THE EVOLVING PICTURE IN OBTAINING GENETICALLY MODIFIED LIVESTOCK

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Abstract: Genetically modified livestock are farm animals that were subject to modification of an endogenous DNA sequence or introduction of exogenous DNA into their genome. Genetically modified livestock have great potential as models for studies of human diseases, for more efficient meat and dairy production, for xenotransplantation, and for production of highly-demanded products for human health. Different methods have been defined for obtention of genetically modified livestock, with varying efficiencies, limitations and advantages. The review aims to describe a brief history on obtention of genetically modified livestock, its major hurdles, current approaches for their obtention, and future perspectives on the technology.
Index terms: Farm animals, GMO, Transformation, Transgenesis, Recombinant DNA.

UMA VISÃO EM EVOLUÇÃO DA OBTENÇÃO DE ANIMAIS DE PRODUÇÃO GENETICAMENTE MODIFICADOS

Resumo: Animais de produção geneticamente modificados são aqueles que tiveram uma sequência de DNA endógena modificada ou um DNA exógeno introduzido em seu genoma. Animais de produção geneticamente modificados apresentam grande potencial como modelo para estudo de doenças humanas, para produção mais eficiente de carne e derivados do leite, para xenotransplante e para produção de produtos sob grande demanda para saúde humana. Diferentes abordagens têm sido descritas para obtenção de animais de produção geneticamente modificados, as quais apresentam eficiências, vantagens e limitações variáveis. O objetivo da revisão é descrever o histórico da obtenção de animais de produção geneticamente modificados, os principais obstáculos, abordagens atuais e perspectiva futuras sobre a tecnologia.

Termos para indexação: Animais de produção, OGM, Transformação, Transgênese, DNA recombinante.

INTRODUCTION

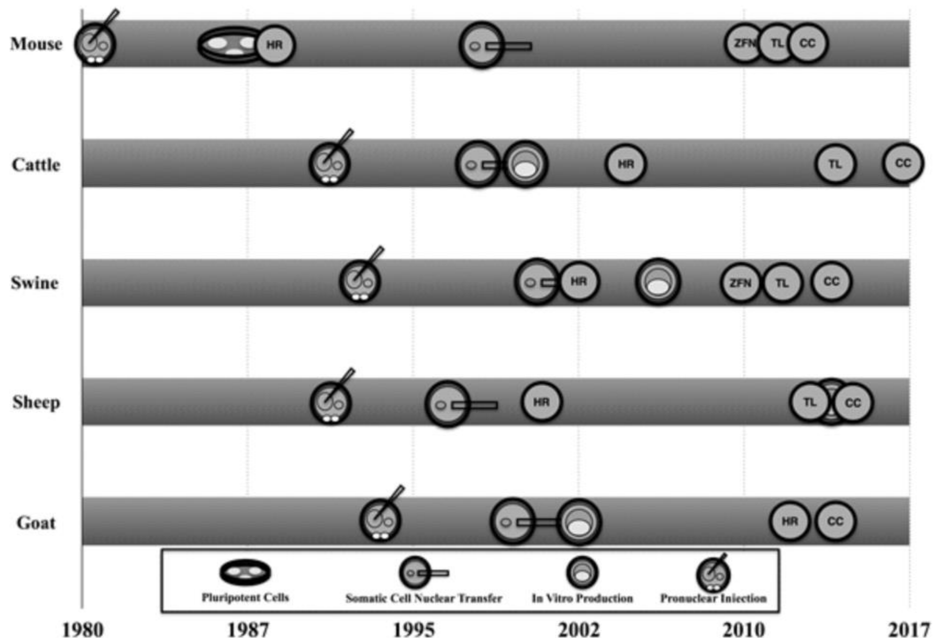
The first influence of humankind on livestock genomes was by the process of domestication over the past 10,000 years (BRUFORD et al., 2003; WIENER; WILKINSON, 2011; LARSON; BURGER, 2013). Another significant event that hugely affected the genetic composition of livestock populations was the advent of breed formation (WIENER; WILKINSON, 2011; WANG et al., 2014). From the twentieth century onward, multiple developments on animal breeding methodologies led to significant progress on livestock quantitative genetic selection (LUSH, 1951; HENDERSON, 1975; GIANOLA; ROSA, 2015), despite limited understanding of the genetic basis of such traits (HAYES et al., 2013; GIANOLA; ROSA, 2015). More importantly, these processes rely on selective breeding to obtain more favorable livestock genotypes, and genetic merit is mostly based on phenotypes alone, genotyping for major genes, or genomic selection based on association between DNA variation and phenotypic data (MEUWISSEN et al., 2001; HAYES et al., 2013; GIANOLA; ROSA, 2015).

The first genetically modified mammalian cell was obtained by co-incubation of naked DNA and rabbit sperm cells (BRACKETT et al., 1971). These sperm cells carrying exogenous DNA were capable of fertilizing eggs and stably transmitting it to the embryo genome (BRACKETT et al., 1971). The production of the first transgenic animal was performed by introduction of simian virus 40 sequences in mouse early embryos, to recapitulate viral-induced oncogenesis in newborn mice (JAENISCH; MINTZ, 1974). Curiously, these retroviral sequences were epigenetically silenced by DNA methylation, and pups were protected from the disease (JAENISCH; MINTZ, 1974; JäHNER et al., 1982). However, these reports did not envision the potential of genetic modification of the mammalian germ-line, as described below.

Later efforts demonstrated the feasibility of genetically modifying the genome by introduction exogenous DNA (transgene) into mouse early embryos (GORDON et al., 1980; BRINSTER et al., 1985; HAMMER et al., 1985; WALL, 1996, 2001). Under similar experimental conditions, genetically modified livestock embryos were produced (BREM et al., 1985; HAMMER

et al., 1985), but live animals were obtained years later (Figure 1). At this stage, cattle had been considered the most difficult livestock species to modify its genome (CLARK, 2002).

Figure 1. Timeline of developments to obtain live-born genetically modified livestock.

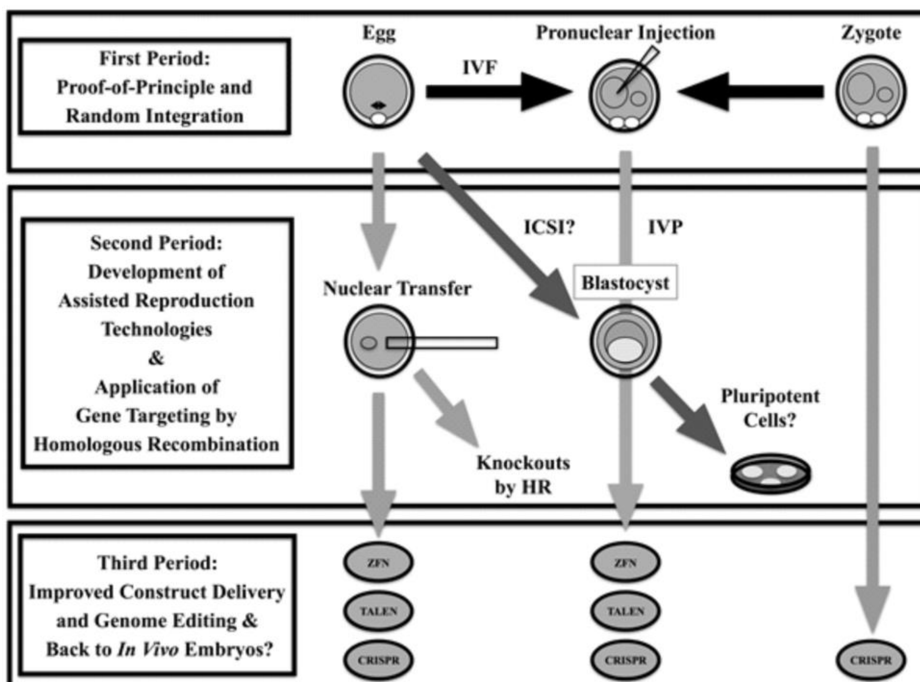


HR: homologous recombination. ZFN: Zinc finger nucleases. TL: TALENs. CC: CRISPR-CAS9.

After these original reports, extensive work has been devoted to improving the generation of genetically modified laboratory and livestock species (EYESTONE, 1994; CAPECCHI, 1989, 2005; FREITAS et al., 2012; POLEJAEVA, 2016; ROGERS, 2016; LOTTI et al., 2017). Several routes were taken to improve the efficiency and type of genetic modification in livestock genomes over almost three decades of intense investigation (NIEMANN; KUES, 2003; POLEJAEVA, 2016; ROGERS, 2016). A careful analysis of the literature suggested that three arbitrary periods can be envisioned during the development of genetically modified livestock (Figure 2). The review aimed to describe these three periods by providing a brief history on genetically modified livestock production (cattle, pigs, sheep, and goats), its

significant hurdles, current approaches for their production, and future perspectives.

Figure 2. The three periods of the development of genetically-modified livestock.



CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats. HR: homologous recombination. ICSI: intracytoplasmatic sperm injection. IVF: *in vitro* fertilization. IVP: *in vitro* production of preimplantation embryos. TALEN: Transcription-Activator Like Endonucleases. ZFN: Zinc Finger Nucleases.

THE FIRST PERIOD: INITIAL REPORTS OF GENETICALLY MODIFIED LIVESTOCK

The introduction of exogenous DNA into zygotes was the first approach developed that led to the small-scale production of genetically modified livestock (EYESTONE, 1994; WALL, 1996, 2001; CLARK, 2002). This procedure, also known as the pronuclear injection (Figure 3A), consists of the introduction of transgene copies into zygote pronuclei by ultra-thin needles using micromanipulators (GORDON et al., 1980; BREM et al., 1985; HAMMER et al., 1985). Embryos can be cultured after injection, to

identify surviving structures, or transferred immediately to recipient females (GORDON et al., 1980; EYESTONE, 1994). Newborn transgenic offspring obtained by pronuclear injection are genotyped for transgene integration and the number of copies (COUSENS et al., 1994; EYESTONE, 1994, 1999). Genetically modified livestock are then raised until puberty and tested for germ-line transmission (EYESTONE, 1994, 1999). Founder germ-line genetically modified animals can then be propagated by natural mating or assisted reproductive technologies (ART) to increase their numbers (EYESTONE et al., 1999; CLARK, 2002; BALDASSARRE et al., 2004).

Although its simplicity and several proof-of-principle reports, pronuclear injection holds several limitations: requires many embryos (e.g., ~150-1,000 injected zygotes per transgenic animal), embryo survival after injection is relatively low, may generate mosaic animals, and requires germ-line transmission testing (EYESTONE, 1994, 1999; CLARK, 2002). Moreover, transgenes form tandem head-to-tail sequences upon injection, may hold variable copy number integration, and its expression may vary due to position effect (PARK, 2007).

Despite the surmountable number of challenges posed to the obtention of genetically modified livestock, such conditions allowed the establishment of initial animal models and set the stage for some relevant applications (Table 1), particularly of pharmaceutical proteins in the milk of goats and cattle (MELO et al., 2007). Under the perspective proposed here, these developments characterize the first period of obtention of genetically modified livestock (Figure 2).

THE SECOND PERIOD: ASSISTED REPRODUCTION TECHNOLOGIES AND GENE TARGETING

The second period is characterized by the substantial development of ART (Figure 2), particularly in cattle (HASLER, 2014; LONERGAN; FAIR, 2016), and less intensively in small ruminants and pigs (PARAMIO; IZQUIERDO, 2014). The *in vitro* production (IVP) of preimplantation embryos became a large-scale tool for commercial operations in cattle, where hundreds of thousands of offspring are born each year (LONERGAN; FAIR, 2016). It offered an attractive approach to generate zygotes for pronuclear injection (EYESTONE, 1999; BALDASSARRE et al., 2003), despite the lower developmental

Table 1. — A survey of key applications of genetically modified livestock.

Major Fields	Key Applications (Period[1,2,3])	Type of Genetic Modification	Method to Obtain GM Animals	References
Animal Models	Development[3]	Gene Targeting	IVP; SCNT	Habermann et al., 2007; Chen et al., 2015
	Disease[3]	Gain-of-Function or Gene Targeting	PI; SCNT	Flisikowska et al., 2016; Rogers, 2016
Livestock Production	Increased Meat Production[3]	Myostatin Knockout	IVP; PI; SCNT	Zhou et al., 2013; Tanihara et al., 2016
	Enriched Milk Composition[1]	Gain-of-Function	PI; SCNT	Bleck et al. 1998; Brophy et al., 2003
	Pollness[3]	Gene Targeting	SCNT	Tan et al., 2013
	Disease Resistance[1-3]	Gain-of-Function or Gene Targeting	SCNT	Kuroiwa et al., 2004; Wu et al., 2015;
	Metabolic Traits[1]	Gain-of-Function	PI	Niemann and Kues, 2003
Biopharming	Human Antibodies[2,3]	Artificial Chromosome	SCNT	Kuroiwa et al., 2002
	Pharmaceutical Recombinant Proteins[1]	Gain-of-Function	PI; SCNT	Schnieke et al., 1997; Melo et al., 2007
Xenotransplantation	Organ Xenografts[2,3]	Gain-of-Function or Gene Targeting	SCNT	Dai et al., 2002; Lai et al., 2002; Weiss et al., 2009
	Human ES-Derived Organs[3]	Gene Targeting	BI	Feng et al., 2015; Wu et al., 2017

BI: Blastocyst Injection. GM: Genetically Modified. PI: Pronuclear Injection. SCNT: Somatic Cell Nuclear Transfer.

potential of IVP embryos (CLARK, 2002).

Another major distinction of the second period was the initial efforts on gene knockouts by homologous recombination in sheep (MCCREATH et al., 2000; DENNING et al., 2001), pigs (LAI et al., 2002; DAI et al., 2002; PHELPS et al., 2003), and cattle (KUROIWA et al., 2004) (Figure 1). This later development was technically-based on pioneered work in the mouse (CAPECCHI, 1989, 2005). Several ART-based methods have been described as alternative routes to pronuclear injection, to obtain of genetically modified livestock (Figure 3), as described below.

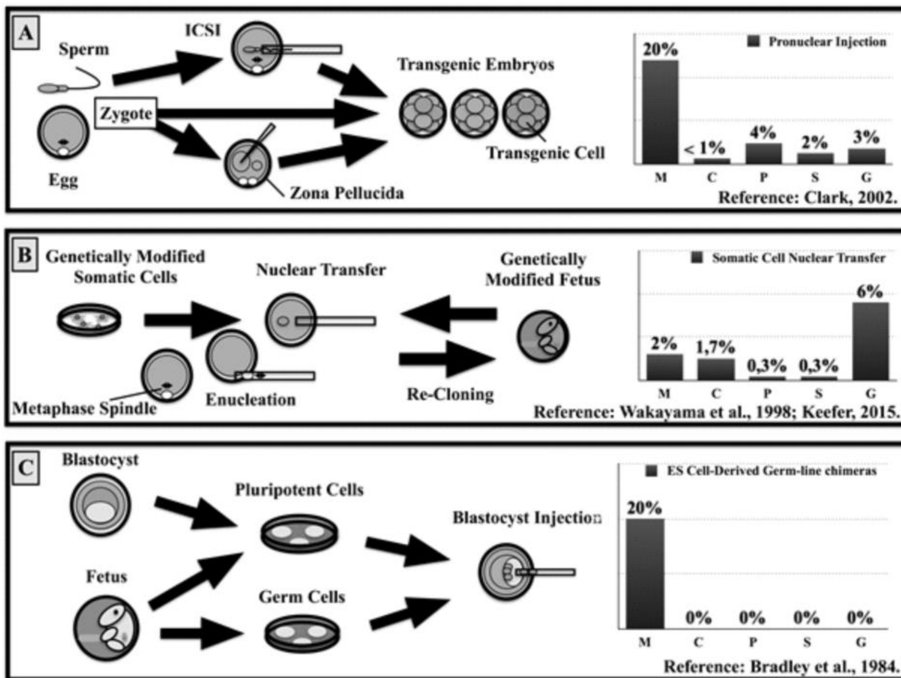
Genetically-modified livestock using sperm-mediated methods

The observation that sperm cells uptake nucleic acid made it as a new method for introduction of foreign DNA into mammalian genomes (BRACKETT et al., 1971) (Figure 3A). However, this first report did not receive much attention, and sperm-mediated method gained broader attention when revisited almost two decades later (LAVITRANO et al., 1989; GANDOLFI, 2000). Proof-of-principle work in several species demonstrated that this approach could be used for obtention of transgenic livestock, even using artificial insemination alone (GANDOLFI, 2000; LAVITRANO et al., 2006).

The process of transgene uptake by sperm cells occurs in two steps (NIU; LIANG, 2008). Firstly, it initiates with exogenous DNA binding to sperm cells and its internalization. Secondly, transgene copies are integrated into the sperm genome in a permanent fashion (NIU; LIANG, 2008). However, the application of this method remains limited by growing skepticism due to its low reproducibility (GANDOLFI, 2000; EGHBALSAIED et al., 2013). A better understanding of the mechanism by which sperm cells uptake DNA molecules and the identification of factors that affect this process should receive more significant attention.

An alternative approach to use sperm cells for transgenesis is through intracytoplasmatic sperm injection (ICSI) into eggs (Figure 3A), as described in the mouse (PERRY et al., 1999; MOISYADI et al., 2009). Physical damage compromises sperm motility but facilitates transgene uptake by sperm cells (PERRY et al., 2001). Although efficient in mice and primates (KIMURA; YANAGIMACHI, 1995; PERRY et al., 1999), ICSI is inefficient and not

Figure 3. Application of assisted reproduction technologies (ART) to obtain genetically modified livestock. Efficiencies are outlined for most-widely used ARTs.



C: cattle. G: goat. M: mouse. S: Sheep. P: Pigs. Pronuclear injection efficiency calculated on transgenic animals per injected zygotes. Somatic cell nuclear transfer efficiency calculated on live animals by reconstructed oocytes. ES-cell derived germ-line chimeras was based on germ-line competent males based on those chimeras bred to non-transgenic controls.

replicable in livestock, since eggs are not readily activated by the process and ICSI embryos have low *in vivo* developmental potential (GARCÍA-ROSELLó et al., 2009; LóPEZ-SAUCEDO et al., 2012). Future developments on ICSI technology in livestock may motivate to revisit it on the future for germ-line modification.

Genetically modified livestock using egg-mediated methods

Another approach is the delivery of transgenes directly into oocytes or eggs (Figure 3A), as described in mouse and cattle systems (CHAN et al., 1998; PERRY et al., 2001; HOFMANN et al., 2004). Initially, retroviral vectors

were used to transfer transgenes to bovine oocytes at high efficiency (CHAN et al., 1998). Infection by retroviruses requires replicative cells, mainly using cells in metaphase, due to the absence of a nuclear envelope. This fact poses metaphase II (MII)-arrested eggs as an attractive cell type (CHAN et al., 1998; HOFMANN et al., 2004). In cattle, oocyte-mediated transgenesis using retrieval vectors was more efficient than pronuclear injection and perivitelline injection in zygotes (CHAN et al., 1998). However, transgene silencing was observed in newborn transgenic calves (CHAN et al., 1998).

The use of lentivirus allows more efficient transgene integration and stable activity in embryonic cells, thus circumventing silencing (HOFMANN et al., 2004). Oocyte-mediated transgenesis in mice permitted the introduction of larger transgenes (10-170 kilobases) or artificial chromosomes into resulting preimplantation embryos and offspring (PERRY et al., 2001). Due to construct size, larger transgenes were co-incubated with sperm heads and delivered into oocytes during ICSI (PERRY et al., 2001). For livestock, this method could now be revisited with the new molecular tools available, as described below.

Genetically modified livestock using somatic cell nuclear transfer

Animal cloning by somatic cell nuclear transfer (SCNT) gave another perspective on the production of genetically modified livestock (Figure 3B). Shortly after these initial reports on cloned mammals (WILMUT et al., 1997; WAKAYAMA et al., 1998; KATO et al., 1998; MOURA, 2012; KEEFER, 2015), several species were cloned using transgenic somatic cells (SCHNIEKE et al., 1997; CIBELLI et al., 1998; BONDIOLI et al., 2001). The exception to this rule was the first report on goat cloning, which already used transgenic donor cells (BAGUISI et al., 1999). A significant advantage of this approach was the possibility to modify primary cells genetically and select for transgenic cell clones before its use for SCNT (HOFMAN et al., 2004; LISAUSKAS et al., 2007). Therefore, cloned transgenic cattle are assured for transgene germ- line transmission (BORDIGNON et al., 2003).

There are two limiting factors on production of transgenic cattle using SCNT. Firstly, primary cultures of somatic cells have a limited replicative capacity (TOMINAGA et al., 2002), thus limiting their expansion and clonal selection (MCCREATH et al., 2000; DENNING et al., 2001). This limitation is often reduced by usage of fetal cells, but multiples rounds of genetic

modification would require recovery of cloned fetuses for SCNT (Figure 3B), in a process often called re-cloning (KUROIWA et al., 2004). Another limiting factor is the low efficiency of SCNT to produce viable offspring (WILMUT et al., 2002; KEEFER, 2015). Although some protocol modifications have increased its efficiency (LOI et al., 2016), more impactful improvements are still in demand to increase SCNT efficiency.

One alternative to increase SCNT efficiency would be to use less differentiated cells as donors, which are more amenable for cellular reprogramming (CIBELLI et al., 1998a; HOCHEDLINGER; JAENISCH, 2002). However, progenitor cells or adult stem cells are difficult to establish primary cultures (CHEN et al., 2015b) and pluripotent stem cells have not been described in livestock (EZASHI et al., 2016; SOTO; ROSS, 2016). Thus, the growing understanding of cellular reprogramming may lead to attractive strategies to improve SCNT efficiency (YAMANAKA; BLAU, 2010; JULLIEN et al., 2011; TAKAHASHI; YAMANAKA, 2015; KRAUSE et al., 2016; LOI et al., 2016).

Genetically modified livestock using pluripotent stem cells

The challenge that is presented by the low nuclear reprogramming efficiency during SCNT could be circumvented by methods using pluripotent cells (Figure 3C). The advent of embryonic stem (ES) cells revolutionized mouse genetics due to their feasibility to introduce exogenous DNA and ease targeted edition of the genome (GOSSLER et al., 1986; CAPECCHI, 1989, 2005). The ES-cell phenotype holds two hallmark biological features: the potential to form any cell type in the body and an unlimited proliferative capacity (EVANS; KAUFFMAN, 1981; MARTIN, 1981; WOBUS; BOHELER, 2005; BUEHR et al., 2008). The ES cell is functionally equivalent to inner cell mass cells of the blastocyst (NAGY et al., 1990), since their introduction into preimplantation embryos leads to ES-derived contribution to all mouse tissues, including the germ-line (BRADLEY et al., 1984; NAGY et al., 1990; SMITH, 2001).

These cellular features allow ES cells to be genetically modified by simples means, such as electroporation or lipofection when found in single cell suspensions (GOSSLER et al., 1986). Transgenic ES cell clones can be isolated and readily expanded by negative and-or positive selection (CAPECCHI

1989, 2005). Injection of transgenic ES cells into mouse blastocysts generates chimeric pups carrying transgenic and non-transgenic cells (GOSSLER et al., 1986; KOLLER et al., 1989). The mating of chimeric transgenic mice with their wild-type counterparts generate transgenic and non-transgenic progeny (GOSSLER et al., 1986). Moreover, injection of transgenic mouse ES cells into tetraploid preimplantation embryos leads to newborn mice fully-derived from ES cells, since tetraploid embryos contribute exclusively to the placenta (NAGY et al., 1990, 1993; EGGAN et al., 2002).

The establishment of livestock ES cells has not been described (EZASHI et al., 2016; SOTO; ROSS, 2016). Livestock ES-like cells are readily obtained (WHEELER, 1994; BEHBOODI et al., 2013). They show morphology of undifferentiated cells and are prone to spontaneous differentiation in embryos bodies or teratoma assays (WHEELER, 1994; CIBELLI et al., 1998b; SAITO et al., 2003; BEHBOODI et al., 2013). However, limited ES-derived tissue contribution *in vivo* has been described and no germ-line transmission for livestock species (WHEELER, 1994; CIBELLI et al., 1998b; SOTO; ROSS, 2016). Culture conditions used for human and mouse ES cells do not maintain livestock ES pluripotency. Thus identification of signaling pathways that contribute to their ES self-renewal is paramount (VERMA et al., 2013; EZASHI et al., 2016; SILVA et al., 2017).

Future research on the more efficient production of livestock tetraploid embryos is also advisable (HE et al., 2013; RAZZA et al., 2016), since raising ES-derived chimeric livestock for germ-line transmission is expected to be expensive, due to high maintenance costs and extended generation intervals required for germ-line transmission testing.

An possible attractive alternative to ES technology in livestock was the development of induced pluripotent stem (iPS) cells (TAKAHASHI; YAMANAKA, 2006; OKITA et al., 2007; WERNIG et al., 2007; OGOREVC et al., 2016; SOTO; ROSS, 2016; SILVA et al., 2017). The combined ectopic expression of different sets of pluripotency-associated genes triggers cellular reprogramming that coverts somatic cells into pluripotent counterparts (TAKAHASHI; YAMANAKA, 2006, 2015; KRAUSE et al., 2016). In cattle and other livestock, iPS cells show morphology, growth traits and *in vitro* differentiation potential resembling pluripotent cells (OGOREVC et al., 2016). However, these iPS cells are dependent on ectopic expression of

reprogramming factors, thus are unlikely to be fully pluripotent, are also expected to rely on yet unidentified self-renewal conditions, and their germ-line contribution also needs to be demonstrated (SOTO; ROSS, 2016; SILVA et al., 2017). Under such circumstances, ES-like can only be destined for SCNT (CIBELLI et al., 1998b), albeit at a not very encouraging efficiency.

THE THIRD PERIOD: NOVEL MOLECULAR TOOLS FOR GENOME EDITING

The third period is marked by some progress on construct delivery systems and the availability of highly efficient molecular tools for genome editing (Figure 2). Although the combination of the targeted locus by homologous recombination in somatic cells and its use for SCNT proved to be reliable (MCCREATH et al., 2000; DENNING et al., 2001), its low efficiency and labor-intensiveness limited its adoption. Therefore, the production of genetically-modified livestock, using gene-targeting remained as a costly technology. At this stage of its development, the two major limiting factors for efficient production of genetically modified livestock remained, namely construct delivery and its integration into the genome. Several efforts were devoted to meet such demands (WALL, 2002), and are described below (Table 2).

The first attempt to genetically increase construct delivery was made using retroviral vectors by co-incubation with cleavage-stage embryos and blastocysts (SQUIRE et al., 1989; KIM et al., 1993; HASKELL; BOWEN, 1995). This approach led to transgenic embryos and fetuses with multiple proviral integrations, suggestive of negligible mosaicism and possible germ-line contribution (HASKELL; BOWEN, 1995). However, retroviral vectors infect only mitotic cells and are subject to silencing in pluripotency cells (CHAN et al., 1998). The advent of lentiviral vectors improved transduction efficiency, including non-dividing cells and are functional in pluripotent cells (HOFMANN et al., 2003, 2004; PARK, 2007). The main disadvantages of such vectors are their limited construct size, an inability for genome editing and it's high transduction efficiency is limited to zygotes (PARK, 2007).

The primary shift brought in the third period came from the availability of designer nucleases (PETERSEN; RIEMANN, 2015; WANG, 2015). Three nuclease-based systems are now routinely used for livestock genome editing, namely zinc finger nucleases (ZFN), transcription activator-like effector

Table 2. — Association of construct delivery strategies and gene editing tools to obtain genetically modified livestock.

Cell Type	Method	Construct Delivery	Gene Editing Tool	Production of Mosaics	Germ-line Transmission	References
Sperm	AI, IVP	CI, VV	No	Yes	Low	Gandolfi, 2000; Lavitrano et al., 2006
Oocyte or Egg	IVP	VV	No	No	Moderate	Hofmann et al., 2004
Zygote	IVV, IVP	MI	No	Yes	Moderate	Eyestone, 1994; Wall, 2001
	IVV, IVP	PI, EP, VV	ZFN, CRISPR, TALEN	No	High	Petersen and Niemann, 2015 Sato et al., 2016
Somatic Cells[#]	SCNT	VV, MI, EP, LP	HR, ZFN, CRISPR, TALEN	No	High	Cibelli et al., 1998; Dai et al., 2002; Kuroiwa et a., 2002; Hofmann et al., 2004; Petersen and Niemann, 2015

AI: Artificial Insemination. CI: Co-Incubation. CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats. IVP: In Vitro Production of preimplantation embryos. EP: Electroporation. HR: Homologous Recombination. IVV: *In vivo*-produced embryos. LP: Liposomes. MI: Microinjection. PI: Pronuclear Injection. SCNT: Somatic cell Nuclear Transfer. TALEN: Transcription-Activator Like Endonucleases. VV: Viral Vectors. ZFN: Zinc Finger Nucleases.

nucleases (TALEN), and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) (WANG, 2015). These nucleases create a double-strand break (DSB) at the desired site in the genome, due to its DNA-binding and cleavage domains (PETERSEN; RIEMANN, 2015). Gene targeting by HR applies relatively long targeting vectors homologous to the gene to be targeted, while the cellular machinery performs the HR event (CAPECCHI, 1989, 2005; PETERSEN; RIEMANN, 2015). The HR approach allows both the generation of gene knock-outs and knock-ins, albeit at low efficiency (PETERSEN; RIEMANN, 2015).

The ZFN technology consists of DNA binding domain formed by at least two zinc finger motifs and a cleavage domain of the FokI endonuclease (WANG, 2015). The ZFN forms a DSB in the targeted site and the cleavage site is repaired by non-homologous end joining (NHEJ) or HR (PETERSEN; RIEMANN, 2015). All dominant livestock species have been subject to genome editing by ZFN (Figure 1), while more reports are expected to be described in the near future.

The transcription activator-like effector (TALE) consists of 33–35 amino-acid repeats with two polymorphisms at positions 12 and 13, which are coined as the repeat variable di-residue (RVD) (PETERSEN; RIEMANN, 2015). Each RVD binds specifically to one nucleotide of genomic DNA, conferring a code for protein-DNA interaction at a single base resolution. Different combinations of TALEs allow the targeted recognition of a single genomic site (WANG, 2015). A TALE can be linked to a FokI endonuclease to form a TALEN, thus allowing targeted edition of the genome (PETERSEN; RIEMANN, 2015; WANG, 2015). TALEN DSB sites are repairs by NHEJ or HR (PETERSEN; RIEMANN, 2015). Gene-edited livestock animals became available shortly after their ZFN-targeted counterparts (Figure 1).

The most recently developed designer nucleases was the clustered regularly interspaced short palindromic repeats / CRISPR-associated protein 9 (CRISPR/Cas9) system(PETERSEN; RIEMANN, 2015; WANG, 2015). This system has a single-guided RNA molecule for targeted sequence recognition and the CAS9 nuclease for DNA cleavage (PETERSEN; RIEMANN, 2015). Although it holds a similar efficiency to ZFN and TALEN systems, it is easier to design, requires less labor and is more cost-effective than previous methods (PETERSEN; RIEMANN, 2015; WANG, 2015). By this fact, gene-edited

livestock has been described in recent years with CRISPR/Cas9 (Figure 1), far more than the other systems, particularly in pigs.

These three designer nucleases may be used in somatic cells destined for SCNT, but more recently in zygotes (Table 2). This advantage may now circumvent the requirement of ART technologies (Figure 1) since in vivo-produced zygotes can be transformed by electroporation or possibly other relatively simple delivery systems (SATO et al., 2016). The production of genetically modified livestock now offers several unprecedented opportunities for basic research, and both agricultural and biomedical industries (Table 1). Several animal models (PERTERSEN; NIEMANN, 2015; SATO et al., 2016), particularly in pigs, became available in the recent years and more are expected to be designed in the foreseeable future. This experimental setup seems robust, but may be challenging for more sophisticated modifications of the genome and for several rounds of gene editing. These may be attractive research topics for the next few years.

CONCLUDING REMARKS

A historical outlook of the obtention of genetically modified livestock was provided by an arbitrary division into three distinct periods. Initial efforts to produce these animals were met with labor-intensive procedures to retrieve eggs or zygotes and to introduce transgenes into them. The development of ARTs, particularly IVP and SCNT, increased the availability of injectable zygotes or allowed more sophisticated editions of the genome, but their low efficiency somewhat counterbalanced their potential. The arrival of new molecular tools made genome edition in livestock a reality. Thus, their usage may circumvent the dependency on ARTs, which have not reached maturity for most livestock species, particularly in pigs. If this trend will stand the test of time, it’s a question that will be answered in the years to come.

REFERENCES

ANZAR, M.; BUHR, M.M. Spontaneous uptake of exogenous DNA by bull spermatozoa. **Theriogenology**, 65: 683-690, 2006.

BAGUISI, A.; BEHBOODI, E.; MELICAN, D.T.; POLLOCK, J.S.; DESTREMPES, M.M.; CAMMUSO, C.; WILLIAMS, J.L.; NIMS, S.D.; PORTER, C.A.; MIDURA, P.; PALACIOS, M.J.; AYRES, S.L.; DENNISTON, R.S.; HAYES, M.L.; ZIOMEK, C.A.; MEADE, H.M.; GODKE, R.A.; GAVIN, W.G.; OVERSTRÖM, E.W.; ECHELARD, Y. Production of goats by somatic cell nuclear transfer. **Nat Biotechnol**, 17: 456-461, 1999.

BALDASSARRE, H.; WANG, B.; KAFIDI, N.; GAUTHIER, M.; NEVEU, N.; LAPOINTE, J.; SNEEK, L.; LEDUC, M.; DUGUAY, F.; ZHOU, J.F.; LAZARIS, A.; KARATZAS, C.N. Production of transgenic goats by pronuclear microinjection of in vitro produced zygotes derived from oocytes recovered by laparoscopy. **Theriogenology**, 59: 831-839, 2003.

BALDASSARRE, H.; WANG, B.; PIERSON, J.; NEVEU, N.; SNEEK, L.; LAPOINTE, J.; COTE, F.; KAFIDI, N.; KEEFER, C.L.; LAZARIS, A.; KARATZAS, C.N. Prepubertal propagation of transgenic cloned goats by laparoscopic ovum pick-up and in vitro embryo production. **Cloning Stem Cells**, 6: 25-29, 2004.

BEHBOODI, E.; LAM, L.; GAVIN, W.G.; BONDAREVA, A.; DOBRINSKI, I. Goat embryonic stem-like cell derivation and characterization. **Methods Mol Biol.**, 1074: 51-67, 2013.

BEVACQUA, R.J.; FERNANDEZ-MARTÍN, R.; SAVY, V.; CANEL, N.G.; GISMONDI, M.I.; KUES, W.A.; CARLSON, D.F.; FAHRENKRUG, S.C.; NIEMANN, H.; TABOGA, O.A.; FERRARIS, S.; SALAMONE, D.F. Efficient edition of the bovine PRNP prion gene in somatic cells and IVF embryos using the CRISPR/Cas9 system. **Theriogenology**, 86: 1886-1896, 2016.

BLECK, G.T.; WHITE, B.R.; MILLER, D.J.; WHEELER, M.B. Production of bovine alpha-lactalbumin in the milk of transgenic pigs. **J Anim Sci.**, 76: 3072-3078, 1998.

BONDIOLI, K.; RAMSOONDAR, J.; WILLIAMS, B.; COSTA, C.; FODOR, W. Cloned pigs generated from cultured skin fibroblasts derived from a H-transferase transgenic boar. **Mol Reprod Dev.**, 60: 189-195, 2001.

BORDIGNON, V.; KEYSTON, R.; LAZARIS, A.; BILODEAU, A.S.; PONTES, J.H.; ARNOLD, D.; FECTEAU, G.; KEEFER, C.; SMITH, L.C. Transgene expression of green fluorescent protein and germ line transmission in cloned calves derived from in vitro-transfected somatic cells. **Biol. Reprod.**, 68: 2013-2023, 2003.

BRACKETT, B.G.; BARANSKA, W.; SAWICKI, W.; KOPROWSKI, H. Uptake of heterologous genome by mammalian spermatozoa and its transfer to ova through fertilization. **PNAS**, 68: 353-357, 1971.

BRADLEY, A.; EVANS, M.; KAUFMAN, M.H.; ROBERTSON, E. Formation of germ–line chimaeras from embryo–derived teratocarcinoma cell lines. **Nature**, 309: 255–256, 1984.

BRINSTER, R.L.; CHEN, H.Y.; TRUMBAUER, M.E.; YAGLE, M.K.; PALMITER, R.D. Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. **PNAS**, 82: 4438-4442, 1985.

BROPHY, B.; SMOLENSKI, G.; WHEELER, T.; WELLS, D.; L'HUILLIER, P.; LAIBLE, G. Cloned transgenic cattle produce milk with higher levels of beta-casein and kappa-casein. **Nat Biotechnol**, 21: 157-162, 2003.

BRUFORD, M.W.; BRADLEY, D.G.; LUIKART, G. DNA markers reveal the complexity of livestock domestication. **Nat Rev Genet**, 4: 900-910, 2003.

BUEHR, M.; MEEK, S.; BLAIR, K.; YANG, J.; URE, J.; SILVA, J.; MCLAY, R.; HALL, J.; YING, Q.L.; SMITH, A. Capture of authentic embryonic stem cells from rat blastocysts. **Cell**, 135: 1287-1298, 2008.

CAPECCHI, M.R. The new mouse genetics: altering the genome by gene targeting. **Trend Genet**, 5: 70-76, 1989.

CAPECCHI, M.R. Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. **Nat Rev Genet**, 6: 507-512, 2005.

CHAN, A.W.; HOMAN, E.J.; BALLOU, L.U.; BURNS, J.C.; BREMEL, R.D. Transgenic cattle produced by reverse-transcribed gene transfer in oocytes. **PNAS**, 95: 14028-14033, 1998.

CHEN, F.; WANG, Y.; YUAN, Y.; ZHANG, W.; REN, Z.; JIN, Y.; LIU, X.; XIONG, Q.; CHEN, Q.; ZHANG, M.; LI, X.; ZHAO, L.; LI, Z.; WU, Z.; ZHANG, Y.; HU, F.; HUANG, J.; LI, R.; DAI, Y. Generation of B cell-deficient pigs by highly efficient CRISPR/Cas9-mediated gene targeting. **J Genet Genomics**, 42: 437-444, 2015A.

CHEN, X.; YE, S.; YING, Q.L. Stem cell maintenance by manipulating signaling pathways: past, current and future. **BMB Rep**, 48: 668-676, 2015B.

CIBELLI, J.B.; STICE, S.L.; GOLUEKE, P.J.; KANE, J.J.; JERRY, J.; BLACKWELL, C.; PONCE DE LEóN, F.A.; ROBL, J.M. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. **Science**, 280: 1256-1258, 1998.

CIBELLI, J.B.; STICE, S.L.; GOLUEKE, P.J.; KANE, J.J.; JERRY, J.; BLACKWELL, C.; PONCE DE LEóN, F.A.; ROBL, J.M. Transgenic bovine chimeric offspring produced from somatic cell-derived stem-like cells. **Nat Biotech**, 16: 642-646, 1998.

CLARK, A.J. Generation of transgenic livestock by pronuclear injection. **Methods Mol Biol**, 180: 273-287, 2002.

COUSENS, C.; CARVER, A.S.; WILMUT, I.; COLMAN, A.; GARNER, I.; O'NEILL, G.T. Use of PCR-based methods for selection of integrated transgenes in preimplantation embryos. **Mol Reprod Dev**, 39: 384-391, 1994.

DAI, Y.; VAUGHT, T.D.; BOONE, J.; CHEN, S.H.; PHELPS, C.J.; BALL, S.; MONAHAN, J.A.; JOBST, P.M.; MCCREATH, K.J.; LAMBORN, A.E.; COWELL-LUCERO, J.L.; WELLS, K.D.; COLMAN, A.; POLEJAEVA, I.A.; AYARES, D.L. Targeted disruption of the alpha1,3-galactosyltransferase gene in cloned pigs. **Nat Biotechnol**, 20: 251-255, 2002.

DENNING, C.; DICKINSON, P.; BURL, S.; WYLIE, D.; FLETCHER, J.; CLARK, A.J. Gene targeting in primary fetal fibroblasts from sheep and pig. **Cloning and Stem Cells**, 3: 221-231, 2001.

EGGAN, K.; RODE, A.; JENTSCH, I.; SAMUEL, C.; HENNEK, T.; TINTRUP, H.; ZEVNIK, B.; ERWIN, J.; LORING, J.; JACKSON-GRUSBY, L.; SPEICHER, M.R.; KUEHN, R.; JAENISCH, R. Male and female mice derived from the same embryonic stem cell clone by tetraploid embryo complementation. **Nat Biotechnol**, 20: 455-459, 2002.

EGHBALSAIED, S.; GHAEDI, K.; LAIBLE, G.; HOSSEINI, S.M.; FOROUZANFAR, M.; HAJIAN, M.; OBACK, F.; NASR-ESFAHANI, M.H.; OBACK, B. Exposure to DNA is insufficient for in vitro transgenesis of live bovine sperm and embryos. **Reproduction**, 145: 97-108, 2003.

EVANS, M.J.; KAUFMAN, M.H. Establishment in culture of pluripotential cells from mouse embryos. **Nature**, 292: 154–156, 1981.

EYESTONE, W.H. Challenges and progress in the production of transgenic cattle. **Reprod Fert Dev**, 6: 647-652, 1994.

EYESTONE, W.H. Production and breeding of transgenic cattle using in vitro embryo production technology. **Theriogenology**, 51: 509-517, 1999.

EZASHI, T.; YUAN, Y.; ROBERTS, R.M. Pluripotent Stem Cells from Domesticated Mammals. **Ann Rev Anim Biosciences**, 4: 223-253, 2016.

FENG, W.; DAI, Y.; MOU, L.; COOPER, D.K.; SHI, D.; CAI, Z. The potential of the combination of CRISPR/Cas9 and pluripotent stem cells to provide human organs from chimaeric pigs. **Int J Mol Sci**, 16: 6545-6556, 2015.

FLISIKOWSKA, T.; KIND, A.; SCHNIEKE, A. Pigs as models of human cancers. **Theriogenology**, 86: 433-437, 2016.

FREITAS, V.J.F.; SEROVA, I.A.; MOURA, R.R.; ANDREEVA, L.E.; MELO, L.M.; TEIXEIRA, D.I.A.; PEREIRA, A.F.; LOPES-JR, E.S.; DIAS, L.P.B.; NUNES-PINHEIRO, D.C.S.; SOUSA, F.C.; ALCâNTARA-NETO, A.S.; ALBUQUERQUE, E.S.; MELO, C.H.S.; RODRIGUES, V.H.V.; BATISTA, R.I.T.P.; DVORYANCHIKOV, G.A.; SEROV, O.L. The establishment of two transgenic goat lines for mammary gland hG-CSF expression. **Small Ruminant Research**, 105: 105-113, 2012.

GANDOLFI, F. Sperm-mediated transgenesis. **Theriogenology**, 53: 127-137, 2000.

GARCÍA-ROSELLÓ, E.; GARCÍA-MENGUAL, E.; COY, P.; ALFONSO, J.; SILVESTRE, M.A. Intracytoplasmic sperm injection in livestock species: an update. **Reprod Domest Anim**, 44: 143-151, 2009.

GIANOLA, D.; ROSA, G.J. One hundred years of statistical developments in animal breeding. **Annu Rev Anim Biosci.**, 3: 19-56, 2015.

GIL, M.A.; CUELLO, C.; PARRILLA, I.; VAZQUEZ, J.M.; ROCA, J.; MARTINEZ, E.A. Advances in swine in vitro embryo production technologies. **Reprod Domest Anim**, 45 (Supplement 2): 40-48, 2010.

GORDON, J.W.; SCANGOS, G.A.; PLOTKIN, D.J.; BARBOSA, J.A.; RUDDLE, F.H. Genetic transformation of mouse embryos by microinjection of purified DNA. **PNAS**, 77: 7380-7384, 1980.

GOSSLER, A.; DOETSCHMAN, T.; KORN, R.; SERFLING, E.; KEMLER, R. Transgenesis by means of blastocyst-derived embryonic stem cell lines. **PNAS**, 83: 9065-9069, 1986.

HABERMANN, F.A.; WUENSCH, A.; SINOWATZ, F.; WOLF, E. Reporter genes for embryogenesis research in livestock species. **Theriogenology**, 68: S116-124, 2007.

HAMMER, R.E.; PURSEL, V.G.; REXROAD, C.E. JR; WALL, R.J.; BOLT, D.J.; EBERT, K.M.; PALMITER, R.D.; BRINSTER, R.L. Production of transgenic rabbits, sheep and pigs by microinjection. **Nature**, 315: 680-683, 1985.

HASKELL, R.E.; BOWEN, R.A. Efficient production of transgenic cattle by retroviral infection of early embryos. **Mol Reprod Dev**, 40: 386-390, 1995.

HAYES, B.J.; LEWIN, H.A.; GODDARD, M.E. The future of livestock breeding: genomic selection for efficiency, reduced emissions intensity, and adaptation. **Trends Genet**, 29: 206-214, 2013.

HE, W.; KONG, Q.; SHI, Y.; XIE, B.; JIAO, M.; HUANG, T.; GUO, S.; HU, K.; LIU, Z. Generation and developmental characteristics of porcine tetraploid embryos and tetraploid/diploid chimeric embryos. **Genomics Proteomics Bioinformatics**, 11: 327-333, 2013.

HENDERSON, C.R. Best linear unbiased estimation and prediction under a selection model. **Biometrics**, 31: 423-447, 1975.

HOCHEDLINGER, K.; JAENISCH, R. Nuclear transplantation: lessons from frogs and mice. **Curr Opin Cell Biol**, 14: 741-748, 2002.

HOFMANN, A.; KESSLER, B.; EWERLING, S.; WEPPERT, M.; VOGG, B.; LUDWIG, H.; STOJKOVIC, M.; BOELHAUVE, M.; BREM, G.; WOLF, E.; PFEIFER, A. Efficient transgenesis in farm animals by lentiviral vectors. **EMBO Reports**, 4: 1054-1060, 2003.

HOFMANN, A.; ZAKHARTCHENKO, V.; WEPPERT, M.; SEBALD, H.; WENIGERKIND, H.; BREM, G.; WOLF, E.; PFEIFER, A. Generation of transgenic cattle by lentiviral gene transfer into oocytes. **Biol Reprod**, 71: 405-409, 2004.

JAENISCH, R.; MINTZ, B. Simian virus 40 DNA sequences in DNA of healthy adult mice derived from preimplantation blastocysts injected with viral DNA. **PNAS**, 71: 1250-1254, 1974.

JäHNER, D.; STUHLMANN, H.; STEWART, C.L.; HARBERS, K.; LÖHLER, J.; SIMON, I.; JAENISCH, R. De novo methylation and expression of retroviral genomes during mouse embryogenesis. **Nature**, 298: 623-628, 1982.

JULLIEN, J.; PASQUE, V.; HALLEY-STOTT, R.P.; MIYAMOTO, K.; GURDON, J.B. Mechanisms of nuclear reprogramming by eggs and oocytes: a deterministic process? **Nat Rev Mol Cell Biol**, 12: 453-459, 2011.

KEEFER, C.L. Artificial cloning of domestic animals. **PNAS**, 112:8874-8878, 2015.

KIM, T.; LEIBFRIED-RUTLEDGE, M.L.; FIRST, N.L. Gene transfer in bovine blastocysts using replication-defective retroviral vectors packaged with Gibbon ape leukemia virus envelopes. **Mol Reprod Dev**, 35: 105-113, 1993.

KIM, G.A.; LEE, E.M.; JIN, J.X.; LEE, S.; TAWEECHAIPAISANKUL, A.; HWANG, J.I.; ALAM, Z.; AHN, C.; LEE, B.C. Generation of CMAHKO/GTKO/shTNFRI-Fc/HO-1 quadruple gene modified pigs. **Transgenic Res**, 26: 435-445, 2017.

KIMURA, Y.; YANAGIMACHI, R. Intracytoplasmic sperm injection in the mouse. **Biol Reprod**, 52: 709-720, 1995.

KOLLER, B.H.; HAGEMANN, L.J.; DOETSCHMAN, T.; HAGAMAN, J.R.; HUANG, S.; WILLIAMS, P.J.; FIRST, N.L.; MAEDA, N.; SMITHIES, O. Germ-line transmission of a planned alteration made in a hypoxanthine phosphoribosyltransferase gene by homologous recombination in embryonic stem cells. **PNAS**, 86: 8927-8931, 1989.

KRAUSE, M.N.; SANCHO-MARTINEZ, I.; IZPISUA BELMONTE, J.C. Understanding the molecular mechanisms of reprogramming. **Biochemical and Biophysical Research Communications**, 473: 693-697, 2016.

KUROIWA, Y.; KASINATHAN, P.; CHOI, Y.J.; NAEEM, R.; TOMIZUKA, K.; SULLIVAN, E.J.; KNOTT, J.G.; DUTEAU, A.; GOLDSBY, R.A.; OSBORNE, B.A.; ISHIDA, I.; ROBL, J.M. Cloned transchromosomic calves producing human immunoglobulin. **Nat Biotech**, 20: 889-894, 2002.

KUROIWA, Y.; KASINATHAN, P.; MATSUSHITA, H.; SATHIYASELAN, J.; SULLIVAN, E.J.; KAKITANI, M.; TOMIZUKA, K.; ISHIDA, I.; ROBL, J.M. Sequential targeting of the genes encoding immunoglobulin-mu and prion protein in cattle. **Nat Genet**, 36: 775-780, 2004.

LAI, L.; KOLBER-SIMONDS, D.; PARK, K.W.; CHEONG, H.T.; GREENSTEIN, J.L.; IM, G.S.; SAMUEL, M.; BONK, A.; RIEKE, A.; DAY, B.N.; MURPHY, C.N.; CARTER, D.B.; HAWLEY, R.J.; PRATHER, R.S. Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. **Science**, 295: 1089-1092, 2002.

LARSON, G.; BURGER, J. A population genetics view of animal domestication. **Trends Genet**, 29: 197-205, 2013.

LAVITRANO, M.; CAMAIONI, A.; FAZIO, V.M.; DOLCI, S.; FARACE, M.G.; SPADAFORA, C. **Cell**, 57: 717-723, 1989.

LAVITRANO, M.; BUSNELLI, M.; CERRITO, M.G.; GIOVANNONI, R.; MANZINI, S.; VARGIOLU, A. Sperm-mediated gene transfer. **Reprod Fertil Dev**, 18: 19-23, 2006.

LISAUSKAS, S.F.; RECH, E.L.; ARAGÃO, F.J. Characterization of transgene integration loci in transformed Madin Darby bovine kidney cells. **Cloning Stem Cells**, 9: 456-460, 2007.

LOI, P.; IUSO, D.; CZERNIK, M.; OGURA, A. A New, Dynamic Era for Somatic Cell Nuclear Transfer? **Trends Biotech**, 34: 791-797, 2016.

LONERGAN, P.; FAIR, T. Maturation of Oocytes in Vitro. **Annu Rev Anim Biosci**, 4: 255-268, 2016.

LóPEZ-SAUCEDO, J.; PARAMIO-NIETO, M.T.; FIERRO, R.; PIñA-AGUILAR, R.E. Intracytoplasmic sperm injection (ICSI) in small ruminants. **Anim Reprod Sci**, 133: 129-138, 2012.

LOTTI, S.N.; POLKOFF, K.M.; RUBESSA, M.; WHEELER, M.B. Modification of the Genome of Domestic Animals. **Anim Biotechnol**, 28: 198-210, 2017.

LUSH, J.L.The impact of genetics on animal breeding. **J Anim Sci**, 10: 311-321, 1951.

MARTIN, G.R. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. **PNAS**, 78: 7634-7638, 1981.

MCCREATH, K.J.; HOWCROFT, J.; CAMPBELL, K.H.; COLMAN, A.; SCHNIEKE, A.E.; KIND, A.J. Production of gene-targeted sheep by nuclear transfer from cultured somatic cells. **Nature**, 405: 1066-1069, 2000.

MELO, E.O.; CANAVESSI, A.M.; FRANCO, M.M.; RUMPF, R. Animal transgenesis: state of the art and applications. **J Appl Genet**, 48: 47-61, 2007.

MEUWISSEN, T.H.; HAYES, B.J.; GODDARD, M.E. Prediction of total genetic value using genome-wide dense marker maps. **Genetics**, 157: 1819-1829, 2001.

MOGHADDASSI, S.; EYESTONE, W.; BISHOP, C.E. TALEN-mediated modification of the bovine genome for large-scale production of human serum albumin. **PLoS One**, 9: e89631, 2014.

MOISYADI, S.; KAMINSKI, J.M.; YANAGIMACHI, R. Use of intracytoplasmic sperm injection (ICSI) to generate transgenic animals. **Comp Immunol Microbiol Infect Dis**, 32: 47-60, 2009.

MOURA, M.T. Pluripotency and cellular reprogramming. **Anais da Academia Pernambucana de Ciência Agronômica**, 8: 138-168, 2012.

NAGY, A.; GóCZA, E.; DIAZ, E.M.; PRIDEAUX, V.R.; IVÁNYI, E.; MARKKULA, M.; ROSSANT, J. Embryonic stem cells alone are able to support fetal development in the mouse. **Development**, 110: 815-821, 1990.

NAGY, A.; ROSSANT, J.; NAGY, R.; ABRAMOW-NEWERLY, W.; RODER, J.C. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. **PNAS**, 90: 8424-8428, 1993.

NI, W.; QIAO, J.; HU, S.; ZHAO, X.; REGOUSKI, M.; YANG, M.; POLEJAEVA, I.A.; CHEN, C. Efficient gene knockout in goats using CRISPR/Cas9 system. **PLoS One**, 9: e106718, 2014.

NIEMANN, H.; KUES, W.A. Application of transgenesis in livestock for agriculture and biomedicine. **Anim Reprod Sci**, 79: 291-317, 2003.

NIU, Y.; LIANG, S. Progress in gene transfer by germ cells in mammals. **J Genet Genomics**, 35: 701-714, 2008.

OGOREVC, J.; OREHEK, S.; DOVč, P. Cellular reprogramming in farm animals: an overview of iPSC generation in the mammalian farm animal species. **J Anim Sci Biotech**, 7: 10, 2016.

OKITA, K.; ICHISAKA, T.; YAMANAKA, S. Generation of germline–competent induced pluripotent stem cells. **Nature**, 448: 313-317, 2007.

PARAMIO, M.T.; IZQUIERDO, D. Current status of in vitro embryo production in sheep and goats. **Reprod Domest Anim**, 49 (Supplement 4): 37-48, 2014.

PARK, F. Lentiviral vectors: are they the future of animal transgenesis? **Physiol Genomics**., 31: 159-73, 2007.

PEREYRA-BONNET, F.; FERNÁNDEZ-MARTÍN, R.; OLIVERA, R.; JARAZO, J.; VICHERA, G.; GIBBONS, A.; SALAMONE, D. A unique method to produce transgenic embryos in ovine, porcine, feline, bovine and equine species. **Reprod Fert Dev**, 20: 741-749, 2008.

PERRY, A.C.; WAKAYAMA, T.; KISHIKAWA, H.; KASAI, T.; OKABE, M.; TOYODA, Y.; YANAGIMACHI, R. Mammalian transgenesis by intracytoplasmic sperm injection. **Science**, 284: 1180-1183, 1999.

PERRY, A.C.; ROTHMAN, A.; DE LAS HERAS, J.I.; FEINSTEIN, P.; MOMBAERTS, P.; COOKE, H.J.; WAKAYAMA, T. Efficient metaphase II transgenesis with different transgene archetypes. **Nat Biotechnol**, 19: 1071-1073, 2001.

PETERSEN, B.; NIEMANN, H. Molecular scissors and their application in genetically modified farm animals. **Transgenic Res**, 24: 381-396, 2015.

PHELPS, C.J.; KOIKE, C.; VAUGHT, T.D.; BOONE, J.; WELLS, K.D.; CHEN, S.H.; BALL, S.; SPECHT, S.M.; POLEJAEVA, I.A.; MONAHAN, J.A.; JOBST, P.M.; SHARMA, S.B.; LAMBORN, A.E.; GARST, A.S.; MOORE, M.; DEMETRIS, A.J.; RUDERT, W.A.; BOTTINO, R.; BERTERA, S.; TRUCCO, M.; STARZL, T.E.; DAI, Y.; AYARES, D.L. Production of alpha 1,3-galactosyltransferase-deficient pigs. **Science**, 299: 411-414, 2003.

POLEJAEVA, I.A.; RUTIGLIANO, H.M.; WELLS, K.D. Livestock in biomedical research: history, current status and future prospective. **Reprod Fertil Dev**, 28: 112-124, 2016.

RAZZA, E.M.; SATRAPA, R.A.; EMANUELLI, I.P.; BARROS, C.M.; NOGUEIRA, M.F. Screening of biotechnical parameters for production of bovine inter-subspecies embryonic chimeras by the aggregation of tetraploid Bos indicus and diploid crossbred Bos taurus embryos. **Reprod Biol**, 16: 34-40, 2016.

ROGERS, C.S. Genetically engineered livestock for biomedical models. **Transgenic Res**, 25: 345-359, 2016.

SAITO, S.; SAWAI, K.; UGAI, H.; MORIYASU, S.; MINAMIHASHI, A.; YAMAMOTO, Y.; HIRAYAMA, H.; KAGEYAMA, S.; PAN, J.; MURATA, T.; KOBAYASHI, Y.; OBATA, Y.; YOKOYAMA, K.K. Generation of cloned calves and transgenic chimeric embryos from bovine embryonic stem-like cells. **Biochem Biophys Res Commun**, 309: 104-113, 2003.

SATO, M.; OHTSUKA, M.; WATANABE, S.; GURUMURTHY, C.B. Nucleic acids delivery methods for genome editing in zygotes and embryos: the old, the new, and the old-new. **Biol Direct**, 11: 16, 2016.

SCHNIEKE, A.E.; KIND, A.J.; RITCHIE, W.A.; MYCOCK, K.; SCOTT, A.R.; RITCHIE, M.; WILMUT, I.; COLMAN, A.; CAMPBELL, K.H. Human factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. **Science**, 278: 2130-2133, 1997.

SILVA, P.G.C.; MOURA, M.T.; BRAGA, V.A.A.; FERREIRA-SILVA, J.C.; NASCIMENTO, P.S.; CANTANHêDE, L.F.; CHAVES, M.S.; OLIVEIRA, M.A.L. Atividade dos genes relacionados à pluripotência em ovinos. **Med Vet** (UFRPE), v, 2017.

SMITH, K.R. Sperm cell mediated transgenesis: a review. **Anim Biotech**, 10: 1-13, 1999.

SMITH, A.G. Embryo–derived stem cells: of mice and men. **Annu Rev Cell Dev Biol**, 17: 435–462, 2001.

SOTO, D.A.; ROSS, P.J. Pluripotent stem cells and livestock genetic engineering. **Transgenic Res**, 25: 289-306, 2016.

SQUIRE, K.R.; EMBRETSON, J.E.; FIRST, N.L. In vitro testing of a potential retroviral vector for producing transgenic livestock. **Am J Vet Res**, 50: 1423-1427, 1989.

TAKAHASHI, K.; YAMANAKA, S. A developmental framework for induced pluripotency. **Development**, 142: 3274-3285, 2015.

TAN, W.; CARLSON, D.F.; LANCTO, C.A.; GARBE, J.R.; WEBSTER, D.A.; HACKETT, P.B.; FAHRENKRUG, S.C. Efficient nonmeiotic allele introgression in livestock using custom endonucleases. **PNAS**, 110: 16526-16531, 2013.

TANG, L.; GONZÁLEZ, R.; DOBRINSKI, I. Germline modification of domestic animals. **Anim Reprod**, 12: 93-104, 2015.

TANIHARA, F.; TAKEMOTO, T.; KITAGAWA, E.; RAO, S.; DO, L.T.; ONISHI, A.; YAMASHITA, Y.; KOSUGI, C.; SUZUKI, H.; SEMBON, S.; SUZUKI, S.; NAKAI, M.; HASHIMOTO, M.; YASUE, A.; MATSUHISA, M.; NOJI, S.; FUJIMURA, T.; FUCHIMOTO, D.; OTOI, T. Somatic cell reprogramming-free generation of genetically modified pigs. **Sci Adv**, 2: e1600803, 2016.

TOMINAGA, K.; OLGUN, A.; SMITH, J.R.; PEREIRA-SMITH, O.M. Genetics of cellular senescence. **Mech Ageing Dev**, 123: 927-936, 2002.

VERMA, V.; HUANG, B.; KALLINGAPPA, P.K.; OBACK, B. Dual kinase inhibition promotes pluripotency in finite bovine embryonic cell lines. **Stem Cells Dev**, 22: 1728-1742, 2013.

WALL, R.J. Pronuclear microinjection. **Cloning Stem Cells**, 3: 209-220, 2001.

WALL, R.J. New gene transfer methods. **Theriogenology**, 57: 189-201, 2002.

WALL, R.J. Transgenic livestock: progress and prospects for the future. **Theriogenology**, 45: 57-68, 1996.

WANG, G.D.; XIE, H.B.; PENG, M.S.; IRWIN, D.; ZHANG, Y.P. Domestication genomics: evidence from animals. **Annu Rev Anim Biosci**, 2: 65-84, 2014.

WANG, Z. Genome engineering in cattle: recent technological advancements. **Chromosome Res.**, 23: 17-29, 2015.

WEISS, E.H.; LILIENFELD, B.G.; MÜLLER, S.; MÜLLER, E.; HERBACH, N.; KESSLER, B.; WANKE, R.; SCHWINZER, R.; SEEBACH, J.D.; WOLF, E.; BREM, G. HLA-E/human beta2-microglobulin transgenic pigs: protection against xenogeneic human anti-pig natural killer cell cytotoxicity. **Transplantation**, 87: 35-43, 2009.

WERNIG, M.; MEISSNER, A.; FOREMAN, R.; BRAMBRINK, T.; KU, M.; HOCHEDLINGER, K.; BERNSTEIN, B.E.; JAENISCH R. *In vitro* reprogramming of broblasts into a pluripotent ES–cell–like state. **Nature**, 448: 318-324, 2007.

WHEELER, M.B. Development and validation of swine embryonic stem cells: a review. **Reprod Fertil Dev**, 6: 563-568, 1994.

WIENER, P.; WILKINSON, S. Deciphering the genetic basis of animal domestication. **Proc Biol Sci**, 278: 3161-3170.,2011.

WOBUS, A.M.; BOHELER, K.R. Embryonic stem cells: prospects for developmental biology and cell therapy. **Phys Rev**, 85: 635-678, 2005.

WU, H.; WANG, Y.; ZHANG, Y.; YANG, M.; LV, J.; LIU, J.; ZHANG, Y. TALE nickase-mediated SP110 knockin endows cattle with increased resistance to tuberculosis. **PNAS**, 112: E1530-1539, 2015.

WU, J.; PLATERO-LUENGO, A.; SAKURAI, M.; SUGAWARA, A.; GIL, M.A.; YAMAUCHI, T.; SUZUKI, K.; BOGLIOTTI, Y.S.; CUELLO, C.; MORALES VALENCIA, M.; OKUMURA, D.; LUO, J.; VILARIñO, M.; PARRILLA, I.; SOTO, D.A.; MARTINEZ, C.A.; HISHIDA, T.; SÁNCHEZ-BAUTISTA, S.; MARTINEZ-MARTINEZ, M.L.; WANG, H.; NOHALEZ, A.; AIZAWA, E.; MARTINEZ-REDONDO, P.; OCAMPO, A.; REDDY, P.; ROCA, J.; MAGA, E.A.; ESTEBAN, C.R.; BERGGREN, W.T.; NUñEZ DELICADO, E.; LAJARA, J.; GUILLEN, I.; GUILLEN, P.; CAMPISTOL, J.M.; MARTINEZ, E.A.; ROSS, P.J.; IZPISUA BELMONTE, J.C. Interspecies Chimerism with Mammalian Pluripotent Stem Cells. **Cell**, 168: 473-486, 2017.

YAMANAKA, S.; BLAU, H.M. Nuclear reprogramming to a pluripotent state by three approaches. **Nature**, 465: 704-712, 2010.

ZHOU, Z.R.; ZHONG, B.S.; JIA, R.X.; WAN, Y.J.; ZHANG, Y.L.; FAN, Y.X.; WANG, L.Z.; YOU, J.H.; WANG, Z.Y.; WANG, F. Production of myostatin-targeted goat by nuclear transfer from cultured adult somatic cells. **Theriogenology**, 79: 225-233, 2013.