47,55,59,61,75,76,84,88], hollow-fiber[32,52,57,77], bubble[93], tubing[15,53,58] and others, are discussed by the authors. The bubble trap removes air bubbles from the perfusate and prevents air embolism. Tubing connects these components, allowing for the seamless flow of the perfusate through the system. The implementation of sample ports is also crucial for obtaining perfusate samples during the MP process[93]. Monitoring MP parameters is also highly significant. Some researchers choose basic manometers to measure pressure[105], while others prefer sophisticated equipment equipped with diverse sensors and data acquisition devices, enabling real-time analysis and display of parameters[75]. Some researchers propose incorporating a dialysis unit into the MP circuit, which could potentially improve preservation outcomes during NMP[27,51].

Perfusate volume and composition

According to the literature, the volume of perfusate used in small animal MP can range from 2 mL to 500 mL, as detailed in Table 4. Although there are no specific recommendations regarding the total perfusion volume, some authors propose reducing it to 50 mL. Notably, when the perfusate includes red blood cells, reducing the volume has been shown to increase hematocrit levels and decrease the release of transaminases[51].

Table 3 Experimental conditions and main findings in small animal liver *ex vivo* machine perfusion studies

Ref.	Animal	WI time before MP	Length of SCS before MP	MP type and length of MP
Kim *et al*[14]	Rats	NA	0 hour	HMP: 48 hours
Dutkowski *et al*[15]	Rats	11.4 minutes 0.8 minutes or 4.2 minutes 0.4 minutes	NA	HMP: 10 hours
Compagnon *et al*[67]	Rats	< 20 seconds	NA	HMP: 24 hours or 48 hours; NMP: 2 hours
Lauschke *et al*[16]	Rats	60 minutes	NA	HMP: 24 hours; NMP (reperfusion): 45 minutes
Lee *et al*[90]	Rats	30 minutes	NA	HMP: 5 hours
Tan *et al*[91]	Rats	NA	30 minutes	HMP: 36 hours
Xu *et al*[92]	Rats	NA	0 hour (before HMP) or 24 hours (before NMP)	HMP: 24 hours; NMP: 1 hour
Bessems *et al*[17]	Rats	NA	NA	HMP: 24 hours; NMP (reperfusion): 1 hour
Dutkowski *et al*[18]	Rats	NA	10 hours	HOPE: 3 hours; NMP (reperfusion): 40 minutes
Tolboom *et al*[19]	Rats	NA	0 hour	NMP: 6 hours
Vairetti *et al*[20]	Rats	NA	NA	4 °C, 10 °C, 20 °C, 25 °C, 30 °C, or 37 °C MP: 6 hours, NMP (reperfusion): 2 hours
Manekeller *et al*[21]	Rats	30 minutes	NA	HMP: 18 hours; NMP (reperfusion): 2 hours
Stegemann *et al*[22]	Rats	30 minutes	NA	HMP: 18 hours; NMP (reperfusion): 2 hours
Ferrigno *et al*[23]	Rats	NA	NA	HMP or SNMP: 6 hours; NMP (reperfusion): 2 hours
Lüer *et al*[24]	Rats	NA	NA	HMP: 18 hours; NMP (reperfusion): 2 hours
Olschewski *et al*[25]	Rats	60 minutes	NA	HMP or SNMP: 6 hours; NMP (reperfusion): 6 hours
Minor *et al*[26]	Rats	30 minutes	NA	HMP: 18 hours; NMP (reperfusion): 2 hours
Tolboom *et al*[27]	Rats	1 hour	NA	SNMP: 5 hours; NMP: 5 hours
Giannone *et al*[28]	Rats	NA	NA	Normobaric or hyperbaric HMP: 24 hours
Perk *et al*[29]	Rats	60 minutes or 90 minutes	NA	NMP: 6 hours
Schlegel *et al*[30]	Rats	30 minutes	4 hours	HMP: 1 hour
Bruinsma *et al*[88]	Rats	NA	0 hour, 24 hours, 48 hours, 72 hours and 120 hours	SNMP: 3 hours
Liu *et al*[31]	Rats	NA	NA	SNMP: 6 hours
Carnevale *et al*[93]	Rats	45 minutes	NA	HMP: 24 hours; NMP (reperfusion): 1.5 hours
Schlegel *et al*[32]	Rats	NA	30 minutes	HMP or HNP: 1 hour
Schlegel *et al*[33]	Rats	30 minutes or 60 minutes	NMP: 0 minute or 15 minutes; HOPE: 4 hours	NMP: 4 hours; HOPE: 1 hour
Bae *et al*[34]	Rats	30-40 minutes	NA	HMP: 8 hours; NMP (reperfusion): 90 minutes
Niu *et al*[68]	Rats	60 minutes	5 hours	NMP: 2 hours
Tarantola *et al*[35]	Rats	NA	6 hours	SNMP, NMP (reperfusion): 6 hours
Bruinsma *et al*[36]	Rats	NA	NA	Loading SNMP: 80 minutes; supercooling: 3-4 days; recovery SNMP: 5 hours
Ferrigno *et al*[37]	Rats	NA	NA	10 °C, 20 °C, 30 °C or 37 °C MP: 6 hours
Jia *et al*[38]	Rats	NA	0 hour or 6 hours	HMP: 6 hours or 0 hour

Westerkamp *et al*[94]	Rats	30 minutes	6 hours	HMP or SNMP or rewarming MP: 1 hour; NMP: 2 hours
Carbonell *et al*[95]	Rats	NA	NA	NMP: 15 minutes to stabilize (all groups); SNMP or NMP: 30 minutes
Op den Dries *et al*[75]	Rats	0 minute or 30 minutes	0 hour or 3 hours	NMP: 3 hours; NMP (reperfusion): 2 hours
Okamura *et al*[39]	Rats	NA	NA	SNMP: 4 hours; NMP: 2 hours
Berardo *et al*[40]	Rats	NA	6 hours	SNMP: 6 hours; NMP: 2 hours
Chai *et al*[89]	Rats	NA	NA	HMP: 2 hours or 12 hours
Zeng *et al*[41]	Rats	30 minutes	NA	HMP: 3 hours; NMP (reperfusion): 2 hours
Beal *et al*[42]	Rats	NA	NA	NMP: 4 hours
Tabka *et al*[69]	Rats	NA	NA	SNMP: 6 hours; NMP (reperfusion): 2 hours
Xue *et al*[43]	Rats	30 minutes	NA	HMP: 3 hours; NMP: 1 hour
He *et al*[78]	Rats	NA	NA	HMP: 3 hours or 6 hours
Gassner *et al*[70]	Rats	30 minutes	NA	NMP: 6 hours
Zeng *et al*[79]	Mice	30 minutes	4 hours	HOPE or HNPE: 1 hour; NMP (reperfusion): 2 hours
Jia *et al*[80]	Rats	NA	NA	HMP: 6 hours
Oldani *et al*[44]	Rats	1 hour	30 minutes	HOPE or NMP: 2 hours
Chin *et al*[45]	Rat	NA	NA	NMP: 6 hours
Gillooly *et al*[46]	Rats	25 minutes	NA	HMP, NMP: 4 hours
Martins *et al*[47]	Rats	NA	12 hours	NMP: 1 hour
Scheuermann *et al*[48]	Rats	NA	0 hour	SNMP or NMP: 4 hours; NMP (reperfusion): 2 hours
Claussen *et al*[71]	Rats	< 15 minutes	< 60 minutes	NMP: 6 hours
Haque *et al*[49]	Rats	NA	NA	NMP: 24 hours
Schlegel *et al*[50]	Rats	30 minutes	4 hours	NMP or HOPE: 1 hour
Nösser *et al*[51]	Rats	NA	81.71 minutes ± 28.44 minutes	SNMP or NMP: 6 hours or 12 hours
Yamada *et al*[96]	Rats	30 minutes	6 hours	SNMP or NMP: 30 minutes, 60 minutes or 90 minutes
Hu *et al*[52]	Rats	30 minutes	NA	HMP: 1 hour, 3 hours, 4 hours, 12 hours, 24 hours; NMP: 2 hours
Von Horn and Minor[53]	Rats	20 minutes	18 hours	NMP: 2 hours
Raigani *et al*[54]	Rats	NA	< 10 minutes	NMP: 6 hours
Yang *et al*[55]	Rats	30 minutes	NA	NMP: 8 hours
Westerkamp *et al*[76]	Rats	NA	4 hours	NMP: 3 hours
De Vries *et al*[86]	Rats	0 h	0 hour or 24 hours or 72 hours	SNMP: 3 hours
Liu *et al*[85]	Rats	0 minute, 10 minutes or 30 minutes	NA	HMP or NMP: 4 hours
Lin *et al*[56]	Rats	NA	1 hour	HMP: 3 hours
Rigo *et al*[57]	Rats	3.10 minutes (0.35) mean (SEM)	NA	NMP: 4 hours
Xu *et al*[97]	Rats	NA	NA	NMP: 4 hours
Carlson *et al*[58]	Rats	NA	< 5 minutes	NMP: 4 hours
Zhou *et al*[81]	Rats	30 minutes	23 hours	HOPE: 1 hour; NMP: 1 hour
Cao *et al*[59]	Rats	30 minutes	NA	NMP: 4 hours
De Stefano *et al*[60]	Rats	60 minutes	No or 34 minutes ± 7 minutes	NMP: 6 hours

Sun *et al*[61]	Rats	30 minutes	NA	NMP: 6 hours
Jennings *et al*[62]	Rats	NA	< 5 minutes	NMP: 4 hours
Wang *et al*[72]	Mice	10 minutes	11 hours	HOPE: 1 hour; NMP (reperfusion): 2 hours
Asong-Fontem *et al*[73]	Rats	NA	24 hours	HOPE: 2 hours; NMP (reperfusion): 2 hours
Shi *et al*[77]	Rats	30 minutes	8 hours	NMP: 2 hours
Von Horn *et al*[63]	Rats	20 minutes	17 hours or 18 hours	HMP: 1 hour; NMP (reperfusion): 2 hours
Zhou *et al*[82]	Rats	30 minutes	0 hour or 23 hours	HOPE: 1 hour; NMP: 1 hour
Luo *et al*[83]	Rats	30 minutes	3 hours or 4 hours	HMP or HOPE: 1 hour; NMP: 2 hours
Ohara *et al*[64]	Rats	60 minutes	NA	NMP: 4 hours
Chen *et al*[65]	Mice	NA	NA	NMP: 12 hours
Fukai *et al*[74]	Rats	30 minutes	NA	HMP: 3 hours; NMP (reperfusion): 90 minutes
Hughes *et al*[84]	Rats	30 minutes	During MP priming	NMP: 4 hours
Bai *et al*[98]	Rats	30 minutes	8 hours	NMP: 2 hours
Von Horn *et al*[66]	Rats	20 minutes	18 hours	Rewarming MP: 2 hours; NMP: 1 hour
Li *et al*[87]	Rats	NA	NA	NMP: 3 hours

WI: Warm ischemia; SCS: Static cold storage; MP: Machine perfusion; HMP: Hypothermic machine perfusion; HOPE: Hypothermic oxygenated machine perfusion; NMP: Normothermic machine perfusion; NA: Not written or not applicable in the main article; HNPE: Hypothermic deoxygenated (nitrogenated) perfusion; SNMP: Subnormothermic machine perfusion.

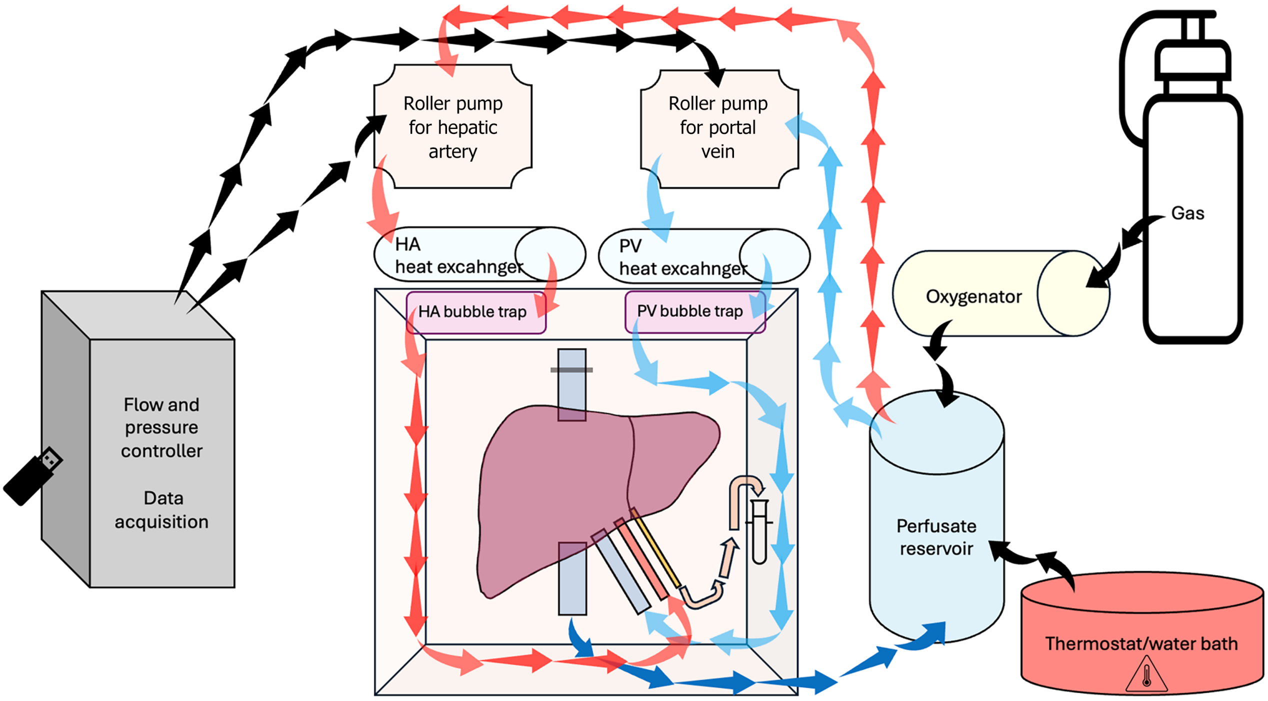

Figure 2 Dual-vessel closed machine perfusion circuit. Perfusate is stored in the perfusate reservoir where the targeted temperature is achieved through tubing connected to the thermos unit (*e.g.*, water bath). The perfusate is oxygenated *via* an oxygenator connected to a gas tank. Oxygenated perfusate, maintained at a specific temperature, is pumped through roller pumps controlled by flow and pressure regulators. Upon passing through the pumps, the perfusate goes through a heat exchanger and bubble traps before reaching its final destination: The portal vein and hepatic artery. Exiting the liver *via* the infrahepatic vena cava, the perfusate returns to the perfusate reservoir through tubing. The common bile duct is cannulated, and the catheter is connected to the tube for bile collection. HA: Hepatic artery; PV: Portal vein.

Table 4 Perfusate compositions in small animal liver *ex vivo* machine perfusion studies

Ref.	Animal weight	Liver weight	Perfusate volume	Perfusate composition
Kim *et al*[14]	200-400 g	NA	200 mL	Cold UW-G solution
Dutkowski *et al*[15]	250-300 g	10.4 ± 0.3 g	500 mL	Modified UW solution: Starch and glutathione was omitted, supplemented with 80 mg/L gentamycin, and 5000 IU/L heparin
Compagnon *et al*[67]	300 ± 50 g	NA	150 mL	HMP: Celsior-HES solution. NMP: KHB buffer with 5% bovine serum albumin
Lauschke *et al*[16]	250-300 g	NA	125 mL	HMP: HTK or Belzer MPS solution supplemented with 6000 IU superoxide dismutase. NMP: KH buffer
Lee *et al*[90]	200-250 g	NA	100 mL	KH solution, then switched to UW solution (starch omitted)
Tan *et al*[91]	180-220 g	NA	120 mL	Modified Hoffmann perfusate: Hydroxyethyl starch 50 g/L, calcium gluconate 80 mmol/L, raffinose 10 mmol/L, KH_2PO_4 25 mmol/L, hydroxyethyl piperazine 10 mmol/L, dexamethasone 12 mg/L, penicillin 2×10^5 units/L, insulin 100 units/L, and with/without 25 mmol/L MgCl and/or 5 mmol/L ATP
Xu *et al*[92]	200-250 g	NA	NA	HMP: UW solution with/without starch. NMP: KH buffer solution containing 112 μmol/L taurocholic acid, and 150 μg/L hyaluronic acid
Bessems *et al*[17]	350 ± 50 g	16.5 ± 0.5 g	250 mL	HMP: The UW-G solution or polysol. NMP: KHB without bovine serum albumin
Dutkowski *et al*[18]	250-300 g	10.8 ± 1.4 g	450 mL	HOPE: Modified starch-free UW solution. NMP: KHB buffer
Tolboom *et al*[19]	250-300 g	9.74-0.81 g	55-60 mL	NMP: Phenol red-free WEM supplemented with 2 IU/L insulin, 40000 IU/L penicillin, 40000 mg/L streptomycin, 0.292 g/L L-glutamine, 10 mg/L hydrocortisone, 1000 IU/L heparin with 25% (v/v) freshly isolated rat plasma and freshly isolated rat erythrocytes to a hematocrit of 16% to 18%
Vairetti *et al*[20]	250-300 g	NA	200 mL	KH solution with 1.25 mmol/L $CaCl_2$ or with 0.25 mmol/L $CaCl_2$
Manekeller *et al*[21]	250-300 g	NA	125 mL	HMP: HTK or Blezer MPS solutions. NMP: KH buffer
Stegemann *et al*[22]	250-300 g	NA	HMP: 125 mL; NMP: 250 mL	HMP: HTK or modified HTK solution (custodiol-N). NMP: KH buffer with 3 g/100 mL of bovine serum albumin
Ferrigno *et al*[23]	375 ± 15 g and 300 ± 10 g	NA	200 mL	KH medium
Lüer *et al*[24]	250-300 g	NA	125 mL	HMP: HTK solution. NMP: WEM solution, supplemented with 3 mg/100 mL of bovine serum albumin
Olschewski *et al*[25]	250-280 g	NA	NA	HMP, SNMP: Lifor solution. NMP: KH solution
Minor *et al*[26]	250-300 g	NA	HMP: 125 mL; NMP: 250 mL	HMP: HTK solution supplemented with 0 μmol/L, 10 μmol/L, 50 μmol/L or 100 μmol/L of dopamine. NMP: WEM solution with 3 mg/100 mL of bovine serum albumin
Tolboom *et al*[27]	250-300 g	NA	55-60 mL	WEM with autologous erythrocytes and plasma. To this were added: Insulin (2 IU/L), penicillin (40000 IU/L)/streptomycin (40000 μg/L), L-glutamine (0.292 g/L), hydrocortisone (10 mg/L), and heparin (1000 IU/L)
Schlegel *et al*[30]	250-300 g	9.83 ± 0.95 g	50 mL	Modified starch free UW-solution
Giannone *et al*[28]	250-300 g	NA	NA	Celsior solution
Perk *et al*[29]	200-300 g	NA	55-60 mL	Phenol red-free WEM supplemented with 2 IU/L insulin (28.85 units/mg), 100000 IU/L penicillin, 100 mg/L streptomycin sulfate, 0.292 g/L L-glutamine, 10 mg/L hydrocortisone, and 1000 IU/L heparin. Fresh rat plasma (25% v/v) and erythrocytes (18%-20% v/v) were collected and added to the perfusate

Bruinsma *et al*[88]	180-250 g	NA	500 mL	WE supplemented with insulin (2 IU/L), penicillin (40000 IU/L)/streptomycin (40000 µg/L), L-glutamine (0.292 g/L), hydrocortisone (10 mg/L)
Liu *et al*[31]	NA	21.4 ± 3.6 g (control) 22.9 ± 5.8 g (defatting group)	200 mL	The control perfusate: Minimum essential medium supplemented with 3% wt/vol bovine serum albumin, 1.07 mmol/L lactic acid, and 0.11 mmol/L pyruvic acid. The defatting perfusate: The control perfusate supplemented with the 6 defatting agents (forskolin, GW7647, scoparone, hypericin, visfatin, and GW501516)
Carnevale *et al*[93]	250-300 g	NA	250 mL	HMP: HTK solution: 100 mmol/L sodium gluconate, 7 mmol/L potassium gluconate, 20 mmol/L sucrose, 30 mmol/L BES, 2.5 mmol/L KH_2PO_4, and 0.03 mmol/L polyethylene glycol (35 kDa), 5 mmol/L $MgSO_4$, 3 mmol/L glutathione, 5 mmol/L adenosine, and 15 mmol/L glycine, together with 0.25 mg/mL streptomycin and 10 IU/mL penicillin G. NMP: KH buffer with 4% dextran added
Schlegel *et al*[32]	250-320 g	9.7 ± 1.5 g	50 mL	Modified starch free UW-solution
Schlegel *et al*[33]	250-320 g	10.14 ± 2.73 g	50 mL	NMP: Diluted full blood or leukocyte and platelet depleted blood perfusate. HOPE: Modified starch-free UW solution
Bae *et al*[34]	300 ± 25 g	NA	100 mL	HMP: KPS-1 solution (Identical to Belzer's UW MPS) or KPS-1, enhanced with α-ketoglutarate, L-arginine, N-acetylcysteine, nitroglycerin, and prostaglandin E1 or KPS-1, with 5.4×10^{-2} mmol/L of α-tocopherol diluted in acetone. NMP: KHB buffer
Niu *et al*[68]	261 ± 4 g	NA	353 ± 11 mL	KH buffer
Tarantola *et al*[35]	375 ± 15 g and 300 ± 10 g	NA	200 mL	KH medium
Bruinsma *et al*[36]	250-300 g	Approximately 10 g	100 mL	3-OMG loading solution: 500 mL phenol-red free WEM, 5 mL of 200 mmol/L L-glutamine (0.292 mg/L), 4 mL of penicillin-streptomycin (5000 IU/mL), 5 mg of hydrocortisone, 5000 U of sodium heparin, 375 U of insulin, 19.42 g of 3-O-methyl glucose (0.2 M)
Ferrigno *et al*[37]	250-300 g	NA	NA	A modified KH buffer
Jia *et al*[38]	250-300 g	NA	60 mL	UW or HTK solutions
Westerkamp *et al*[94]	290-320 g	NA	100 mL	HMP, SNMP, rewarming MP: Belzer MPS. NMP: 25 mL of human red blood cell concentrate (final hematocrit 25%), 53.9 mL of WEM solution, 20 mL of human albumin (200 g/L), 1 mL of insulin (100 IU/mL), and 0.1 mL of unfractionated heparin (5000 IU/mL)
Carbonell *et al*[95]	225-250 g	NA	NA	KH buffer: 118 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 2.5 mmol/L $CaCl_2$, 25 mmol/L $NaHCO_3$, 20 mmol/L HEPES
Op den Dries *et al*[75]	303 ± 4 g (mean ± SEM)		100 mL	20 mL of human red blood cell concentrate (final hematocrit 15%-20%), 59 mL of WEM solution, 20 mL of human albumin (200 g/L), 1 mL of insulin (100 IU/mL), and 0.1 mL of unfractionated heparin (5000 IU/mL)
Okamura *et al*[39]	250-300 g	NA	300 mL	SNMP: Polysol solution. NMP: KH buffer
Berardo *et al*[40]	250-300 g	NA	NA	KH buffer
Chai *et al*[89]	250-300 g and 600-630 g	NA	80 mL	UW solution with/without 0.165 mg/L of metformin
Zeng *et al*[41]	250-300 g	NA	150 mL	HTK solution
Beal *et al*[42]	250-350 g	NA	300 mL	86 mL of 25% albumin, 184 mL of WEM, 30 mL of penicillin/streptomycin (10 IU/mL penicillin and 0.01 mg/mL streptomycin), insulin (50 IU/L), heparin (0.01 IU/mL), L-glutamine (0.292 g/L), and hydrocortisone (0.010 g/L). Addition of 625 IU/mL pegylated-catalase
Tabka *et al*[69]	250-300 g	NA	SNMP: 150 mL;	SNMP: Celsior with/without Ang IV. NMP: KBB

			NMP: 140 mL	enriched with 5% albumin
Xue *et al*[43]	250 ± 10 g	NA	HMP: 100 mL; NMP: 250 mL	HMP: HTK solution. NMP: KH buffer with 4% dextran
He *et al*[78]	250-300 g	NA	NA	HTK solution
Gassner *et al*[70]	280-350 g	13.6 g ± 2.15 g	50 mL	NMP: Low-glucose DMEM supplemented with the rat erythrocyte concentrate and 12.5 mL strain specific rat plasma
Zeng *et al*[79]	20-24 g	NA	50 mL	HTK solution
Jia *et al*[80]	250-300 g	NA	60 mL	HTK solution
Oldani *et al*[44]	176 (155-193) g	NA	50 mL	HOPE: IGL-1 solution with 150 IU heparin. NMP: 25 mL Fischer rat blood with 25 mL normal saline, and with 150 IU heparin
Chin *et al*[45]	200-250 g	NA	150 mL	DMEM supplemented with 200 mmol/L L-glutamine, 10% v/v FBS, 5% with bovine serum albumin, 8 mg/L dexamethasone, 2000 IU/L heparin, 2 IU/L insulin, and 5×10^6 engineered rat fibroblasts
Gillooly *et al*[46]	Approximately 300 g	NA	100 mL	99 mL WEM with 10 IU insulin, and lipid nanoparticles (50 nM siRNA)
Martins *et al*[47]	320-350 g	NA	NA	50% plasma-Lyte and 50% KH solution
Scheuermann *et al*[48]	296 g ± 8 g (mean ± SEM)	NA	100 mL	SNMP, NMP: 80 mL of WEM supplemented with 5% bovine serum albumin, 20 mL of type O + human RBCs, 0.2 IU insulin, 29.2 mg L-glutamine, 1 mg hydrocortisone, and 500 IU heparin. NMP: KH buffer supplemented with 5% bovine serum albumin
Claussen *et al*[71]	280-350 g	NA	50 mL	10 mL of the erythrocyte concentrate, 35 mL of DMEM, 5 mL of strain specific rat plasma supplemented with 1000 IU heparin and 12 mmol/L glycine
Haque *et al*[49]	250-300 g	NA	500 mL	NMP: 950 mL of William's E media, 20 g of bovine serum albumin, 20 g of polyethylene glycol 35000, 20 mg of dexamethasone, 2 mL of heparin, 1 mL of regular insulin, 10 mL penicillin-streptomycin, 10 mL of antibiotic-antimycotic, and 2.2 g of sodium bicarbonate
Schlegel *et al*[50]	250-320 g	9.8 0.6 g	100 mL	HOPE: Belzer MPS. NMP: Belzer MPS or diluted heparinized blood
Nösser *et al*[51]	398.87 ± 133.12 g	14.15 ± 2.66 g	250 mL, 100 mL, 80 mL, 50 mL	SNMP: 500 Ml DMEM supplemented with 100 µg/mL penicillin and streptomycin, 4 mmol/L L-glutamine/L-alanine, 1 µM human insulin, 14 ng/mL glucagon, 1 µM dexamethasone. NMP: The isolated RBCs suspended in DMEM, isolated rat plasma (10% of the total volume), and 500 IU of heparin
Yamada *et al*[96]	260-350 g	DCD 10.12 ± 0.93 g	NA	KH buffer
Hu *et al*[52]	250-300 g	NA	150 mL	HTK solution
Von Horn and Minor [53]	250-300 g	NA	200 mL	Aqix RS-I solution
Raigani *et al*[54]	NA	NA	500 mL	High-glucose DMEM supplemented with 10% v/v FBS, 2% v/v penicillin-streptomycin, and 3% w/v bovine serum albumin. Defatting cocktail agents include 10 µM forskolin, 1 µM GW7647, 1 µM GW501516, 10 µM scoparone, 10 µM hypericin, 0.4 ng/mL visfatin, 0.8 mmol/L L-carnitine, and additional amino acids
Yang *et al*[55]	200-220 g	NA	NA	60 mL DMEM/F12 (1:1) containing 20% FBS and 1% penicillin-streptomycin solution (penicillin 10000 IU/mL, streptomycin 10000 µg/mL), 20 mL of fresh blood, 5 IU/mL of heparin, 2 IU/L of insulin, and 2.5 µg/mL of dexamethasone
Westerkamp *et al*[76]	270-300 g	NA	100 mL	25 mL human red blood cell concentrate, 53.9 mL WEM solution, 20 mL human albumin (200 g/L), 1 mL insulin (100 IU/mL), and 0.1 mL unfractionated heparin (5000 IU/mL)

De Vries *et al*[86]	200-250 g	NA	500 mL	WEM supplemented with sodium bicarbonate (2.2 g/L), dexamethasone (24 mg/L), insulin (5 IU/L), heparin (2000 IU/L), and bovine serum albumin (10 mg/mL)
Liu *et al*[85]	NA	NA	70 mL	HMP: UW solution with 250 IU heparin, 20000 IU penicillin and 2 mg hydrocortisone. Addition of CIRP competitive inhibitor (C23, 300 ng/mL). NMP: 20 mL rat blood, 50 mL WEM, 250 IU heparin, 20000 IU penicillin, 2 mg hydrocortisone, 0.4 IU insulin, and 0.0292 g glutamine
Lin *et al*[56]	180 ± 20 g	NA	50 mL	HTK solution with or without various defatting agents (10 mmol/L forskolin; 1 mmol/L GW7647; 10 mmol/L hypericin; 10 mmol/L scoparone; 0.4 ng/mL visfatin; 1 mmol/L GW501516)
Rigo *et al*[57]	200-250 g	10.35 g (0.41) mean (SEM)	70 mL	NMP: 20 mL of fresh rat blood, 50 mL of complete phenol red-free WEM, supplemented with 11.6 mmol/L glucose, 50 IU/mL penicillin, 50 μg/mL streptomycin, 5 mmol/L L-glutamine, 1 IU/mL insulin, 1 IU/mL heparin, and 2 mEq of sodium bicarbonate
Xu *et al*[97]	Approximately 500 g	Approximately 15 g	60-110 mL	Whole blood-based perfusate with different defatting components
Carlson *et al*[58]	331 ± 16 g (mean ± SEM)	NA	95 mL	WEM, 3250 IU each penicillin/streptomycin, 0.65 mmol/L sodium pyruvate, 1.30 mmol/L L-glutamine, 1% human albumin, 500 IU heparin, 15 mg papaverine, 1 mg insulin, 1.25 mg hydrocortisone, and 30 mL of leukoreduced, packed RBCs
Zhou *et al*[81]	250-300 g	NA	NA	NA
Cao *et al*[59]	200-220 g	NA	80 mL	DMEM/F12, 20 mL rat blood, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 5 IU/mL heparin
De Stefano *et al*[60]	200-250 g	Control group: 17.10 (1.93). WI group: 15.17 (0.83)	150 mL	100 mL of phenol red-free WEM, supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin, 0.292 g/L L-glutamine, 1 IU/mL insulin, 1 IU/mL heparin, and 50 mL of recently expired (max 5 days) human red blood cell
Sun *et al*[61]	200-220 g	NA	2 mL	Normal saline or single cell suspension containing 1×10^7-3×10^7 BMMSCs
Jennings *et al*[62]	320 ± 11 g (mean ± SEM)	NA	130 mL or 146 mL	WEM (65 mL for pRBC oxygen carriers and 50 mL for oxyglobin) with 3250 U each penicillin/streptomycin, 500 U heparin, 1 mg insulin, 1.25 mg hydrocortisone, and 15 mg papaverine. In addition, sodium pyruvate 0.65 mmol/L, L-glutamine 130 mmol/L, and human albumin 1%. The oxygen carriers added to the perfusate were human pRBC (30 mL), rat pRBC (30 mL), or oxyglobin (46 mL)
Wang *et al*[72]	NA	NA	HOPE: 50 mL; NMP: 30 mL	HOPE: HTK solution. NMP: 7.5 mL blood, 22.5 mL KHB solution containing 2% FBS, 60 mg glucose, 1 IU insulin, and 150 IU heparin
Asong-Fontem *et al*[73]	NA	NA	NA	HOPE: IGL-2 or PERF-GEN (Belzer MPS) solutions. NMP: Williams medium E supplemented with insulin 2 U/L, penicillin (40000 U/L)/streptomycin (40000 μg/L), L-glutamine, hydrocortisone 10 mg/L and heparin (1000 U/L)
Shi *et al*[77]	320-350 g	NA	36 mL	24 mL whole heparinized blood supplemented with 10% sodium citrate, 1% penicillin and streptomycin, and 12 mL circuit priming solution with 45% lactated ringer, 5% sodium bicarbonate and 50% hydroxyethyl starch
Von Horn *et al*[63]	250-300 g	NA	150 mL	HMP: Aqix RS-I solution. NMP: WEM supplemented with 3 mg/100 mL of bovine serum albumin
Zhou *et al*[82]	250-300 g	NA	NA	NA
Luo *et al*[83]	250-300 g	NA	HMP: 150 mL; NMP: 180 mL	HMP: HTK solution. NMP: DMEM/F12, 20% FBS, 1% penicillin streptomycin solution (penicillin 10000 IU/mL, streptomycin 10000 mg/mL), 5 IU/mL of heparin, 2 IU/L of insulin, and 2.5 mg/mL of

				dexamethasone. Full rat blood (30-45 mL) was reconstituted up to a total volume of 180 mL perfusate
Ohara *et al*[64]	250-350 g	NA	100 mL	Rat whole blood
Chen *et al*[65]	34 ± 4 g (mean ± SEM)	Approximately 1 g	300 mL	WEM was supplemented with 20% FBS, 1% penicillin/streptomycin, 5000 IU/L heparin, 50 IU/L insulin, and 0.010 g/L hydrocortisone
Fukai *et al*[74]	NA	NA	300 mL	HMP: UW-MPS solution. NMP: KHB solution
Hughes *et al*[84]	250-300 g	14 ± 0.2 g	150 mL	Phenol red-free WEM with addition of 25% bovine albumin, rat RBCs, 2 IU/L insulin, 40000 IU/L penicillin, 40000 μg/L streptomycin, 0.292 g/L L-glutamine, 10 mg/L hydrocortisone, 1000 IU/L heparin, and bicarbonate 75%
Bai *et al*[98]	360-380 g	NA	36 mL	24 mL whole heparinized blood supplemented with 10% sodium citrate, 1% penicillin, and streptomycin and 12 mL circuit priming solution with 45% lactated ringer, 5% sodium bicarbonate, and 50% hydroxyethyl starch
Von Horn *et al*[66]	250-300 g	NA	NA	Rewarming MP: Diluted Steen solution or Belzer MPS. NMP: WEM supplemented with 3 mg/100 mL of bovine serum albumin
Li *et al*[87]	250-350 g	NA	100 mL	90 mL WEM with 10 mL rat blood cells supplemented with 10 mg/L hydrocortisone, 5 IU/mL heparin, 1 IU/mL insulin, 5 mmol/L L-glutamine, 40 IU/mL penicillin, 40 μg/mL streptomycin, and with/without 10 mmol/L epigallocatechin gallate

MP: Machine perfusion; HMP: Hypothermic machine perfusion; NMP: Normothermic machine perfusion; NA: Not written or not applicable in the main article; SNMP: Subnormothermic machine perfusion; DCD: Donated after circulatory death; WI: Warm ischemia; HOPE: Hypothermic oxygenated machine perfusion; UW: University of Wisconsin; UW-G: University of Wisconsin-gluconate; HES: Hydroxyethyl starch; KHB: Krebs Henseleit bicarbonate; HTK: Histidine Tryptophan Ketoglutarate; Belzer MPS: Belzer machine perfusion solution; KH: Krebs Henseleit; KH_2PO_4: Potassium dihydrogen phosphate; MgCl: Magnesium chloride; ATP: Adenosine triphosphate; WEM: William's E Medium; $CaCl_2$: Calcium chloride; $MgSO_4$: Magnesium sulfate; KPS: Kidney perfusion solution; 3-OMG: 3-O-methyl-D-glucose; NaCl: Sodium chloride; KCl: Potassium chloride; $NaHCO_3$: Sodium bicarbonate; HEPES: 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid; Ang IV: Angiotensin IV; KBB: Krebs bicarbonate buffer; DMEM: Dulbecco's modified Eagle's medium; IGL: Institute George Lopez; siRNA: Small interfering RNA; FBS: Fetal bovine serum; RBCs: Red blood cells; CIRP: Cold-inducible RNA-binding protein; BMMSC: Bone marrow mesenchymal stem cell; pRBC: Packed red blood cell.

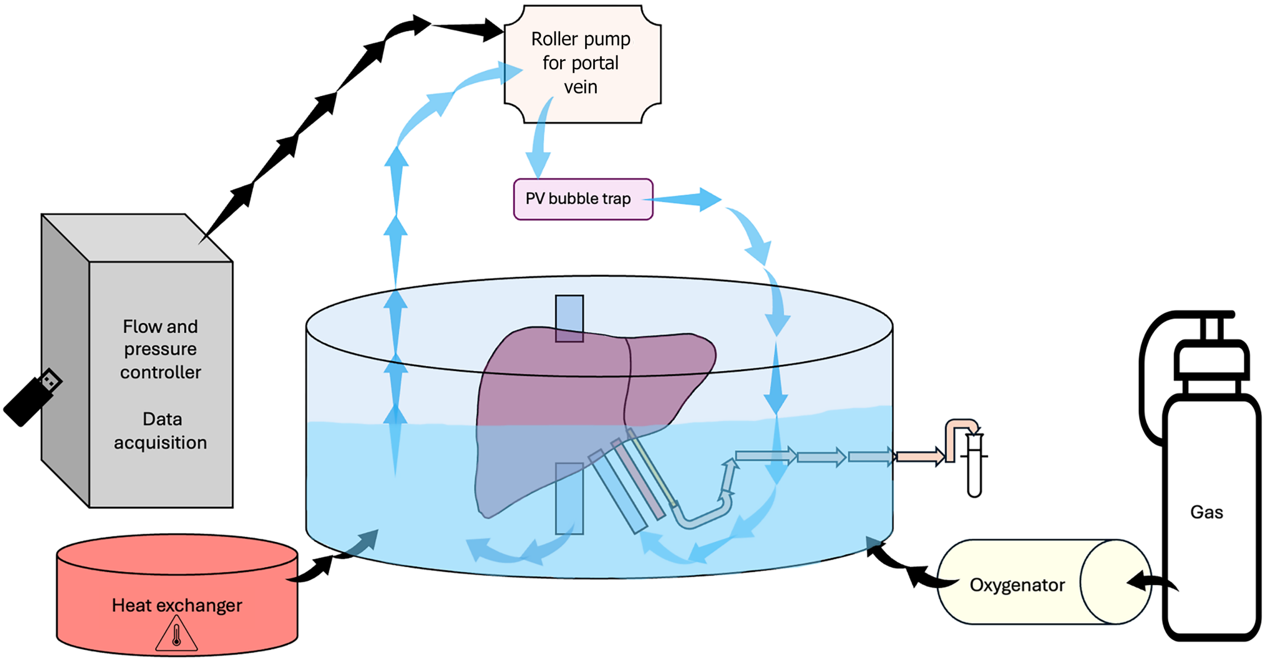

Figure 3 Single-vessel machine perfusion circuit. The liver is placed inside an organ chamber, which serves as a perfusate reservoir. The perfusate is oxygenated *via* an oxygenator, and the targeted temperature is achieved *via* a thermos unit. The perfusate from the organ chamber is then taken up by a roller pump,

which is controlled by a flow and pressure controller. After passing through the roller pump, the perfusate goes through a bubble trap and into the portal vein. Upon perfusing the liver, the perfusate exits the liver freely *via* the vena cava. The common bile duct is cannulated, and the catheter is connected to the tube for bile collection. PV: Portal vein.

There is a wide range of suggested perfusate compositions for HMP and SNMP in the literature. Some of the most commonly used perfusates are Krebs Henseleit bicarbonate, UW solution, HTK solution, Belzer MPS, WEM and others. Some researchers use modified perfusates by omitting starch[15,18,30,32,33,90], while others argue that starch-containing solutions enhance endothelial cell function and reduce hepatocellular damage compared to starch-free solutions[92]. In all three types of MP authors usually supplement perfusate with antibiotics and heparin[73,83-85,87,98]. Supplementation with other various medications is less common in HMP and SNMP setting as liver function is suppressed in lower temperatures[109]. However, some authors suggest supplementation in order to improve MP effectiveness and improve liver preservation outcomes. For instance, adding dopamine has been shown to potentially improve HMP effectiveness [26]. Additionally, supplementation with low-dose tacrolimus has resulted in better graft function and survival[32]. Adding α-tocopherol to the perfusate has demonstrated a reduction in inflammatory cytokines[42], while the inclusion of metformin in the perfusion solution has decreased rat liver injury[89]. Furthermore, perfusate supplementation with IGL-2 has been reported to reduce transaminases and significantly lower levels of glycocalyx proteins, *CASP3*, and *HMGB1*, indicating its protective role in preserving fatty livers[73]. Supplementation with angiotensin IV has been found to decrease the median effect concentration value and improve endothelium-dependent relaxation of HA rings[69].

For NMP, authors typically use various perfusates mixed with whole fresh blood, artificial blood, red blood cells, or other oxygen carriers. During NMP perfusate supplementation with erythrocytes reduces cell damage and improves liver function[51]. However, some protocols have demonstrated that 12-hour erythrocyte-free NMP in mice has no significant impact on histological structure[65]. Supplementation with additional medications such as insulin, glucose, heparin, hydrocortisone, albumin, and amino acids is more frequent in NMP to better mimic the *in vivo* liver environment. Some researchers have added pegylated-catalase to the base perfusate, which has reduced liver preservation injury[42]. Glycine treatment has synergistically preserved the integrity of both normal and donation after DCD liver grafts[70]. Supplementation with metamizole has led to higher bile production, lower transaminase levels, and reduced necrosis in liver and bile duct tissue[71]. Several researchers have investigated perfusate supplementation with bone marrow mesenchymal stem cells (BM-MSCs), which have shown promise in enhancing liver quality in rat DCD livers by reducing oxidative stress, improving mitochondrial function, lowering reactive oxygen species and free ferrous ion levels, and repairing the morphology and function of donor livers[55,61]. Additionally, BM-MSCs modified with heme oxygenase 1 have inhibited natural killer cell and cluster of differentiation 8^+ T cell activation, thus reducing acute graft rejection[59]. Other medications, such as defatting agents, are used when researchers aim not only to bridge the time until LTx but also to treat conditions like fatty liver disease[56,73,87,97].

Table 4 represents various perfusate compositions and volumes proposed by the authors. To highlight the importance of adjusting perfusate volume according to liver weight, we included the animal and liver weights. This provides a more accurate representation of the relationship between organ weight and perfusate volume used.

MP parameters

Ex vivo rat MP studies are published with a variety of temperature, flow, and pressure settings, although detailed descriptions are missing in several studies. Table 5 represents possible MP parameters used in small animal liver MP models.

Perfusion applied *via* PV exhibits varying flow settings depending on the type of MP employed: In HMP, the potential flow settings range from 0.1 mL/minute/g to 0.5 mL/minute/g liver; In SNMP, the range extends from 1 mL/minute/g to 12 mL/minute/g liver; And in NMP, the range spans from 1 mL/minute/g to 30 mL/minute/g liver. Another feasible approach is to tailor the flow based on targeted pressure levels[29,44,46,94]. However, HA flow and pressure descriptions are less common, as the majority of authors prefer single-vessel MP. In NMP, HA flow settings range from 4 mL/minute to 6 mL/minute or from 0.1 mL/minute/g to 0.25 mL/minute/g liver weight, while in SNMP, it is suggested to be 0.1 mL/g of liver/minute. Some authors propose setting PV and HA flow rates in a 3:1 ratio[77].

As there are three main types of MP, literature reveals significant inconsistency regarding the specific temperatures associated with these terms[13]. Most authors perform HMP at 4 °C, though some use lower temperatures between 0 °C and 4 °C, and a few employ temperatures as high as 12 °C. NMP is generally conducted at 37 °C, with slight variations of up to 2 degrees in some studies. SNMP exhibits the most diverse temperature settings, ranging from 20 °C to 35 °C. Table 5 presents a range of temperature settings employed in small animal HMP, SNMP, and NMP.

DISCUSSION

Over the past two decades, MP has been considered a significant advancement in the field of transplantation[110]. While it has shown superiority over standard SCS and the potential to enhance suboptimal livers, many of its possible applications remain unexplored. Utilizing small animal liver MP presents an excellent opportunity to investigate these potential roles. Rat liver MP is an ideal model for investigating possible MP applications due to its lower costs compared to large animal studies[49]. In rats, around 10% of the liver's blood supply is arterial and 90% is venous, compared to larger animals. Most literature suggests that perfusion through the PV alone is sufficient and that LTx can be done without

Table 5 Parameters used in small animal liver *ex vivo* machine perfusion studies

Ref.	HMP			SNMP			NMP		
	Temperature	Flow	Pressure	Temperature	Flow	Pressure	Temperature	Flow	Pressure
Kim *et al*[14]	4 °C	0.5 mL/minute/g liver	11.2 ± 0.4 mmHg						
Dutkowski *et al*[15]	3-6 °C	0.43-0.44 mL/minute/g liver	4.48-0.47 mmHg						
Compagnon *et al*[67]	4 °C	0.1-0.4 mL/minute/g liver	PV, VC: ≤ 1 mmHg; HA: ≤ 11 mmHg				37 °C	3 mL/minute/g wet liver	NA
Lauschke *et al*[16]	4 °C	0.5 mL/minute/g	NA				37 °C	3 mL/g/minute	NA
Lee *et al*[90]	4-5 °C	0.4 mL/minute/g liver	< 3 mmHg						
Tan *et al*[91]	6-8 °C	0.1 mL/minute/g	NA						
Xu *et al*[92]	4 °C	0.4 mL/minute/g liver	NA				37 °C	NA	NA
Bessems *et al*[17]	4 °C	< 1 mL/minute/g liver	< 20 cmH_2O				37.1 °C ± 0.4 °C	< 3 mL/minute/g liver	< 20 cmH_2O
Dutkowski *et al*[18]	3-5 °C	2.75-3.25 mL/minute	4.4 ± 0.5 mmHg				37 °C	15 mL/minute	NA
Tolboom *et al*[19]							37.5 °C	1.8 mL/minute/g ± 0.12 mL/minute/g wet liver	12-15 cmH_2O
Vairetti *et al*[20]	4 °C, 10 °C	NA	NA	20 °C, 25 °C, 30 °C	NA	NA	37 °C	Initial: 1 mL/minute/g, increased to 4 mL/minute/g	NA
Manekeller *et al*[21]	4 °C	NA	NA				37 °C	3 mL/g/minute	NA
Stegemann *et al*[22]	4 °C	0.5 mL/minute/g	NA				37 °C	3 mL/g/minute	NA
Ferrigno *et al*[23]	4 °C or 8 °C	4 mL/minute/g	7.4 ± 0.6 mmHg (lean, 4 °C), 8.7 ± 2.1 mmHg (fat, 4 °C), 6.9 ± 0.8 mmHg (lean, 8 °C), 7.1 ± 0.9 mmHg (fat, 8 °C)	20 °C	4 mL/minute/g	7.3 ± 0.8 mmHg (lean) 7.5 ± 1.6 mmHg (fat)	37 °C	4 mL/minute/g	NA
Lüer *et al*[24]	4 °C	0.5 mL/minute/g	NA				37 °C	3 mL/g/minute	NA
Olschewski *et al*[25]	4 °C or 12 °C	1 mL/minute/g liver	NA	21 °C	1 mL/minute/g liver	NA	37 °C	3 mL/minute/g liver	NA
Minor *et al*[26]	4 °C	0.5 mL/minute/g	NA				37 °C	3 mL/g/minute	NA
Tolboom *et al*[27]				20 °C or 30 °C or 37 °C	2 mL/minute/g	10-14 cmH_2O	37 °C	2 mL/minute/g	10-14 cmH_2O
Giannone *et*	4 °C	1	NA						

al[28]		mL/minute/g liver							
Perk *et al*[29]							37 °C	Pressure dependent	10-12 cmH$_2$O
Schlegel *et al* [30]	4 °C	Pressure dependent	≤ 3 mmHg						
Bruinsma *et al*[88]				Room temperature	NA	NA			
Liu *et al*[31]				20 °C	1 mL/minute/g	NA			
Carnevale *et al*[93]	5.0 °C ± 0.5 °C	0.23 mL/minute/g liver	40 mmH$_2$O (25% of the NMP PV pressure)				37 °C	NA	NA
Schlegel *et al* [32]	4 °C	Pressure dependent	≤ 3 mmHg						
Schlegel *et al* [33]	4 °C	PV: 1-2 mL/minte	PV: 3 mmHg				37 °C	HA: 6 mL/minute; PV: 15 mL/minute	PV: 8 mmHg
Bae *et al*[34]	4 °C	3.5 mL/minute/g of liver	NA				37 °C	NA	NA
Niu *et al*[68]							37 °C	20 mL/minute	NA
Tarantola *et al*[35]				20 °C	4 mL/minute/g	NA	37 °C	4 mL/minute/g	NA
Bruinsma *et al*[36]				21°C	8-12 mL/minute	10-15 cmH$_2$O			
Ferrigno *et al* [37]	10 °C	2.6 mL/minute/g	5.8 ± 0.2 mmHg	20 °C or 30 °C	2.6 mL/minute/g	4.9 ± 0.1 mmHg, 4.9 mmHg ± 0.2 mmHg	37 °C	2.6 mL/minute/g	4.2 ± 0.1 mmHg
Jia *et al*[38]	4 °C	1.4 mL/minute	NA						
Westerkamp *et al*[94]	8 °C	Pressure dependent	PV: 3 mmHg; HA: 25 mmHg	20 °C	Pressure dependent	PV: 4 mmHg; HA: 40 mmHg	37 °C	Pressure dependent	PV: 11 mmHg. HA: 110 mmHg
Carbonell *et al*[95]				22 °C or 26 °C	3 mL/minute/g	NA	37 °C	3 mL/minute/g	NA
Op den Dries *et al*[75]							37 °C	PV: 22.6 ± 0.8 mL/minute; HA: 5.3 ± 0.4 mL/minute	PV: < 11 mmHg. HA: < 110 mmHg
Okamura *et al*[39]				20-24 °C	PV: 1 mL/g-liver/minute; HA: 0.1 mL/g-liver/minute	NA	37 °C	PV: 3 mL/g-liver/minute	NA
Berardo *et al* [40]				20 °C	NA	Starting: 6-7 mmHg	37 °C	NA	Starting: 6-7 mmHg
Chai *et al*[89]	4 °C	4 mL/minute	NA						
Zeng *et al*[41]	0-4 °C	0.5 mL/g/minute	NA				36-37 °C	Pressure dependent	10.3 mmHg
Beal *et al*[42]							37 °C	1-2 mL/minute	10-16 cmH$_2$O
Tabka *et al* [69]				20 °C	0.5 mL/g/minute	NA	37 °C	NA	NA
Xue *et al*[43]	0-4 °C	0.23	NA				36-37 °C	3	NA

		mL/minute/g						mL/minute/g	
He *et al*[78]	4 °C	1.4 mL/minute	NA						
Gassner *et al* [70]							37 °C	1 mL/minute/g liver weight	4-9 mmHg
Zeng *et al*[79]	Approximately 4 °C	0.2 mL/minute/g	< 2 mmHg				36.5 °C ± 0.5 °C	2.5 mL/minute/g	NA
Jia *et al*[80]	4 °C	1.4 mL/minute	4.61 mmHg (mean)						
Oldani *et al* [44]	4 °C	Pressure dependent	4 mmHg				37 °C	Pressure dependent	8 mmHg
Chin *et al*[45]							37 °C	Initial: 5 mL/minute, then pressure dependent	5 mmHg
Gillooly *et al* [46]	4-7 °C	Pressure dependent	10 mmHg				37 °C	Pressure dependent	10 mmHg
Martins *et al* [47]							32 °C or 37 °C	NA	NA
Scheuermann *et al*[48]				25 ± 0.1 °C or 30 °C	1.80 mL/minute/g liver	NA	37 °C	1.80 mL/minute/g liver. Reperfusion: 2.84 ± 0.04 mL/minute/g liver	NA
Claussen *et al* [71]							37 °C	PV: 1 mL/minute/g liver. HA: 0.1 mL/minute/g liver	PV: 5.65-9 mmHg. HA: 48.8-110 mmHg
Haque *et al* [49]							37 °C	25-30 mL/minute	< 12 mmHg
Schlegel *et al* [50]	10 °C	1-2 mL/minute	≤ 3 mmHg				37 °C	15-18 mL/minute	12 mmHg
Nösser *et al* [51]				21 °C	1 mL/g wet liver/minute	5.0 mmHg	37 °C	1 mL/g wet liver/minute	5.0 mmHg
Yamada *et al* [96]				20-25 °C	NA	NA	37 °C	NA	NA
Hu *et al*[52]	0-4 °C	0.5 mL/g/minute	NA				36.5 °C ± 0.5 °C	NA	10.3 mmHg
Von Horn and Minor [53]							35-42 °C	NA	Initial: 3 mmHg. Later: 5 mmHg
Raigani *et al* [54]							37 °C	NA	10-12 mmHg
Yang *et al*[55]							35-38 °C	2 mL/g wet liver/minute	10-12 mmH$_2$O
Westerkamp *et al*[76]							37 °C	NA	PV: 11 mmHg. HA: 110 mmHg
De Vries *et al* [86]				21 ± 1 °C	≤ 25 mL/minute	5 mmHg			
Liu *et al*[85]	4 °C	PV: 8 mL/minute	NA				37 °C	PV: 8 mL/minute; HA: 4 mL/minute	NA
Lin *et al*[56]	4 °C	< 0.15	< 3 mmHg						

		mL/minute/g							
Rigo *et al*[57]							37 °C	1.1-1.3 mL/minute/g	8-10 mmHg
Xu *et al*[97]							37 °C	NA	NA
Carlson *et al* [58]							37 °C	1.8 mL/minute/g	NA
Zhou *et al*[81]	NA	NA	NA				NA	NA	NA
Cao *et al*[59]							36-38 °C	1.5 mL/minute/g wet liver	10-14 cmH$_2$O
De Stefano *et al*[60]							37 °C	NA	12-16 mmHg
Sun *et al*[61]							NA	NA	NA
Jennings *et al* [62]							37 °C	1.8 mL/minute/g	NA
Wang *et al* [72]	4 °C	NA	NA				36.5 ± 0.5 °C	NA	NA
Asong-Fontem *et al* [73]	5 °C ± 3 °C	NA	NA				37 °C	NA	NA
Shi *et al*[77]							38 °C	PV: 5-15 mL/minute. PV and HA flow ratio 3:1	PV: 8-10 mmHg. HA: 90-100 mmHg
Von Horn *et al*[63]	8 °C	NA	5 mmHg				37 °C	3 mL/g minute	NA
Zhou *et al*[82]	NA	NA	NA				NA	NA	NA
Luo *et al*[83]	4 °C	1.2 mL/minute	NA				35-37 °C	2 mL/g/minute	NA
Ohara *et al* [64]							37 °C	PV: 1 mL/minute/g liver. HA: 0.25 mL/minute/g liver	NA
Chen *et al*[65]							37 °C	1 mL/minute	7-10 mmHg
Fukai *et al*[74]	7-10 °C	0.5 mL/minute/g liver	4-6 cmH$_2$O				37 °C	NA	8-12 cmH$_2$O
Hughes *et al* [84]							36-37.5 °C	PV: 1.2 mL/g-liver/minute; HA: 0.2 mL/g-liver/minute	NA
Bai *et al*[98]							NA	NA	NA
Von Horn *et al*[66]				Risen from 8 to 35 °C	5.5 ± 1.4 mL/g/minute or 5.2 ± 1.1 mL/g/minute	Risen from 3 mmHg to 6 mmHg	37 °C	3 mL/g/minute	NA
Li *et al*[87]							37 °C	Pressure dependent	< 4 mmHg

HMP: Hypothermic machine perfusion; NMP: Normothermic machine perfusion; NA: Not written or not applicable in the main article; SNMP: Subnormothermic machine perfusion; PV: Portal vein; VC: Vena cava; HA: Hepatic artery.

reconstructing the HA. Moreover, rat liver explant surgery and transplantation operating times can be under 1 hour, making the process faster, cheaper, and easier compared to large animals. Additionally, the wide availability of inbred rat strains allows researchers to avoid immunological compatibility issues during MP and transplantation[19].

For liver explant surgeries in experimental animals, ensuring proper anesthesia is crucial for maintaining the welfare and stability of the subjects throughout the procedure. Researchers employ various anesthesia protocols tailored to meet experimental requirements and ethical considerations. One common approach involves isoflurane inhalation for sedation, combined with subcutaneous administration of analgesics[21,44,71,76,90]. Alternatively, some researchers opt for intramuscular injection of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) to induce anesthesia[16,22,24,26]. Conversely, other authors utilize intraperitoneal administration of anesthetics such as sodium pentobarbital at different dosages ranging from 20 mg/kg to 60 mg/kg[35,43,52,82,89,95], or chloral hydrate at varying concentrations (5% 10 mL/kg or 10% 3 mL/kg or 500 mg/kg)[41,55,93]. Each method aims to ensure adequate sedation and pain management for the animals undergoing liver explant surgery, thereby facilitating a successful experimental procedure with minimal discomfort or distress to the subjects.

The first description of rat orthotopic LTx dates back to 1979 and has since remained the gold standard, despite its complexity and long learning curve[111,112]. Although various alternative techniques were proposed to simplify the surgery and make it easier to learn, liver explant surgery remains complex and demands meticulous training[99,113,114]. In the study by Tolboom *et al*[19], a surgeon with prior experience of over 100 orthotopic liver transplants in rats, performed all surgeries. Specific expertise in liver surgery, prior training, and a deep understanding of the procedure are crucial for successful outcomes[115]. Unfortunately, many studies lack detailed descriptions of liver explant surgery and information about surgeons' training backgrounds.

Currently, no studies have definitively analyzed which MP setup closed or open circuit is superior. Both configurations are shown to be effective in preserving liver function according to the articles reviewed in this study. A closed circuit, while requiring additional cannulation and precise surgery to prevent perfusate leakage and ensure continuous perfusion, may offer certain advantages. In contrast, an open circuit simplifies the procedure by not requiring VC cannulation. Dual vessel MP, involving both the PV and HA, is generally superior to PV-only perfusion, as it better supports the vascularization of the biliary tree and ensures adequate oxygen delivery[64]. However, it is also more expensive and necessitates advanced techniques to avoid damage to arterial intima[106]. Some authors suggest that single-vessel PV or retrograde perfusion is sufficient for liver preservation. Perfusion *via* the HA alone is less advantageous because this artery supplies roughly one-tenth of the liver[67]. Dual vessel MP offers superior outcomes but at a higher cost and complexity, whereas single-vessel PV or retrograde perfusion can be sufficient in many cases. Bile duct cannulation is essential and should always be performed, as it allows for the measurement of bile output and the assessment of bile composition, which are critical markers of biliary viability[107].

The heterogeneity of MP setups makes it difficult to select the best one. Key considerations in setting up the apparatus include: Ensuring the organ chamber accommodates the liver position without bending its edges and remains close to physiological conditions, positioning cannulas connected to the tubing close to the anatomical position of vessels, ensuring adequate oxygenation and temperature control, utilizing pumps and sensitive sensors for necessary flow and pressure adjustments, and incorporating bubble traps into the system to prevent air embolisms. We recommend avoiding excessively large tubing or perfusate reservoirs, as miniaturization is crucial for the successful establishment of small animal MP[70].

The choice of perfusate is as critical as the setup of the perfusion apparatus. Compositions and volumes of perfusates proposed by different authors vary significantly, yet few studies have examined the relative superiority of one perfusate over another[17,21]. Future research should focus on identifying the optimal perfusate composition. Regardless of the chosen perfusate composition, we recommend adjusting the perfusate potential of hydrogen (pH) to the normal range before connecting the liver to the MP circuit. Additionally, mild acidosis of the perfusate is believed to enhance cytoprotection during hypothermia, which can be considered an added advantage[17]. Therefore, it is advisable to maintain the perfusate within the normal pH range and avoid alkalosis.

HMP and SNMP do not require the addition of red blood cells or hourly supplementation of the perfusate because liver metabolic activity is suppressed and the risk of clotting and hemolysis is higher at lower temperatures[109]. During NMP, supplementation with red blood cells or other oxygen carriers is crucial for adequate oxygen delivery to the cells [116]. Additionally, during NMP, consideration should be given to hourly supplementation with heparin, insulin, amino acids, or other additives throughout the entire perfusion process as at 37 °C the liver is metabolically active and should be provided with substances similar to *in vivo* conditions[19].

CONCLUSION

This review highlights the current state of small animal liver MP systems, emphasizing their benefits and challenges in experimental research. Rat liver MP models offer a cost-effective and accessible alternative to larger animal studies, with simplified perfusion processes and shorter operative times, making them valuable for LTx and preservation research. However, significant challenges remain, particularly in dual-vessel perfusion, which enhances vascularization and oxygenation but requires advanced surgical skills and additional resources. Furthermore, the lack of a standardized protocol limits reproducibility across studies. Maintaining adequate perfusate volume is another challenge, as the small blood volume of rats often falls short of the fluid requirements of the perfusion apparatus. Closed-circuit systems may improve preservation through continuous perfusion but require meticulous handling, while open-circuit setups simplify procedures at the cost of reduced control over perfusion parameters. Likewise, perfusate composition remains an area of ongoing investigation, as factors such as red blood cell content, heparin use, and pH adjustments significantly impact liver viability, particularly in NMP perfusion. In conclusion, while dual-vessel closed-circuit MP offers superior physiological outcomes, single-vessel and open-circuit approaches may still be suitable for many studies, depending on research objectives. Future efforts should focus on optimizing perfusate composition and refining MP setups to improve reproducibility and minimize animal use. Establishing a standardized protocol will be crucial for advancing small animal

MP research, facilitating faster implementation, and reducing the number of animals required for experimentation. We hope this review serves as a valuable resource for researchers seeking to streamline their MP protocols and enhance experimental efficiency.

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