Biotechnological potential of stem cells in food-producing animals: A review

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Abstract

Animal biotechnology related to production has contributed to many improvements in agriculturally important traits in livestock. Although, artificial insemination was the first assisted reproductive technology used to enhance farm animal reproduction and propagate superior genetics, reproductive cloning and the development of transgenic animals are the most current of these new technologies. Reproductive biotechnology can be used to propagate superior genetics in considerably less time than traditional breeding methods. Genetic manipulation based on the selection of phenotypic traits has been long practiced in animal agriculture. Artificial insemination, embryo transfer and *in vitro* fertilization have added significant improvement in livestock development. Cloning shortens the generation period without altering the genetic composition, thereby helps to provide superior livestock that can replace low-productive animals. Cloning can be done by using stem cell, usages of stem cells in reproductive cloning serves to be useful biotechnology to create a transgenic animal.

Key words: Cloning, Livestock, Stem cell, Transgenic animal

Introduction

Stem cells are unspecialized or undifferentiated cells that can transform into any cell type i.e. specialized or differentiated cells. Stem cells can be isolated from an embryo, fetus, or adult tissue or a cell that can reproduce themselves for a longer period of time under

certain conditions (Table 1) (Zakrzewski *et al.*, 2019). They are categorized as totipotent, pluripotent, or multipotent cells depending on their stage of development and differentiation (Table 2) (Weissman and Wagers, 2004). This review will target the applications of stem cells to increase the productive trait of livestock.

Table 1. Types of stem cells (Based on sources)

Origin	Stem cell type
Blastocyst (ICM)	Embryonic stem cell (ESC)
The gonadal ridge of a fetus (e.g. Primordial germ cells) from 5-10 weeks of fetus	Embryonic germ (EG) cells
Embryonal carcinoma (e.g. PGC), the component of testicular tumors of adult	Embryonic carcinoma (EC) cells
Umbilical cord	Umbilical cord stem cells
Placenta of newborn	Placental stem cells
Amniotic fluid	Amniotic fluid stem cells
Adult tissues: e.g. liver, brain, skin, cornea, retina, skeletal muscle, dental pulp, pancreas, endothelial progenitors (EPCs), etc.	Adult stem cells

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Table 2. Types of stem cell (Based on potency)

Type	Description	Example
Totipotent	Each cell can develop into a new individual	Cells from early embryo (1-3 days)
Pluripotent	Cells that can give rise to all adult cell types	Some cells of a blastocyst (5-9 days)
Multipotent	Differentiated cells & cells of other tissue	Adult stem cells
Unipotent	Cells from only one cell type	Spermatogonial stem cells (sperm)/oocyte

Application of stem cells

The effective use of stem cells in livestock production has shown to be a valuable technique (Table 3). Stem cells have applications in a various field such as-

Use of stem cell in human: ESC can be used for cardiac repair (heart disease), neurological disorders, diabetes, spinal cord injury, Parkinson's and genetic diseases (Hassan *et al.*, 2009).

Therapeutic use of animal ES cells: Domestic animals are immunologically and physiologically more similar to humans than rodent models, and domestic animals may be used as a model for studying human pathology (Vandamme *et al.*, 2014). Animal ES cells can be used as a model for human research (Blastocyst complementation) (Crane *et al.*, 2019)

Use of stem cell in livestock: ESC can be used to improve the efficiency of the somatic cell nuclear transfer (SCNT)/cloning technique, production of transgenic and chimeric animals, gene targeting and genome editing, production of germ cells to treat infertility, screening of drug and chemicals, as well as for the studies on early embryonic development, DNA methylation/genomic imprinting (Vijayalakshmy *et al.*, 2018).

Application of stem cells in food-producing animals:

- ✓ Transgenic animals: Embryonic stem cell-mediated gene transfer
- ✓ Gene targeting (Myostatin)
- ✓ Cloning
- ✓ *In vitro* meat production/cultured meat production (Yadav *et al.*, 2012)

Table 3. Biotechnological potential of stem cells in food-producing animals

Species	Potential application	Reference
Cattle/Buffalo	Enhancing milk and meat production, enhancing biopharming through transgenic milch animals; reduction in emissions of greenhouse gases; production of meat <i>in vitro</i>	Singh <i>et al.</i> , 2009; Niemann <i>et al.</i> , 2011; Singh <i>et al.</i> , 2011
Pig	Developing cells, tissues and organs for xeno-transplantation, enhancing biopharming through transgenic animals, production of pork <i>in vitro</i>	Niemann <i>et al.</i> , 2011
Goat and Sheep	Enhancing biopharming through transgenic animals, <i>in vitro</i> mutton/chevon production and conservation of genetic diversity through repopulation of endangered species	Mauro <i>et al.</i> , 2010; Azari <i>et al.</i> , 2011
Poultry	Transgenic chicken with higher growth rate, increase in dark and white muscle, producing low cholesterol egg	Mozdziak <i>et al.</i> , 2003; Ricks <i>et al.</i> , 2003; Li <i>et al.</i> , 2008

Role of stem cells in food producing animal

Transgenic animals

In the 1980s, several transgenic animals were reported to have been created. Several approaches are used to produce transgenic animals. The micro-injection approach is widely utilized, although it has some disadvantages, including low efficiency and variable expression patterns. So, various alternative techniques such as sperm-mediated DNA transfer (Chang *et al.*, 2002), intracytoplasmic injection (Umeyama *et al.*, 2012), nuclear transfer (Carvalho *et al.*, 2019) technologies, injection or infection of oocytes and/or embryos by different types of viral vectors (Clark and Whitelaw, 2003) and ribonucleic acid (RNA) interference technology (Xu *et al.*, 2019) have been developed.

Transgenic animals are organisms that have had incorporated cloned DNA sequences (transgenes) that have been transferred using genetic engineering techniques (including gene transfer and gene substitution). Several types of transgenic animals like transgenic cow, bull, sheep, pig, etc. were produced for the better and good quality breed with increased milk yield and meat production. Genetically modified animals are proving ever more vital in the development of new treatments and cures for many serious diseases (Feng *et al.*, 2020).

Advantages of transgenic animals for enhancing animal productivity:

- ✓ Increase the growth performance
- ✓ Improve the feed conversion efficiency
- ✓ Increase the muscle mass and mutton production
- ✓ Improve the nutritional quality of milk
- ✓ Improve the wool quality (fineness, luster)

Methods for producing transgenic animals:

The introduction of a foreign gene or genes into an animal is the primary principle in the development of transgenic animals. Transmission of the foreign genes through germ lines must be there to ensure that every cell of the animal contains the same modified genetic material.

Embryonic stem cell-mediated gene transfer:

In 1981, the term embryonic stem cells (ES cells) were used to denote a cell line isolated directly from mouse embryos while the term embryonal carcinoma cells (EC) were derived from teratocarcinoma. ES cells are harvested from the inner cell mass (ICM) of mouse blastocysts. They can be grown in culture and retain their full potential to produce all the cells of the mature animal, including its gamete (Zhang *et al.*, 2020).

Gene targeting based transgenesis: Gene targeting is a technology that specifically modifies a particular gene in the chromosome through homologous recombination and integrates extrinsic genes into the specific target site. Gene targeting technology overcomes random integration events and is therefore ideal for the modification and reconstruction of biological genetic materials (Miao, 2013).

Embryonic stem cell gene targeting: ESC gene targeting takes advantage of embryonic stem cells' totipotency to insert modified extrinsic genes into ESCs that can then be inserted into animal blastula/blastocysts. The ESCs will participate in the formation of host embryos, construct chimera until species chimerism, and transmit the extrinsic gene to the next generation and produce transgenic animals (Suchy and Nakauchi, 2017).

Benefits	Risks
1. The desired characteristic may be introduced for an animal that requires few feed supplements as well as medical treatments.	1. Insertion of a foreign gene may upset the expression of the genome.
2. A desired characteristic of offspring could be established in one generation.	2. Normal reproduction may result in a transgene being released to the wild population.
3. The characteristic required can be chosen with greater specificity and accuracy.	

ESC gene targeting technology was used to build a mouse model with a hypoxanthine-guanine phosphoribosyltransferase (HPRT) knockout. Through gene targeting, current techniques can not only knock out specific genes but also transfer extrinsic genes into the animal genome to obtain gene knock-in animals. Targeted disruption of the myostatin (*mstn*) gene in yellow catfish was performed using engineered zinc-finger nucleases (ZFNs), and six *mstn*^{nju6/+} and 14 *mstn*^{nju7/+} offspring were obtained. p53 (also known as Tp53) gene knockout rats were generated by homologous recombination in embryonic stem (ES) cells. The entire process requires ~1 year of completion, from the derivation of ES cells to the generation of knockout rats (Hall *et al.*, 2009).

ES cells provide a system for studying the whole cellular population. By the utilization of ES cells as a carrier and the modification of ES cells *in vitro*, all issues including the numbers, the loci, and the expression extent of integrated genes, the stability of inserted genes, and the screening of genes can be finished on a cellular level. Currently, homologous recombination in ES cells becomes one common technique to modify any gene site in the mouse chromosome and is applied to study gene function and disease models. However, ES cells from domestic animals have not been obtained for gene targeting so far, which thereby limits the application of gene targeting for production such as mammary gland bioreactor (Monzani *et al.*, 2016).

Transgenic Cattle:

- ✓ The transgenic cow produces lactoferrin and interferons (Li *et al.*, 2014).
- ✓ Myostatin gene is used for double muscling in cattle (Monin and Sante-Lhoutellier, 2014).
- ✓ Transgenic cattle containing two extra copies of kappa casein increase the 13% more proteins (Laible *et al.*, 2016).
- ✓ Lactose-free milk (transgene lactase) has been produced (Shepelev *et al.*, 2018).

- ✓ Resistance to bacterial infections/prion free cow has been produced (Richt *et al.*, 2007)

Transgenic Sheep:

- ✓ Producing α -antitrypsin in their milk (Viglio *et al.*, 2020)
- ✓ Myostatin gene used for double muscling (Miar *et al.*, 2014)

Transgenic Pig:

- ✓ Containing a human metallothionein promoter or porcine growth hormone gene construct referred to significant improvements in economic traits including growth rate, body fat/muscle ratio (Ju *et al.*, 2015).
- ✓ Containing spinach desaturase gene in their genome which produces a large amount of non-saturated fatty acids in its pork which when consumed by humans reduces the risk of stroke and coronary disease (Niemann, 2004).

Transgenic Goat: Tissue plasminogen activator, antithrombin III (He *et al.*, 2018), spider silk (Decker, 2018), lysozyme (Yu *et al.*, 2013), and lactoferrin (Zhang *et al.*, 2015) has been produced in their milk.

Transgenic Fish: A superfish has been produced by inserting the growth hormone gene into eggs and enhanced the body weight more than 10-11 times faster than the normal fish (Kurdianto *et al.*, 2016).

Cloning

Nuclear cloning is a process in which a diploid nucleus is injected into an enucleated egg and allows them to develop up to the blastocyst stage or full term. It is a technique to create genetically identical organisms (Rogers, 2016).

Types of cloning: a) Embryo cloning: to produce monozygotic twins or triplets from a fertilized embryo- single cell can develop an embryo, b) Reproductive cloning: to create clone animals, c) Therapeutic cloning: to produce stem cells for regenerative medicine.

A clear advantage of cloning is to conserve breeds or species (particularly those are endangered), thereby maintaining or increasing genetic diversity in the population (Bazer *et al.*, 2012). Cloning can also allow castrated male animals that possess good traits (e.g., high-quality meat, as well as unusually high rates of lean tissue growth and feed efficiency) to pass their genetic traits to offspring.

The United States Food and Drug Administration (FDA, 2018) published an article stating that “meat and milk from clones of cattle, pigs, goats, and the offspring of clones from any species traditionally consumed as food, are as safe to eat as food from conventionally bred animals”.

ES cells have 10-20 folds higher efficiency as somatic cell nucleus donors in cloning than cumulus cells (Jaenisch *et al.*, 2002). Genetic modification by homologous recombination is higher in ES cells than in somatic cells, hence there is higher efficiency of transgenic animal production by nuclear transfer (Burgio, 2018).

***In vitro* meat production/ cultured meat production**

Meat produced from cell cultures is a prominent alternative for traditional meat derived from live animals (Slade, 2018). This approach gained increasing attention in public opinion, popular media, animal welfare organizations, the scientific community, and among investors (Stephens *et al.*, 2018), particularly after the production of the first clean meat prototype (Post and van der Weele, 2014).

According to an FAO survey, livestock accounts for 14.5 percent of global greenhouse gas emissions, 30 percent of Earth's terrain, and 8% of global freshwater (Gerber *et al.*, 2013). Traditional meat processing systems cannot be considered viable with global meat demand predicted to double by 2050.

Food-borne illnesses such as swine and avian influenza are often caused by intensive factory farming and poor animal health

conditions. They also contribute to the spreading of *E.coli*, salmonella, and campylobacter, which can be found in meat (Anomaly, 2015). Overuse of antibiotics induces the selection of antimicrobial-resistant (AMR) strains, a major human health concern (Tang *et al.*, 2017). Ethical concerns regarding factory farms (Herzog, 2016) and the slaughter of animals are another drive behind clean meat research (Sharma *et al.*, 2015). These issues could be avoided and food safety could be enhanced by processing meat in a sterile and controlled environment.

Methods of production of *in vitro*/cultured meat: For the realization of the concept, two major technologies have been created. The biotechnological approach, which broadly includes cell and tissue culture, is at the core of the scheme and based on their design, they are also popularly known in the jargon of biotechnology as ‘Scaffold based’ and ‘Self-organizing techniques’, respectively (Edelman *et al.*, 2005).

1. Self-organizing technique: The first method involves using an explant from a donor animal's muscle, which is then multiplied in a nutrient medium. The origins of the term can be traced back to the early 21st century when tissue-engineering methods were used to grow meat (Benjaminson *et al.*, 2002). They placed skeletal muscle explants from goldfish (*Carassius auratus*) in diverse culture media and observed a varied pattern of growth, concerning an increase in surface area over 7 days. The results based on the medium were as - fetal bovine serum: 13.8%, fish meal extract: 7.1%, shiitake extract: 4.8%, maitake extract: 15.6%. Explants were also put in a culture of dissociated *Carassius* skeletal muscle cells, which resulted in a 79 percent increase in the explant surface region. The self-organizing technique helps to create structured meat i.e. meat produced will have a well-defined 3-D structure, just as the natural conformation of meat. The same can be achieved using the principles of tissue engineering for *de novo* synthesis of muscle tissue (Edelman *et al.*, 2005).

2. Scaffold-based technique: The second method of culturing meat involves suitable stem cells which can be obtained from a variety of tissues. The scaffold-based technique involves proliferating embryonic myoblasts or adult skeletal muscle satellite cells, adding them to a scaffold or carrier, and then perfusing them with a culture medium in a bioreactor (Kosnik *et al.*, 2003). The scaffold-based technique operates on the concept of appropriate muscle cells proliferating on a scaffold in the presence of a culture medium in a bioreactor. Myofibers are produced as a result of this culturing, which can then be harvested, processed, and consumed as meat or meat products (Bhat and Fayaz, 2011).

There are currently two elaborated propositions for using cell culture to create meat in the lab. One has been written by Vladimir Mironov for NASA (Wolfson, 2002), while the other by Willem van Eelen who holds a worldwide patent for a similar system (Van Eelen *et al.*, 1999). Vladimir Mironov proposed that cells be grown on collagen spheres where they would attach and differentiate in a bioreactor. Willem van Eelen proposed using collagen meshwork instead of spheres and regularly refreshing the culture medium. Collagen may be replaced by other edible proteins or artificial substrates if the culture medium is percolated through the meshwork (Van Eelen *et al.*, 1999). After harvesting, it can also use two-dimensional monolayers of muscle cells sandwiched together (Bhat and Fayaz, 2011). This method is expected to be suitable for the production of processed ground meat products, but not for the production of highly structured meats like steaks. To produce organized muscle tissue, a more refined approach such as Self Organising Technique is needed (Edelman *et al.*, 2005). The meat is majorly composed of skeletal muscle which consists of several cell types. Proliferation, differentiation, and fusion of embryonic

myoblasts or satellite cells result in the development of skeletal muscle fibers (Langelaan *et al.*, 2010). Several cell types have been proposed for the *in vitro* meat production system by several authors; these are Myosatellite cells, ESCs, and Adult stem cells. Satellite cells, also known as myoblasts, are responsible for muscle regeneration and can quickly differentiate into myofibrils. Stem cells, on the other hand, can differentiate into various cell types and are predominantly located in the early stages of organism growth (Williams, 2012).

Conclusion

Stem cells are a powerful tool for improving livestock performance and *in vitro* meat production in a controlled, animal-free environment. It will necessitate a change in optimization parameters, with a greater emphasis on low-cost, animal-free biomaterials, effective farm animal cell expansion, intramuscular fat tissue engineering, and analysis methods that emphasize nutritional value and organoleptic properties. The use of stem cell technology would assist in dealing with limited resource supply and a related rise in the demand for food production. Producers can elicit rapid genetic change to create the next generation of superior animals. Transgenic methodologies may also help both farmers and consumers by incorporating new genes into a population. Cloning and precise placement of genetic information will undoubtedly become the most useful approach to generate potential transgenic livestock because it is vastly more productive than other types of transgenesis. Cloned and transgenic livestock will continue to be developed, but consumer acceptance is needed to realize their full potential and effect on animal health and food security.

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