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Xenotransplantation: The End of The Organ Shortage Crisis?

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Abstract

Xenotransplantation refers to the transfer of animal tissues or organs to humans. This procedure is being explored as a potential solution to address the shortage of human organs available for transplantation. In the UK, there are currently around 7,000 individuals waiting for a transplant, and sadly, over 430 people have lost their lives while waiting as of 2023. However, the NHS has facilitated 4,600 transplants so far, saving many lives and improving the quality of life for countless others[29]. In the US, as of 2019, the situation is far worse, with 115,000 people on the waiting list and 20 people dying every day. Every 10 minutes, an additional person joins the list[10]. Xenotransplantation offers a solution.

Keywords: MHC class I, Non-HLA, Organ donation, Pig, Transplantation, Genetic engineering, Xenotransplantation.

Brief History of Xenotransplantation:

During the early 1900s, medical professionals tried to perform xenotransplantation by replacing damaged human organs with organs obtained from frogs, pigs, or primates. However, these initial procedures were unsuccessful, and further research was halted until the reason for the failed transplants could be determined[4,9]. In 1944, Peter Medawar made a significant discovery that post-transplantation, the recipient's immune system identifies organs as foreign and attacks them, resulting in transplant rejection and failure[34]. In 1954, Dr Joseph Murray accomplished the first successful kidney human-to-human transplant between identical twin brothers. Due to genetic similarity, the receiving twin accepted the organ without immune suppression. Nonetheless, successful transplants between non-identical donors were difficult to achieve until antirejection measures were developed[34]. In the early 1960s, scientists discovered the first immunosuppressive drugs. Researchers then set out to determine whether these drugs could also prevent the rejection of xenotransplants[34]. In 1963, Dr Thomas Starzl transplanted baboon kidneys into six human recipients with survival periods ranging from 19

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to 98 days. Despite further attempts over the following decades, success rates remained non-existent[34]. Since the mid-1990s, scientists have focused on genetically modifying donor animals to prevent organ rejection, which brings us closer to the possibility of xenotransplantation[5,34].

What is Organ Rejection and the Types of Organ Rejection

Organ rejection can be defined as a biological process whereby the recipient's immune system recognises the transplanted organ as a foreign tissue and initiates an immune response to eliminate it. This process is akin to an immune response directed against an unwanted infection. Organ rejection (organ failure) is a significant challenge that limits the success of organ transplantation procedures and is a subject of ongoing research in the field of transplant immunology[14,17].

In the case of a transplant, hyperacute rejection can occur within minutes if the antigens are incompatible. Immediate removal of the tissue is crucial to prevent fatality. Acute rejection can take place within the first week to three months, as the recipient's immune system is activated against the donor tissue. Chronic rejection is a gradual process that occurs over years as the recipient's immune system slowly damages the donor tissue[17,18]. No organ rejection and prevention of organ failure are the main objectives of xenotransplantation.

The cellular plasma membrane is coated with multiple unique molecules, providing the cells with a unique fingerprint. The immune system therefore can capably distinguish whether a cell is ‘self,’ ‘foreign,’ or cancerous. The surface molecules central to this role are human leukocyte antigens HLA, specifically the MHC class I and its antigen-presenting pathway[3]. Non-HLA molecules play a less critical role yet contribute to cell recognition[22].

All nucleated cells present small fragments of digested glycoproteins via the Major Histocompatibility Complex (MHC) class I at the cell surface. The displayed proteins in MHC class I are receptors for CD8+ T cells, ensuring appropriate cell functions in signalling when encountering a compromised state[15,27].

The larger proteins are broken down into peptides by proteasome enzymes in the cytoplasm. Only peptides sized 8 to 9 amino acids long are transferred into the lumen of the Endoplasmic Reticulum (ER) via Transporter Associated with antigen Presentation (TAP). In the lumen of

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the ER, those peptides are loaded onto MHC class I molecules, forming peptide-MHC class I complexes. Finally, peptide-MHC class I complexes are transported out of ER by the Golgi apparatus to fuse with the cells' plasma membrane for CD8+ T cell receptors (TCR)[27].

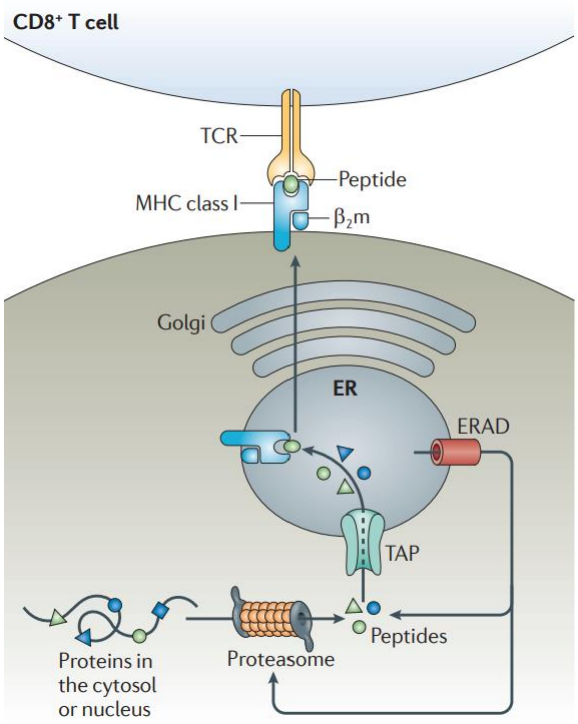

Figure 1.1. MHC class I antigen-presenting pathway[27].

There are 20 naturally used amino acids[26]. This means that a peptide, presented on the cell plasma membrane can potentially have 20^8 (= 25,600,000,000) to 20^9 (=512,000,000,000) possible combinations, allowing CD8+ T cells to discriminate against cells of its own, same species and different species.

Non-HLA antibodies and Xenotransplants

Non-HLA antibodies can be classified into two categories, alloantibodies, and autoantibodies. Alloantibodies specifically target polymorphic antigens that differ between the donor and recipient, while autoantibodies recognise self-antigens[44]. The development of antibodies

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against non-HLA autoantigens is associated with rejection and reduced long-term graft survival. Immunological recognition of cells is mainly governed by the MHC molecules. The immune system also recognises specific molecules on the cell surface unrelated to the MHC class I receptors like perlecan, angiotensin type 1 receptor, and collagen which play a significant role in the process of antibody-mediated acute and chronic rejection[44]. The role of non-HLA antibodies in antigen presentation and immune surveillance has been extensively researched in immunology and organ transplantation, but the knowledge remains incomplete[44].

Role of genetic engineering in Xenotransplantation

Genetic engineering has revolutionised how we understand and manipulate living organisms. Altering the genes involved in cell surface molecules within the animal organ of choice can trick the immune system into believing the cell is 'self.' This is the essence of xenotransplantation.

Two major real-life cases have involved heart xenotransplantation: David Bennet, Sr, who lived two months after xenotransplantation, and Lawrence Faucette, who lived six weeks after xenotransplantation[19,35]. Genetically engineered pig hearts were used in both cases, but why were pig hearts used instead of the hearts of chimpanzees or baboons? Which are more genetically similar to us[1,20,30,39]?

The domesticated pig, scientifically known as *Sus scrofa domesticus,* lives up to 20 years and can reach up to 240cm. Pig organs, especially the heart, have a striking similarity to their human counterparts[16,41,42]. Unlike our primate cousins, pigs have a higher breeding potential, are widely available, and are not endangered. The low maintenance costs and extensive genetic engineering experience with pig cells make them an attractive alternative. Furthermore, using pig organs for medical research and transplantation poses fewer ethical objections, as pigs are commonly raised as farm animals. This introduces interest in developing pig-to-human organ transplantation to address the shortage of human organs available for transplantation[12,43].

In the case of Mr Bennet's heart xenotransplantation, four genes were removed, and six genes were added. Three genes (GGTA1, CMAH, and β4GalNT2) involved in adding carbohydrate

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molecules to the plasma membrane have been knocked out to prevent hyperacute rejection[6,32,40]. The growth hormone receptor (GHR) gene was removed from the pig's heart to ensure it would grow to an adequate size for human recipients during xenotransplantation to prevent fatal compression of the heart[2,13,16].

Six genes have been introduced for the heart to be recognised as less foreign to the immune system. The Human CD55 and Human CD46 genes mimicked the necessary surface signals to prevent humoral xenograft rejection and arterial blood clots through eliminating antibody binding and natural killer cell adhesion. Furthermore, human thrombomodulin and human endothelial C receptors were added to prevent thrombotic microangiopathy, as pig thrombomodulin does not bind well to human thrombomodulin. Finally, the inclusion of the Human heme oxygenase-1 gene helped to reduce inflammation and apoptosis. At the same time, the human CD47 gene aided the suppression of T cells and macrophages by human phagocytes. This improves the compatibility of the organ with the recipient's body[32].

The good and the bad:

Despite its potential, xenotransplantation still presents numerous challenges. Xenotransplantation advocates argue that the procedure can provide an efficient organ pool, hence saving lives and reducing suffering. However, assuming that xenotransplantation is successful, the patient will likely have to take long-term immunosuppressants with unclear long-term effects. Human-to-human organ transplant studies have observed that missing a single dose of medication can increase the risk of rejection via reactivating the immune system. Furthermore, patients who use immunosuppressants are at a higher risk of infections, cancer, and high blood pressure. Some patients also experience digestive issues like Gastrointestinal discomfort as a side effect[7,25,38]. Using immunosuppressants means that zoonotic infections can potentially worsen long-term health outcomes. Microorganisms like the porcine endogenous retrovirus (PERV) can infect both human and pig cells as well as cause cancer[8]. This highlights the need for continued research like CRISPR genetic engineering PERV virus out of pigs and vigilant monitoring technologies such as PCR, droplet digital PCR, DNA and RNA analysis to prevent the spread of such infections and mitigate their impact on human health[23,24,31].

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There is an indication that some individuals who receive animal organs may experience a shift in their self-perception, potentially impacting their identity. While this phenomenon can occur with human organ transplants as well, it may be a more significant issue with animal organs, given the recipient's awareness that they are receiving a non-human organ. This loss of identity threatens the fundamental principle of autonomy that underlies all medical interventions[11,21,31,33].

Additionally, animal welfare is a crucial concern regarding genetic modification and xenotransplantation. These practices often involve subjecting animals to various forms of suffering, such as isolation, monitoring, and invasive investigations. Additionally, the long-term effects of genetic modifications on animals are not yet fully understood. There is also the potential for many animals to be subjected to cloning, which raises ethical concerns. For instance, a disease could be highly effective at damaging or killing a specific clone line of Xeno-pig-organs. This could pose a problem for both the patient's health and the pigs as some may have already received xenotransplants from these pigs. Infected pigs are usually subjected to euthanasia. As such, it is essential to carefully consider the impacts of genetic modification on animal welfare and take steps to mitigate any adverse effects[21,31].

Accurately estimating the risk of zoonosis to both the recipient and society is challenging, making vigilant post-operative monitoring necessary. As a precaution against the potential risk of zoonosis to the broader public, recipients may need to restrict physical relationships, daily activities, and socialisation. These limitations could even involve staying home, resulting in temporary detention. By consenting to xenotransplantation, the individual would enter a binding contract for a lengthy period, potentially without the ability to withdraw. This contract could include restrictions or even deprivation of human rights, impeding the ability to give fully informed consent, even for an informed patient. It is difficult to anticipate future limitations on one's freedom, further restricting the ability to provide informed consent[21,31].

For example, Jewish and Islamic individuals may exhibit a lower acceptance rate of pig organs due to religious beliefs. As a result, alternative sources of organs may need to be explored for these communities to ensure equitable access to life-saving medical procedures.

Xenotransplantation regulations are evolving globally, with the US, Japan, EU, and the UK having specific regulations[37]. Academic groups and biotechnology companies, for example, United Therapeutics Corporation, are actively researching and developing genetically

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modified pigs to provide compatible organs for human transplantation[28,36]. Researchers are addressing safety, efficacy, immune responses, infection transmission, and long-term survival and function of xenotransplants through clinical trials[28,36].

Xenotransplantation is a promising solution to address the shortage of human organs available for transplantation. With thousands of people waiting for transplants in the UK and the US, the need for viable organ donors is critical. Though initial attempts at xenotransplantation were unsuccessful, advances in medical research and genetic engineering have brought us closer to making this a viable option. The immune system plays a crucial role in organ rejection, which limits the success of transplant procedures. However, by genetically modifying donor animals, we may be able to prevent organ rejection and save countless lives. It is worth reemphasising that the longest an organ has survived following xenotransplantation in humans is two months, indicating the tremendous amount of further research and practical applications to consider it a standard healthcare procedure sustaining humanity.

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References:

1. Britten, R. J. (2002). Divergence between samples of chimpanzee and human DNA sequences is 5%, counting indels. *Proc Natl Acad Sci U S A*, *99*(21), 13633-13635. https://doi.org/10.1073/pnas.172510699
2. Chaban;, R., & Cooper, D. K. C. (2022). The First Clinical Pig Heart Transplant and the Future of Cardiac Xenotransplantation. Retrieved 11/03/2024, from https://www.acc.org/latest-in-cardiology/articles/2022/09/21/08/42/the-first-clinical-pig-heart-transplant-and-the-future-of-cardiac-xenotransplantation
3. Chaplin, D. D. (2010). Overview of the immune response. *The Journal of allergy and clinical immunology*, *125*(2 Suppl 2), S3-23. https://doi.org/10.1016/j.jaci.2009.12.980
4. Cooper, D. K. C., Ekser, B., & Tector, A. J. (2015). A brief history of clinical xenotransplantation. *Int J Surg*, *23*(Pt B), 205-210. https://doi.org/10.1016/j.ijsu.2015.06.060
5. Cooper, D. K. C., Gaston, R., Eckhoff, D., Ladowski, J., Yamamoto, T., Wang, L., Iwase, H., Hara, H., Tector, M., & Tector, A. J. (2017). Xenotransplantation—the current status and prospects. *British Medical Bulletin*, *125*(1), 5-14. https://doi.org/10.1093/bmb/ldx043
6. Cowan, P. J., Aminian, A., Barlow, H., Brown, A. A., Chen, C. G., Fisicaro, N., Francis, D. M., Goodman, D. J., Han, W., Kurek, M., Nottle, M. B., Pearse, M. J., Salvaris, E., Shinkel, T. A., Stainsby, G. V., Stewart, A. B., & d'Apice, A. J. (2000). Renal xenografts from triple-transgenic pigs are not hyperacutely rejected but cause coagulopathy in non-immunosuppressed baboons. *Transplantation*, *69*(12), 2504-2515. https://doi.org/10.1097/00007890-200006270-00008
7. Deathridge, J. (n.d.). *Transplant Immunology*. British Society for Immunology. Retrieved 11/03/2024 from https://www.immunology.org/policy-and-public-affairs/briefings-and-position-statements/transplant-immunology
8. Denner, J. (2021). Porcine Endogenous Retroviruses and Xenotransplantation, 2021. *Viruses*, *13*(11). https://doi.org/10.3390/v13112156
9. Ekser, B., Li, P., & Cooper, D. K. C. (2017). Xenotransplantation: past, present, and future. *Curr Opin Organ Transplant*, *22*(6), 513-521. https://doi.org/10.1097/MOT.0000000000000463
10. Finger Lakes Donor Recovery Network. (2018). *Organ Donation and Transplant Statistics*. Retrieved 11/03/2024 from http://www.donorrecovery.org/learn/organ-donation-transplant-statistics/
11. Golden, L. E., DiMartini, A. F., & Dew, M. A. (2021). Dissociative Identity Disorder in a Non-Directed Kidney Donor. *J Acad Consult Liaison Psychiatry*, *62*(4), 478-479. https://doi.org/10.1016/j.jaclp.2021.02.005
12. Groth, C. G. (2007). The potential advantages of transplanting organs from pig to man: A transplant Surgeon's view. *Indian J Urol*, *23*(3), 305-309. https://doi.org/10.4103/0970-1591.33729
13. Hansen, J. (2022). The 10-gene pig and other medical science advances enabled UAB's transplant of a pig kidney into a brain-dead human recipient. Retrieved 11/03/2024, from https://www.uab.edu/news/research/item/12567-the-10-gene-pig-and-other-medical-science-advances-enabled-uab-s-transplant-of-a-pig-kidney-into-a-brain-dead-human-recipient
14. Hassanein, M., & Augustine, J. J. (2024). Chronic Kidney Transplant Rejection. In *StatPearls*. https://www.ncbi.nlm.nih.gov/pubmed/31747169
15. Hewitt, E. W. (2003). The MHC class I antigen presentation pathway: strategies for viral immune evasion. *Immunology*, *110*(2), 163-169. https://doi.org/10.1046/j.1365-2567.2003.01738.x
16. Hinrichs, A., Riedel, E. O., Klymiuk, N., Blutke, A., Kemter, E., Langin, M., Dahlhoff, M., Kessler, B., Kurome, M., Zakhartchenko, V., Jemiller, E. M., Ayares, D., Bidlingmaier, M., Flenkenthaler, F., Hrabe de Angelis, M., Arnold, G. J., Reichart, B., Frohlich, T., & Wolf, E. (2021). Growth hormone receptor knockout to reduce the size of donor pigs for preclinical xenotransplantation studies. *Xenotransplantation*, *28*(2), e12664. https://doi.org/10.1111/xen.12664

DOI: https://doi.org/10.57898/biosci.204

17. Justiz Vaillant, A. A., Misra, S., & Fitzgerald, B. M. (2024). Acute Transplantation Rejection. In *StatPearls*. https://www.ncbi.nlm.nih.gov/pubmed/30571031
18. Justiz Vaillant, A. A., & Mohseni, M. (2024). Chronic Transplantation Rejection. In *StatPearls*. https://www.ncbi.nlm.nih.gov/pubmed/30571056
19. Kotz, D. (2022). IN MEMORIAM: David Bennett, Sr. Retrieved 11/03/2024, from https://www.medschool.umaryland.edu/news/2022/in-memoriam-david-bennett-sr.html
20. Kovacs, A. (n.d.). *How Much DNA Do Humans Share With Other Animals and Plants?* Retrieved 11/03/2024 from https://thednatests.com/how-much-dna-do-humans-share-with-other-animals/
21. Krishna, M., & Lepping, P. (2011). Ethical debate: ethics of xenotransplantation [Report Viewpoint essay]. *British Journal of Medical Practitioners*, *4*, 46+. https://link.gale.com/apps/doc/A271053183/AONE?u=anon~16ccb5e8&sid=googleScholar&xid=4f23711b
22. Lammerts, R. G. M., Altulea, D., Hepkema, B. G., Sanders, J. S., van den Born, J., & Berger, S. P. (2022). Antigen and Cell-Based Assays for the Detection of Non-HLA Antibodies. *Front Immunol*, *13*, 864671. https://doi.org/10.3389/fimmu.2022.864671
23. Lu, T.-f., Sun, B., Yu, T.-y., Wu, Y.-j., Zhou, J., & Wu, S.-g. (2022). Porcine Endogenous Retroviruses: Quantification of the Viral Copy Number for the Four Miniature Pig Breeds in China [Original Research]. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.840347
24. Mazurek, U., Kimsa, M. C., Strzalka-Mrozik, B., Kimsa, M. W., Adamska, J., Lipinski, D., Zeyland, J., Szalata, M., Slomski, R., Jura, J., Smorag, Z., Nowak, R., & Gola, J. (2013). Quantitative analysis of porcine endogenous retroviruses in different organs of transgenic pigs generated for xenotransplantation. *Curr Microbiol*, *67*(4), 505-514. https://doi.org/10.1007/s00284-013-0397-3
25. National Kidney Foundation. (2015). *Immunosuppressants*. Retrieved 11/03/2024 from https://www.kidney.org/atoz/content/immuno
26. National Research Council. (1989). *Recommended Dietary Allowances: 10th Edition*. The National Academies Press. https://doi.org/doi:10.17226/1349
27. Neefjes, J., Jongsma, M. L., Paul, P., & Bakke, O. (2011). Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol*, *11*(12), 823-836. https://doi.org/10.1038/nri3084
28. Newman, M. E. (2023). Johns Hopkins Medicine to Receive $21.4 Million to Advance Viability of Animal Organs for Transplants and Enable Human Clinical Trials. Retrieved 11/03/2024, from https://www.hopkinsmedicine.org/news/newsroom/news-releases/2023/03/johns-hopkins-medicine-to-receive-214-million-to-advance-viability-of-animal-organs-for-transplants-and-enable-human-clinical-trials#:~:text=All%20News%20Releases-,Johns%20Hopkins%20Medicine%20to%20Receive%20%2421.4%20Million%20to%20Advance%20Viability,and%20Enable%20Human%20Clinical%20Trials
29. NHS Blood and Transport. (n.d.). *Organ donation and transplantation*. Retrieved 11/03/2024 from https://www.nhsbt.nhs.uk/what-we-do/transplantation-services/organ-donation-and-transplantation/#:~:text=Thanks%20to%20our%20amazing%20donors
30. Piorkowska, K., & Ropka-Molik, K. (2021). Pig Genomics and Genetics. *Genes (Basel)*, *12*(11). https://doi.org/10.3390/genes12111692
31. Rollin, B. E. (2020). Ethical and Societal Issues Occasioned by Xenotransplantation. *Animals : an open access journal from MDPI*, *10*(9). https://doi.org/10.3390/ani10091695
32. Ryczek, N., Hryhorowicz, M., Zeyland, J., Lipinski, D., & Slomski, R. (2021). CRISPR/Cas Technology in Pig-to-Human Xenotransplantation Research. *Int J Mol Sci*, *22*(6). https://doi.org/10.3390/ijms22063196
33. Sarkar, S., Grover, S., & Chadda, R. K. (2022). Psychiatric Assessment of Persons for Solid-Organ Transplant. *Indian J Psychiatry*, *64*(Suppl 2), S308-S318. https://doi.org/10.4103/indianjpsychiatry.indianjpsychiatry_1015_21

DOI: https://doi.org/10.57898/biosci.184

34. Science Learning Hub. (2011). History of xenotransplantation. Retrieved 11/03/2024, from https://www.sciencelearn.org.nz/resources/1214-history-of-xenotransplantation
35. Seiler, B. (2023, 31/10/2023). *In Memoriam: Xenotransplant Patient Lawrence Faucette* https://www.umms.org/ummc/news/2023/announcing-the-passing-of-lawrence-faucette
36. Shah-Neville, W. (2023). *Saving lives with xenotransplantation: how biotechs are solving the transplant shortage crisis*. Labiotech. Retrieved 11/03/2024 from https://www.labiotech.eu/in-depth/xenotransplantation-organ-shortage-crisis/
37. Solbach, T., Kwisda, S., Kremer, M., & Hamer, E. (2023). Animal material for human surgery is poised to deliver an immense return on investment - Xenotransplantation. https://www.strategyand.pwc.com/de/en/industries/pharma-life-science/xenotransplantation.html
38. Tielemans, M. M., van Boekel, G. A. J., van Gelder, T., Tjwa, E. T., & Hilbrands, L. B. (2019). Immunosuppressive drugs and the gastrointestinal tract in renal transplant patients. *Transplant Rev (Orlando)*, *33*(2), 55-63. https://doi.org/10.1016/j.trre.2018.11.001
39. Walters, E. M., & Prather, R. S. (2013). Advancing swine models for human health and diseases. *Mo Med*, *110*(3), 212-215. https://www.ncbi.nlm.nih.gov/pubmed/23829105
40. Wang, R. G., Ruan, M., Zhang, R. J., Chen, L., Li, X. X., Fang, B., Li, C., Ren, X. Y., Liu, J. Y., Xiong, Q., Zhang, L. N., Jin, Y., Li, L., Li, R., Wang, Y., Yang, H. Y., & Dai, Y. F. (2018). Antigenicity of tissues and organs from GGTA1/CMAH/beta4GalNT2 triple gene knockout pigs. *J Biomed Res*, *33*(4), 235-243. https://doi.org/10.7555/JBR.32.20180018
41. Wang, W., He, W., Ruan, Y., & Geng, Q. (2022). First pig-to-human heart transplantation. *Innovation (Camb)*, *3*(2), 100223. https://doi.org/10.1016/j.xinn.2022.100223
42. Wickline, K. (2014). *Sus scrofa - Wild Boar*. University of Michigan. Retrieved 11/03/2024 from https://animaldiversity.org/site/accounts/information/Sus_scrofa.html
43. Xi, J., Zheng, W., Chen, M., Zou, Q., Tang, C., & Zhou, X. (2022). Genetically engineered pigs for xenotransplantation: Hopes and challenges. *Front Cell Dev Biol*, *10*, 1093534. https://doi.org/10.3389/fcell.2022.1093534
44. Zhang, Q., & Reed, E. F. (2016). The importance of non-HLA antibodies in transplantation. *Nature Reviews Nephrology*, *12*(8), 484-495. https://doi.org/10.1038/nrneph.2016.88

DOI: https://doi.org/10.57898/biosci.184