REVIEW

Perspectives of pluripotent stem cells in livestock

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Abstract

The recent progress in derivation of pluripotent stem cells (PSCs) from farm animals opens new approaches not only for reproduction, genetic engineering, treatment and conservation of these species, but also for screening novel drugs for their efficacy and toxicity, and modelling of human diseases. Initial attempts to derive PSCs from the inner cell mass of blastocyst stages in farm animals were largely unsuccessful as either the cells survived for only a few passages, or lost their cellular potency; indicating that the protocols which allowed the derivation of murine or human embryonic stem (ES) cells were not sufficient to support the maintenance of ES cells from farm animals. This scenario changed by the innovation of induced pluripotency and by the development of the 3 inhibitor culture conditions to support naïve pluripotency in ES cells from livestock species. However, the long-term culture of livestock PSCs while maintaining the full pluripotency is still challenging, and requires further refinements. Here, we review the current achievements in the derivation of PSCs from farm animals, and discuss the potential application areas.

Key Words: Livestock; Cellular reprogramming; Chimera; Cell-therapy; Ontogenesis; Pluripotency

Core Tip: The successful derivation of pluripotent stem cells (PSCs) from livestock represents an ideal model for the progress of veterinary, biomedical and regenerative medicine. The inherent properties of self-renewal and differentiation make PSCs an ideal raw biomaterial for innovative approaches in artificial reproductive techniques, cell-based therapy, disease modelling, drug testing, organ generation, breed conservation and *in vitro* meat production. In this review, we present the current status of PSCs application for the development of livestock farming and their potential applications for human welfare.

INTRODUCTION

Pluripotent stem cells (PSCs) have the capability to self-renew and to develop into the three primary germ cell layers and therefore can form all cells and tissues of the adult body. There are two sources for obtaining PSCs, embryonic stem (ES) cells developed from an embryo, and induced pluripotent stem (iPS) cells derived *via* reprogramming of somatic cells (Figure 1). The process of fertilization, parthenogenetic activation, or nuclear transfer (NT), can lead to zygote formation followed by rapid cleavage divisions, which eventually results in the blastocyst stage with two different cell compartments, the outer trophectoderm and the inner cell mass (ICM). After zygote formation, the embryo undergoes several genetic and epigenetic changes, such as DNA de-methylation and re-methylation, replacement of protamines to histones, telomere extension, histone reprogramming, and first activation of the embryonic genome[1]. The resulting ICM cells in the blastocyst have a transient cellular pluripotency and will later form the embryo proper, and thus are able to develop into all somatic cells of an organism. The first successful derivation of cell cultures from the ICM, which maintain these pluripotent properties *in vitro*, was described in 1981 with murine cells, which were termed ES cells[2,3]. Under specific *in vitro* conditions, such as the culture on feeder cell layers, the pluripotent status of ES cells becomes locked in the Petri dish. The ES cells showed an unlimited proliferative capacity, were able to be maintained in an undifferentiated state of potency (naïve pluripotency), and could be triggered to differentiate into any cell type. Consequently, ES cells developed into an important arsenal for developmental biology, and new reproduction approaches, such as blastocyst complementation assays and generation of cell chimeric animals, or *in vitro* differentiation of desired cell types, including gametes[4,5].

However, translation of the protocols for the derivation of ES cells to livestock species is painfully slow. Almost a decade later in 1990, putative ES cells from the early stages of embryos were reported in domestic livestock species such as sheep, pig and cattle; however, these cells could be maintained only for a few passages[6,7]. Later, ES cell-like lines have been derived from many species of livestock such as pig[8,9], cattle[10-13], sheep[14,15], goat[16,17], horse[18], and buffalo[19,20]; however, detailed characterizations suggested that these putative ES cell cultures seem to be in a primed status of cellular potency.

NT describes the transplantation of a somatic cell or nucleus in an enucleated oocyte, subsequently, the re-constructed zygote is activated and cultured up to the blastocyst stage. This requires successful reprogramming of the donor nucleus by factors accumulated in the cytoplasm of the recipient oocyte. The NT-derived blastocyst can then be used to derive ES cells from the ICM ('therapeutic cloning')[21]. NT-ES cell lines have been established in mice[22-24], cattle[21], buffalo[25] and non-human primates[26]. In livestock, NT-ES cells could be derived from genomically selected high value animals with potential use in reproductive cloning or for conservation using cryopreservation of these cell lines[27].

Alternatively, parthenogenetically derived embryos are equally valuable for the generation of ES cells. The first parthenogenetic embryos derived ES (pES) cell lines were established from mice[28]. Thereafter, it was established in other farm animals such as in pig[29], horse[18], sheep[14], cattle and buffalo[30-33]. Muzaffar *et al*[32] successfully
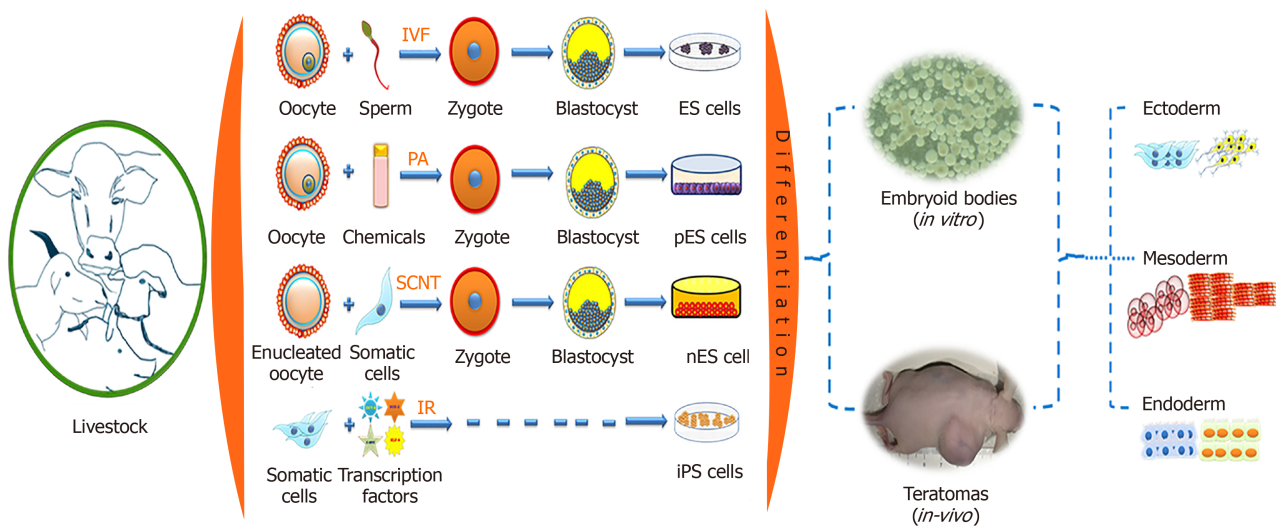
Figure 1 Derivation of pluripotent stem cells from livestock and their differentiation properties. IVF: *In vitro* fertilization; ES Cells: Embryonic stem cells; PA: Parthenogenetic activation; pES Cells: Parthenogenetically derived embryonic stem cells; SCNT: Somatic cell nuclear transfer; nES Cells: Nuclear transfer derived embryonic stem cells; IR: Induced reprogramming; iPS cells: Induced pluripotent stem cells.

established buffalo ES cell lines from blastocysts derived from *in vitro* fertilization, parthenogenesis, and NT. These results suggested that the cell line generated from parthenogenetically derived embryos maintained the ES cell properties and could be used as a model to study the effects of imprinting. However, isolation and characterization of ES cells from livestock species is technically still challenging, as the derived lines showed variable expression patterns of pluripotency markers[34] and may undergo spontaneous differentiation; limited or failed contribution of the transferred ES cells to a chimeric organism in blastocyst complementation assays suggested a limited cellular potency[11,35,36].

In 2008, the 3 inhibitor (3i) approach resulted in the first isolation of authentic rat ES cells[37]. Basically, the 3i approach is an ES culture medium supplemented with three inhibitors of metabolic pathways, which interact with cellular potency: CHIR99021 (GSK3 kinase inhibitor), PD184352 (ERK 1-2 kinases inhibitor), and SU5402 [fibroblast growth factor (FGF) tyrosine kinase receptor inhibitor]. The application of this strategy may allow the generation of genuine PSCs from livestock.

A recent approach is the reprogramming of somatic cells to iPS cells by forced expression of a set of key reprogramming factors such as octamer-binding transcription factor 4 (Oct4), Nanog, MYC proto-oncogene (c-Myc), Kruppel-like factor 4 (Klf4), *etc*[38-41]. Similar to ES cells, iPS cells are characterized by their self-renewal ability, morphological resemblance, expression of stemness gene, epigenetic state, and their differentiation potential toward all somatic cell types including the germ-line. This technique has been swiftly and widely adopted in farm animal species such as pig[42-47], sheep[48-50], goat[51,52], dog[53-56], cattle[57,58], and horse[59-64]. At present, bona fide ES cells from livestock are not yet available, but long-term stable iPS cells have been generated from many species and allow the further optimization of culture conditions.

The majority of generated livestock iPS cells showed classical features of pluripotency, such as *in vivo* differentiation and teratoma formation. It has been claimed that porcine iPS cells can contribute to chimera formation[45]. Similarly, ovine iPS cells also formed chimeric lambs after aggregation with early embryonic stages[65-67]; however, the efficiency of chimera contribution warrants further studies. Nevertheless, the results represent advancements in iPS cell technology and promoted the molecular understanding of livestock pluripotency.

PSCs can also be derived from germline stem cells, a class of unipotent stem cells which reside in testis tissue. Observations made by several researchers that neonatal and adult mouse testis contains spermatogonial stem cells (SSCs) or male germline stem cells, which are phenotypically similar to ES cells, are capable of differentiating into three embryonic germ lineages *in vitro*, form teratomas, and showed germline contribution and transmission[68-71], suggested that the SSCs may retain the ability to generate PSCs. The PSCs derived from these approaches are a useful tool for examining the molecular mechanisms of pluripotency in the male germline. Potentially, the SSCs will be useful for cell-based therapies which benefit males.

Similar to SSCs, female germline stem cells (FGSCs) have been proposed to reside in the ovary. Wang *et al*[72] derived FGSCs from ovaries, which exhibited properties similar to those of ES cells in terms of stemness gene expression and differentiation potential. These novel approaches provide new opportunities to study germ cell biology and opens the possibility of using these cells for genetic diseases in various cell lineages and provides a foundation for personalized regenerative applications. Recent research directed at producing artificial germ cells from stem cells of non-germ line cells may offer the possibility of treating infertility in the future[73].

PSCs derived from ES cells, iPS cells or the germ cell lineage may have great potential in veterinary medicine, cell-based therapies, production of pharmaceutical molecules *via* transgenic animals, multiplication of elite animals, conservation of endangered animals and as model animals for effective biomedical applications[74-80]. Previously, several review articles on livestock iPS cells have been published which described methods of cellular reprograming and their potential applications[78-83]. Here, we provide the most current achievements in PSCs from livestock, and discuss the potential applications of PSCs for the development of livestock farming and their potential applications for human welfare.

CURRENT STATE OF ES CELLS FROM LIVESTOCK

In general, putative livestock ES cells have been derived from early embryonic stages applying either standard culture systems developed for the culture of murine or human ES cells. However, most livestock ES cells did not maintain robust self-renewal and typically failed to perform in teratoma and blastocyst complementation assays[84,85]. This may be due to differences in the ontogenesis of cell lineage formation between rodent and livestock species. In rodent embryonic development, the first cellular differentiation is initiated at the late morula stage where the outer cells develop into an epithelial structure, followed by blastocoel formation. This leads to the development of two cell lineages, the trophectoderm (TE) and ICM[86]. The ICM differentiates further into the epiblast and the primitive endoderm or hypoblast. The epiblast, hypoblast and TE are common in all mammalian blastocysts, but the timing since fertilization in livestock embryos is relatively delayed compared to the mouse[34,87-89].

In porcine and bovine embryos, the hypoblast cells form on 7/8 d post-fertilization, and the epiblast forms on day 12[90,91]. Therefore, the time points used to isolate ES cells are not equivalent between livestock and rodent counterparts[92]. Also differences in the molecular pathways that control pluripotency between murine and domestic animals have been elucidated[93-95].

The current concept of different pluripotency states, which are termed naïve and primed, is likely to provide new approaches for the derivation of livestock ES cells. A steady progress has been made toward optimizing culture conditions for the derivation of stable and highly potent porcine ES cells[8,96]. More recently, porcine ES cells have been claimed to give rise to the chimeric contribution using a modified medium supplemented with basic fibroblast growth factor and leukemia inhibitory factor; however, follow-up studies with respect to contribution to the germline and formation of functional gametes are warranted[97,98]. Similarly, canine ES cells were derived; these canine ES cells expressed all the pluripotent markers, showed long-term self-renewal, and formed teratomas[99]. Thus, these cell lines exhibited most hallmarks of genuine ES cells, which represent a step toward pre-clinical therapies in large animal models.

However, these efforts on establishing livestock ES cells have not been turned into bona fide ES cell lines that are competent in germ line transmission. Multifactorial reasons may contribute to the lack of success for this relevant aim. First, significant differences in the initial embryonic development in livestock from that in rodents, second, the established pluripotency markers may be less distinct for livestock ES cells, and third, the pluripotency states, naïve *vs* primed, are pretty poorly defined in livestock species[84,100]. Several reviews have been published on the topic of ES cells in livestock covering isolation stages, culture condition, differentiation, proliferation properties and characterization[85,89,92,101-103]. These reviews summarized our knowledge on livestock ES cells. Table 1 summarizes some of the most recent observations that have been reported for ES cells from livestock.

Table 1 Most recent examples of embryonic stem cells successfully generated from livestock

Species	Embryonic stage	Culture medium and condition	Expression of pluripotency markers	Long-term culture (passage)	Karyotype	*In vitro* differentiation to EBs	*In vivo* differentiation to teratoma	Germ line transmission	Ref.
Cattle	CDX2-KD blastocysts	KO-DMEM, 2 mM glutamine, 1% MEM-NEAAs, 20 ng/mL hrFGF, 20 ng/mL hrLIF, 0.1 mM β–mercaptoethanol, 15% FBS + MEF under 37°C, 5% CO_2	Yes	37	Normal	Yes	Yes	No	[272]
	Blastocysts	CTFR medium contains low fatty acid BSA, 20 ng/mL hFGF2, 2.5 μM IWR1 + MEFs under 37°C, 5% CO_2	Yes	> 70	Normal	No	Yes	No	[145]
Buffalo	Blastocysts	KO-DMEM, 15% KSR, 2 mM L-glutamine, 50 μg/mL gentamicin sulfate, 1% MEM-NEAAs, 0.1 mM β-mercaptoethanol, 1000 IU/mL mLIF, 5 ng/mL FGF2 + BFF under 37°C, 5% CO_2	Yes	135	Normal	Yes	No	No	[20]
	Blastocysts	DMEM, 20% FBS, 2 mM L-glutamine, 0.1 mM β-mercaptoethanol, 2% NEAA, 1% ITS, 50 μg/mL gentamycin sulfate, 30 ng/mL LIF, 40 ng/mL bFGF + BFF under 38.5°C, 5% CO_2	Yes	15	Normal	No	Yes	No	[273]
Ovine	Blastocysts	DMEM high glucose, 2 mM L-glutamine, 1 mM Na-Pyruvate, 0.1 mM β-mercaptoethanol, 0.1 mM NEAAs, 10 ng/mL LIF, 20 mg/mL insulin, 1000 IU/mL penicillin, 10 mg/mL streptomycin + STO under 38.5°C, 5% CO_2	Yes	No	Normal	Yes	No	No	[274]
	Blastocysts	DMEM/F12 supplemented with N2, B27, GSK3 inhibitor (CHIR99021), rhbFGF + OEF or MEF under 38.5°C, 5% CO_2	Yes	30	No	Yes	Yes	No	[275]
Caprine	Blastocysts	DMEM, 20% FCS, 1000 IU/mL mLIF, 1% NEAAs 0.1 mM β-mercaptoethanol, 2 mM l-glutamine + GFF under 38.5°C, 5% CO_2	Yes	15	Normal	Yes	No	No	[17]
	Blastocysts	DMEM, 0.1 mM 2-mercaptoethanol, 0.1 mM MEM-NEAAs, 2 mM L-glutamine, 10% FBS, 1000 U/mL hLIF + GFF under 37°C, 5% CO_2	Yes	120	Normal	Yes	Yes	No	[16]
Porcine	Blastocysts	1:1 ratio of 1. α-MEM medium supplemented with 10% KSR, 0.05 mM β-mercaptoethanol, 1% NEAAs, 1% antibiotic-antimycotic, 4 ng/mL EGF, 10 μL/mL 100 × ITS, 1000 U/mL mLIF, 2 ng/mL bFGF and, 2. DMEM/F-10-based medium supplemented with 15% heat-inactivated FBS, 0.2 mM β-mercaptoethanol, 1% NEAA, 1% antibiotic–antimycotic and 2 ng/mL bFGF + MEFs under 37°C, 5% CO_2	Yes	19	Normal	Yes	No	No	[276]
	Blastocysts	α-MEM, 20% KSR, 20 ng/mL bFGF, 20 ng/mL EGF, 10 ng/mL Activin-a, 1% ITS, 1 mM MEM-NEAAs, 55 μM β2-mercaptoethanol + STO at 38.5°C, 5% CO_2	Yes	21	Normal	Yes	Yes	No	[277]
	Blastocysts	DMEM, 20% KSR and N2B27 medium, 1% NEAAs, 2 mM L-glutamine, 1% PS, 0.1 mM b-mercaptoethanol, 3 mM	Yes	139	Normal	Yes	Yes	No	[278]

		CHIR99021, 1 mM PD0325901, 2 mM SB, and 50 ng/mL vitamin C + MEFs under 38.5℃, 5% CO_2							
Equine	Blastocysts	DMEM/F12, 15% FCS, 1000 U/mL hLIF, 15% FBS + MEF under 38.5℃, 5% CO_2	Yes	28	Normal	Yes	No	No	[279]
	Blastocysts	KO-DMEM, 15% FBS, 0.1 mM NEAAs, 2 mM L-glutamine, 1% ITS, 100 µg/mL streptomycin, 100 IU/mL penicillin, 0.1 mM β-mercaptoethanol, hLIF, hbFGF + MEF under 38.5℃, 5% CO_2	Yes	15	No	No	No	No	[280]
Canine	Blastocysts	KO-DMEM/Ham's F12, 15% KSR, 1 × GlutaMAX, 1 × NEAAs, R3IGF1, 0.1 mM 2-mercaptoethanol, 10 ng/mL hrLIF, 4 ng/mL rhFGF2, 0.5 µM, MEK inhibitor PD0325901, 3 µM GSK3β inhibitor CHIR99021 + MEFs under 37℃, 5% CO_2	Yes	--	Normal	Yes	Yes	No	[281]
	Blastocysts	KO-DMEM or DMEM/-12, 0.1 mM β-mercaptoethanol, 5 µM thymidine, 15 µM cytidine, 15 µM guanosine, 15 µM adenosine and 15 µM uridine nucleosides, 0.2 mM GlutaMax, 0.1 mM NEAAs, penicillin (100 IU/mL), streptomycin (50 µg/mL), 10 ng/mL hLIF, 4 ng/mL hbFGF, 15% FBS or KSR + MEFs under 37.5℃, 5% CO_2	Yes	30	Normal	Yes	Yes	No	[99]

CDX2-KD: CDX2 gene knockdown; KO-DMEM: knockout Dulbecco's modified Eagle's medium; DMEM: Dulbecco's modified Eagle's medium; MEM-NEAA: Minimum Essential Medium-non-essential amino acids; hrFGF: Human recombinant fibroblast growth factor; hrLIF: Human recombinant leukemia inhibitory factor; hFGF: Human fibroblast growth factor; DMEM/F12: Dulbecco's modified Eagle's medium/nutrient mixture F-12; FBS: Fetal bovine serum; MEF: Mouse embryonic fibroblast; mLIF: Mouse leukemia inhibitory factor; FGF2: Fibroblast growth factor 2; ITS: Insulin–transferrin–selenium; LIF: Leukemia inhibitory factor; bFGF: Basic growth factor; rhbFGF: Recombinant human basic fibroblast growth factor; OEF: Ovine embryonic fibroblast; GFF: Goat fetal fibroblast; hLIF: Human leukemia inhibitory factor; PS: Penicillin-streptomycin; BSA: Bovine serum albumin; FCS: Fetal calf serum; KSR: Knockout serum replacer; EGF: Epidermal growth factor; BFF: Buffalo fetal fibroblast; STO: Sandos inbred mouse-derived 6-thioguanine-and ouabain-resistant; SB: SB431542 inhibitor.

CURRENT STATE OF iPS CELLS FROM LIVESTOCK

A ray of hope was the development of reprogramming techniques to obtain iPS cells from somatic cells[38]. Recent advances in iPS cells technology may overcome the bottleneck of establishing pluripotent cells from livestock species, and demonstrate that iPS cells showed advanced level of pluripotency, such as the ability to differentiate *in vitro* into multi lineages and *in vivo* into teratomas and chimeras[79,104]. The production of chimeric livestock from iPS cells would open the possibility to genetically engineer farm animals to improve traits of agricultural importance, and the generation of biomedical models[45,67,105].

The current knowledge on stemness gene regulation in ES cells helps to execute iPS cells in a better way, and could allow the development of approaches closer to clinical application. Transcriptional profiling of ES cells revealed that factors such as Oct4, Nanog, sex determining region Y-box 2 (Sox2), Klf4, c-Myc and Lin28 are essential to maintain pluripotency[106,107]. Among these factors, Oct4, Nanog and Sox2 have been identified as core transcription factors, showing both spatial and temporal expression in cultured PSCs and pluripotent cells of the ICM[108-110]. These core transcription factors also play pivotal roles in regulation of the pluripotent gene expression and

simultaneous suppression of many genes related to differentiation[111,112]. They exert their functions by co-occupying their target genes, and can bind at their own and each other's promoters to form an interconnected auto-regulatory loop[111,113]. These three factors function collaboratively in an auto-regulatory circuitry fashion to maintain their own expression and maintain the pluripotency of ES cells[106,114]. Additionally, Nanog was identified as the key factor, which regulates the establishment of the pluripotent epigenome[115,116]. The role and mechanism of these transcription factors in the reprogramming of livestock somatic cells to iPS cells have been reviewed[78,81,104,117].

Several strategies have been applied to deliver core reprogramming factors such as genes, mRNAs and proteins into somatic cells for the derivation of iPS cells. Alternatively, the replacement of reprogramming factors by small chemical agents has also been assessed. Commonly, retro- and lenti-viral approaches were employed for cellular reprogramming of different types of somatic cells from livestock such as pig[105], cattle[57,118-120], sheep[67], goat[51,121], dog[54,56], horse[63] and buffalo[65]. Recently, for the first time, cat iPS cells were created using disarmed retroviruses with the coding sequences for human Oct4, Sox2, Klf4, cMyc, and Nanog[122]. The expression of ectopic factors can be temporally confined by employing inducible promoters or viral promoters, which are epigenetically silenced. Shortcomings of the viral approach include the limited cargo capacity of approximately 7 kb for the transduced genes, the induction of innate immune responses, potential genotoxic effects, which limit the translation into clinical trials[123], and increased safety methods.

To evade these safety concerns, remarkable technological progress has resulted in the establishment of non-integrating viral and non-viral approaches, but limited attempts have been made to apply these to cells from livestock species. For example, the non-integrating adeno- and Sendai-viruses demonstrated efficient production of human, murine and canine iPS cells[124-127]. Apart from viral-mediated derivation of livestock iPS cells, non-viral approaches such as plasmid vectors, recombinant proteins, transposons, minicircle DNAs, small molecules, and mRNAs are in use to eliminate the risk of genomic alteration and enhance the prospects of iPS cells. In this regard, bovine iPS cells were successfully derived by plasmid[128], and transposon systems[58,129,130]. Similarly, porcine iPS cells were also established using episomal[131,132], and transposon systems[47], the transposon systems have been attempted to derive equine[59] and buffalo[66] iPS cells. Detailed information on approaches to generate transgene-free iPS cells has recently been reviewed by Haridhasapavalan *et al*[82]. Most recent studies of iPS cells derived from livestock are shown in Table 2. In addition, extensive overviews of iPS cells produced from a wide range of animal species including livestock with their prospective applications and limitations have been recently reviewed[85,103,133]. Most recently, our group presented the potential applications of transposon-mediated derivation of iPS cells for cell-based therapies[83].

POTENTIAL APPLICATIONS OF PSCs FROM LIVESTOCK

Reproduction

A long-standing goal of PSCs research is the differentiation into functional germ cells, and their application for *in vitro* fertilization to obtain sexually recombined genotypes. In combination with the readout of genomic trait values from few cells *via* single nucleotide polymorphism chips and whole genome sequencing techniques this will dramatically improve the breeding process[134]. In mammals, germ cells originate from PGCs, the PGCs are initially specified outside the post-implantation embryo through gradients of Wnt family member 3 (WNT3) and bone morphogenetic proteins (BMPs)[135-137]. After that PGCs migrate to the genital ridges, where they settle and ultimately make gametes. The PSCs are principally immortal, with a high proliferative rate, and the ability to differentiate into gametes that could enable *in vitro* breeding schemes for accelerated genetic improvement in livestock. Under the conventional breeding scheme of dairy animals, the generation interval for sire(s) or dam(s) of bulls is approximately ± 5 years. This time period could be considerably reduced to about 2.5 years using genomic selection approaches[134,138]. Recently, a proposed parental embryos to offspring embryos breeding system will require approximately 2 mo to finish one round of selection, and annual genetic gain will increase approximately 10-fold as compared to the standard genomic selection in dairy animals[134,139]. However, the feasibility of applying this approach to farm animals needs to be proven in field studies.

So far the proof of principle has been provided in rodents, where sperm and oocytes were generated by the differentiation of male or female PSCs[140,141]. These works

Table 2 Most recent examples of induced pluripotent stem cells successfully generated from livestock

Species	Cell type	Culture medium and condition	Expression of pluripotency markers	Long-term culture (passage)	Karyotype	*In vitro* differentiation to EBs	*In vivo* differentiation to teratomas	Germline transmission	Ref.
Cattle	Fetal fibroblasts	DMEM/F-12, 20% KSR, 1 mM l-glutamine, 0.1 mM NEAAs, 0.1 mM mercaptoethanol, 100 U/mL penicillin, 100 μg/mL streptomycin, 8 ng/mL bFGF, 1000 U/mL hLIF on MEF at 37℃ and 5% CO_2	Yes	40	Normal	Yes	Yes	No	[58]
	Fetal fibroblasts	KO-DMEM, 15% FBS, 2 mM L-glutamine, 1% NEAAs, 0.1 mM β-mercaptoethanol, 10^6 U/mL hLIF, 10 ng/mL bFGF on STO at 37℃ and 5% CO_2	Yes	50	Normal	Yes	Yes	No	[130]
Buffalo	Fetal fibroblasts	DMEM high glucose, 20% ESC-FBS, 2 mM l-glutamine, 1% NEAAs, 0.1 mM β-mercaptoethanol, 10 ng/mL bFGF, 10 ng/mL LIF on MEF at 37℃ and 5% CO_2	Yes	10	Normal	Yes	Yes	No	[65]
	Fetal fibroblasts	DMEM/F-12, 20% KSR, 0.1 mM NEAAs, 1 mM L-glutamine, 0.1 mM mercaptoethanol, 100 U/mL penicillin, 100 μg/mL streptomycin, 10 ng/mL bFGF, 1000 U/mL hLIF on gelatine at 37℃ and 5% CO_2	Yes	15	Normal	Yes	No	No	[66]
Ovine	Embryonic fibroblasts	DMEM, 20% FBS, 1% ITS, 0.1 mM 2-β mercaptoethanol, 1 mM NEAAs, 2 mM glutamine, 4 ng/mL bFGF, 1000 U/mL mLIF on MEFs at 37℃ and 5% CO_2	Yes	17	Normal	Yes	Yes	Yes (formation of ICM in tetraploid)	[50]
	Embryonic fibroblasts	KO-DMEM, KSR, 0.1 mM NEAAs, 2 mM L-glutamine, 0.1 mM 2-mercaptoethanol, 8 ng/mL hFGF2, 1000 U/mL mLIF on SNL at 37℃ and 5% CO_2	Yes	23	Normal	Yes	Yes	Yes (live-born chimeric lambs)	[67]
Caprine	Fetal fibroblasts	DMEM/F12, 20% KSR, 1 mM L-glutamine, 0.1 mM 2-mercaptoethanol, 1% NEAAs, 2% sodium bicarbonate solution, 1000 IU/mL 2i/LIF, 4 ng/mL bFGF on STO at 37℃ and 5% CO_2	Yes	30	Normal	Yes	Yes	No	[282]
	Embryonic fibroblasts	KO-DMEM, 20% KSR, 1% NEAA, 1% L-glutamine, 0.1 mM EAA, 1% penicillin /streptomycin, 10 ng/mL FGF2 on GEF at 37℃ and 5% CO_2	Yes	22	Normal	Yes	No	No	[283]
Porcine	Embryonic fibroblasts and microvascular pericyte cells	LCDMV medium contains 50% neurobasal medium, 50% DMEM/F12, 1 × N2, 0.5 × B27, 5% KSR, 10 ng/mL LIF, 1 μM CHIR99021, 2 μM (S)-(+)-dimethindene maleate, 2 μM minocycline hydrochloride, 40 μg/mL vitamin C on MEF at 37℃ and 5% CO_2	Yes	28	Normal	Yes	Yes	Yes (chimeric formation in post-implantation pig conceptuses)	[284]
	Sertoli cells	DMEM/F12, 10% KSR, 10% FBS, 1 mM l-glutamine, 1 mM antibiotic, 1% NEAAs, 0.1 mM β-mercaptoethanol, 10 ng/mL bFGF, 10 ng/mL hLIF on MEF at 37℃ and 5% CO_2	Yes	50	Normal	Yes	Yes	No	[285]
Equine	Fetal fibroblasts	DMEM/F12, 20% KSR, 10 ng/mL bFGF, 1% penicillin/streptomycin, 10 ng/mL hLIF on MEF at 37℃	Yes	25	--	Yes	No	No	[286]

	Cell source	Medium							Ref.
		and 5% CO$_2$							
	Fetal fibroblasts	DMEM, 20% FBS or KO-DMEM, 20% KSR, 2 mM l-glutamine, 0.1 mM β-mercaptoethanol, 0.1 mM MEM-NEAAs, 1% penicillin–streptomycin, 8 ng/mL hbFGF, 1000 U/mL hLIF on SNL at 37°C and 5% CO$_2$	Yes	30	Normal	Yes	Yes	No	[61]
Canine	Embryonic fibroblasts	Serum-free N2B27-based medium, 4 ng/mL hbFGF on MEF at 37°C and 5% CO$_2$	Yes	50	Normal	Yes	No	No	[287]
	Fetal fibroblasts	KO-DMEM/F12, 20% KSR, 2 mM L-glutamine, 0.1 mM NEAAs, 0.1 mM β-mercaptoethanol, 0.1 mM bFGF on MEF at 37°C and 5% CO$_2$	Yes	15	Normal	Yes	Yes	No	[288]

DMEM: Dulbecco's modified Eagle's medium; KO-DMEM: Knockout Dulbecco's modified Eagle's medium; DMEM/F12: Dulbecco's modified Eagle's medium/nutrient mixture F-12; NEAAs: Non-essential amino acids; bFGF: Basic fibroblast growth factor; hLIF: Human leukemia inhibitory factor; MEF: Mouse embryonic fibroblast; KSR: Knock-out serum replacement; FBS: Fetal bovine serum; LIF: Leukemia inhibitory factor; ESC-FBS: Embryonic stem cells-fetal bovine serum; ITS: Insulin–transferrin–selenium; mLIF: Mouse leukemia inhibitory factor; hFGF2: Human fibroblast growth factor 2; EAAs: Essential amino acids; hbFGF: Human basic fibroblast growth factor; STO: Sandos inbred mouse-derived 6-thioguanine-and ouabain-resistant.

support the notion that this approach might be translatable to farm animals. However, before the proposed breeding system can be applied in farm animals a series of obstacles need to be overcome. Earlier, it was observed that monkey ES cells could differentiate into primordial germ cell-like cells (PGCLCs) and their differentiation ability was further improved by supplementing a conditioned medium from testicular or ovarian cells with recombinant BMP4, retinoic acid (RA), or stem cell factor[142,143]. Another study showed the possibility of spermatogonial stem cell transplantation in a non-human primate infertility model[144]. The findings laid the basis for the development of future germ cell regeneration in livestock. Recently, the derivation of stable bovine ES cells was reported, which could offer a technical basis for the auxiliary establishment of *in vitro* germ cell induction in farm animals[145]. Porcine iPS cells have been successfully differentiated into PGCLCs, and xenotransplantation of these cells into the testes of infertile immune-deficient mice resulted in immunohistochemically identifiable germ cells[146,147]. Another study revealed that porcine PGCs could be derived from the posterior pre-primitive-streak epiblast by upregulation of SOX17 and B-lymphocyte-induced maturation protein 1 through activation of WNT and BMP signaling pathways[148]. A number of comprehensive studies dedicated to bovine germ cell differentiation suggested that RA and/or BMPs are important for induction of PSCs[149]. The significance of RA in gametogenesis and meiosis induction has also been reported in buffalo[150,151].

Apart from reduction of the generational interval using *in vitro* breeding in livestock, the idea of generating gametes *in vitro* may translate to treatments of infertility, understanding the complexity of gametogenesis, and it could also be a source for regenerative medicine[78,152]. Improvements in germ cells differentiation of PSCs from livestock will further fuel the enthusiasm of researchers working on farm animals (Figure 2). If robust and field-applicable protocols for *in vitro* germ cell
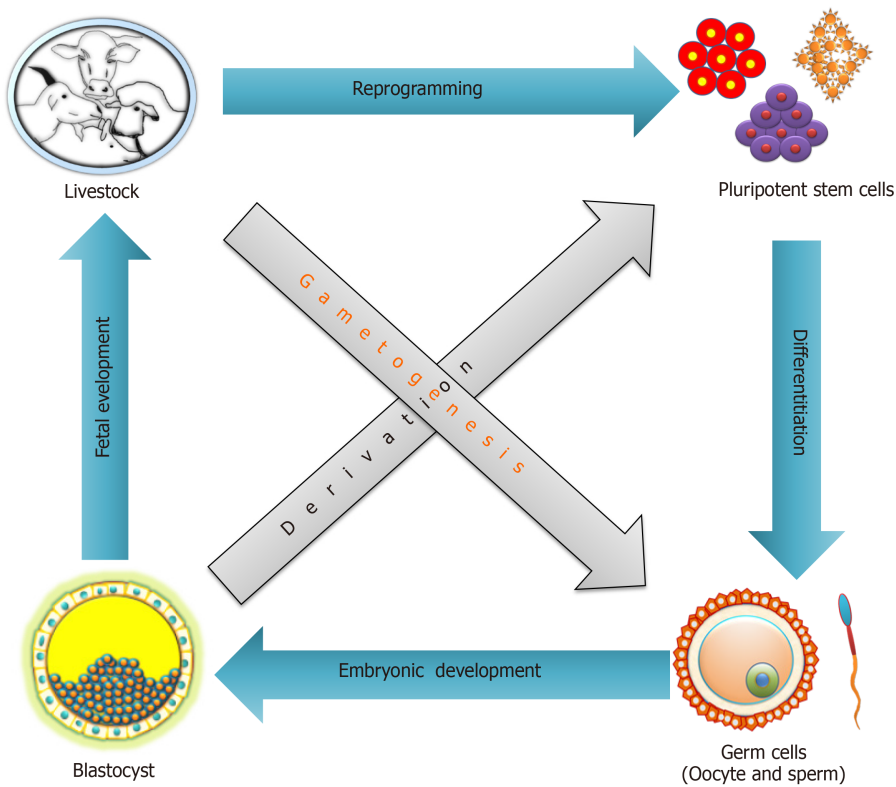
Figure 2 Involvement of pluripotent stem cells in the reproductive cell cycle through reprogramming, differentiation and development.

differentiation of livestock PSCs could be developed, an *in vitro* breeding program will rapidly be implemented.

More recently, haploid stem cells (hSC), having a single set of chromosomes, are considered excellent tools to study gene function (due to having a single copy) and obviate the mutation effect[153]. To date, hSCs have been derived from mouse, rat, monkey and humans[153,154]. In nature, ova and sperm are haploid cells. Experimentally it has been shown that it is possible to generate murine hSCs containing only the maternal genome or the paternal genome through either parthenogenetic or androgenetic embryos. Recently, it was demonstrated that fertile adult mice can be produced after fertilization of a sperm with an ovum derived from haploid ES cells[155], supporting the significance of haploid stem cells as a new tool to quickly generate genetic models for the direct transmission of genomic modifications at the organism level.

Genetic engineering

The genetic engineering of animals refers to adding, changing or removing certain DNA sequences, and the inheritance of these modifications to the next generation. The self-renewal and differentiation ability of PSCs make these cells an attractive tool for genetic engineering for various downstream applications. The self-renewal property of PSCs means they are theoretically immortal *in vitro through* symmetric cell divisions, which could provide a possibility for genetic modification and screening of cells, carrying the intended gene modifications.

Usually, genetic modification requires several generations and a large number of animals that could be overcome using PSCs especially in livestock *via* contribution to the germline[156]. The PSCs can be genetically modified *in vitro* and then injected into an embryo where they contribute to the germline, resulting in transgenic offspring (Figure 3) and thus reducing the required number of animals to produce the line founders[157].

In murine ES cell-based targeted mutagenesis, homologous recombination (HR) approaches allow the loss-of-function, gain-of-function experiments of desired loci, but also more complex genetic modifications, such as large genetic recombinations, as well as spatial and temporal expression patterns[158-160]. Using this technology, mouse ES cells can be screened and single colony-derived cells produced to employ for blastocyst complementation. However, in livestock due to the lack of bona fide ES
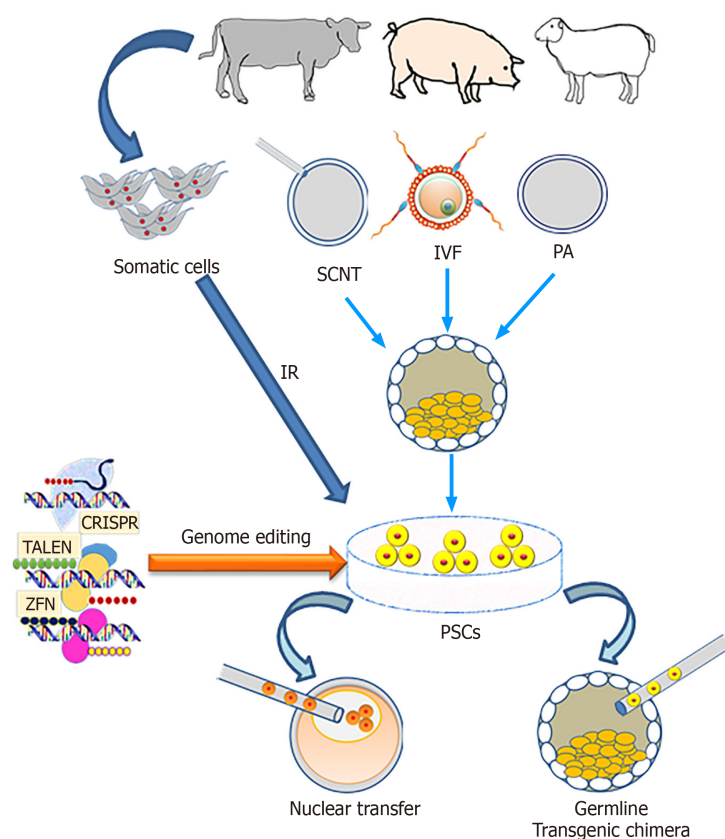
Figure 3 Outline of the production of transgenic livestock using pluripotent stem cells. IR: Induced reprogramming; SCNT: Somatic cell nuclear transfer; IVF: *In vitro* fertilization; PA: Parthenogenetic activation; PSCs: Pluripotent stem cells; CRISPR: Clustered regularly interspaced short palindromic repeats; TALEN: Transcription activator-like effector nucleases; ZFN: Zinc finger nucleases.

cells, the efficiency of HR events is very low. Richt *et al*[161] produced cattle with knock-out of the prion gene using somatic cell nuclear transfer (SCNT) combined with HR in somatic cells. Details of several genetic engineering methods used for successful creation of modified PSCs including ES and iPS were reviewed[162].

Previously, many workers reported that the use of ES cells as donor nuclei in SCNT resulted in higher birth rates as compared to differentiated somatic cells[25,163], which may be due to the un-differentiated state of PSCs. An advantage of this approach is that homozygous transgenic founders are produced in one generation. The use of iPS cells derived from livestock to produce offspring by SCNT has so far resulted in low success rates. Attempts to clone pigs and sheep using iPS cells resulted in very low efficiencies[121,164,165], whereas in murine experiments cloned animals were produced with similar efficiency using iPS or ES cells[163,166].

It was assumed that the forced expression of exogenous factors in iPS cells may hamper the nuclear reprogramming of donor cells during SCNT[121]. For this reason the possible applications of PSCs for generating genetically modified livestock have been limited. The recent discovery of site-specific nucleases such as zinc finger nucleases, transcription activator-like effector nucleases and CRISPR-Cas9 has allowed us to overcome the bottlenecks of genetic engineering in livestock[167-171]. The detailed description of site-specific nucleases is beyond the scope of the current manuscript, and interested readers can find excellent reviews elsewhere[156,172].

Models for cell therapy

Blood stem cell transplantation is a well-established clinical treatment of leukemias[173]. Nowadays, the feasibility and translation of innovative cell therapies, based on PSCs, is actively studied. In general, cell therapy is the transfer of cells into a patient to heal lesions or cure a disease, which cannot be addressed adequately by existing pharmaceutical interventions. For this purpose, cells may originate either from the patient (autologous cells) or a donor (allogeneic or heterogenic cells). The cells used in cell therapy should have the capability to proliferate *in vitro*, and to differentiate into specific cell types in a patient. In the last few years, PSCs have received considerable

attention for innovative cell therapies, and has resulted in significant progress in the understanding of their characteristics and therapeutic potential in different lineages. PSCs are considered ideal candidates for cell therapy to achieve tissue repair, or to restore and replace diseased cells.

Using PSCs a large number of animal models has already been treated for numerous diseases to assess the effectiveness of innovative cell-therapies[174-176]. The use of ES cells in cell-therapies is limited due to difficulties in patient-specific derivation, immune-rejection and ethical considerations, whereas derivation of iPS cells has overcome these concerns. Before the clinical application of iPS cell-based therapies is approved, they should be properly assessed using appropriate simulated animal models. Traditionally, laboratory animals (rodents) are used as models due to available knock-out or knock-in gene mutants which demonstrate disease phenotypes. Rodent models for cell-therapies do not always accurately mimic the genetically heterogeneous human situation[177,178]. The use of large animal models seems to be more suitable to analyze efficacies and risks in longitudinal pre-clinical tests and regenerative studies using cell-based-therapy[78,179,180]. Large animal models more closely match human patients in terms of life-span, metabolism, physiology, pathophysiology and biomechanics[181-184]. Large animal models will also permit determination of the effective cell dose, to track the fate of transplanted cells, and to assess their functional integration in the host organ[185]. In addition, large animal models also offer comparative models for research due to naturally occurring diseases, such as cancer[186,187].

Among livestock, the pig is considered a suitable animal model for pre-clinical evaluation of the efficacy and safety of novel cell therapies[179,188,189]. For example, porcine iPS cells can be differentiated *in vitro* into cells of the rod photoreceptor lineage, which were capable of integration into the retina, and generated outer segment-like projections[186,190]. Similarly, improvement of cardiac functions was documented in pig, but also in sheep, dog and rabbit models by assessing the efficacy of cell transplantations. The transplanted cells include skeletal myoblasts, bone marrow cells, cardiac stem cells, and endothelial stem cells[191-193]. Porcine models have also been used for the transplantation of human iPS cell-derived cardiovascular cells for the treatment of acute myocardial infarction, in which improvements were observed in myocardial wall stress, and contractile performance[194]. In addition, porcine iPS cell–derived endothelial cells were transplanted into a murine myocardial infarction model; the results showed an improved myocardial function by paracrine activation[195]. van der Spoel *et al*[196] analyzed the published reports of pre-clinical studies involving large animals for ischemic heart disease cell therapies and they concluded that large animal models allow prediction of the outcome of clinical trials for efficacy and safety. Hence, large animal models are useful targets for assessing the potential of iPS cell therapies to treat diseases, which are caused by the degeneration of specific cell populations, such as Alzheimer's disease, Huntington´s disease (HD), spinal muscular atrophy, retinitis pigmentosa, and diabetes[80,186].

It is well established that PSCs (including ES cells and iPS cells) are able to differentiate into any cell type *in vivo* or *in vitro* under suitable conditions, whereas the differentiation and reprogramming potentials of some adult stem cells are still under investigation[197]. This indicates that cells have the potential to switch from one cell type to another under the expression of some pluripotent related genes. Recently, progress made in cellular reprogramming and transdifferentiation suggests that it will be possible to generate cells from autologous sources without immunologic rejection and ethical consideration for therapeutic and regeneration purposes[197].

Conservation of valuable and endangered breeds

The objective of animal conservation is to maintain biodiversity because elimination of even a single species can interfere with the functioning of an ecosystem[198,199]. The protection of viable populations in their natural habitat (*in situ*) is one of the best methods for biodiversity conservation. *In situ* conservation allows the propagation of a small population using multidisciplinary approaches including genetic and ecological characterizations, but is sometimes insufficient for maintaining adequate genetic diversity[200]. Ex situ conservation approaches have been adopted with the aim of establishing viable populations through cryopreservation of animal genetic resources such as sperm, oocytes, somatic cells, and tissues of valuable domestic breeds and for conservation of endangered wild species. Earlier efforts in wildlife cryo-conservation were generally focused on spermatozoa and embryos[201,202]. More recently, somatic cell bio-banking has emerged as an attractive option for cryo-conservation of endangered and valuable farm animal breeds aiming to revive those in the future using assisted reproduction technologies[203-205]. Advancements made in nuclear transfer and stem cell

technologies, and the ability to reprogram differentiated somatic cells into embryonic or germ cell lineages prompted the interest in storing somatic cells for offspring production in future[206-208]. Further advancements in cryobiology may make it possible to cryopreserve different types of cells including somatic cells. Among somatic cells, fibroblast cells are preferable due to abundant availability in skin-tissue and easy establishment in cell culture[203,205]. Primary fibroblast cells derived from livestock such as cattle, buffalo, sheep, goat, and pig have been successfully cryopreserved and are being used for various purposes, including SCNT[205,209-212].

However, the efficiency rates of SCNT in many domestic and wild animals are low, and cloning experiments present a bottleneck in this approach. In mouse cloning, the use of pluripotent blastomeres as donor cells has significantly improved cloning efficiency and decreased the incidence of developmental abnormalities[213]. For endangered species, the availability of oocytes and embryos is often restricted, whereas the generation of iPS cells from somatic cells offers a more practical source of stem cells with less moral and ethical restrictions[214]. The morphology of iPS cells from wild and valuable domestic animals resemble those of ES cells[74,215]. The derivation of iPS cells from skin fibroblasts of endangered primate, silver-maned drill and white rhinoceros[74], snow leopard[215], orangutan[216] and endangered felids such as Bengal tiger, serval and jaguar[217], indicate the feasibility of derivation of iPS cells from threatened species. The generated iPS cells could be expanded for banking as a genetic resource, or used in the animal cloning process to produce viable offspring. Alternatively, iPS cells could be differentiated to derive mature and functional oocytes and spermatozoa, which might be used for *in vitro* fertilization to produce offspring. Furthermore, the availability of iPS cells from diverse species would help to accelerate research progress on evaluating phylogeographic structure, paternity determination, delineating subspecies, assessing gene flow and genetic variation related information, which could be critical for decision-making in managing both *ex situ* and *in situ* wildlife populations[218]. More recently, Hildebrandt *et al*[219] successfully generated ES cells and embryos from the critically endangered northern white rhinoceros. These achievements strengthen the beliefs that modern biotechnologies or iPS cell techniques in collaboration with cloning technology will allow the generation of more offspring from selected parents to ensure genetic diversity and may reduce the interval between generations. In future, the advancements of reproductive techniques and the new knowledge can only be employed when cryopreserved raw biomaterials (germ cells/somatic cells) are maintained, otherwise these would be lost forever[214].

PSCs FROM LIVESTOCK FOR HUMAN HEALTH

Drug testing and disease modelling
Human medicine requires animal models to test any new drug, as *in vitro* systems are still not able to model the pathophysiology of a whole organism. However, many preclinical studies of new therapies conducted on rodents and non-human primates failed due to the fact that they do not allow the prediction of safety and effectiveness in human patients[178,186]. For example, rodent models fail to simulate the basic physiological functions of heart diseases due to their faster heart rate. In contrast, large animals are more similar to humans with regard to their life span, physiology, metabolism, and pathophysiology[179,181,183,220]. The generation of PSCs from livestock[45,58,98] is economically valuable and critically important for the establishment of disease models, testing of new drugs and for the production of medically useful substances such as enzymes and growth hormones[221]. Additionally, animal disease models and animal iPS cells allow the establishment of informative assays to test the efficacy of new compounds, their toxicity, and dosing[222].

Previously, it was demonstrated that iPS cells can be differentiated into lineages of cardiomyocytes and hepatocytes, which were used for disease modelling and drug screening[223-225]. Presently, several biotechnological tools are available to generate disease models using either ES cells or iPS cells, creating new possibilities for their use in drug testing[226,227]. In many cases, somatic cells are also exploited for drug testing and validation, thus promoting new drug discoveries. In addition, the validation of existing drugs in new iPS cell models is performed. The availability of patient-specific iPS cells is crucial to discover new personalized therapeutics.

Large animals such as the domesticated pig have been recognized to be important models for studying colorectal cancer, cardiovascular diseases, cystic fibrosis, diabetes, osteosarcoma, Duchenne muscular dystrophy and Alzheimer's disease[228]. Similarly, cattle have become a relevant model for studying human female fertility *vis-a-vis* the

effects of ageing on fertility[229], uterine infection[230], and ovarian function[231]. In addition, dogs have natural occurring genetic diseases such as hemophilia B[232]. For other diseases that do not occur spontaneously in animals, transgenic animals have been generated such as monkeys with HD and cystic fibrosis-diseased pigs[233]. Induced PS cells generated from HD monkeys were differentiated into neuronal cells *in vitro,* and showed typical HD-like features; thus, representing an attractive model for investigating HD pathogenesis and therapy[234,235]. Recently, CRISPR-Cas9 was used to generate large animal models of neurodegenerative diseases that can more realistically mimic human disease progression[236]. The derivation of iPS cells from patients with congenital heart disease, and differentiation of these cells into cardiomyocytes has been anticipated to serve as a model system to study disease pathogenesis and for drug discovery[225,237].

Chimera formation and growth of human organs in livestock

A chimera is a composite organism that is composed of at least two genetically different cell populations[238,239]. It can be produced by combining blastomeres from a minimum of two individual embryos, by aggregating two or more sectioned embryos, or by injecting PSC cells into a blastocyst[240]. Tarkowski *et al*[241] were the first to demonstrate that the aggregation of two sectioned mouse embryos after transfer into the uterus of a surrogate could result in the development of healthy and fertile chimeric animals. If the cells used for chimera generation have differentiation potency they can contribute to form chimera and chimerism rates depend on the potency of the cells. Presently, ES and iPS cells are preferred for aggregation or injection into early embryos due to their pluripotency and ability to contribute to multiple organs of the resulting chimera[5,242-244].

Concurrently, scarcity and demand for human organ donors have motivated scientists to examine options other than donation from deceased patients, such as the possibility of growing human organs in animals. The availability of human PSCs suggested the possibility of producing human organs in animals *via* the chimera route (Figure 4). As proof of principle it was demonstrated that the combination of mouse/rat cells resulted in viable chimeras, with the possibility to direct the one cell type into an organ-specific lineage. To achieve this one species carries a defective gene (Pdx1) necessary for pancreas development. By complementing the cells with the defective Pdx1 gene, with fully competent PSCs from the other rodent species, a chimera with a functional pancreas may develop. A pancreas formed from rat PSCs has been observed in a mouse host, and a pancreas formed from mouse PSCs in a rat host[242,244]. Interestingly, a rat-sized pancreas formed from mouse PSCs, suggested that it could be possible to produce human organs (xenogeneic in nature) in various animal species[244].

Earlier, rat-mouse[242], human-mouse[245], and sheep-goat[246] chimeras were documented. However, blastocyst injection has failed to introduce stem cells into primate embryos[247]. Aggregation of rat-mouse[248], sheep-goat[249], and cattle-buffalo[250] embryos were able to form interspecies chimeras. In spite of the lower survival rate of chimeric embryos produced by the aggregation method compared with blastocyst injection, the chimerism rates have been observed to be higher[251]. Considering the higher rates of chimerism, the human-animal chimeras could be an organ resource, aggregation is also a desirable choice when the embryo and stem cells are in a good growth condition.

The generation of human organs in animal models would have a significant impact in the field of regenerative medicine, since the shortage of donor organs is a major bottleneck. For the generation of human organs, human PSCs would be injected into blastocysts acquired from carrier animals that should be genetically modified to block the development of a particular organ (Figure 4). Thus, only human cells might predominantly contribute to the development of that organ[76]. Previously, human iPS cells were employed to created chimeras upon integration in porcine and bovine blastocysts[5,244]; however, with very limited colonization of early fetuses by the human cells. Yang created human-mouse chimeras overexpressing functional human coagulation factor IX that could be a suitable candidate for hemophilia B treatment[252]. The feasibility of growing complete human organs using a chimeric approach and the proposed immune tolerance (if autologous donor cells are used) still needs to be substantiated.

More recently, the advent of precision genome editing tools like CRISPR/Cas9, efficiently generating the mutation that leads to organ deficiencies in larger animal models further widening the possibility to create human-animal chimeras[253]. The CRISPR/Cas9 mediated zygote genome editing, already successfully documented for mouse and larger livestock species[254], will likely be an effective tool for chimera
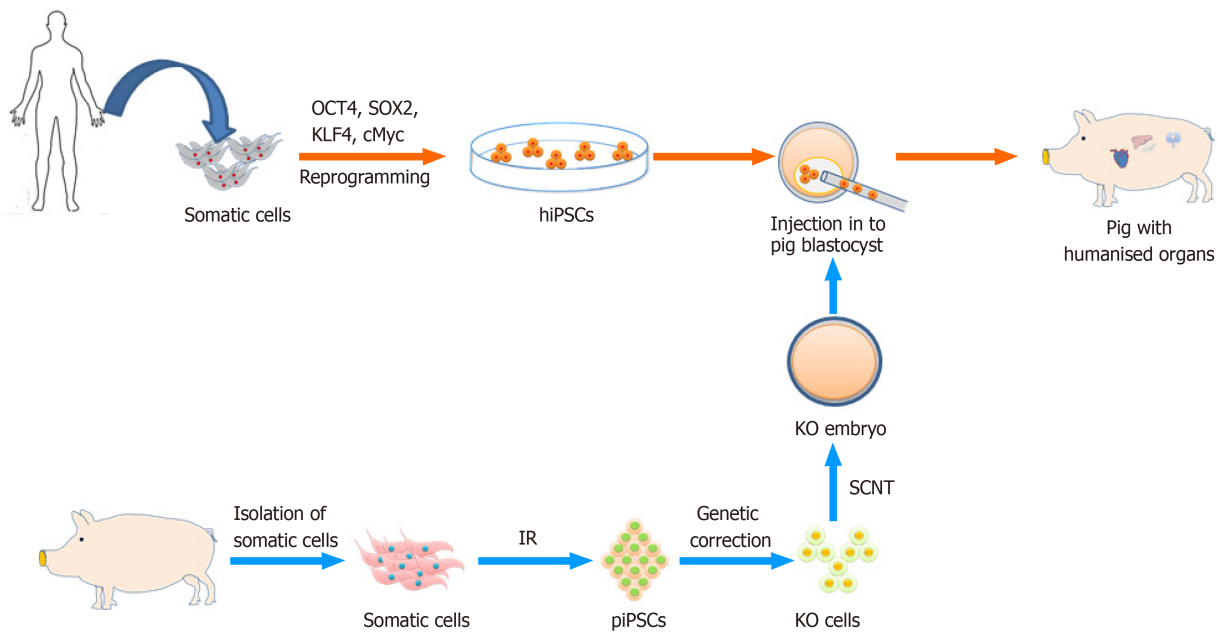
Figure 4 Outline of the production of humanized organs in livestock by chimera formation. OCT4: Octamer-binding transcription factor 4; SOX2: Sex determining region Y-box 2; KLF4: Kruppel-like factor 4; c-MYC: MYC Proto-Oncogene; hiPSC: Human induced pluripotent stem cells; piPSC: Porcine induced pluripotent stem cells; IR: Induced reprogramming; KO cells: Knock-out cells; SCNT: Somatic cell nuclear transfer.

production[5]. These results open up horizons in the field of regenerative and personalized medicine, but also impose big challenges of ethical concern with low efficiency of human-animal chimeras. Debate on existing frameworks for the ethical assessment of chimeric animal research involving human tissue and their risk minimization has been documented[255,256]. In general, human-animal chimeras might be developed into a strategy to overcome organ shortage, but also a model for studies on organ development; pathogenesis, immunologic defenses, drug screening and toxicity testing. However, this requires us to overcome species-specific incompatibilities, such as differences in placental structures, cell cycle, and growth factor dependencies.

PSCs FROM LIVESTOCK FOR *IN VITRO* MEAT PRODUCTION

The production of meat *in vitro* using livestock PSCs is proposed as a clean and prominent alternative to slaughtering animals[257]. Bovine stem cells were used to make the world's first burger from *in vitro* meat, which was served during a London press conference held in 2013[258]. This event was proclaimed as beneficial to reduce the global burden of the livestock industry, and was associated with environmental, ethical, and human health impacts[221,259]. The production of high-quality meat depends on the types of stem cells; source of ingredients and its composition. Among these, myoblast or satellite cells, and recently iPS cells are most important[260]. More recently, cattle umbilical cord blood cells have been reprogrammed to generated iPS cells, subsequently differentiated into muscle and fat cells[214,261]. Recent advancements have been made in the generation of stable bovine[145,262] and porcine PSCs[98], which could potentially be differentiated into skeletal muscle[263,264]. These PSCs form a cell bank with an unaltered and stable karyotype, and may eliminate further dependence on animals for cell isolation. However, this technology is in its infancy, and facing a number of challenges, such as whether *in vitro* meat will have the same taste as real meat, and whether this technology will be able to produce sufficient quantities in a cost-effective and clean way[265-267].

CONCLUSION

Challenges and perspectives

The PSCs comprise of ES cells derived from embryos and iPS cells obtained from reprogramming of somatic cells. The derivation of ES cells from embryos of livestock

such as cattle, buffalo, sheep, goat, pig, horse, cat and dog had a relatively long and until recently unfruitful history. By contrast, the successful generation of iPS cells from livestock species is more promising and a straightforward technology. Commonly, the use of reprogramming vectors that integrate into the host cell genome, and are continuously expressed is a major bottleneck in the utility of iPS cells. To evade this hurdle, the use of non-integrating viral- and non-viral approaches for iPS cells generation resulted into safe and clinical grade cells for further downstream applications[82,268]. Various technical hurdles remain to be overcome for iPS cell technology to fully expand its potential, but remarkable achievements in recent years have led to clinical applications, provided new ways for the development of disease models, and improved patients' treatments in a more adequate and personalized manner[269]. Looking ahead, the results of on-going clinical trials of iPS cells will deliver valuable information for preparing future strategies for cell-based therapy, drug testing, organ generation and disease modelling[237,270]. Pre-clinical testing of these approaches with livestock PSCs and large animal models are crucial for achieving these aims and successful translation into clinical therapies.

In future, PSCs along with novel upcoming technologies will synergically transform cellular reprogramming, differentiation and banking, which is necessarily connected to the industrialization of processes. More recently, massive progress has been achieved by the latest technologies in which ex-vivo and *in vivo* gene editing allowed efficient removal of the gene(s) responsible for the development of particular organs and the creation of new chimeric organs[271]. These approaches have the potential to innovate the field, but issues of safety and ethics need to be addressed in bringing the application of PSCs from bench to bedside.

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