# An article reviewing the truth about animal transplants to humans: Is it possible or just scientific fiction?

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**Abstract** __ Nowadays there is a constant increase in the number of patients with end stage organ failure who are in need for allotransplants. This demand is exhibited both worldwide and in KSA and this increase in organ demand is faced with a decrease in supply with the consequence of losing many patients in the waiting list. Xenotransplantation, may be a promising solution to the allograft shortage, by using other species organs as an alternative. This review aims mainly at determining the gap between organ availability and the required needs especially in KSA and to track the revolution of xenotransplantation from its start until now and if there were any successful attempts and to search for new approaches and techniques which might improve its outcome and finally to conclude based on clinical trials if it can really happen one day or just a fantasy. This review showed no apparent success in xenotransplantation, for example the maximum survival period achieved after liver xenotransplantation didn't exceed 70 days, however, the preclinical trials in NHPs showed a high success rate using genetically modified pigs. At last, the International Xenotransplantation Association congress held in Australia 2015 and that held in USA in 2016 have reported an improvement in the survival records using multitransgenic pigs in NHPs. This might point to the possibility of successful xenotransplantation taking place in the fore coming years. Although xenotransplantation carries many disadvantages they can probably be overcome by future modulations and even the ethical aspects and cultural acceptance can change over the years and the huge demand might force the society to accept it later on.

## 1 INTRODUCTION

In the last decade an increasing demand for human donors worldwide has become evident due to the shortage of human organs for the required transplants which made it impossible for the transplant group to meet all these needs. The statistics show that 21 patients in the United States die each day because of lack of organs needed for transplantation. (1)

SCOT (Saudi Center for Organ Transplantation) faces many challenges and obstacles to cover the demand for human organs. Table-1 illustrates statistics demonstrating the gap between the supply and demand for the different organs in Saudi Arabia in 2016. (2)

The gap between human organs requirements and it's availability continues to rise despite the public education effort on organ donation. The statistics show that large number of patients on the waiting list die every day. So this article will discuss xenotransplantation , which maybe-if it can be applicable- an ideal way to solve the problem of waiting list of patients who need organ transplantation and a may be a cheaper method for those threatened by impending death due to organ failure and an alternative carrying hope for saving lots of lives.

Current medical researches in the area of xenotransplantation aim at improving the quality of human lives of those suffering from organ failure.

Xenotransplantation, the transfer of animal cells, tissues or organs into human recipients, may become a biomedical reality (3). The development of protocols which modify the immune system to prevent both graft rejection and graft vs. host disease, and advances in surgical techniques might open the door to a field with the potential to save thousands of human lives each year. Also genetic engineering used to modify animal transplants to be similar to the human transplant might just make what we see today not applicable a hope for future generations.

The aim of the review is to:

1- Highlight and focus on the increasing numbers of patients in need of transplants worldwide and in Saudi Arabia

2- Track the journey of revolution of xenotransplantation from its beginning until now.

3- Search for Innovative techniques and procedures that might make xenotransplantation in the future an actual success.

4- Reach a possible conclusion from all the above

## REVIEW OF LITERATURE

### 2 Xenotransplantation, past and present:

The concept of using animal organs, cells and tissues in transplantation is not new, and has started 300 years or more ago with a surprisingly large number of clinical attempts. The problems that need overcoming are the immunological barrier, the risk of infection transmission, ethical issues, and the competency of the transplanted organ in the human environment (4). Skin, blood, liver and heart transplantations from animal to human have all been experimented.

### 2.1 HEART XENOTRANSPLANT

In 1964, the first clinical heart transplantation was attempted by James Hardy. He used some chimpanzees for donation. The recipient was a semi-comatosed patient with an atheromatous vascular disease and his legs were amputated which made him less than ideal for transplantation and most probably would not have been accepted for organ transplantation in our days. However, Hardy used a chimpanzee heart for transplantation, but the size of heart was not large enough to support the circulation so the patient rapidly died as result of heart failure within a couple of hours. (5)

### 2.2 BLOOD XENOTRANSFUSION:

In the 17th century the first trial of blood transfusion from animals to humans took place and the result was successful and continued for a number of years until the early 1900s , but the risk of transferring infectious agents made blood transfusion from animals less popular except in strong cases and from animals which live in highly clean houses.

Recently, in view of advantage according to blood xenotransplantation include:

1- Microorganisms-free so decrease the possibly of infection
2- The progress made in genetic engineering. Finally, pig RBCs have a large possibility to be available for clinical transfusion. (5)

### 2.3 CORNEAL XENOTRANSPLANTATION:

There were several efforts to use corneas of animals to humans but the first clinical attempt was done by Kissam in 1838. In the 19th century, corneas from different sources including pigs, fish, sheep, rabbits and dogs were used. The loss of immediate revascularization, and thus an absence of hyperacute rejection makes pig corneas more likely to achieve acceptance compared to pig organ xenografts.

The results of corneal xenotransplantation when applied on nonhuman primates showed a high chance of success. The efforts in these days aim at developing genetically-engineered pigs to expand the sources of corneas for clinical transplantation. (6)

### 2.4 KIDNEY XENOTRANSPLANTATION:

In 1960s the idea of using animal kidney for treatment of renal failure was presented by Keith Reemtsma. The first experiment used a chimpanzee kidney. He carried out 13 of these transplants. The results showed that within 4 to 8 weeks most transplants failed due to either rejection or from an infectious complication. However, there is one patient that lived more than 9 months and lived a healthy life but died suddenly as a result of an acute electrolyte disturbance. (7)

Another study in 1964 was applied on six patients suffering from uremia as a complication of glomerulonephritis or pyelonephritis. They were treated with heterografts from East African baboons. Follow up revealed that the patients lived for 19 to 98 days after heterotransplantation. Four died with the baboon kidneys still in place. In the other two cases the heterografts were removed after 60 and 49 days respectively (8)

## 3 REQUIREMENTS AND PREREQUISITES OF XENOTRANSPLANTATION

### 3.1. Choice of the animal donor

THE "IDEAL" DONOR ANIMAL

There are many characteristics that should exist in the animals we consider to be suitable organ donors for humans.

*First,* the animal should be near to human regarding physiology and anatomy for the purposed organ to function well in the human body.

*second,* there should be no risk of infection transmission from animal to human, on the contrary the animal donor should exhibit resistance against human infections especially viral,

*In addition* they should be cheap in feeding and breeding, with short periods of pregnancy and multiple births.

*Finally,* the ethical argument should be minimal in the chosen animal.

Monkeys are most similar to humans physiologically and anatomically, Also they may have resistance to some human diseases. For example (hepatitis B and HIV virus)

However, there are ethical obstacles in using monkey organs in xenotransplantation because of the infectious risks. Herpes- 8 - is an example for the viruses found in monkeys and lethal to humans in a few days. Finally, the idea of raising large enough numbers of monkeys pathogen-free, to satisfy clinical demands is likely to be ethically prohibited.

Of all animals pigs have many characteristics that make them more suitable to use in xenotransplantation such as being large litters, 4 months of gestation which is a very short time, physiological / anatomical similarities to humans, long history of providing medicinals, previous usage of many of their products as porcine insulin and also their clotting factors in addition to skin and cardiac prothesis for humans. However major differences between human and porcine physiology in areas such as coagulation cascade and Immunological barriers may represent a considerable obstacle (9).

### 3.2 A consent should be obtained from the recipient explaining the possible complications

### 3.3 Should submit to rules and legislations of every country

### 3.4 Should be culturally acceptable in wide regions of the world.

## 4 COMPLICATIONS OF XENOTRANSPLANTATION:

### 4.1 Immunity barrier

Rejection Reactions:

Hyperacute rejection, acute vascular rejection, T cell response and chronic xenograft rejection are four immunological barriers that we need to overcome either wise xenotransplantation will become unsuccessful and ineffective(10).

Hyperacute rejection:

Is a rejection that usually develops immediately after the implantation of a vascular graft .Galactose-alpha-1,3-galactose (alpha-Gal) is a main antigen on cell membranes of pigs which evokes the immune system of humans to recognize it as a foreign body and produce xenoreactive antibodies.

Endothelial activation causes many complications such as ischemia, thrombosis, and rapid rejection which leads to rejection of the transplanted organ. The endothelial activation happens in combination with activated complement and antibodies .This problem can be overcome by methods that inhibit the activation of complement system.

one of the suggested solutions is the expression of a human protein in the pigs . For example, human Decay Accelerating Factor (hDAF) is a protein that prevents human complement activation. There was an experiment showing how insertion of this protein into the membrane of pig endothelial cells, will prevent lysis and activation of the cells.

Another conceivable solution for hyperacute rejection is to incredibly reduce the expression of "a-gal" from pigs by thumping out the 1,3 galactosyl transferase quality, which is required for the expression of "a-gal". This has not yet been accomplished in pigs. (10)

Acute vascular rejection:

Acute rejection starts to occur few days or weeks after xenotransplantation and is a main cause of its failure.

Xenograft invasion by host inflammatory cells, monocytes and natural killer cells with the production of xenoreactive antibodies which precipitate acute vascular rejection. Subsequently, the activated endothelial cells will lead to thrombosis, compromised blood supply and rejection.

The available solution for this problem is the administration of immunosuppressive drugs which increase the survival of the transplanted organ. Another solution is using genetic engineering which aims to suppress the inflammatory response.

T-Cell Response:

There are two types of T cell response allogeneic and xenograft while allogenic can be controlled the xenograft may be difficult with the use of immunosuppressive. There is a new chance to promote tolerance in the donor in cases of pig to primate transplants.

Tolerance is a state of unresponsiveness of the immune system to tissue that have the capacity to elicit an immune response. There are two successful approaches toward the induction of tolerance:

i. mixed hematopoietic chimerism
ii. thymus transplantation .

Such tolerance is the hope of transplantation in general and may be aided in the xenogenic arena by further genetic engineering of the source animal. (11)

Chronic Xenograft Rejection:

There is a possibility of rejection after months or years even if all previous rejections were overcome, this type is known as "chronic" rejection . The proliferation of smooth muscle cells and obstruction of the lumens of blood vessels are main features of this rejection. (10)

Overcoming the complications of Rejection Reactions:

To overcome the complications genetic engineering has been used which is a new technique aiming to overcome both physiological and immunological barriers of xenotransplantation. Now more than 40 genetic alterations are available. Also five or six of these manipulations have already been implemented in pigs. (12)

One of approaches aims to protect organs from hyperacute rejection which occurs as a result of antibodies and activated complement, by monitoring human complement-mediated injury.

However, when the surface of vascular endothelial cells of the transplanted organ expresses proteins which are known as human complement-regulatory proteins this will prevent complement activation so It is recommended that pigs must be genetically modified to express transgenes for human CRPs of which there are several types (eg CD59, CD46, CD55). There is a group which adopted this original suggestion at 1990, by using microinjection they expressed a human CRP (CD55) in the pronucleus of a fertilized egg directly.

The results of the experiment after many studies in cases of transplants from pigs to non-human primates such as baboons, show prolonged period of survival for the kidney and heart grafts to days or weeks. (13)

According to genetically modified pigs this was the first proof of the efficacy of this technique in overcoming the barriers of xenotransplantation.

Suppressing the inflammatory response is a second aim of pig genetic engineering. One of the main causes of graft failure is (SIXR) "systemic inflammatory response in xenograft recipients" which is an inflammatory response to pig organs by Interleukin-6

which plays an important role in this response as well other chemokines and cytokines.

Consequently introducing pigs with human anti-inflammatory gene for example, (A20 or hemeoxygenase-1) may therefore be beneficial to improve outcomes of xenotransplantation but it has not yet been clarified in vivo.

Furthermore, there are many benefits in applying pig genetic engineering such as correcting coagulation dysfunction, suppressing the adaptive immune response and deleting Gal antigens. (13)

### 4.2 Infections:

Infection is another aspect to worry about when talking about xenotransplantation. Porcine endogenous retroviruses (PERVs) are a major microbiological risk when using porcine tissues, organs and even cells. Their transmission is possible because:

1) they are generated by normal pig cell
2) the genome of all pig strains has many copies of it
3) they are able to infect human cells in vitro
4) they have immunosuppressive properties .

There are three subgroups of PERVs ,the first two are (PERV-B and PERV-A) which can be found in the genomes of all pigs and can infect human cells, The third type is PERV-C which is not present in all pig genomes in addition it is ectropismic and restricted to pig cells.

Recently, In vitro the scientists discovered the ability of PERV-C type to infect human cell lines and this brought more focus to that species (14)

However, In one study, 24 patients which had some neurological diseases received fetal porcine neuronal cells for treatment no proof of PERV provirus integration in the DNA from PBMCs of all neuronal transplant recipients was found . Also there was no proof of transmission of porcine endogenous retroviruses from neuronal cells of fetal pig to human cells in vitro.

However, the virological barriers to organ transplantation from animal to human are not as serious as initially perceived. (15)

Strategies to overcome the Infections barrier:

i. Selection of PERV-C-Free Low-Producer Animals
The selection of the donor animals is the essential tool to prohibit transmission of pigs retroviruses , including PERV. (pig tissues analyses by Western blot, and RT-PCR ) are methods to measure virus expression. Low expression of PERV type A and C and absence of PERV-C pro-viruses should be selected .

ii. Vaccination against PERVs : should be applied to prevent PERV transmission
iii. PERV Expression Inhibited by Genetic engineering (RNA Interference ) (16)

### 4.3 Ethical view of xenotransplantation :

The ethical issues related to using animals in medical research are complicated because it is a mixture of different points of views.

As mentioned above the genetic engineering of pigs might be the key to xenotransplantation's success. However there are arguments against this method depending on that it: (17)

- Contradicts with the innate order of the world .
- Might alter the nature of animals.
- Break the boundaries between species.
- Might change our opinion about living beings.
- Can affect the environment and other animals especially if inter-breeding occurs.

On the other hand, there are psychological and social aspects that may affect patients which include: (17)

- What will be the patient´s feeling and thinking when he has pig organs inside his body
- How can the patient accept some of the steps that are needed after transplantation as limited physical contact with others to decrease possibility of infection.

Religious aspects that prohibit eating meat from pigs will sure be an obstacle towards the popularity of xenotransplantation from pigs (18)

Finally, Working Party of the Institute of Medical Ethics. has a book which is Lives in ''the Balance: The ethics of using animals in biomedical research''

He talked about this ethical issue and he produced the following statement:

The use of animals in research and in xenotransplantation though undesirable may sometimes be the only alternative to save lives however this depends on the benefits acquired that justify the use of the animal but not every improvement in human health or scientific addition should justify the use of animals especially if there are serious risks involved so at the end the achievable benefits are weighed against the adverse effects of using the animal. (19)

## 5 RESULTS OF PREVIOUS CLINICAL AND PRECLINICAL TRIALS:

One study applied in 1964 on six renal failure patients who received baboon kidneys, the results showed graft loss in two patients and death of four patients, between 19 and 60 days after transplantation. (20)

First xenotransplantation applied on a baby girl was in 1985 suffering from hypoplastic left heart syndrome (HLHS), she received baboon heart after 20 days she died by humoral response. (21)

For liver transplantation, in 1993 the clinical experiment done on a 35 year old patient with abnormal liver function tests, hepatitis B virus (HBV) and human immunodeficiency virus (HIV) using baboon liver , the patient survived for 70 days before he died by angioinvasive aspergillus infection. (22)

In pre-clinical trials the transplantation applied on non-human primate most animals being used are monkeys of different species, For example rhesus macaque , p.anubis, and baboon.

In kidneys transplantation from pigs to NHPs a maximum survival period of 310 days has been achieved by using organs of pigs genetically modified. The modification included GalT-KO .

Two other studies one exhibited 229 days survival period and the other showed 136 survival period by using multitransgenic.

Lung xenotransplantation faces severe complications so the possibility of success is lower than other organs. The maximum survival period which has been achieved with genetically modified pigs is 109 hours by an experiment done in 2007. Concerning heart transplantation from Pigs to NHPs, the result showed 2.5-year as a maximum survival period with GalT-KO pig engineering in combination with immunosuppressive drugs.

Overall, the pig xenografts show high survival rate in NHPs, which raises the probability of success of pigs organ transplants in humans after genetic manipulation (23)

## IN SUMMARY :

Although xenotransplantation seems an apparent solution to organ shortage and carries a lot of hope to desperate patients but it is not free of disadvantages and this is illustrated in Table-2. And in the end every case needing transplantation should be dealt with individually calculating the benefits, risks and reaching a final decision of whether xenotransplantation can be of help to the patient or worsen the case. (24) (25).

## CONCLUSION:

Xenotransplantation has many benefits and it may be a solution for organ transplantation waiting lists which are a major cause of losing lives each day while waiting for a transplant. The increase in organ demand with the decrease in organ supply makes it a challenge provoking many scientists to try to solve this issue.

As mentioned above, the three major problems hampering success in transplantations from pigs to humans are:

1- The immune barrier
2- The risk of microorganisms transmission
3- The ethical argument related mainly to society and to the future recipients.

For overcoming these obstacles the scientists are keen to find solutions to make xenotransplantation enter reality, and to prolong survival of xenotransplanted organs

One of the most sufficient techniques is genetic engineering of pigs which provided us with several genetically modified types aiming at overcoming the immune barrier. Genetic manipulation has made it possible to add, delete or exchange genes from one species to another.

Other obstacles facing xenotransplantation are infection risks as previously mentioned there are many ways to decrease this risk by vaccination, genetic engineering, and selection of PERV-C-Free

The last obstacle is the ethical issue which can be solved by increasing the awareness of the society about the importance of using animal organs in humans suffering from chronic illnesses and impending death, and direct their attention about new techniques that may alleviate their fears and concerns

Overall, even with all these obstacles there are some successful trials which have been applied in vivo and in vitro. Finally, xenotransplantation could take place in the near future.

## ACKNOWLEDGMENT:

I would thank Dr. Enas M Qasem for assistance and her comments that greatly improved this research.

## TABLES:

Table-1 : Statistics of the gap between the supply and demand for the different organs in Saudi Arabia in 2016.
Shaheen FA . (2016 Jul) (2)

| Organs | Incident patients on the waiting list (cases/PMP per year) | Organs available (organs/PMP per year) | Gap |
|---|---|---|---|
| Kidney | 200 | 40 | 160 |
| Liver | 40-50 | 9 | 30-40 |
| Heart | 15-20 | 1.5 | 14-18 |
| Corneas | 100 | 2 | 98 |

Numbers are per million populations.
PMP, per million populations

Table 2: Advantages and disadvantages of xenotransplantation .
Chapman L (24) Groth CG (25) .

| Pros | Cons |
|---|---|
| Larger supply of organs : one of the main goals of xenotransplantation is making organ transplant available and provide unlimited tissue and organ . Also to decrease the number of death on waiting list. | Risks shorter life spans : typically, the animals have short life span than human so the risk of organs dying prematurely , which may lead to frequent transplant for patient to overcome this problem. |
| Supply exogenous infection-free sources : Some animals have ability to fight off human diseases. | Xenosis: which mean (transmitting infectious agents from xenografts into humans ) |
| Solving the problems related to the black market for organ donation : these markets cause huge issue around the world , especially from third-world who sell their organs to gain money . Also the kidnapped children for the harvesting their organs. Xenotransplantation can end this type of trade. | Moral issues: including the religious beliefs as Islamic and Jewish people forbid the consumption of pig , animal rights , and psychological .aspects that may affect patients |
| Using animal organs with genetic engineering : to overcome the graft rejection , thereby making less dependent on immunosuppressive agents. | Risk of rejection: is the greatest obstacle to xenotransplantation. Recently there are many techniques may be used to reduce the risk. |

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International Journal of Scientific & Engineering Research, Volume 8, Issue 8, August-2017
ISSN 2229-5518