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Review Article

Embryonic Stem Cells in Cattle

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Contents

Because of the potential use of embryonic stem cells (ESC), especially for genetic modifications, there is great interest in establishing domestic animals-related ESCs. Unfortunately, despite considerable efforts, validated ESC lines in species other than mice and primates are yet to be isolated. In this paper, we will summarize the current knowledge on bovine ESCs in an attempt to understand why derivation of domestic animal ESC is still unsuccessful and we will discuss some promising future approaches.

Introduction

Embryonic stem cells (ESCs) were first isolated from mouse embryos over 20 years ago (Evans and Kaufman 1981; Martin 1981). These cells, which are able to self-renew indefinitely and to differentiate *in vitro* and *in vivo* into derivatives of all three germ layers, were initially used to study the differentiation process. Yet, soon after, it became clear that ESCs provided an efficient route for precise modification of the genome by gene targeting. The first mutant mice derived from genetically engineered ESCs were created, opening the doors to a new era of animal transgenesis (Capecchi 1989).

The successful isolation and multiple applications of mouse ESC (mESC) resulted in numerous efforts aimed to establish ESCs in other species. Livestock production, for example, would greatly benefit if farm animals ESCs were established, in order to efficiently create genetically modified farm animals bearing improved production traits or increased disease resistances. Furthermore, ESC lines from farm animals such as pigs, sheep or cattle will enable to create transgenic animals for modelling human diseases without some of the limitations from the mouse model, i.e. short life span or a physiology and anatomy very different from humans. Unfortunately, although considerable effort has been exerted to isolate and maintain ESC lines from domestic animals, validated ESC lines in species other that mice and primates are yet to be established.

Issues that need to be investigated are many, including the identification of species-specific mechanisms underlying pluripotency and markers (for review, see Keefer et al. 2007). A further hurdle is that most of the current empirical approaches to obtain ESC from farm animals closely followed procedures that were developed in humans and mice. Nevertheless, data extrapolation from these species has produced unsatisfactory results and might have misled researchers from finding the functional pathways that control pluripotency in ungulates.

In this paper, we analyse some of the currently available data on bovine ESCs (bESC) to have a better understanding of the failure to establish domestic animals-validated ESC lines. We also describe some new approaches that will perhaps enable the establishment of these cell lines.

The Stage and the Source of Embryos

Embryonic stem cell lines have been successfully isolated from mouse, monkey and human blastocysts, although outstanding derivations have also been made using embryos at pre-compaction stages (Eistetter 1988; Delhaise et al. 1996; Strelchenko 1996; Mitalipova et al. 2001). Most attempts to isolate and culture bESCs have been done with day 7–9 bovine blastocysts (Stice et al. 1996; Strelchenko 1996; Cibelli et al. 1998; Iwasaki et al. 2000; Betts et al. 2001; Saito et al. 2003; Roach et al. 2006; Muñoz et al. 2008) although ESC-like cells have also being isolated from day 12–14 embryos (Gjørret and Maddox-Hyttel 2005). Yet, the optimal timing of bovine pre-implantation development to derive ESCs is still unknown.

Attempts to derive bESC from zygotes and early cleavage stage embryos mostly failed (Strelchenko 1996; Mitalipova et al. 2001), while only a single bovine embryonic cell line, generated from a two-cell embryo, has been cultured over 3 years (Mitalipova et al. 2001). Yet, when bovine morulae were used as starting material, efficient colony formation rates ranged over 60–70% (Stice et al. 1996; Strelchenko 1996). The former author found that the embryonic stage (morulae and Day-7 blastocysts) or source (*in vivo* and *in vitro*) used did not influence the efficiency in establishing bESC colonies. These results are nevertheless contrary to studies reporting that Day-8 hatched blastocysts yield a higher proportion of epiblast colonies than inner cell masses (ICMs) isolated from Day-9 blastocysts (41% and 13%, respectively) (Talbot et al. 1995). Although bovine embryos have been used at different pre-implantation stages to isolate bESC, the lack of standardized and detailed protocols leads to an absence of homogeneous criteria to define efficiency of colony formation. This hurdle precludes comparisons between published data, making it impossible to conclude which developmental stage entails the best starting material to isolate ESCs. An alternative approach to answer this question might be to extrapolate available data on mouse embryonic development and mESC derivation; yet

several species-specific differences must be carefully considered prior to doing so. In mouse embryos, the first differentiation event occurs at the late morula stage, when the outer cells adopt an epithelial structure. This event is followed by the formation of the blastocoel, which marks the divergence of the first two lineages: trophectoderm (TE) and inner cell mass. Upon blastocyst expansion, differentiation continues with the ICM forming two further lineages: the epiblast and the primitive endoderm or hypoblast. Although epiblast, hypoblast and TE lineages will be present in blastocysts of all mammalian embryos, the time between fertilization and formation is shorter in mouse than in other species. Therefore, in order to isolate ESC from the same type of cells used to derive mESC, i.e. epiblast cells (Brook and Gardner 1997), we must consider differences in the time scale of development and make sure that embryonic stages equivalent to the murine counterparts are used. In the pig blastocyst a defined epiblast is not present before hatching, while in mice the epiblast is already formed in blastocysts enclosed in the zona pellucida. Therefore, derivation of ESCs from early murine or porcine blastocysts is not equivalent. In addition to differences in time scale of development, recent data have reported important differences in pathways that control pluripotency vs extra-embryonic lineage restriction in mouse (Rossant 2007) and bovine (Degrelle et al. 2005) (see below characterization of bESC). Therefore any data extrapolation between mouse and late-implanting species such as cattle should be carefully considered.

Bovine embryos from different sources have been used to isolate bESCs. Yet the only published experiment aimed to compare the feasibility of *in vitro*- and *in vivo*-derived embryos for the isolation of pluripotent cells was undertaken by Talbot in 1995. Such a study demonstrated that *in vivo-derived* blastocysts, especially from early hatching blastocysts, were shown to be a source of pluripotent epiblasts superior to their *in vitro*-produced (IVP) counterparts.

The basis for any advantage by *in vivo*-produced blastocyst to produce ESC lines is not known, although a number of differences in morphology, metabolic rates, gene expression and susceptibility to cooling damage (Smith et al. 2005; Lonergan et al. 2006) have been reported between *in vivo-derived* and IVP bovine embryos. It is possible that the reduced number of cells present in the ICM of IVP bovine embryos (Van Soom et al. 1996) might affect survival of the ICM in culture, hindering the chances to establish ESC lines from IVP embryos. In fact, Anderson et al. (1994) assumed that the factor that may affect survival of porcine ICMs in culture was the number of cells of the ICMs.

In vivo-derived embryos might be a better source of pluripotent cells, but their use as a starting material to isolate ESC is expensive and laborious. Therefore, it would be advisable to improve the procedures to derive ESCs from IVP embryos, as well as the ability of IVP-ICMs to yield ESCs. An obvious way to progress in this aspect would be increasing numbers of cells in these ICMs.

Characterization of Bovine ESC

Morphology, as well as the capacity to differentiate *in vitro* through embryoid body (EB) formation, was one of the two defining criteria initially used to identify bESC cultures. Other traits such as small size, rounded shape or high nucleus to cytoplasm ratio were used to define bESC lines. Yet, cells belonging to TE and visceral endoderm, which usually can be found in blastocysts or isolated ICMs primary cultures, may be confounded with ESC if solely morphological features are used as evaluating criteria. Bovine blastocyst-derived TE and endoderm cell lines have been thoroughly characterized not only by morphological criteria but also by the expression tissue-specific marker. For instance, transferrin is a definitive marker for bovine blastocyst-derived endoderm cell lines (Talbot et al. 2000). Therefore, the combined use of morphological criteria and the analysis of extra-embryonic markers is suggested to truly identify bESC and/or rule out the presence of TE or visceral endoderm cells in ESC cultures.

A useful strategy to characterize ESC lines is to analyze the expression of pluripotency-related molecular markers. Unfortunately, until now, no specific markers have been identified in bovine. Therefore, markers associated to pluripotency in other species (heterospecific pluripotency markers) such as stage-specific embryonic antigens (SSEA-1, -3, -4) have been used to characterize bESC. SSEAs are developmentally regulated cell surface antigens expressed by murine and human pluripotent cells (Fig. 1). mESCs strongly express SSEA-1 (Solter and Knowles 1978; Gooi et al.

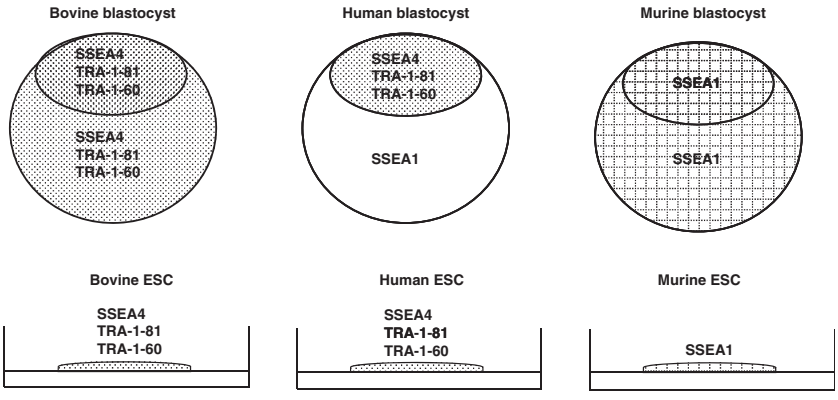

Fig. 1. Expression of stage specific embryonic antigens SSEA-1 and SSEA-4 and keratin sulphate-associated antigens TRA-1-60 and TRA-1-81 in human and murine blastocyst and embryonic stem cells and in bovine blastocysts and bovine ESC-like cells. Data from human and mouse ICM and trophectoderm are from Henderson et al. (2002); data from human and mouse embryonic stem cells are from Boiani and Schöler (1997). Data from bovine are from our lab

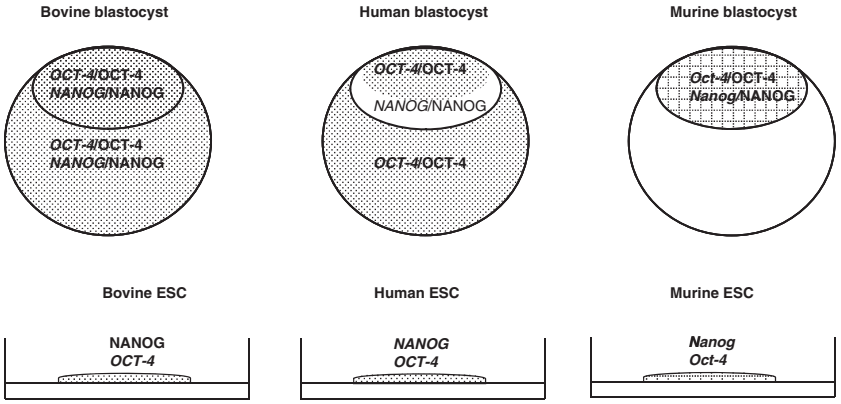

Fig. 2. Expression of oct-4 and nanog mRNA and proteins in bovine, human and murine blastocyst and embryonic stem cells. Data from bovine are from Kirchhof et al. (2000), Degrelle et al. (2005), Gjørret and Maddox-Hyttel (2005), Wang et al. (2005) and Muñoz et al. (2008). Data from human are from Adjaye et al. (2005), Cauffman et al. (2005), Chambers et al. (2003) and Kimber et al. (2008). Data from mouse are from Palmieri et al. (1994), Kirchhof et al. (2000), Chambers et al. (2003) and Hatano et al. (2005)

1981), whereas differentiated mESCs are characterized by the loss of SSEA-1 expression and in some instances, by the appearance of SSEA-3 and SSEA-4 (Solter and Knowles 1979). In contrast, hESCs typically express SSEA-3 and SSEA-4, but not SSEA-1 and their differentiation is characterized by down-regulation of SSEA-3 and SSEA-4 and up-regulation of SSEA-1 (Andrews et al. 1984; Fenderson et al. 1987). Undifferentiated hESCs also express the keratin sulphate-associated antigens TRA-1-60 and TRA-1-81 (Andrews et al. 1984). In bovine, a positive staining for SSEA-1, SSEA-3 and SSEA-4 was reported in three embryonic cell lines derived from pre-compaction embryos (Mitalipova et al. 2001). Similarly, SSEA-1 expression was also detected by Saito et al. (2003), while none of the bovine ES-like cells analysed by these authors were found positive for SSEA-3 or SSEA-4. In contrast, Wang et al. (2005) reported a positive SSEA-4 staining in the absence of SSEA-1 staining in five ESC lines.

We have reported positive staining for SSEA-4, TRA-1-60 and TRA-1-81 in bESC-like cells (Muñoz et al. 2008). Unfortunately, the above antigens were not only present in the ICM of bovine blastocysts but also in the TE (Fig. 1). Therefore in bovine, these markers are not specific for undifferentiated and/or pluripotent cells. The use of such markers to characterize bESC may mislead researchers into isolating and culturing TE-derived cells instead of ESCs.

The expression of SSEAs, in the TE of bovine blastocysts, was unexpected, considering that for a long time SSEAs have been used to characterize 'undifferentiated' bESCs. Nevertheless, it was not totally surprising as bovine TE cells show a slow differentiating phenotype characterized by the co-expression of epiblast-specifying genes (*OCT-4, SOX-2, NANOG*) and proteins (OCT-4, NANOG) and trophoblast-specific genes (*CDX-2, HAND1, ETS-2, IFN-TAU, C12*) (Kirchhof et al. 2000; Degrelle et al. 2005; Muñoz et al. 2008). Therefore in bovine, the expression of markers which are associated to pluripotency in other species (SSEA-4, TRA-1-60, TRA-1-81, *OCT-4, NANOG*) is not restricted to pluripotent cells (Figs 1 and 2), which is a warning to validate any pluripotency marker before its heterospecific use.

An additional difficulty to characterize bESC is that available antibodies currently used to characterize ESCs are produced using mouse or human proteins as immunogens. Therefore, their ability to cross-react with the appropriated bovine protein should be evaluated before their use.

Cell Culture Conditions

Culture conditions close to those established for murine ESC culture were successfully used to derive monkey (Thomson et al. 1995) and human ESCs (Thomson et al. 1998). Nevertheless, it soon became evident that some factors required for the maintenance of mESC pluripotency were not only dispensable in maintaining hESC pluripotency but were also detrimental. As an example, this occurred with BMP4, a member of the transforming growth factor-β (TGF-β) family involved in controlling mESC differentiation that induces differentiation of human ESCs into trophoblast cells (Xu et al. 2002). Since then, considerable amount of data have been published over differences between mouse and human pluripotency maintaining factors and signalling pathways (for review see Renard et al. 2007; Fig. 3). Until now, following a similar approach to primate ESC isolation, most attempts to culture bESC have been inspired by the original culture methods for mESC of Evans and Kaufman (1981).

Bovine ESCs are usually cultured on mouse embryonic fibroblasts (primary MEF or transformed STO cells). Culture media consists of Dulbecco's Modified Eagle's Medium supplemented with foetal bovine serum, L-glutamine, 2-β mercaptoethanol and different growth factors, mostly leukaemia inhibitory factor (LIF) and epidermal growth factor (EGF) (for comparison of some bESC culture conditions see Table 1).

Yet it is likely that culture conditions suitable to maintain mESC could be inadequate to maintain undifferentiated bESC. Preliminary studies by Keefer (2007) showed that the bovine ICM and its primary outgrowths express the LIF receptor and gp130 transducer. Yet, LIF did not improve the establishment and maintenance of ESCs from other ungulates (see Vackova et al. 2007 for review) although its presence in pig ESC culture medium prevented EB formation (Brevini et al. 2007). It can be speculated that, such as in hESC, stimulation of the STAT3 pathway by LIF might not induce proliferation of ungulate ESCs. Similarly, some growth factors found to suppress differentiation of mESCs [such as TGB-β, EGF or insulin-like growth factors (IGFs)] did not inhibit differentiation of porcine

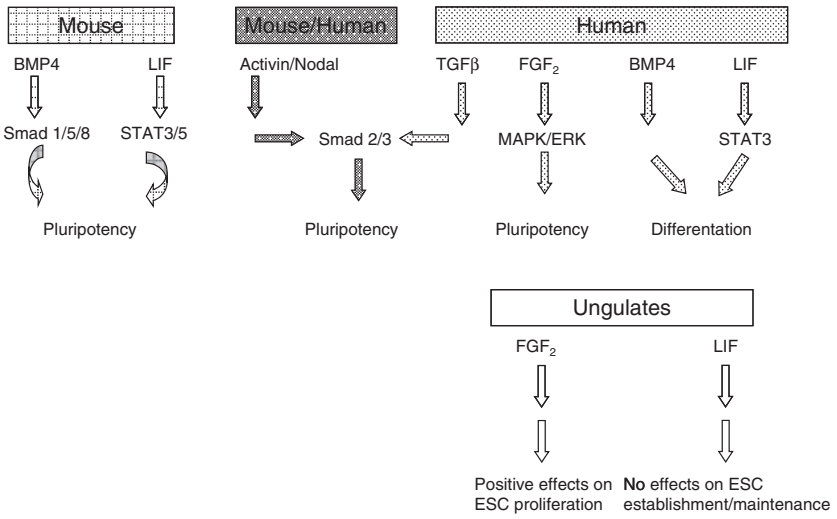

Fig. 3. Pluripotency and differentiation mediating pathways in murine, human and embryonic stem cells. Data from murine and human are from Renard et al. (2007). Data from ungulates were reviewed by Vackova et al. (2007)

Table 1. A selection of reports on the culture media employed to derive bovine embryonic stem cells

Reference	Feeder layer	Cell culture media
Talbot et al. (1995)	Murine STO cells	DMEM-M 199 FBS β-ME L-Glutamine Non-essential amino acids Nucleosides
Mitalipova et al. (2001)	Murine embryonic fibroblasts	Alpha-MEM FBS β-ME L-Glutamine
Saito et al. (2003)	Murine STO cells	MEM FBS β-ME hLIF hEGF
Wang et al. (2005)	Murine embryonic fibroblasts	Knock-out DMEM FBS β-ME L-Glutamine Non-essential amino acids hLIF bFGF
Gjørret and Maddox-Hyttel (2005)	Murine STO cells	Knock-out DMEM FCS β-ME L-Glutamine MEM amino acids hLIF bFGF
Muñoz et al. (2008)	Bovine embryonic fibroblasts	DMEM FCS MEM non-essential amino acids bFGF

bFGF, basic fibroblast growth factor; β-ME, β-mercaptoethanol; DMEM, Dulbecco's Modified Eagle's Medium; FBS, foetal bovine serum; FCS, foetal calf serum; hEGF, human epidermal growth factor; hLIF, human leukaemia inhibitory factor; MEM, Minimum Essential Medium.

stem cells (Hochereau-de Reviers and Perreau 1993; Prelle et al. 1994).

The inability of currently used culture conditions to meet the nutrient or growth factor requirements of bESC is one of the possible explanations for the failure to isolate these cells. Identification of specific pluripotency signalling pathways will help to determine which growth factors are beneficial or which ones are inappropriate for establishing a successful bESC culture.

In Vitro and *In Vivo* Differentiation of bESC

The differentiation ability of bESC *in vitro* is evaluated by EB formation. EBs are aggregates of stem cells whose development is reminiscent of early embryogenesis. Maintenance of bESC in a suspension culture (Strelchenko 1996) or in the absence of a feeder layer (Saito et al. 2003; Wang et al. 2005) initiates the formation of EBs. The EBs are composed of two layers of cells, ectoderm-like cells covered by a thin layer of endoderm like cells, with heterogeneous cellular particles within the cavity. The cells of bovine EBs give rise to a wide variety of differentiated cell types, including derivatives of the three germ layers (Saito et al. 2003; Wang et al. 2005). This ability is a proof of their pluripotent-differentiation character *in vitro*.

The ability of bESC to participate in the embryogenesis has been proven only after a short propagation period *in vitro*. Chimeric transgenic calves have been born after injection of bESC (passage 3) into cleavage stage embryos (Cibelli et al. 1998), embryo aggregation with bESC (passage 9–13) (Iwasaki et al. 2000). Yet integration of ESCs into the germ line, one of the properties used to define ESCs, have not been achieved in any of these experiments.

Calves were also successfully cloned using ES-like cells as donor nuclei (short cultured ICMs or passage 14–18) (Sims and First 1994; Saito et al. 2003), but the use of ESC in nuclear transfer can not be taken as a proof of pluripotency as cloned animals have been produced from fully differentiated somatic cell nuclei (Kato et al. 2000; Wakayama and Yanagimachi 2001).

Conclusions

The above summarized data indicate that although it is possible to isolate bESC, suitable conditions for

preventing spontaneous differentiation and senescence of these cells are yet to be established. Similarly, validated ESC lines have not been produced yet in other domestic species (for review see Keefer et al. 2007).

Possible explanations for this failure are many, including inadequate starting material or misleading characterization of cell lines because of the inadequate use of heterospecific markers. It is likely that the inability to optimize culture conditions might have been the biggest obstacle precluding success.

Until now empirical approaches, based almost exclusively on protocols developed for derivation of mouse stem cells, have been used to design cell culture environments for ES derivation in other species. Nevertheless, taking in account the increasing differences reported in the mechanisms and factors that regulate pluripotency and self-renewal among mammals (for review see Renard et al. 2007), it has become clear that alternative and/or complementary strategies will have to be applied.

Several techniques are currently used to identify the gene expression profile of ESCs. These techniques include serial analysis of gene expression, subtractive hybridization, representational difference analysis, cDNA microarrays and oligonucleotide microarray technologies. The use of the above tools have allowed to identify unique expression patterns of transcripts found in mouse and human stem cells in comparison with differentiated counterparts. The genes and pathways that appear to be related to stemness in mouse and human ESCs are good candidates to analyse basic mechanism governing pluripotency in mammalian ESCs. Furthermore, future transcriptomic analysis among ESCs from different species will help to identify species-specific pluripotency related genes and pathways which could be used to design tailored cell culture environments that enable ESC derivation from other species.

Nuclear transfer could also provide an alternative approach to improve ESC derivation (Renard et al. 2007). Studies with different strains of mice have shown that the isolation of ESC can be hampered by the use of different genotypes (Kawase et al. 1994). Thus in species where the genetic background variability is high, the use of genetically identical ICMs isolated from blastocysts obtained by nuclear transfer could simplify the screening for cell culture environments that would yield stable ESC lines.

All the data gathered actually so far and that may be collected in the near future about ESCs will help unravel the mystery of the pluripotent state enabling the establishment of ESCs from domestic animals.

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Conflicts of interest

Authors of this paper have no conflicts of interest.

Author Contributions

All authors have contributed equally to write this paper.

References

Adjaye J, Huntress J, Herwig R, BenKahla A, Brink TC, Wierling C, Hultschig C, Groth D, Yaspo ML, Picton HM, Gosden R, Lehrach H, 2005: Primary differentiation in the human blastocyst: comparative molecular portraits of inner cell mass and trophectoderm cells. Stem Cells **23,** 1514–1525.

Anderson GB, Choi SJ, Bondurant RH, 1994: Survival of porcine inner cell masses in culture after injection into blastocysts. Theriogenology **42,** 204–212.

Andrews PW, Damjanov I, Simon D, Banting GS, Carlin C, Dracopoli NC, Fogh J, 1984: Pluripotency embryonal carcinoma clones derived from human teratocarcinoma cell line Tera-2: differentiation *in vivo* and *in vitro*. Lab Invest **50,** 147–162.

Betts D, Bordignon V, Hill J, Winger Q, Westhusin M, Smith L, King W, 2001: Reprogramming of telomerase activity and rebuilding of telomere length in cloned cattle. Proc Natl Acad Sci U S A **98,** 1077–1082.

Boiani M, Schöler HR, 1997: Regulatory networks in embryo-derived pluripotent stem cells. Nat Rev Mol Cell Biol **6,** 872–884.

Brevini TA, Antonini S, Cillo F, Crestan M, Gandolfi F, 2007: Porcine embryonic stem cells: facts, challenges and hopes. Theriogenology **68,** S206–S213.

Brook FA, Gardner RL, 1997: The origin and efficient derivation of embryonic stem cells in the mouse. Proc Natl Acad Sci U S A **94,** 5709–5712.

Capecchi MR, 1989: Altering the genome by homologous recombination. Science **244,** 1288–1292.

Cauffman G, Van de Velde H, Liebaers I, Steirteghem A, 2005: Oct-4 mRNA and protein expression during human preimplantation development. Mol Hum Reprod **11,** 173–181.

Chambers I, Colby D, Robertson M, Nichols J, Lee S, Tweedie S, Smith A, 2003: Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell **113,** 643–655.

Cibelli JB, Stice SL, Golueke PJ, Kane JJ, Jerry J, Blackwell C, Ponce de León FA, Robl JM, 1998: Transgenic bovine chimeric offspring produced from somatic cell-derived stem-like cells. Nat Biotechnol **16,** 642–646.

Degrelle SA, Campion E, Cabau C, Piumi F, Reinaud P, Richard C, Renard JP, Hue E, 2005: Molecular evidence for a critical period in mural trophoblast development in bovine blastocysts. Dev Biol **288,** 448–460.

Delhaise F, Bralion V, Schuurbiers N, Dessy F, 1996: Establishment of an embryonic stem cell line from 8-cell stage mouse embryos. Eur J Morphol **34,** 237–243.

Eistetter HR, 1988: A mouse pluripotent embryonal stem cell line stage-specifically regulates expression of homeo-box containing DNA sequences during differentiation *in vitro*. Eur J Cell Biol **45,** 315–321.

Evans MJ, Kaufman MH, 1981: Establishment in culture of pluripotent cells from mouse embryo. Nature **292,** 154–156.

Fenderson BA, Andrews PW, Nudelman E, Clausen H, Hakamori S, 1987: Glycolipid core structure switching from globo- to lacto- and gangli-series during retinoic acid-induced differentiation of TERA-2-derived human embryonal carcinoma cells. Dev Biol **122,** 21–34.

Gjørret JO, Maddox-Hyttel P, 2005: Attempts towards derivation and establishment of bovine embryonic stem cell-like cultures. Reprod Fertil Dev **17,** 113–124.

Gooi HC, Feizi T, Kapadia A, Knowles BB, Solter D, Evans MJ, 1981: Stage-specific embryonic antigen involves alpha 1 goes to 3 fucosylated type 2 blood group chains. Nature **292,** 156–158.

Hatano SY, Tada M, Kimura H, Yamaguchi S, Kono T, Nakano T, Suemori H, Nakatsuji N, Tada T, 2005:

Pluripotential competence of cells associated with Nanog activity. Mech Dev **122,** 67–79.
Henderson JK, Draper JS, Baillie HS, Fishel S, Thomson JA, Moore H, Andrews PW, 2002: Preimplantation human embryos and embryonic stem cells show comparable expression of stage-specific embryonic antigens. Stem Cells **20,** 329–337.
Hochereau-de Reviers MT, Perreau C, 1993: *In vitro* culture of embryonic disc cells from porcine blastocysts. Reprod Nutr Dev **33,** 475–483.
Iwasaki S, Campbell KH, Galli C, Akiyama K, 2000: Production of live calves derived from embryonic stem-like cells aggregated with tetraploid embryos. Biol Reprod **62,** 470–475.
Kato Y, Tani T, Tsunoda Y, 2000: Cloning of calves from various somatic cell types of male and female adult, newborn and fetal cows. J Reprod Fertil **20,** 231–237.
Kawase E, Suemori H, Takahashi N, Okazaki K, Hashimoto K, Nakatsuji N, 1994: Strain difference in establishment of mouse embryonic stem (ES) cell lines. Int J Dev Biol **38,** 385–390.
Keefer CL, Pant D, Blomberg L, Talbot NC, 2007: Challenges and prospects for the establishment of embryonic stem cell lines of domestic ungulates. Anim Reprod Sci **98,** 147–168.
Kimber SJ, Sneddon SF, Bloor DJ, El-Bareg AM, Hawkhead JA, Metcalfe AD, Houghton FD, Leese HJ, Rutherford A, Lieberman BA, Brison DR, 2008: Expression of genes involved in early cell fate decisions in human embryos and their regulation by growth factors. Reproduction **135,** 635–647.
Kirchhof N, Carnwath JW, Lemme E, Anastassiadis K, Schöle H, Niemann H, 2000: Expression pattern of Oct-4 in preimplantation embryos of different species. Biol Reprod **63,** 1698–1705.
Lonergan P, Fair T, Corcoran D, Evans AC, 2006: Effect of culture environment on gene expression and developmental characteristics in IVF-derived embryos. Theriogenology **65,** 137–152.
Martin GR, 1981: Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A **78,** 7634–7638.
Mitalipova M, Beyhan Z, First N, 2001: Pluripotency of bovine embryonic cell line derived from precompacting embryos. Cloning **3,** 59–67.
Muñoz M, Rodriguez A, De Frutos C, Caamaño JN, Díez C, Facal N, Gómez E, 2008: Conventional pluripotency markers are unspecific for bovine embryonic derived cell-lines. Theriogenology **69,** 1159–1164.
Palmieri SL, Peter W, Hess H, Schöler HR, 1994: Oct-4 transcription factor is differentially expressed in the mouse embryo during establishment of the first two extraembryonic cell lineages involved in implantation. Dev Biol **166,** 259–267.
Prelle K, Wobus AM, Wolf E, Neubert N, Holtz W, 1994: Effects of growth factors on the *in vitro* development of porcine inner cell masses isolated by calcium ionophore. J Reprod Fertil **13,** 41.
Renard JP, Maruotti J, Jouneau A, Vignon X, 2007: Nuclear reprogramming and pluripotency of embryonic cells: Application to the isolation of embryonic stem cells in farm animals. Theriogenology **68,** S196–S205.
Roach M, Wang L, Yang X, Tian XC, 2006: Bovine embryonic stem cells. Methods Enzymol **418,** 21–37.
Rossant J, 2007: Stem cells and lineage development in the mammalian blastocyst. Reprod Fertil Dev **19,** 111–118.
Saito S, Sawai K, Ugai H, Moriyasu S, Minamihashi A, Yamammoto Y, Hirayama H, Kageyama S, Pan J, Murata T, Kobayashi Y, Obata Y, Yokoyama KK, 2003: Generation of cloned calves and transgenic chimeric embryos from bovine embryonic stem-like cells. Biochem Biophys Res Commun **309,** 104–113.
Sims M, First NLM, 1994: Production of calves by transfer of nuclei from cultured inner cell mass cells. Proc Natl Acad Sci U S A **91,** 6143–6147.
Smith SL, Everts RE, Tian XC, Du F, Sung LY, Rodriguez-Zas SL, Jeong BS, Renard JP, Lewin HA, Yang X, 2005: Global gene expression profiles reveal significant nuclear reprogramming by the blastocyst stage after cloning. Proc Natl Acad Sci U S A **102,** 17582–17587.
Solter D, Knowles BB, 1978: Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). Proc Natl Acad Sci U S A **75,** 5565–5569.
Solter D, Knowles BB, 1979: Developmental stage-specific antigens during mouse embryogenesis. Curr Top Dev Biol **13,** 139–165.
Stice SL, Strelchenko N, Keefer CL, Matthews L, 1996: Pluripotent bovine embryonic cell lines diret embryonic development following nuclear transfer. Biol Reprod **54,** 100–110.
Strelchenko N, 1996: Bovine pluripotent stem cells. Theriogenology **45,** 131–140.
Talbot NC, Powell AM, Rexroad CE Jr, 1995: *In vitro* pluripotency of epiblasts derived from bovine blastocysts. Mol Reprod Dev **42,** 35–52.
Talbot NC, Caperna TJ, Edwards JL, Garrett W, Wells KD, Ealy AD, 2000: Bovine blastocyst-derived trophectoderm and endoderm cell cultures: interferon tau and transferrin expression as respective *in vitro* markers. Biol Reprod **62,** 235–247.
Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Becker RA, Hearn JP, 1995: Isolation of a primate embryonic stem cell line. Proc Natl Acad Sci U S A **92,** 7844–7848.
Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM, 1998: Embryonic stem cell lines derived from human blastocysts. Science **282,** 1145–1147. Erratum in: Science 1998 *282,* 1827.
Vackova I, Ungrova A, Lopes F, 2007: Putative embryonic stem cell lines from pig embryos. J Reprod Dev **53,** 1137–1149.
Van Soom A, Boerjan M, Ysebaert MT, De Kruif A, 1996: Cell allocation to the inner cell mass and the trophectoderm in bovine embryos cultured in two different media. Mol Reprod Dev **45,** 171–182.
Wakayama T, Yanagimachi R, 2001: Mouse cloning with nucleus donor cells of different age and type. Mol Reprod Dev **58,** 376–383.
Wang L, Duan E, Sung L, Jeong B, Yang X, Tian C, 2005: Generation and characterization of pluripotent stem cells from cloned bovine embryos. Biol Reprod **73,** 149–155.
Xu RH, Chen X, Li DS, Li R, Addicks GC, Glennon C, Zwaka TP, Thomson JA, 2002: BMP4 initiates human embryonic stem cell differentiation to trophoblast. Nat Biotechnol **20,** 1261–1264.

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