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OBM Transplantation

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Review

Review of Heterotopic Heart Transplantation Models in Rats

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Academic Editor: Prashant Vaijyanath

Special Issue: Orthotopic and Heterotopic Transplantation of the Heart

OBM Transplantation
2025, volume 9, issue 1
doi:10.21926/obm.transplant.2501242

Received: October 21, 2024
Accepted: March 09, 2025
Published: March 14, 2025

Abstract

Heterotopic heart transplantation (HHT) in rats is a valuable tool for cardiac transplantation research. While abdominal HHT has been established in rats for nearly 90 years, novel approaches involving cervical and femoral techniques have also been explored. The abdominal HHT model, despite its long history, is technically demanding and has a steep learning curve. In contrast, the cervical HHT model enables surgeons with more basic microsurgical skills to achieve competency more quickly while producing comparable outcomes. The femoral HHT model offers the possibility of retransplantation but is equally, if not more, technically challenging than the abdominal model. This article aims to review the existing models available to researchers and provide a guidance for each technique.

Keywords

Heart transplantation; heterotopic; rat

1. Introduction

Cardiac transplantation research covers a broad range of important topics, including immunosuppressive strategies, mechanisms of graft rejection, and novel preservation methods. Rat models provide a practical and cost-effective option for such studies due to their versatility and the availability of various genetic strains. These models are especially useful for investigating ischemia-reperfusion injury, acute and chronic rejection, and innovative preservation techniques. The diversity of available strains allows researchers to minimize confounding variables and better tailor experimental setup to specific research goals.

While foundational studies have explored various aspects of rat cardiac transplantation [1-12], including technical modifications and experimental outcomes, a comprehensive review comparing the distinct surgical models and their specific advantages and disadvantages remains lacking. This presents a challenge for researchers seeking the most appropriate model for their studies.

Rather than focusing on the pathophysiological aspects such as graft rejection or ischemia-reperfusion injury, this review centers on the practical surgical techniques and post-operative monitoring crucial for ensuring successful outcomes in heterotopic heart transplantation (HHT). We provide a detailed comparison of the abdominal, cervical, and femoral HHT models in rats, examining each technique's procedural intricacies and outcomes, while also highlighting their strengths, limitations, and potential for future research applications. By offering practical guidance for each HHT model, this review aims to assist researchers in selecting the most suitable model for their specific experimental goals, thereby advancing the field of cardiac transplantation research.

2. Historical Background

The evolution of HHT techniques in rats has significantly advanced our understanding of cardiac transplantation biology. The development of different models has enabled researchers to explore complex physiological processes, such as graft rejection and ischemia-reperfusion injury, under controlled experimental conditions.

The first reported HHT model in rats was introduced by Abbott et al. in 1964, involving end-to-end anastomoses between the donor aorta and the recipient aorta, as well as the donor pulmonary artery and the recipient inferior vena cava [1]. Despite being technically successful, this model often led to paraplegia due to spinal cord ischemia. In 1969, Ono and Lindsey [2] made a critical advancement by introducing end-to-side anastomoses, which significantly reduced the risk of paraplegia while maintaining efficient oxygenated blood flow to the coronary arteries. This technique has remained the gold standard for HHT in rats [3-7], despite its technical complexity and potential for prolonged warm ischemia.

In 1971, Heron introduced a novel cervical heart transplant technique, offering further improvements in surgical outcomes [8]. This technique utilizes end-to-end anastomoses between the donor aorta and the recipient's right common carotid artery (RCCA), as well as the donor pulmonary artery and the recipient's external jugular vein (EJV). This approach reduced operative and warm ischemia times, allowing for greater success rates among surgeons with limited

microsurgical training [9, 10]. However, the mandatory ligation of the right common carotid artery poses a risk of cerebral ischemia in the recipient [11].

In 1985, Rao and Lisitza introduced a femoral HHT model that facilitated retransplantation, a critical advancement for long-term transplantation studies [12]. This model, by preserving longer vascular stalks, allows for easier proximal division and subsequent retransplantation, expanding its utility in experimental retransplantation research.

All the models described above fall under the category of non-volume-loaded HHT, in which the transplanted heart lacks significant hemodynamic workload. However, in 1999, Asfour et al. introduced the volume-loaded heterotopic heart transplantation model to address the issue of myocardial atrophy observed in non-volume-loaded HHT models [13]. In this model, the right ventricle loads the left ventricle via a direct anastomosis between the donor's pulmonary artery and the recipient's left atrium. This configuration enables the transplanted heart to maintain a more physiological workload, potentially influencing graft adaptation and myocardial function over time. However, despite its potential advantages, reports on short-term survival rates, surgical difficulty, and complications are limited. Due to the lack of sufficient data for direct comparison with other HHT models, this review primarily focuses on non-volume-loaded HHT models with extensively documented outcomes.

3. Operative Technique

Cardiac transplantation in rats involves several critical steps, beginning with donor preparation and graft procurement, followed by recipient preparation and actual graft transplantation. These steps are essential for ensuring successful transplantation and minimizing variability across experiments.

3.1 Donor Preparation and Graft Procurement

While the basic procurement technique is consistent across the discussed HHT methods [1-3, 5-8], variations exist in anticoagulation protocols and the administration of cardioprotective solutions due to the lack of specific guidelines. To provide clarity, we briefly introduce the protocols employed at our facility. Proper donor preparation is crucial for achieving consistency in the rat transplantation model.

3.1.1 Anticoagulation

Effective anticoagulation is crucial in HHT procedures to prevent thrombus formation during graft procurement. Whereas, many studies do not provide details on heparin dosage, dilution or waiting times post-administration, we administer 500 units of heparin (or 1.0-1.2 u/g for smaller rodents) into the abdominal IVC immediately after opening the abdomen, followed by chest exposure. This approach allows for at least 5 minutes before graft procurement, ensuring sufficient anticoagulation to prevent thrombus formation.

3.1.2 Cardioprotective Solution

To ensure optimal graft preservation, cardioprotective solutions are commonly administered either via the ascending aorta (AAo) or the IVC. Both techniques are commonly used in many studies,

but no study has compared these procedures. Retrograde cardioplegia via the IVC is considered to be a straightforward method, however, despite its complexity and time requirements, antegrade cardioplegia is preferred in some studies for its efficiency in quickly inducing cardiac arrest and preserving myocardial function. Though, the detailed method of antegrade administration via the AAo is less frequently described in the literature. In our facility, we favor the antegrade method, where the aorta is first ligated with 6-0 silk, followed by injecting the cardioprotective solution into the aorta with 27 G needle to induce cardiac arrest. We primarily use 5 ml of University of Wisconsin (UW) solution as the cardioprotective solution.

3.2 Recipient Preparation and Graft Transplantation

Once the donor heart is procured and adequately preserved, the focus shifts to preparing the recipient and performing the graft transplantation. Each HHT model—abdominal, cervical, and femoral—presents unique challenges and variations in surgical technique. Once transplanted, However, these models share a common flow of oxygenated blood [11]. The pathway begins in the recipient's arterial system, flowing through the donor thoracic aorta and coronary arteries, then returning from the coronary sinus to the right atrium, ventricle, and finally out through the donor pulmonary artery into the recipient's venous system. Regarding anticoagulation during recipient surgery, we could not find any studies that used heparin. The use of heparin in the recipient may reduce the risk of thrombus during and after the procedure, but it could increase post-operative bleeding risks. Below, we review cardiac transplantation techniques.

3.2.1 Abdominal HHT

The most commonly used technique for abdominal HHT is the Ono technique [2]. This method is characterized by performing the arterial anastomosis first, followed by the venous anastomosis, with both anastomoses typically being at the same level and of the same size [2]. The drawback of this approach is the high level of technical skill and speed required as clamp times exceeding 30 minutes lead to worse outcomes (Figure 1A).

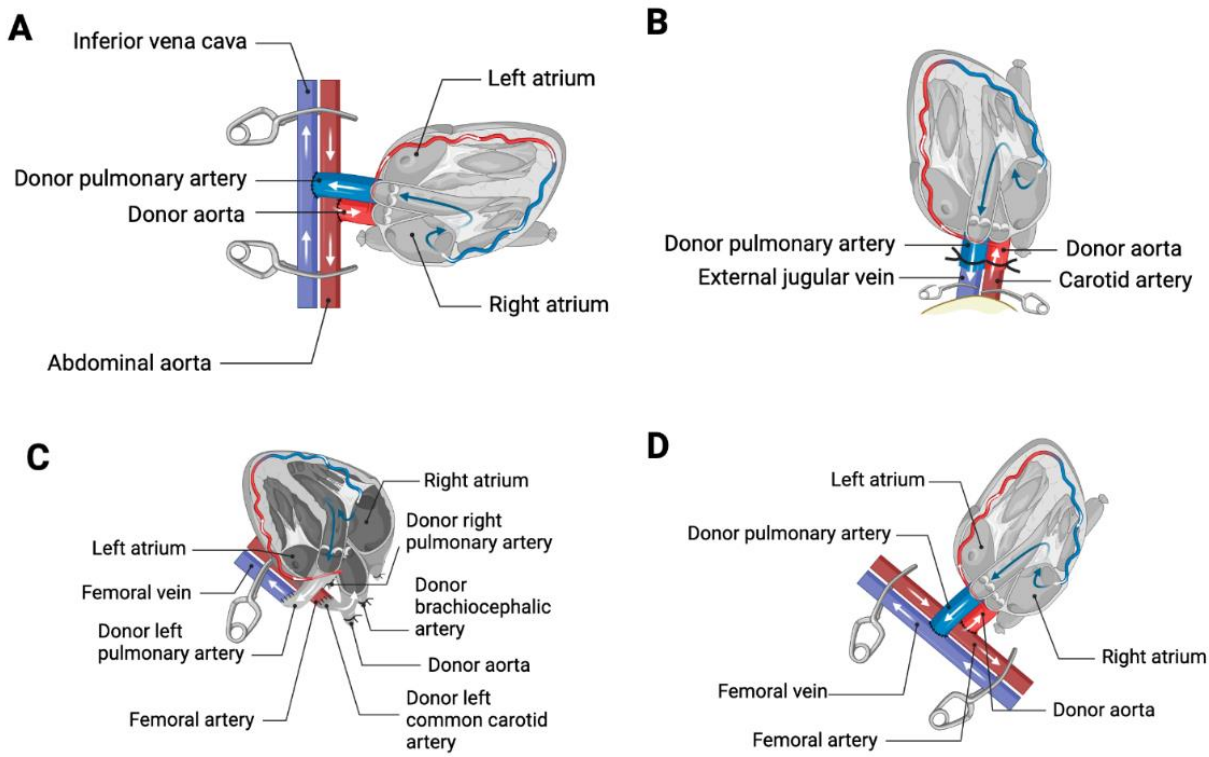

Figure 1 Schematic illustration of heterotopic heart transplantation in the abdominal position, (B) cervical position, (C) femoral position, (D) femoral position prepared for planed retransplantation. (Created in BioRender. Kawago, K. (2025) https://BioRender.com/l45j006).

3.2.2 Cervical HHT

Heron [8] introduced a novel technique for cervical HHT, utilizing an extra-luminal Teflon prosthesis for blood vessel anastomosis. This quick and reliable non-suture technique involves end-to-end connections between the donor aorta and the recipient's right common carotid artery (RCCA), as well as the donor pulmonary artery and the recipient's external jugular vein (EJV). This technique offers several advantages, including reduced operative time and warm ischemia time, making it more accessible for surgeons with limited microsurgical experience. However, a significant drawback is the mandatory ligation of the RCCA, which increases carotid artery thrombosis, occurring in 10-15% of rat recipients (Figure 1B).

3.2.3 Femoral HHT

Rao and Lisitza [12] introduced a femoral HHT model designed to facilitate retransplantation experiments. This model preserves longer vascular stalks, allowing for easier proximal division and retransplantation, making it particularly useful for studies requiring multiple transplantations. The groin location offers advantages such as easier access for monitoring heart function and managing potential complications. Overall, this model aims to enhance research in transplantation biology by providing a reliable system for studying retransplantation outcomes. However, this approach again requires a very high level of technical skill and speed, given potential for long ischemia times (Figure 1C, 1D).

4. Comparative Outcomes of HHT Models

While some data is limited due to insufficient reporting, outcomes from various technical papers are summarized in Table 1, which provides an overview of key metrics such as mortality rates, anastomosis times, and complications [2, 6, 8, 12, 14-16]. To address gaps in the data, later studies that make minor modifications to the original techniques have been included, as these reflect contemporary practices in the field.

Table 1 Summary of outcomes presented in technique papers.

Study	*Technique*	*Transplants performed*	*Complications (%)*	*Graft failure (%)*	*Thrombosis (%)*	*Mortality rate (%)*	*Anastomosis Time (min)*
Ono and Lindsey		161	n/a	n/a	n/a	8.7	n/a
Wang, et al.	Abdominal	100	8	5	2	5	21
Ma and Wang		56	0	0	0	0	21.6 ± 2.8
Heron	Cervical	59	10	0	10	25	15**
Rao and Lisitza: Initial		20	0	0	0	0	120**
Rao and Lisitza retransplant	Femoral	30	16.6	16.6	0	6.6	120**
Gordon, et al.		12	16.6	8.3	8.3	0	30 ± 12

**denoted values are for entire operative time (procurement and transplant). Anastomosis time was not reported in these studies.

Ono and Lindsey documented the highest number of transplants performed, with a mortality rate of 8.7%. However, they did not report complications or anastomosis time [2]. In contrast, Wang et al., who made slight modifications to the Ono and Lindsey technique, reported an anastomosis time of 21 minutes and a reduced mortality rate of 5% [6]. Ma and Wang conducted a head-to-head comparison of sutured cervical and abdominal transplants, noting a similar abdominal anastomosis time to that reported by Wang et al. [15]. Heron provided details on complications, mortality, and total operative time, but did not specify anastomosis time [8].

Rao and Lisitza [12, 15] indicated a total operative time of 120-150 minutes for their femoral HHT model, whereas Gordon et al. [15], who slightly modified the femoral technique, reported an anastomosis time of 30 minutes. Rao and Lisitza also noted a higher complication and mortality rate in their retransplantation cohort [12, 15]. None of the studies commented on time-to-competency, although most emphasized the need for highly skilled microsurgeons.

Overall, these outcomes demonstrate the importance of technical refinements in improving survival rates and reducing operative times. Further research is needed to optimize these techniques and explore their applicability in retransplantation and other experimental settings.

5. Postoperative Monitoring

Postoperative monitoring of the transplanted heart involve several techniques applicable to all HHT models. These methods include Palpation, electrocardiography (ECG), ultrasound, magnetic resonance imaging (MRI), and 18F-FDG positron emission tomography (PET). Each of these techniques offers unique advantages and precautions.

5.1 Palpation

The strength of cardiac contractility is typically assessed using a 4-point scale, where 4+ indicates the strongest contraction and 1+ indicates the weakest [14]. This evaluation can be considered to have subjective aspects. Palpation is more commonly employed in femoral and abdominal models. In cervical transplantation, visual confirmation of contractility is often sufficient. Palpation in the cervical model may risk disrupting the anastomosis due to the sutureless technique.

5.2 Electrocardiography (ECG)

While this method is reliable, locating appropriate electrode positions in abdominal HHT can be challenging. The mobility of the transplanted heart and artifacts caused by intestinal peristalsis can significantly affect the quality of the ECG recordings. These factors make it difficult to obtain consistent data, as the movement of the heart can lead to variations in the ECG waveform, even when recorded from the same heart at different time points. In contrast, in cervical HHT, electrode placement is easier and less affected by such factors, but care must be taken to avoid damaging the graft.

5.3 Ultrasound

Standard ultrasound focusing on left ventricular ejection fraction (LVEF) remains widely used in heart transplant research. However, its accuracy can be affected by insufficient blood flow into the

donor heart's left ventricle. The Spencer model, which introduces artificial aortic regurgitation, helps maintain left ventricular compliance, allowing for better LVEF assessment [3].

5.4 Magnetic Resonance Imaging (MRI)

MRI offers detailed imaging of myocardial structure and tissue composition. T1-weighted imaging is useful for fibrosis detection, while T2-weighted imaging highlights edema and inflammation. Due to the depth of the abdominal HHT model, ECG synchronization can be challenging, whereas cervical and femoral models offer easier localization. Additionally, contrast-enhanced MRI, including delayed enhancement (DE) sequences, can provide valuable information about myocardial viability and scarring by highlighting areas of ischemia and necrosis.

5.5 18F-FDG Positron Emission Tomography (PET)

For assessment of cardiac viability, 18F-FDG PET is a valuable tool [17-19], allowing differentiation between viable and nonviable myocardium in the transplanted heart. This technique provides high sensitivity in detecting metabolic activity, offering a clear indication of myocardial health. However, several methodological limitations must be considered. First, this technique requires fasting to reduce background glucose uptake and enhance myocardial signal. While some studies indicate rats can tolerate 24 hours of fasting, others suggest that 4 hours is sufficient to achieve optimal imaging results. Although 4 hours of fasting is generally safe, it can suppress the basal metabolic rate by up to 30%, leading to a decrease in body temperature. Second, as scans require general anesthesia, there is a risk of further temperature reduction. Additionally, anesthesia may affect glucose metabolism, potentially influencing the imaging results. To mitigate these risk, it is crucial to maintain body temperature within the range of 36.5-38°C using a heating pad throughout the procedure, ensuring stable conditions for accurate imaging.

6. Discussion

Rat cardiac transplantation models continue to play a pivotal role in advancing our understanding of transplantation biology. These models not only provide a cost-effective platform for studying critical topics such as immunosuppression, ischemia-reperfusion injury, and graft rejection, but they also allow for the testing of novel preservation strategies and therapeutic interventions. Additionally, a diverse range of functional monitoring methods, along with *ex vivo* studies, enhances our understanding of the entire transplantation process.

The comparative analysis of different HHT models, as discussed in this review, highlights the importance of procedural refinements in improving surgical outcomes. For instance, the Ono and Lindsey technique, despite its complexity, remains the gold standard for abdominal HHT, while modifications such as those introduced by Wang et al. have shown potential to further reduce operative times and improve survival rates. Selecting the appropriate model depends on the specific research goals.

Rat cardiac transplant models are particularly valuable across several key research areas. First, they facilitate the investigation of transplant rejection mechanisms, allowing researchers to explore both acute and chronic rejection processes and the associated immune responses against transplanted organs. In the abdominal and femoral HHT models, employing non-absorbable sutures

aligns with clinical standards, ensuring an acceptable level of inflammatory response, unlike the cervical model, which may introduce more foreign body reactions. Additionally, these models enable the testing of immunosuppressive therapies, where the efficacy and safety of new drugs and regimens can be evaluated in a controlled environment. HHT models also provide critical insights into ischemia-reperfusion injury, shedding light on the physiological and molecular responses involved, which are essential for transplant success.

Rat models enable the selection of genetically similar strains, effectively minimizing the risk of acute rejection [1, 2, 8, 12]. While alternative experimental conditions can simulate ischemia-reperfusion injury, organ transplantation allows for increased ischemia time, with the potential for varying cold and warm ischemia durations [20]. Notably, the cervical HHT model allows for easier manipulation of warm ischemia time due to shorter operative duration, paving the way for studies investigating new preservation techniques.

7. Future Perspectives

The potential for advancements in rat cardiac transplantation models is promising. Future studies will benefit from integrating advanced imaging techniques, such as MRI and 18F-FDG PET, to enhance the functional monitoring of transplanted hearts. These modalities offer detailed insights into graft viability, fibrosis, and ischemia, providing a more comprehensive understanding of graft health post-transplantation. Moreover, the use of genetically modified rat models in conjunction with these advanced imaging modalities opens new avenues for studying specific molecular pathways involved in graft rejection and organ preservation. Such approaches will help in identifying novel biomarkers and therapeutic targets for improving long-term transplant outcomes. As the field evolves, these models will remain integral to unraveling complex biological responses and improving transplant outcomes.

8. Conclusion

In conclusion, the variety of rat HHT models provides invaluable opportunities for advancing our understanding of transplantation through research. Each model presents unique strengths and considerations, allowing researchers to tailor their investigations to specific objectives and expertise levels. The abdominal HHT model, while technically demanding and time-consuming, serves as a versatile option for various small animal studies. Among the different HHT models, the femoral model offers unique advantages for retransplantation studies due to its longer vascular stalks, while the cervical model requires less technical expertise, easier access for monitoring and reduced operative times. These models will continue to drive innovations in cardiac transplantation research. This comprehensive overview aims to serves as a valuable resource for researchers and to inspire further innovations in transplantation science.

Author Contributions

Koji Kawago: participated in conceptualizing the project, performance of research, and writing and editing of the paper. John Farag: participated in conceptualizing the project, performance of research, data collection and analysis, and writing and editing of the paper. Yujiro Kawai: participated in performance of research, editing of the paper. Umayr Syed: participated in

performance of research. Eric Pfrender: participated in performance of research. Satoshi Miyairi: participated in conceptualizing the project. Yasuhiro Shudo: participated in conceptualizing the project, final approval of the version to be published.

Funding

American Heart Association, Career Development Award (23CDA1038803), Shudo Y (PI). American Society of Transplantation (1349899), Shudo Y (PI).

Competing Interests

The authors declare no conflicts of interest.

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