LESSON 19:
TRANSGENIC ANIMALS

Welcome students, in this lecture let us discuss about some of the transgenic animals which are produced by various methods which we discussed in the last classes.

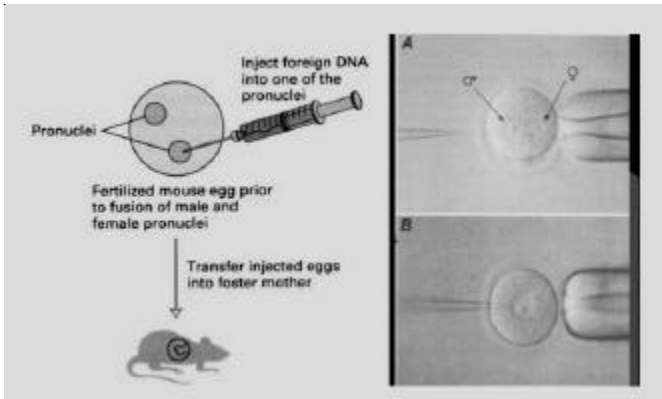
In this regard, from this lecture let us see what are the different animals produced and what was the protocol involved in the production of these animals.

Objective

- Introduction to the Transgenic animals and various types of Transgenic animals produced.
- Advantages and Disadvantages (Technical & Ethical issues) Involved in the production of Transgenic animals.

Introduction

Transgenic Mice and Other Animals



One of the first reports of transgenic animal is published in Dec 1982, involved transfer of growth hormone (GH) gene (from rat) fused to the promoter for the mouse metalotheonine 1 (MT) Gene since than many transgenic animals including those in cattle, sheep, goats, pigs, rabbits, chicken and fish have been produced and will be utilized in future for a variety of purpose including

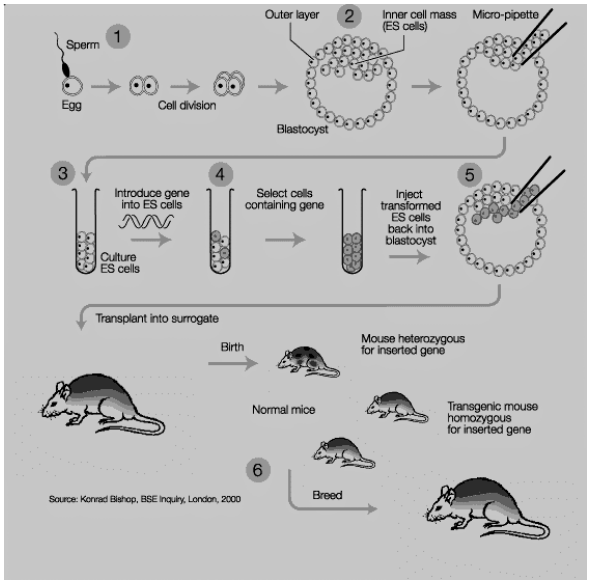
1. Their efficiency in utilizing a feed
2. Ability to give a leaner meat
3. Their ability to grow to markedable size sooner
4. Their resistance to certain diseases. More than this recently efforts are being made to use transgenic animal as living Bioreactor.

Transgenic animals produced for this purpose will secret valuable recombinant proteins and pharmaceuticals into their milk, blood and urine which can be used for extraction of these drugs.

This new possibility of manufacturing drugs through transgenic animals is often described as molecular farming or molecular pharming.

Although initially many experiments leading to the production of transgenic animals did not give exactly commercially attractive results, success has been obtained in some recent cases, some of the cases will be discussed.

Mechanism Involved in the Preparation in the Preparation of Transgenic Mice



Transgenic Sheep

Dolly

- The rate of transgenesis in sheep is very low (0.1% - 0.2%) This can be improved, if only transgenic viable embryo (after necessary checking) are transferred to surrogate eves (female sheep).
- Embryos at 8-16 cell stage can be split into two parts one for continued culture and the other for detection of integrated genes using a polymerase chain reaction.
- Although micro injection is most common for DNA delivery, gene targeting may be increasingly used in the future. In this approach embryonic stem cells in culture are transfected with vector which target the gene to particular site by homologous recombination this technique though successfully used in mice ,has yet to be applied to be sheep where as cells will have to be isolated first.
- The first reports of transgenic sheep were published by j.p.simons of Edinburgh. Two transgenic ewes were produced ,each carrying about 10 copies of humanity hemophilic factor ix gene (c DNA) fused with the 10.5kb BLG gene (b lactoglobulin) BLG gene was used ,because it necessary for specific expression of gene in mammary glands. Consequently, the had a tissue specific expression of gene and ewes secreted human factor 1X into their milk this human factor IX is active even though the expression of trans gene is low . the transgenic ewes were born in early summer of 1986 and were success mated same year in December . In 1987 each ewe gave birth to single lambeach lamb inherited BLG-F IX trans gene and secreted factor IX in the milk . This programme of the production of transgenic animals by J.P.simons at Edinbergh was funded by pharmaceuticals proteins ltd. U.K due to its commercial appeal.
- Recombinant DNA technique can also be used to increase the ability of the sheep for wool growth .for this purpose, genes essential for synthsis of some important amino acids found in keratin proteins of wool , have been cloned and introduced in embryos to produce transgenetic sheep . for instance genes (cysE and cysM) for two enzymes (serine acetyl transferase = SAT and O- acetylserine sulphydralase = OAS) involved in cysteine biosynthesis were isolated from bacteria and cloned in vector these genes were introduced in sheep cells , ultimately leading to the production of transgenic sheep where as genes are expressed . Growth hormone (GH) genes also have been introduced and can be used to promote body weight .other genes involved in wool production have also been cloned and will be used for transgenesis thus increasing the potential of wool production through genetic engineering.

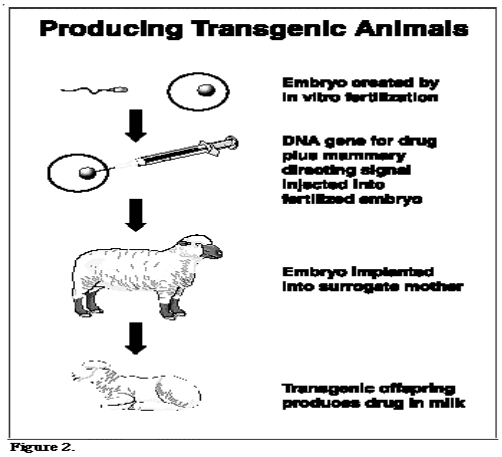
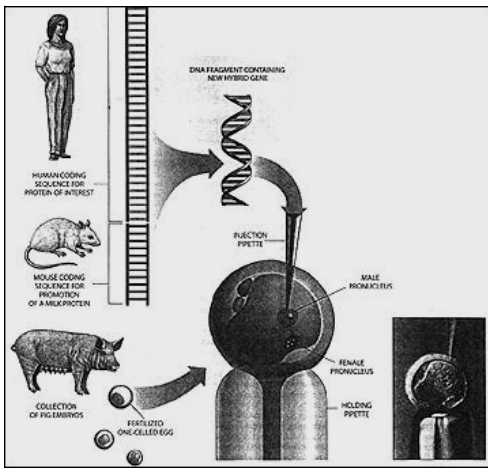


Figure 2.



Transgenic Pigs

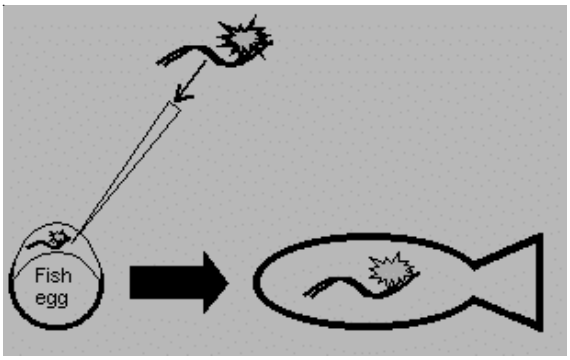
- The efficiency of the production of the transgenic pigs is still very low compare to that of production of transgenic mice. In mice 2.5% to 6% of the micro injected eggs developed into transgenic mice but in pigs this frequency was as low as 0.6%. Even as many as 7,000 eggs were injected. This low frequency transgenic pigs carrying a growth hormone gene from bovine or human and sheep globin gene have been produced at agriculture research service, BeltSalive, USA.
- The pigs carrying an “hGH” gene showed different levels of expressions and only 66% of this animals showed detectable levels of human growth hormone and bovine growth hormone in their plasma.
- The animals grow little faster but did not become a large similarly pigs with a sheep globin gene did not show any expression of the transgene for unknown reasons. In these transgenic pigs, however the modest increase of 10 to 15% in daily weight and 16 to 18% in feed efficiency was observed which is though lower to those in mice but are comparable to those obtained due to daily injection with pig growth hormone.

- It was also observed that there was marked reduction in the subcutaneous fat in some of these transgenic pigs suggesting the possibility of producing a linear meat with a lower fat content.
- This result may have a significant impact on the 9.5$ billion annual pig industry in USA.
- It is also reported that long term elevation of growth hormone was generally detrimental to health. The pigs had incidents of gas ulcer, arthritis and several other diseases. Therefore techniques will have to be developed to manipulate better the transgene expression by a variety of methods.

Transgenic Cows

- In earlier attempts for the production of transgenic cows, embryos or fertilized oocytes produced in vivo were utilized.
- Fertilized oocytes or proembryos were surgically retrieved from super ovulated and artificially inseminated cows, microinjected zygotes were then transferred by surgery either directly into the oviduct of recipient cows or into temporary hosts like sheep or rabbits. In view of two surgical operations this method is labour intensive and more expensive.
- In The Netherland recently a technique has been developed for in vitro embryo production. In this new procedure, oocytes obtained from the ovaries of slaughter house cows, were matured and fertilized invitro.
- Their pronuclei were microinjected with a construct containing a bovine alpha S1 casein promoter driving a cDNA encoding the antibacterial human iron binding protein, lactoferrin.
- The embryos were cultured to morula/blastula stage and then non-surgically transferred to recipient females. Two of the 18 calves born from 103 transferred to recipient females. Two of the 19 calves born from 103 transferred zygotes were transgenic (one male and other female). This procedure may facilitate the use of cows as bioreactors at the commercial level

Transgenic Fish



Different types of Transgenic fish:-

Attempts to produce transgenic fish started in 1985 and some encouraging results have been obtained.

The genes that have been introduced by mircroinjection in fish include the following

1. Human or rat gene for growth hormone
2. Chicken gene for delta crystalline protein
3. E.Coli gene for beta galactosidase
4. E.Coli gene for neomycin resistance
5. Winter flounder gene for antifreeze protein
6. Rainbow trout gene for growth hormone.

- The technique of microinjection has been successfully used to generate transgenic fish in many species such as common carp, catfish, goldfish and salmon in other animals usually direct microinjection of cloned DNA into male pronuclei of fertilized eggs has proved very successful but in most fish species studied so far, pronuclei can not be easily visualized so that the DNA needs to be collected and placed into a separate dry container.
- Fertilization is intiated by adding water and sperm to eggs, with gentle stirring to facilitate the fertilization process.
- Egg shells are hardened in water. About 10^6 to 10^8 molecules of linearized DNA in a volume of 20 ml or less are microinjected into each egg within the first few hours after fertilization. Following microinjection, eggs are incubated in appropriate hatching trays and dead embryos are removed daily.
- Since in fish, fertilization is external, in vitro culturing of embryos and their subsequent transfer into foster mothers is not required. Further, the injection into the cytoplasm is not as harmful as that into the nucleus, so that the survival rate in fish is much higher.
- Human growth hormone gene transferred to transgenic fish allowed growth that was twice the size of their corresponding non transgenic fish.
- Similarly antifreeze protein gene was transferred in several cases and its expression was studied in transgenic salmon.
- It was shown that the level of AFP gene expression is still too low to provide protection against freeze. There is also a report of the production of transgenic zebrafish from an Indian laboratory .
- In this attempt a plasmid containing rat growth hormone gene was microninjected into fertilized zebrafish eggs and its presence confirmed in adult fish.

The above are the various transgenic animals which are produced, research is going to improve further better qualities in animals and plants

Current Research Work on Transgenic Animals

Students now we knew about animal tissue culture but we need practical oriented research work we have to now what's going in the world

I am giving some web site address of journals:

1. Pressure grows for inquiry into welfare of transgenic animals

   Ehsan Masood
   SUMMARY: london Mounting public concern over the increasing use of animals in genetics research is fuelling calls for a national expert commission to investigate the...
   CONTEXT: london Public concern in Britain over the increasing use of animals in genetics research is fuelling calls for a commission of inquiry to investigate the welfare of transgenic animals. The calls come in the wake of the UK government's.....
   Nature□388, 311 - 312 (24 Jul 1997) News
2. Environmental impact tops list of fears about transgenic animals

   Erika Check
   Description: Washington A committee convened by the US National Academy of Sciences has.....
   CONTEXT: Washington A committee convened by the US National Academy of Sciences has expressed strong concerns that transgenic animals could damage their wild surroundings. The panel was asked by the Food and Drug Administration (FDA) to list the.....
   Nature□418, 805 (22 Aug 2002) News

Note