Review

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Review

Bovine Pluripotent Stem Cells: Current Status and Prospects

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Abstract: Pluripotent stem cells (PSCs) can differentiate into three germ layers and diverse autologous cell lines. Being among the most commonly used large domesticated hoofed animals and serving as a vital source of food and bioreactors, cattle are among the many species in which PSCs have been developed. Bovine PSCs (bPSCs) are the "seed cells" of regenerative science and have immense potential to advance species propagation, genetic engineering, and disease treatment. Moreover, they can aid in screening new drugs for efficacy and toxicity, model human diseases, and establish a sustainable agricultural system that can cater to the needs of the growing global population. Establishing stable bPSCs *in vitro* has been a critical scientific challenge, and researchers have made numerous attempts to address this issue. This review primarily delves into the current research progress of bPSCs, the challenges faced, and their potential applications.

Keywords: cattle; embryonic stem cells; potential stem cells; induced pluripotent stem cells; reprogramming

1. Introduction

"Pluripotency" refers to the potential of a cell to differentiate into any cell type in the body[1]. Initially, life scientists observed pluripotency during human embryonic development, where embryonic stem cells (ESCs) existed in the inner cell mass (ICM). The ESCs manifest pluripotency by forming three germ layers, which develop into different organ systems with physiological structures and functions. However, cells in the ICM cannot self-renew due to being globally hypomethylated, resulting in transient embryonic pluripotency, but artificial culture can maintain pluripotency *in vitro*. Unlike ectoderm, pluripotent cells cultured in a stable system can self-renew indefinitely and retain the ability to differentiate across multiple lineages. These methods for maintaining pluripotency include *in vitro* culture of embryonic germ cells, somatic cell nuclear transfer (SCNT), and cell reprogramming (restoring pluripotency of cells at later developmental stages)[2,3]. Among others, the artificially transformed pluripotent cells exhibit molecular and functional properties similar to embryonic ectoderm.

Pluripotent stem cells (PSCs), including ESCs from preimplantation embryos and induced pluripotent stem cells (iPSCs) obtained through somatic cell reprogramming, have the advantages of self-renewal and pluripotency[4]. Self-renewal means that PSCs can generate new cells indefinitely, and the new cells have the same properties as the original cells, implying the immortalization of PSCs *in vitro*. At the same time, pluripotency indicates that PSCs can differentiate into three germ layers and multiple cell types, except that they cannot develop into extraembryonic endoderm and trophectoderm[5]. These properties make them uniquely attractive for engineering genomes. They can be used for genome modification and screening of cells with correct genome modifications, generating embryonic chimeras and passing the engineered genome to the next generation, contributing to germline optimization, and for donor nuclei of SCNT to improve reprogramming efficiency[6].

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As the first established PSCs, ESCs were initially developed from mouse ICM and have been subsequently explored in other species, including humans. From an agricultural perspective, establishing ESCs in domestic animals can make generating transgenic livestock more efficient. In addition, ESCs are a valuable resource in many areas of biotechnology and biomedicine as experimental models for studying incurable or inherited diseases and developing therapies. Although ESCs provide great potential for multiple fields and numerous efforts have been made, they are less successful in domestic animals, especially in large livestock like cattle[7].

The introduction of iPSCs has opened up a new possibility for the practical use of stem cells. Nuclear transplantation of mature cells can recharacterize them as fertilized eggs, demonstrating the potential of somatic cells to be reprogrammed into PSCs. By ectopic expression of a few key transcription factors, such as OCT4, KLF4, SOX2, and C-MYC, somatic cells can be reprogrammed to form iPSCs similar to ESCs. The pluripotency, proliferative capacity, viability, and demethylation of iPSCs can be activated and maintained, with the ability to differentiate into multicellular lineages *in vitro* and to form teratomas and chimeras *in vivo*. Additionally, iPSCs possess the advantages of ESCs and are free of ethical controversy. These features expand the use of stem cells in regenerative medicine[8]. Up to now, iPSC lines have been extensively studied and characterized in human and mouse models. Several iPSCs have been established from cattle, but reproducible results still need to be improved due to interspecies differences. Since the basic mechanisms of pluripotency activation may follow a similar pattern in mammals, the recent advances in iPSCs cellular technology may break the bottleneck in establishing ESCs from cattle. The chimeric livestock generated with iPSCs can serve as biomedical models and change the status of agricultural science and technology[9].

2. Sources of PSCs

Mouse are the classic model animal for biological experiments; their preimplantation embryo is relatively well-studied compared with other mammals (e.g., cattle). Preimplantation development is the only developmental stage that can be studied *in vitro*. In a mouse preimplantation embryo, the totipotent zygote gives rise to the first three cell lineages of the embryo: trophectoderm, epiblast, and primitive endoderm. Preimplantation embryonic development is relatively conservative in mice, humans and most mammals. After fertilization, the 1-cell embryos develop quickly without transcription, undergo a series of cleavage divisions generating relatively symmetric blastomeres without growth, compact tightly to form the morulae, and finally generate the blastocysts. During this mitotic cleavage process, there are increasingly distinct differences between the oocytes and the new cells in early embryos. After three rounds of cleavage divisions, an 8-cell embryo formed with polarization and densification. Subsequently, clear cell differentiation occurs during the mulberry embryo, followed by the internalization of some of the cleavage spheres to form the pluripotent ICM cells. In contrast, other cells remain outside the embryo to form the trophoblast ectoderm [10]. The emergence of these two types of cells represents the first genealogical differentiation during embryo development. As the embryo develops into the central cell ball of the blastoderm, the ICM differentiates into epiblast and primitive endoderm. The developmental program of the mulberry embryo into the uterine cavity is not static. It is regulated by different division patterns and flexible cell fate to compensate for cell loss[11]. The progeny of the ICM in the early ectoderm then transitions from the naïve state to the primed state during post-implantation development of the mouse embryo [12]. Therefore, PSCs can be categorized into three types: Naïve, formative, and primed. Naïve PSCs correspond to the blastocyst ICM stage and primed PSCs correspond to the post-implantation ectoderm stage[13]. Naïve PSCs are capable of forming somatic chimeras and germline chimeras; primed PSCs have a biased differentiation potential and weak chimerism under conventional conditions; formative PSCs are in-between, chimerism-capable but less efficient than naïve PSCs, and can make a direct response to germ cell induction [14]. PSCs in different states differ in cell morphology, gene expression, epigenetic features, and developmental potential performance [15]. Compared with primed PSCs, naïve PSCs are more advantageous, with more vital proliferation ability, less demanding culture conditions, and high plating efficiency when dispersed into single cells, making them the most desirable PSCs. The naïve-like PSCs obtained from the transformation

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of primed PSCs have similar advantages as naïve PSCs and are essential models for the study of differentiation pathways. It has been demonstrated that naïve and primed PSCs can be transformed into each other and passed on stably under appropriate culture conditions. (Figure 1).

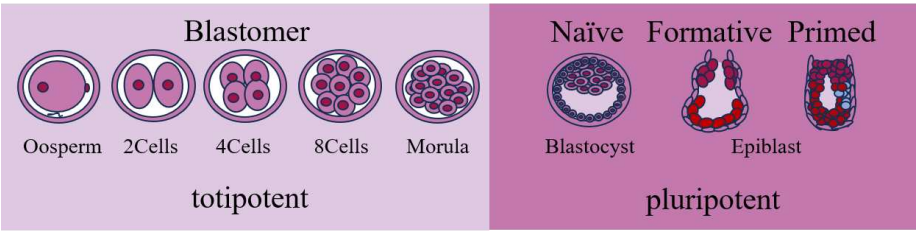

Figure 1. Early embryonic development.

It indicates the characteristics of early embryonic development. Embryonic cells with totipotent or pluripotent potentials are accordingly categorized into different pluripotent states.

3. Establishment of bESCs

ESCs are derived from the ICM of the preimplantation blastocysts and can proliferate indefinitely *in vitro* after stable culture, always maintaining pluripotency and the ability to give rise to a wide range of cell types and tissues. Mouse ESCs (mESCs) were obtained from mouse embryos in 1981[16], and ESCs derived from human blastocysts (hESCs) were isolated in 1998[17]. Accordingly, researchers found that mESCs and hESCs differ in culture conditions and characteristics. mESCs depend on leukemia inhibitory factor (LIF) and bone morphogenetic protein 4 (BMP4) signaling and have a dome-shaped clone, which can proliferate indefinitely. In contrast, hESCs depend on fibroblast growth factor 2 (FGF2)/ activin signaling have a flattened clone morphology, and are incapable of single-cell transmission. Mouse ectodermal stem cells (mEpiSCs) are derived from post-implantation embryos and have similar characteristics to hESCs and are equivalent to hESCs[18]. Researchers have classified mESCs as a naïve state and hESCs equivalent to mEpiSCs as a primed state. naïve ESCs are characterized by the expression of pluripotency genes, such as OCT4, SOX2, and NANOG, and have two active X chromosomes in female cells, which can rapidly differentiate into triturated germ layers after injected into the host blastocysts. X chromosome activation is an epigenetic hallmark of PSCs. Through the pathway and glycogen synthase kinase-3beta (GSK3B) inhibitor CHIR99021 and mitogen-activated protein kinase (ERK) inhibitor PD0325901, this so-called "2i" can maintain mESCs in the naïve state. Primed ESCs are in a more advanced developmental stage than Naïve ESCs and express pluripotency genes such as OCT4, SOX2, and NANOG, which have similar but not identical gene expression profiles[19].

Researchers have attempted to apply the culture systems of mESCs and hESCs directly to cattle, establishing bESCs by various methods such as in vitro fertilization (IVF) and SCNT. In 1992, SAITO first attempted to obtain bESCs using the mESC system, isolating the ICM from in vivo-derived embryos, using feeder layer cells, and supplementing LIF and other growth factors[20]. 2005 WANG borrowed the culture system of hESCs and added bFGF[21]. However, long-term studies have found that it is not feasible to establish bESCs directly using mouse and human culture systems, and most of the reported bESC lines failed standard pluripotency tests, i.e., *in vitro* embryoid body formation, *in vivo* teratoma assay, and chimera formation[22]. In addition, they have poor derivation efficiency, limited proliferative capacity, and no longer express pluripotency markers after prolonged passages, probably because hoofed animals, rodents, and primates share only major pluripotent epigenetic traits.

Microarray analysis of bESCs from different sources revealed that bovine pluripotency-associated genes are closely related to the TGFβ, WNT, and LIF signaling pathways and may be associated with BMP signaling. LIF inhibits the differentiation activity of mESCs, maintains them in a totipotent state, and stimulates their self-renewal, and STAT3 is the essential signaling transducer after LIF activation and mediates most of the cellular effects [23]; unlike in mice, signaling between

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LIF and STAT 3 may be disconnected in cattle, which may be the reason why true bESCs could not be generated in the previous culture system using LIF, and STAT3 may be the key to the establishment [24]. Inactivation of the WNT pathway is essential for the development of preimplantation and early post-implantation embryos as it stabilizes cellular activity through inhibition of GSK 3 β to stabilize cytoplasmic β-linker proteins, and the addition of classical WNT inhibitors is critical for the derivation and proliferation of bESCs. bFGF helps to maintain bESCs, but the LIF and ERK inhibitor PD98059, which is known to promote pluripotency of mESCs, was ineffective in the maintenance of bESCs, while CHIR99021 and PD0325901 effectively promote the expression of stem cell markers by bESCs [25]. However, the complete signaling pathway for the maintenance of bESCs has still not been reported. In 2018, Bogliotti used a culture system supplemented with inhibitors of the FGF2 and Wnt signaling pathways to obtain a stable primed bESCs cell line that was able to pass on for an extended period, maintained normal morphology, karyotype, transcriptome, and epigenetic features after 70 generations, and produced teratomas, which did not result in chimerism but was still a breakthrough [26]. Subsequently, Soto conducted an in-depth study of this culture system, optimizing it for a more straightforward one that does not require a feeder layer. It ensured the standardization the establishment of bESCs and broadened the potential use of bESCs [27] (Figure 2).

Compared to rodents and primates, it is still complex and challenging to establish stable bESCs. Current culture systems still need to succeeded in building gene networks that can maintain the pluripotency of bESCs stably. Establishing bESCs also relies on the hESCs and mESCs systems, and establishing of bESCs requires new programs. Meanwhile, bESCs are expected to facilitate the process of genome editing, accelerate molecular breeding programs for economic traits, and provide a platform for the study of preimplantation development in cattle. Large farm animals, physiologically and morphologically similar to humans, are essential models for clinical stem cell therapy and for modeling human diseases.

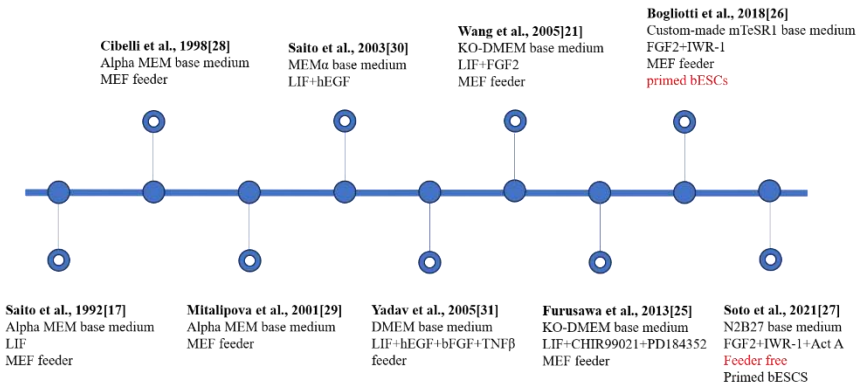

Figure 2. Establishment and characteristics of bovine embryonic stem cells. .

The main components of the medium used are the substrate and the pluripotent state of cells[17,21,25–31]. MEF, inactivated mouse embryonic fibroblast; KO-DMEM, knockout DMEM; Act-A, Activin A.

4. Establishment of biPSCs

In 2006-2007, Yamanaka's team reprogrammed mouse adult fibroblasts into iPSCs by overexpressing transcription factors (POU5F1, SOX2, KLF4, and c-MYC) [32]. Subsequently, human iPSCs were also obtained by the same method through dermal cells [33]. Since then, scientists have attempted to apply the method to livestock such as pigs, cows, sheep, and dogs to obtain iPSCs [34]. biPSCs were first established in 2011 from bovine embryonic fibroblasts [35], and currently, the method of reprogramming bovine somatic cells continues to advance. biPSCs that can be stably passed on for 50 generations were established by Talluri in 2015[36]. In 2017, single-cell-derived

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biPSCs that can be stably transmitted for more than 50 generations were obtained, expressing essential pluripotency genes and producing mimics and teratomas[37].

The choice of vector is crucial in determining the success of reprogramming. The use of retroviral and lentiviral vectors has been widely reported. The advantages of using viral vectors are high efficiency and simple and easy operation. However, the use of viral vectors causes the reprogramming genes to be continuously expressed in the cells, which hinders the study of further differentiation of iPSCs. Meanwhile, the safety of the resulting iPSCs is also worth considering, and their introduction into animals is likely to induce tumors [38]. To compensate for these drawbacks, scientists have used adenoviral vectors and Sendai virus vectors, which allow reprogrammed genes to be delivered and expressed transiently within the cell and minimize the possibility of genomic integration. There are also reports of using non-viral delivery, transposon-based delivery systems, and protein transduction[39]. Each method has advantages and disadvantages, and overall reprogramming efficiency is low. Traditional reprogramming techniques drive direct cell fate shifts through the overexpression of endogenous transcription factors, making the induction process challenging to control. Chemical reprogramming, on the other hand, utilizes exogenous chemical small molecules to mimic external signaling stimuli to drive cell fate transformation in a staged manner, which is highly controllable and is expected to regulate cell fate accurately, reverse cell identity and functional status, and make reverse development possible. Up to now, the chemical reprogramming approach has gained significant progress in both mice and humans, and this approach may become a new direction for the future development of bovine somatic cell reprogramming[40,41].

Different combinations of transcription factors may improve the efficiency and quality of iPSCs generation. The combinations of OCT4, KLF4, SOX2, C-MYC (OSKM) [42]; OCT4, KLF4, SOX2, C-MYC, NANOG (OSKMN) [43] and OCT4, KLF4, SOX2, C-MYC, NANOG, LIN28A (OSKMNL) have been commonly used. Most reports have used a protocol with at least four transcription factors, but there are also reports that biPSCs can be generated from testicular germ cells using only OCT4[44]. The efficiency of reprogramming bovine fibroblasts into biPSC has been reported to be significantly improved by adding SV40 large T antigen [45]. biPSCs could be obtained from bovine MSCs using a combination of overexpression of lysine-specific demethylase 4A (KDM4A) and other reprogramming factors [46]. It means new reprogramming factors related to pluripotency can improve reprogramming efficiency. Three main sources of transcription factors are used for reprogramming: human, mouse, and bovine[47,48]. Three main sources of transcription factors used for reprogramming: human, mouse, and bovine. Analysis of the homology of transcription factors and proteins from mouse, human, and bovine revealed that bovine and human homologies are close, and the use of bovine-derived transcription factors for reprogramming will also improve the efficiency of bovine somatic cell reprogramming to a certain extent. Molecularly, reprogramming remodels somatic transcriptomic and chromatin programs to the state of ESCs, including processes such as cell silencing and X-chromosome activation, demethylation of OCT4 and NANOG promoter regions, and genome-wide resetting of histone H3 lysine 4 and 27 trimethylation [49–51]. A key question by transcription factor-induced reprogramming is how these several transcription factors function to bring about such changes. As a result, researchers are constantly exploring unknown reprogramming factors and trying to understand the mechanisms by which they work. Recently, it has been found that chromatin remote interactions play an important role in regulating cell fate decisions. CTCF (CCCTC-binding factor) is a key regulator in the reprogramming process, with a two-stage action: CTCF up-regulation can act as a chromatin insulator to repress somatic genes, thus reducing the interaction between enhancer-promoter and somatic genes, and then CTCF helps to maintain the chromatin-induced reprogramming process. Subsequently, CTCF helps maintain chromatin accessibility and activate pluripotency genes [52]. However, the mechanism of how the different reprogramming stages are regulated to accomplish reprogramming is currently unknown. Gene expression can be regulated at every stage of gene product manufacture. Transcriptional regulation acts as an on/off switch, and post-transcriptional mechanisms can act as a rheostat to improve the output of gene expression, which plays a crucial role in pluripotency through RNA-

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binding proteins, RNA-processing mechanisms, and regulatory RNA molecules. The study of post-transcriptional mechanisms can help obtain higher-quality iPSCs[53].

The culture system of biPSCs is the key to obtaining high-quality biPSCs, ensuring their stability, and maintaining their pluripotency, so it is crucial to explore the combination of various growth factors and the concentration of their use. Scientists have made many attempts at the composition of the culture system. naïve and primed biPSCs have different gene expression profiles and can maintain self-renewal and renewal through different signaling pathways[54]. bFGFs trigger protein kinase C (PKC) signaling by self-phosphorylation and activation of FGF receptors, thus regulating cell self-renewal, metabolism, survival, proliferation, and differentiation. bFGFs regulate cellular self-renewal, metabolism, survival, proliferation, and differentiation by triggering protein kinase C (PKC) signaling through autophosphorylation and activation of FGF receptors[55].On the other hand, LIF inhibits the differentiation activity of iPSCs and promotes cellular self-renewal, activating pluripotency through three pathways: STAT3, PI(3) kinase, and MAPK. In addition, inhibitors PD0325901 and CHIR99021 inhibited MAPK and GSK-3 signaling, maintaining naïve pluripotency by suppressing the expression of FGF receptors and DNA methyltransferases[56]. Reversal of FGF 2 action is critical in controlling the interconversion of primed and naïve states and vice versa. Combining LIF with FGF under inhibitor-free conditions to block GSK3B and MEK signaling resulted in primed biPSCs, and naïve biPSCs were obtained when LIF was used in combination with CHIR99021, PD0325901(2i) medium, or with BMP4[57]. Using an autologous feeder layer may circumvent some of the challenges in the composition of the medium[58,59]. It has been claimed that the use of a BEF feeder layer enhances the reprogramming of bovine somatic cells, is more conducive to the long-term maintenance of clonal morphology and pluripotency than the use of a MEF feeder layer, and also improves the efficiency of the conversion of primed biPSCs to naïve biPSCs in the transformation medium[60]. Successful conversion systems of primed iPSCs to naïve iPSCs in other animals will also provide more lessons for cattle[61,62]. In addition, Bessi found that a hypoxic environment was detrimental to maintaining pluripotency in biPSCs by comparing oxygen levels during and after bovine-induced reprogramming [63]. Establishing a more complete freezing system can also further ensure the excellent quality of cells after recovery. [64].

Different tissue cells have different gene modifications and expression patterns, and the difficulty of inducing them to enter the "critical state" varies. Therefore, the source of iPSCs affects reprogramming efficiency. Moreover the differentiation status of the cells is a central factor in reprogramming efficiency, and progenitor cells are more likely to be reprogrammed than terminal cells[65]. biPSCs have been reported to be derived from somatic cells such as embryonic fibroblasts, amniotic cells, mammary cells, and supporting cells from adult cattle and fetuses [66–69]. It was shown that epigenetic disorders in bovine fibroblasts prevented complete reprogramming. Compared to bovine fibroblasts, bovine amniotic membrane-derived cells and mammary epithelial cells were more easily reprogrammed, and neural stem cells and testicular germ cells could be reprogrammed into biPSCs with OCT4 alone [70].

However, despite many efforts, attempts to maintain biPSCs by promoting self-renewal have not been entirely successful, and the obtained biPSCs do not meet the criteria for PSCs, have leaky expression of reprogramming factors and low reprogramming efficiency, do not maintain pluripotency in long-term cultures, and have a limited developmental potential (not able to generate chimeras) as measured in *vitro* and in *vivo*. However, these works will lay the foundation for future studies of biPSCs and stem cell signaling pathways, the establishment of bovine stem cell lines, and the production of transgenic cattle. These findings represent advances in biPSCs technology and broaden the understanding of bovine pluripotency molecules.

5. Establishment of bovine expanded potential stem cells

Chimeras can test the developmental potential of donor cell lineages in normal tissue development and are therefore considered the gold standard for assessing stem cell pluripotency [71]. Both bESCs and biPSCs have limited developmental potential and ability to contribute to extraembryonic tissues, especially the placenta, and therefore do not meet the gold standard. Unlike

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ESCs established from blastomeres, expanded potential stem cells (EPSCs) are derived from 4 and 8-cell embryos. Based on the successive establishment of mouse, human, and porcine expansion potential stem cells (EPSCs), ZHAO successfully established wild-type and somatic cell nuclear transplanted bovine EPSCs (bEPSCs) derived from preimplantation embryos [72]. bEPSCs express high levels of pluripotency genes, are stably propagated in a feeder layer-free culture environment, are genetically stable during long-term culture, and allow for effective and precise gene editing. bEPSCs possess a rich transcriptomic profile of early preimplantation embryos. They can differentiate in vitro into cells of all three germ layers and in chimeras into embryonic (fetal) and extraembryonic cell lineages. Importantly, genetically modified bEPSCs can be used as donors for somatic cell nuclear transplantation, potentially significantly advancing biotechnology and agriculture.

6. Challenge

Although reported, establishing primed bESCs provides limited information about the species-specific mechanisms required to maintain the pluripotent state. Reports on the in-depth mechanism of bovine pluripotency are still need to help us obtain bESCs quickly, and bESCs are still constrained by the difficulties of isolation, culture, and lineage establishment[26]. In the study of livestock ESCs, the study of pig ESCs has recently made remarkable progress. A more in-depth study of the process of pluripotency regulation in the early bovine embryo by referring to the lineage establishment method of pig ESCs and combining it with the characteristics of bovine early embryonic development will help to stabilize the establishment of bESCs.

Generating pluripotent cells through reprogramming that circumvents restrictions such as sex, age, and reproductive status is an attractive option for specific applications such as genetic preservation. However, methods known to increase reprogramming efficiency in mouse and human cells have not helped generate biPSCs. Currently used media for biPSCs do not maintain endogenous expression of pluripotent TFs, and attempts to maintain biPSCs by promoting self-renewal have not been successful. Thus, the biPSCs reported to date are only partially reprogrammed cells, and establishing biPSCs remains challenging[73]. Viral integration during reprogramming of somatic cells results in karyotypic instability and genomic alterations, thus producing iPSCs less efficiently, and improving the production of iPSCs to make them reproducible and safe is essential[74]. In addition, studies have yet to analyze how this transcription factor binds and functions in biPSCs cells. Given their different origins, they have different binding modes in these cells. They may also play transient roles during reprogramming, which the final pluripotent state cannot analyze. There is a need to study the intermediate phases of the process to understand the contribution of each factor to the different steps of the reprogramming as well as an in-depth characterization of the state of the iPSCs, which will lead to elucidating the molecular nature of the reprogramming. Reprogrammed somatic cells are primarily unable to silence exogenous factors, and the resulting biPSCs usually carry exogenous genes, which are also necessary for the self-renewal and maintenance of homeostasis of biPSCs[75]. Therefore, we urgently need to explore how to activate endogenous pluripotency genes so they are no longer dependent on exogenous factors[76]. Pluripotency is one of the characteristics of iPSCs, and the criteria for the identification of stem cell pluripotency include six aspects: *in vitro* differentiation, teratoma assay, chimeric assay, germline assay, tetraploid compensation, and single-cell chimerism. Currently, there is a lack of assays on the actual pluripotency status of biPSC cells, and thus, the ability of biPSCs to down-differentiate and generate bPGCLCs is still limited. In addition, it is crucial to establish a cryopreservation system for biPSCs and evaluate the effects of cryopreservation on their recovery, proliferation and differentiation ability, pluripotency, and apoptosis.

7. Conclusion and outlook

bPSCs contribute to cattle breeding and reproduction[77]. While traditional livestock breeding takes a long time, stem cell breeding can bypass this stage by obtaining bPSCs *in vitro* and inducing them to differentiate into germ cells, and then combining them with *in vitro* fertilization and monosperm microinjection to produce fertilized eggs, which can effectively shorten the

intergenerational interval, improve the efficiency of breeding, accelerate the breeding process, and facilitate the reproduction of good breeds to obtain genetically advantageous cattle with enhanced resistance to diseases. bPSCs may also be used to produce cell-cultured meat, thus eliminating ecological threats to the farming industry and public health risks such as the spread of zoonotic diseases. bPSCs may also be used to produce cell-cultured meat, thus eliminating the ecological threat to the farming industry and the public health risk of spreading zoonotic diseases.

Studying the *in vitro* differentiation process of bPSCs can be used to model *in vivo* differentiation. The ability of bPSCs to recapitulate the developmental process *in vitro* allows bPSCs to be used in developmental biology research. By developing a three-dimensional culture system, stem cells can give rise to structures that resemble whole organs (organoids), which shows great promise in modeling human development and disease and in biomedical research and regenerative medicine [78]. bPSCs can also be used as a tool for evolutionary analysis, and the acquisition of multispecies iPSCs may help us to analyze interspecies cells *in vitro*, compare interspecies differences, and elucidate the process by which organisms acquire diversity and complexity.

The gradual accumulation of genetic mutations is the basis of cancer development, and the similarity between the reprogramming process of somatic cells and cancer initiation implies that there may be similar molecular mechanisms between the two. In addition, the epigenetic instability induced by the forced expression of reprogramming factors can be used for research on cancer development. Mutual advances in the two fields could contribute to a deeper understanding of cancer development.

The application value of biPSCs is also reflected in disease modeling. Disease modeling refers to establishing experimental objects or related materials with human disease manifestations in medical research to promote understanding of diseases. However, due to technical and financial constraints, most of the research is conducted using animal models. biPSCs benefit disease modeling, significantly reduce the use of experimental animals, and safeguard animal welfare. For example, cows have a similar reproductive cycle to humans, which makes it possible to model human ovarian and uterine diseases. biPSCs can also be used for drug screening and gene editing of animals. PSCs can produce gene-edited animals, which can obtain xenogeneic organs that do not cause human immune rejection and help treat diseases.

The research of bPSCs has excellent potential for application in various fields. bPSCs have essential significance for in-depth research, and it is believed that after overcoming all the difficulties, bPSCs will bring more possibilities.

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