Development of a Partial Heart Transplant Model With Immunosuppression for Neonatal Applications: First Studies of Ice-Free Vitrification and Nanowarming

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BACKGROUND: While medical advancements have improved the management of congenital heart disease, a subset of newborns diagnosed with severe cardiac abnormalities continue to experience high mortality rates (40-50%). Neonatal partial heart transplantation (PHT), a novel surgical intervention, offers a potential solution to this challenging problem. This procedure involves transplanting a portion of a donor heart containing either the pulmonary valve and/or aortic valve into a neonate's diseased heart in conjunction with immunosuppression with the objective of long-term valve growth reducing or removing the need for further valve replacements with improved patient survival. The first two patients have already demonstrated PHT valve growth, 3 more patients have since been treated and we are eagerly awaiting outcomes.

HYPOTHESIS: Partial heart transplantation with a viable pulmonary valve and artery will grow in a neonatal piglet transplant model over a period of 60 days or until the recipient bodyweight has doubled.

METHODS: Thirteen piglets were employed, 4 recipient wild type (WT), 4 WT blood donors, 1 unoperated WT control, and 4 GFP-transgenic PHT donor piglets, ~6 weeks of age and weighing ~10kg. The donors and recipients were blood group matched and swine leukocyte antigen (SLA) documented. The PHTs were either cryopreserved by vitrification or kept fresh at 4°C in culture medium. The vitrified PHTs were gradually infiltrated to final concentrations of 3.10 M DMSO, 3.10M formamide, and 2.21 M 1-propanediol ± ≤0.6M disaccharides in Euro-Collins solution at 4°C using methods as previously described. Magnetic nanoparticles (mNPs from Ferrotec, EMG-308) were added in the final infiltration step. The valves were left in an isopentane bath at >-135C overnight and then storage below -135°C for >7 days. Rapid nanowarming, ~45 seconds, was performed by inserting samples into the radio frequency coil of a 6.0 kW inductive heating system. Nanowarmed vitrified (N&#3f2) and fresh PHTs (N&#3f2) were then transplanted in recipient WT pigs on circulatory bypass (Fig. 1). The pigs were maintained on triple immune suppression with tacrolimus titrated to a trough of 5-10 ng/mL, mycophenolate 20 mg/Kg BID,

and methylprednisone 1 mg/Kg QD (equivalent to an orthotopic heart transplant protocol) for the duration of the study. The PHT was examined by echocardiography at baseline post transplantation and subsequently every week during each experiment. Viability of valve leaflets was determined using the alamarBlue method for both fresh and vitrified PHTs with and without transplantation.

RESULTS: Fresh and vitrified PHT leaflets without transplantation and at explant had similar viability (not significantly different). Both fresh and one vitrified PHT annulus demonstrated growth during the study similar to the unoperated control that demonstrated 73% growth (Figure 2A). The second vitrified valve recipient experienced mycoplasma-induced endocarditis and demonstrated less annulus growth (Figure 2A). The leaflets of the fresh PHTs appeared normal (Fig. 2B) while the vitrified PHTs demonstrated some thickening of the spongiosa and increased elastin tissue on the ventricularis (Fig. 2C-D).

DISCUSSION: We have successfully established a neonatal piglet model for the investigation of living, viable, PHT transplantation with immunosuppression. The fresh PHTs appear to be growing (0.64 and 0.92 cm or 74 and 94%, respectively), similar to clinical observations in the first PHT recipient patients, one of two cryopreserved by vitrification PHTs also grew well with the recipient (0.59 cm or 73%). The second vitrified PHT only grew 0.3 cm or 30%. We believe this was due to a mycoplasma infection but it could also be that our vitrification method needs further optimization for pediatric sized valves since the method was originally developed for tissues from more mature 60-90 Kg pigs. Further studies are in progress.

CONCLUSIONS: We have established a PHT model in piglets and demonstrated valve annulus growth in 3 of 4 PHT recipients similar to an unoperated control. Further vitrified PHT studies are needed and possibly method optimization for PHT banking.

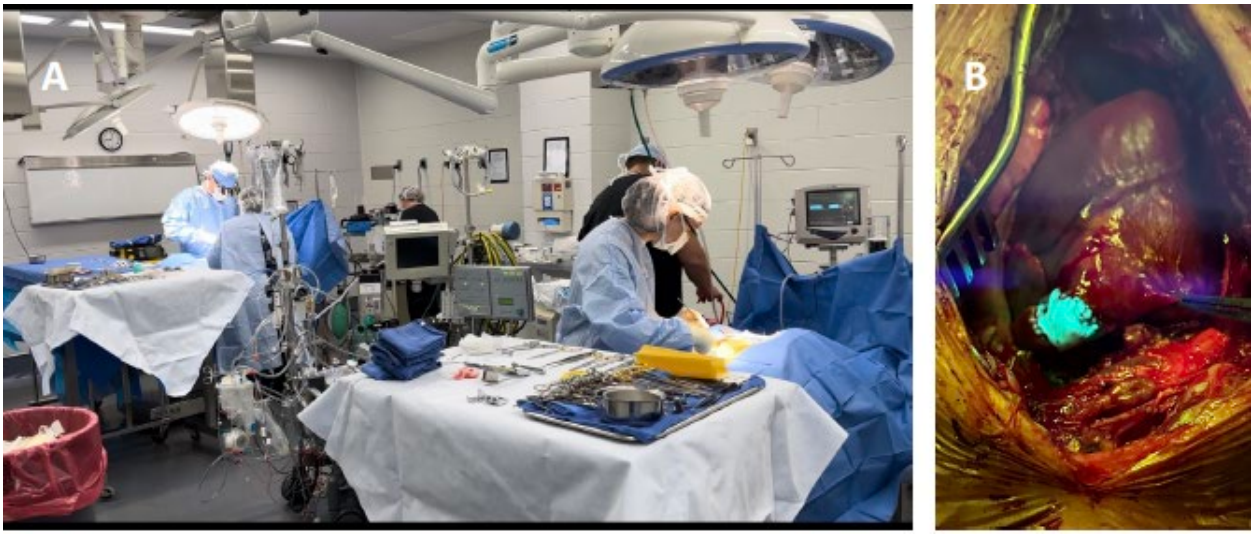

Figure 1: A) Surgery suite at the beginning of a PHT procedure to illustrate complexity. The blood donor table is front right, recipient table far left with bypass and monitoring equipment in the middle. The direct costs of each procedure from donor procurement to explant analysis are approximately $50,000 per experiment. **B) Recipient heart just prior to closing at the end of a procedure** with the florescent GFP-transgenic donor PHT in place under florescent light. Experiments in progress will demonstrate the presence of GFP positive donor cells in the WT recipient at explant. The donor cells should survive because the recipient is immunosuppressed.

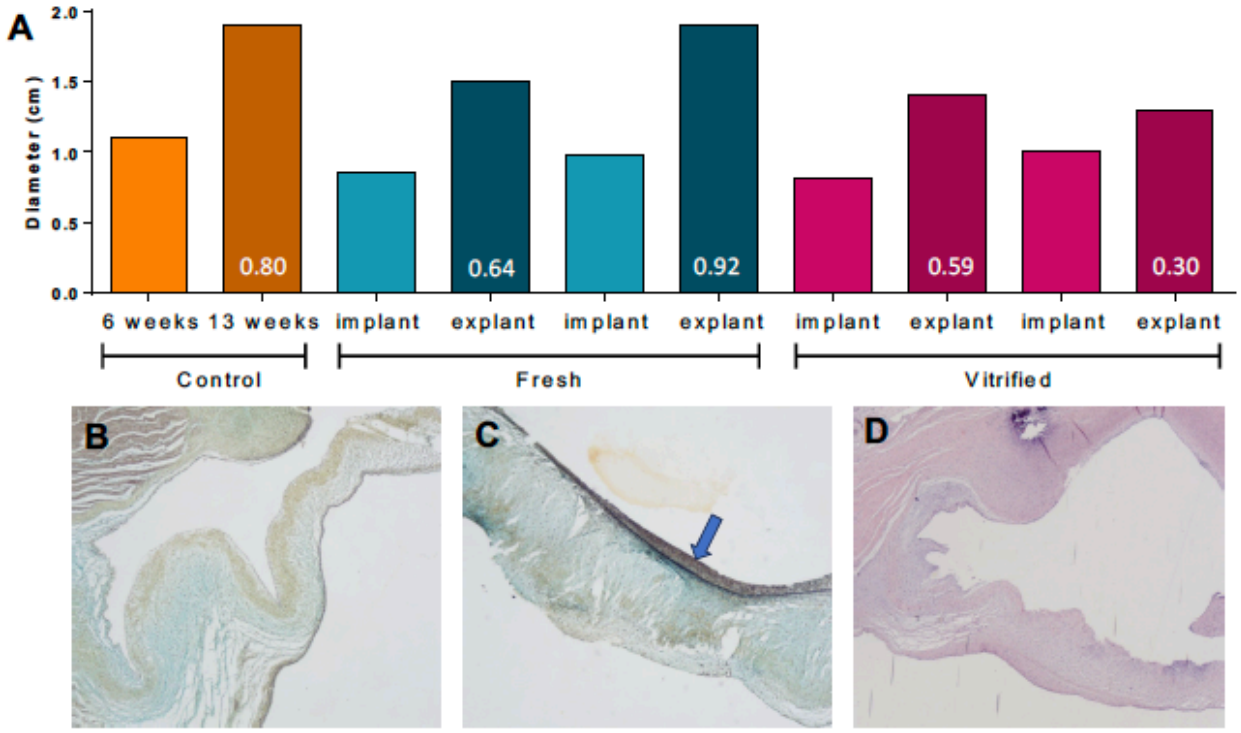

Figure 2: A) First and last echo measurements of annulus diameter demonstrating growth of Fresh (N=2) and Vitrified (N=2) PHTs compared with an unoperated control (N=1). Results presented in cms with growth shown at base of the last echo bar for each animal. The second vitrified PHT recipient had mycoplasma endocarditis that may have caused the lower 0.30 cm annulus growth. **B) Unoperated control leaflet** (4X pentachrome stain), **C) Vitrified leaflet** (4X pentachrome stain) illustrating thickening and increased elastin (black), and **D) Overview of the vitrified PHT** showing leaflet at the bottom (2X hematoxylin and eosin stain).