View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

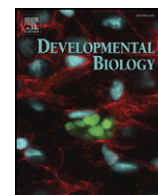
provided by Elsevier - Publisher Connector

Developmental Biology 322 (2008) 1–10

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology



Review

Trophoblast stem cell derivation, cross-species comparison and use of nuclear transfer: New tools to study trophoblast growth and differentiation

Maite Rielland, Isabelle Hue, Jean-Paul Renard, Jouneau Alice *

INRA, UMR 1198 Biologie du Developpement et Reproduction, F-78350 Jouy en Josas, France
ENVA, UMR 1198 Biologie du Developpement et Reproduction, F-78350 Jouy en Josas, France
CNRS, FRE 2857, F-78350 Jouy en Josas, France

ARTICLE INFO

Article history:
Received for publication 20 February 2008
Revised 4 July 2008
Accepted 9 July 2008
Available online 22 July 2008

Keywords:
Trophoblast
Epiblast
Stem cells
Reprogramming
Proliferation
Niche

ABSTRACT

The trophoblast is a supportive tissue in mammals that plays key roles in embryonic patterning, foetal growth and nutrition. It shows an extensive growth up to the formation of the placenta. This growth is believed to be fed by trophoblast stem cells able to self-renew and to give rise to the differentiated derivatives present in the placenta. In this review, we summarize recent data on the molecular regulation of the trophoblast *in vivo* and *in vitro*. Most data have been obtained in the mouse, however, whenever relevant, we compare this model to other mammals. In ungulates, the growth of the trophoblast displays some striking features that make these species interesting alternative models for the study of trophoblast development. After the transfer of somatic nuclei into oocytes, studies in the mouse and the cow have both underlined that the trophoblast may be a direct target of reprogramming defects and that its growth seems specifically affected. We propose that the study of TS cells derived from nuclear transfer embryos may help to unravel some of the epigenetic abnormalities which occur therein.

© 2008 Elsevier Inc. All rights reserved.

Introduction

Trophoblast is an essential extra-embryonic tissue that arises during development of mammals. It supports embryonic patterning, foetal growth and nutrition. It gives rise to the foetal part of the placenta. Although being a temporary organ, disorders affecting the placenta may have long term effects (Godfrey, 2002). Trophoblast constitutes by itself an interesting cellular model due to its properties of extensive and fast growth, invasiveness, and cell migration. Intriguingly, it seems to be more easily affected than the embryo proper by the consequences of reprogramming of nuclear activity through nuclear transfer. Indeed, a recurrent phenotype displayed by clones in different mammalian species is placentomegaly. Is reprogramming “more difficult” in this tissue (Yang et al., 2007)?

In the mouse, trophoblast stem (TS) cells have been isolated *in vitro* from pre- and early post-implantation embryos (Tanaka et al., 1998). They can self-renew indefinitely in the presence of specific growth factors and in their absence readily differentiate into the different cell types present in the foetal part of the placenta.

In this review we will describe the development of the mouse trophoblast lineage during the early stages when it remains mostly undifferentiated and stem cells can be isolated: so from its origin up to the end of gastrulation. We will review the molecular regulation involved in the control of growth and differentiation of TS cells. Trophoblast growth in other species such as the ungulates displays specific characteristics that we will compare with the mouse. In some of these species, trophoblast cell lines have been isolated, the stem cell nature of which has been neither questioned nor demonstrated so far. This will be discussed here as an alternative hypothesis to understand trophoblast proliferation establishment and maintenance across mammals. At last we will emphasize the usefulness of mouse TS cell models to understand some placental growth disorders such as those found after nuclear transfer.

Specification of the trophoblast

The first visible differentiation event occurs at blastocyst stage in the mouse embryo, with the appearance of an epithelial sheet of cell (the trophoblast) surrounding a cavity and an inner cell mass (ICM) (for a review, see Yamanaka et al., 2006). The trophoblast is a multipotent tissue that will give rise to the few differentiated cell types in the foetal part of the placenta. The transcription factor Cdx2 is a key marker of the first lineage separation (Niwa et al., 2005). In absence of Cdx2, a blastocyst-like structure can initially form but soon degenerates. It indicates that although essential for maintenance of the trophoblast, Cdx2 may not be the first trigger of its initial formation. Indeed, recent studies suggest that cellular mechanisms such as polarization of the cells after asymmetric division in the morula play a triggering role and are initially independent of *Cdx2* expression (Dietrich and Hiiragi, 2007; Honda et al., 2008; Ralston and

* Corresponding author. UMR Biologie du Développement et Reproduction, Bat 230, INRA, 78352 Jouy-en-Josas cedex, France. Fax: +33 1 34 65 23 64.
E-mail address: alice.jouneau@jouy.inra.fr (J. Alice).

0012-1606/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2008.07.017

Rossant, 2008). Recently, a factor belonging to the TEAD family, Tead4, has been found to act at an earlier stage of trophoblast specification, but is not involved in the process of polarization (Yagi et al., 2007; Nishioka et al., 2008). Interestingly, this gene is only required during a narrow window of development, before implantation, and in its absence the trophoblast lineage does not appear. Its positioning in the complex model of trophoblast emergence and more specifically, its relationship with Cdx2 remains to be elucidated. Initially present in all cells of the morula, Cdx2 starts to be more concentrated in the nuclei of the outer cells than in the inner cells as epithelialisation progresses and blastocoel forms (Dietrich and Hiiragi, 2007; Strumpf et al., 2005). The transcription factor *Oct-4* is initially expressed in all cells of the morula and later become restricted to the ICM and then to the epiblast. Cdx2 and Oct-4 have been shown to reciprocally inhibit each other in embryonic stem cells in culture (Niwa et al., 2005; Smith et al., 2005). This mutual inhibition can be envisaged *in vivo* as a safety mechanism to lock the lineage segregation. So far, the order of events leading to the establishment of the first two lineages at blastocyst stage is not completely clear. Some actors are probably missing, that could make the link between the cellular and mechanistic processes of polarization, the blastocoel formation and the network of transcription factors that give the genetic identity of both lineages.

Apart from *Cdx2* and *Tead4*, another transcription factor encoding gene has been shown to be essential for the first steps of trophoblast development, *Eomes* (Table 1). This T-box transcription factor is required slightly later than Cdx2 in the embryo and although its expression is not initially dependant on Cdx2, the latter stimulates its expression (Niwa et al., 2005; Strumpf et al., 2005). In its absence, the blastocyst can be formed and maintained, but does not implant (Russ et al., 2000).

Maintenance of the trophoblast identity and more specifically, of its proliferation ability requires additional transcription factors (Table

Table 1
Characteristics of genes expressed in the mouse trophoblast lineage and importance for TS cell derivation

Gene name	Expression pattern in embryo and trophoblast lineage	Phenotype of mutant embryos	TS derivation from mutant	References
Tead4	Starts at 2-cell stage, after implantation is restricted to trophoblast lineages	Die at 3.5 dpc, no blastocyst formation	No	(Hattori et al., 2007; Nishioka et al., 2008)
Cdx2	Polar and mural TE at 3.5 dpc, proximal ExE	No TE determination	No (from blastocyst)	(Beck et al., 1995; Niwa et al., 2005)
Eomes	TE, proximal ExE and chorion, posterior epiblast and primitive streak	Arrest at blastocyst stage, no implantation	No (from blastocyst)	(Ciruna and Rossant, 1999; Strumpf et al., 2005)
Elf5	ExE at 5.5 dpc and after	Loss of ExE at E5.5	No (from blastocyst)	(Donnison et al., 2005)
Sox2	ICM, epiblast, ExE, chorion	Die before 6.0 dpc, loss of epiblast and Exe	No (from blastocyst)	(Avilion, 2003)
Foxd3	ICM and epiblast, a subset of cells in ExE, primary and secondary TGC	Die at 6.5 dpc, loss of epiblast, expansion but precocious differentiation of Exe	No (from blastocyst and ExE)	(Tompers et al., 2005)
Ets2	Restricted to TE from 5.0 to 6.75 dpc, then in primitive streak after 7.75 dpc	Die at 8.0 dpc, loss of ExE, smaller EPC	No	(Georgiades and Rossant, 2006; Yamamoto et al., 1998)
Esrrb	ExE at 5.5 dpc, chorion at 7.5 dpc	Die at 10.5 dpc, reduced proliferation of trophoblast, no placental development	No (from blastocyst)	(Luo et al., 1997; Tremblay et al., 2001)
Dll1	ExE at 6.5 dpc	Die at 10 dpc, abnormal placenta development	Yes but abnormal differentiation	(Papadaki et al., 2007)
Fgf signaling pathway				
Fgf4	ICM, epiblast	Die before 5.5 dpc just after implantation	ND	(Feldman et al., 1995; Goldin and Papaioannou, 2003)
Fgfr2	Blastocyst, then restricted to ExE	Die at 4.5 dpc just after implantation	ND	(Arman et al., 1998; Goldin and Papaioannou, 2003)
Frs2	Polar and mural TE, ExE	Die at 8.5 dpc, defect in A-P polarity	No (from blastocyst and ExE)	(Gotoh et al., 2005)
Ptpn11 (Shp2)	Ubiquitous	ICM death, reduced number of TGC	No	(Yamanaka et al., 2006)
Erk2	Ubiquitous, but P-Erk2 is present in EPC and ExE	Die at 8.0 dpc, no ExE and EPC	No (from blastocyst and ExE)	(Corson et al., 2003; Saba-El-Leil et al., 2003)
Tgfb signaling pathway				
Nodal	ICM and epiblast, then posterior epiblast	Die at 7.5 dpc, no primitive streak, defect in A-P polarity, defect in ExE molecular patterning (see text)	ND	(Brennan et al., 2001; Guzman-Ayala et al., 2004; Takaoka et al., 2006; Varlet et al., 1997)
Activin A	Decidua	ND	ND	(Chen et al., 2006; Crossley et al., 1995)
Furin and Pace 4	ExE	Double mutant: defect in primitive streak formation and A-P polarity	ND	(Beck et al., 2002; Guzman-Ayala et al., 2004)
Smad2	Ubiquitous (P-Smad2 throughout the embryo at 5.5 dpc–8.5 dpc)	Die at 8.5 dpc, size reduction, defect in ExE, defect in gastrulation and visceral endoderm patterning	ND	(Brennan et al., 2001; de Sousa Lopes et al., 2003; Weinstein et al., 1998)
Wnt3	Posterior epiblast at 5 dpc then primitive streak	Die at 8 dpc, no primitive streak	ND	(Ben-Haim et al., 2006; Liu, 1999)
Bmp4	At 3.5 dpc in ICM and polar TE, at 6.5 restricted to ExE	Die before 9.5 dpc, size reduction, impaired mesoderm formation and patterning of anterior visceral endoderm	ND	(Goldman et al., 2006; Soares et al., 2005; Winnier et al., 1995)
Acvr1B (ALK4)	In epiblast and ExE between 5.5 dpc and 7.5 5 dpc	Die between 8.5 and 9.5 dpc, ExE and epiblast intertwined and disorganised, defect in visceral endoderm	ND	(Gu, 1998; Chang et al., 2002; Erlebacher et al., 2004)
AcvR2B (ActRIIB)	In epiblast and ExE since 6.0 dpc	Post-natal lethality: cardiac and intestine defects	ND	(Feijen et al., 1994; Oh and Li, 1997; Song et al., 1999; Chang et al., 2002; Erlebacher et al., 2004)

Index: AP – Antero-Posterior; EPC – Ecto Placental Cone; ExE – Extra-Embryonic Ectoderm; dpc – day post-coitum; ND – not determined; TGC – Trophoblast Giant Cells; TE – trophectoderm.

1). Esrrb belongs to the family of estrogen-related orphan receptors (Giguere et al., 1988). In embryos, *Esrrb* is expressed in the extra-embryonic ectoderm until the end of gastrulation (Pettersson et al., 1996). Embryos deficient in *Esrrb* die at E10.5 from an arrest in trophoblast development (Luo et al., 1997). Thus *Esrrb* appears to be involved in late trophectoderm or trophoblast (TE) maintenance. Two transcription factors containing a DNA-binding ETS domain, Ets2 and Elf5, are also important for the maintenance of proliferation in the trophoblast. In their absence, the Exe cannot be maintained and disappears, Elf5 being required earlier than Ets2 (Wen et al., 2007; Donnison et al., 2005). Finally, two transcription factors have been shown to be essential for both trophoblast and epiblast development, *Sox2* and *Foxd3*. In absence of either of the two factors, the epiblast is lost and the extra-embryonic ectoderm differentiates completely into trophoblast giant cells, thus, pluripotent populations are not maintained in the embryo (Avilion, 2003; Hanna et al., 2002; Tompers et al., 2005).

Development and maintenance of the trophoblast in the early post-implantation mouse embryo

ICM cells segregate to separate the pluripotent epiblast from the second extra-embryonic tissues, the primitive endoderm (Fig. 1A). Implantation in rodents is concomitant to this event. The polar trophoblast overlying the epiblast actively proliferates along with the epiblast to form an "egg cylinder" shaped embryo at E5 (Fig. 1A). The development of a cup-shaped embryo, specific to rodents, and the concomitance of implantation and gastrulation (in rodents and primates; Eakin and Behringer, 2004; Viebahn, 1999) imply some specific constraints for cell proliferation and differentiation processes. Until the formation of the chorion (mid-gastrulation period at E7.5), the polar trophoblast (called the extra-embryonic ectoderm or Exe) remains directly in contact with the epiblast.

Role of Cdx2

We have seen that Cdx2 is clearly essential for trophoblast specification in the mouse. It can induce trophoblast specification if its expression is forced in ES cells (Niwa et al., 2005). What is its role after? Does it play a role in controlling self-renewal or preventing differentiation? Paradoxically, Cdx2 has been initially discovered for its role as a tumour-suppressor in the gut (Guo et al., 2004). The protein is subjected to serine 60 phosphorylation via the MapK pathway, which modulates its transactivating activity (Rings et al., 2001). Interestingly, the phosphorylated form is localized in the proliferating cells of the crypt in the gut, whereas the non-phosphorylated form is present in more differentiated cells, where it regulates the expression of differentiation genes. As the activated MapK P-Erk1/2 is present in the blastocyst and the Exe (Corson et al., 2003; Wang and Jaenisch, 2004), Cdx2 is probably subjected to phosphorylation in the early embryo, which may also modulate its transactivation properties. The presence of ser60P-Cdx2 has been detected in the blastocyst (Liu et al., 2004) but the localization of the different isoforms of Cdx2 in the Exe awaits further investigation. The genes regulated by Cdx2 in the trophoblast are not known, except

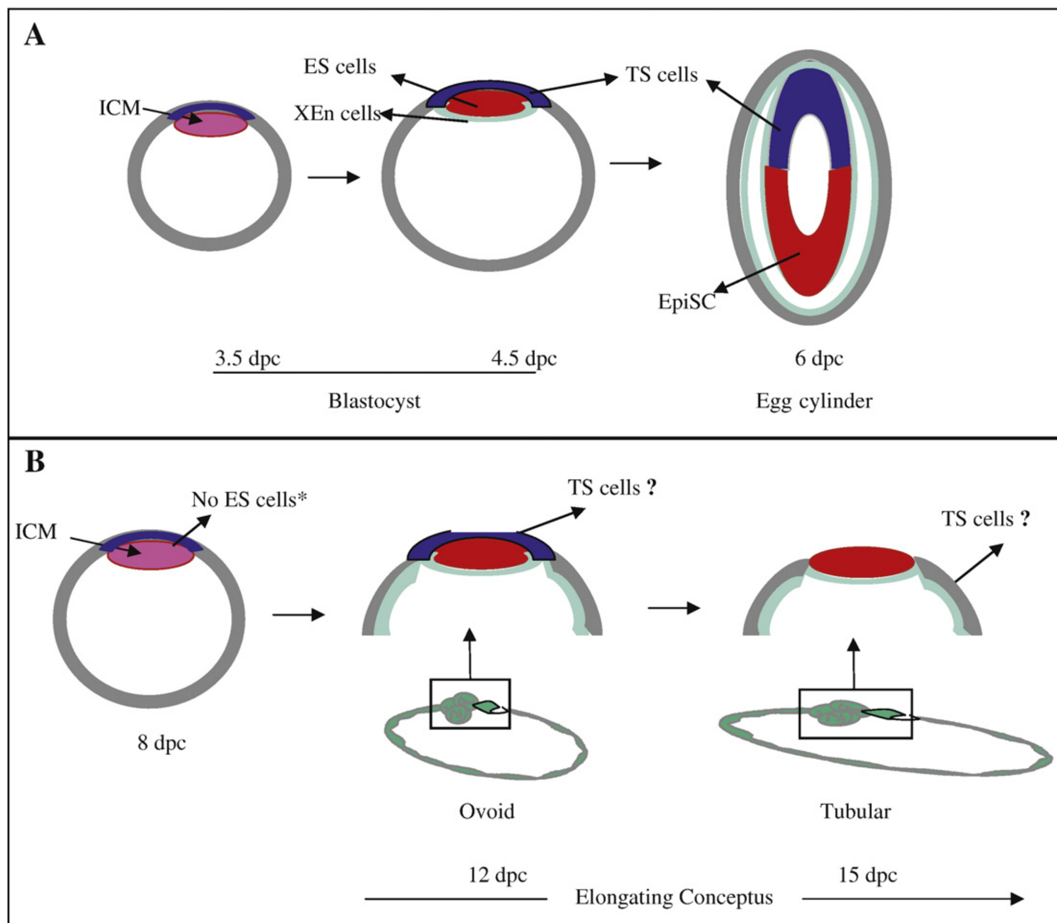


Fig. 1. Changes in the morphology in the mouse embryo (A) during early post-implantation development and in bovine embryo (B) during pre-implantation development. Embryologically equivalent stages are depicted. The tissues of origin of the different embryonic stem cells are shown. These simplified drawings do not take into account the mesoderm layer present in the bovine embryo at tubular stage (Eakin and Behringer, 2004) and are not represented at scale. *Up to now, no true ES cells have been isolated from bovine embryos. In dark blue, polar trophectoderm (then mouse extra-embryonic ectoderm and ruminant Rauber's layer). In gray, mural trophectoderm (then mouse giant cells) and bovine trophoblast (then bovine binucleated cells). In light blue, primitive endoderm, then visceral and parietal endoderm. In red, epiblast.

Bmp4, a TGF-β family growth factor for the early epiblast and then involved in the mesoderm formation (see Fig. 2, Gotoh N., personal communication; Gotoh et al., 2005; Winnier et al., 1995). *Cdx2* is expressed in the proliferating trophoblast in the mouse, and disappears when this tissue further differentiates into derivatives such as giant cells or spongiotrophoblasts in the placenta (Simmons and Cross, 2005). Whether its role is to directly control trophoblast cell proliferation or to inhibit terminal differentiation remains however to be determined.

Interaction of the trophoblast with the epiblast and its role in the growth and patterning of the mouse embryo

An exquisitely regulated network of interactions takes place between the epiblast and the overlying trophoblast layer in the mouse (Fig. 2) and is essential for the growth and patterning of the embryo as well as the prevention of precocious differentiation of the Exe (Guzman-Ayala et al., 2004). The role of different growth factors belonging to the FGF and TGF-β families are central actors in this crosstalk (Table 1). First, in the blastocyst, *Fgf4* is expressed in the ICM and early epiblast, and its receptor *Fgfr2* is mainly found in the trophoblast (weak expression in the ICM) (Arman et al., 1998; Feldman et al., 1995). Expression of *Fgfr2* by TE cells and the secretion of Fgf4 by ICM cells are necessary to maintain a proliferating status in the polar trophoblast (Arman et al., 1998; Chai et al., 1998). The Fgf signaling pathway leads to activation of Erk1/2 signaling molecules by phosphorylation. P-Erk1/2 has been detected in the blastocyst and then in the Exe, proximal to the epiblast, in addition to scattered cells of the epiblast (Corson et al., 2003; Wang et al., 2004). More specifically, Erk2 has been shown to be essential for trophoblast maintenance as mutants deficient for *Erk2* do not form an Exe (Saba-El-Leil et al., 2003). Although not directly shown in the mouse, Fgf signaling through MapK activation stimulates the expression of *Cdx* genes in Xenopus (Keenan et al., 2006). It is, therefore, probable that P-Erk2 activates the transcription of *Cdx2* in the mouse trophoblast.

Nodal, encoding a member of the TGF-β family which shares receptors and downstream effectors with Activin, starts to be expressed in the ICM of blastocyst and then in the epiblast of early post-implantation embryos (Takaoka et al., 2006; Varlet et al., 1997). Later it will be expressed in the spongiotrophoblasts of the placenta after E10 (Ma et al., 2001). Nodal is active both in the epiblast and in the Exe. Interestingly, its activity in the epiblast requires its cleavage by Spc proteases that are secreted by the Exe (Beck et al., 2002; Ben-Haim et al., 2006; Brennan et al., 2001). Both Nodal and Fgf signaling are essential to maintain the expression of *Cdx2*, *Eomes* and *Esrrb* in the Exe (Guzman-Ayala et al., 2004). In their absence, the Exe starts to express differentiation genes such as *Ascl2* (*Mash2*), a marker of spongiotrophoblasts, one of the trophoblast derivatives (Guillemot et al., 1994).

Fig. 2. The crosstalk between the epiblast and the extra-embryonic ectoderm patterns the epiblast and maintains a TS cell niche in the Exe. From Guzman-Ayala et al. (2004); Ben-Haim et al. (2006). Nodal p, m = precursor and mature form of Nodal, respectively. The simple arrows indicate gene activation. Double thick arrows mean ligand–receptor interaction.

Uterine decidual cells surrounding the early implanted embryos are another source of growth factors, as they secrete two members of the TGF-β superfamily, Activin A and Tgf-β1 (Feijen et al., 1994; Manova et al., 1992). The heterodimeric receptors for both Nodal and Activin, *Acvr1b* (*Alk4*) and *Acvr2b* (*ActRIIB*), have been shown to be expressed in the epiblast and Exe after E5.5 (Feijen et al., 1994; Gu et al., 1998). Moreover, the presence of activated downstream effectors of the Nodal/Activin pathway (PSmad2 and PSmad3) in the blastocyst and in the entire post-implantation embryos from E5 to E7 clearly supports the view that the whole signaling pathway is active *in vivo* (Gao et al., 2003; James et al., 2005).

Development of the trophoblast in other species: the ruminant embryo as another model?

The ruminant embryo gastrulates well ahead of implantation and does not give rise to a cup-shaped embryo but to a flat disc within a long conceptus (Fig. 1B). In this species as well as in other ungulates, the trophoblast elongates dramatically up to the onset of implantation (pig: Carnegie et al., 1985; cow: Chang, 1952; sheep: Stroband et al., 1984). The initially round-shaped embryo becomes ovoid, then tubular and at last filamentous. This incredibly long (more than 15 cm in the cow) trophoblast starts to form loose contacts with several points of the uterus, which signals the onset of implantation. The epiblast develops as a disc and is initially overlaid by a layer of cells called Rauber's layer, anatomical equivalent of the mouse polar trophoblast, which disappears soon after the elongation has started (Betteridge and Fléchon, 1988; Greenstein et al., 1958).

The trophoblast which elongates in the bovine embryo is the anatomical equivalent of the mouse mural trophoblast so that the contact between trophoblast and epiblast is restricted to a bordering ring of cells (see Fig. 1B). In this case, it seems unlikely that the proliferation in the elongating trophoblast could be only sustained by factors emanating from the ICM cells. Growth and patterning should be regulated differently between rodents and ruminants, either by different processes or similar processes but different gene networks. Interestingly, the pluripotent marker *Oct-4* is expressed in the mural trophoblast up to the ovoid stage (Degrelle et al., 2005; van Eijk et al., 1999). The bovine trophoblast layer which covers the embryonic disc maintains the expression of two markers of pluripotent embryonic cells, *Oct-4* and *Nanog*, but does not show any expression of *Eomes* while *Cdx2* is only weakly expressed (Degrelle et al., 2005). As expected from the mutual inhibition of expression of *Oct-4* and *Cdx2* once TE and ICM lineages are established, the latter is hardly detectable in the bovine polar trophoblast before its disappearance. In the mouse, apoptosis is increased in outer cells of the *Cdx2*-null mutant blastocyst (Strumpf et al., 2005), therefore, down-regulation of Cdx2 in the bovine polar trophoblast may be the reason of the degeneration of this cell layer. Alternatively, Cdx2 may not be an essential marker of proliferating trophoblast in non-rodents species. In the rhesus monkey blastocyst, no staining has been detected in the trophoblast using an antibody against Cdx2 (Vandevoort et al., 2007).

The mechanisms sustaining the extensive growth of the trophoblast in ruminants have not yet been directly studied. However, recent analyses of the transcriptome at the early elongation stages have revealed in ungulates (cow, sheep, pig) the expression of gene sets related to cell proliferation (reviewed in Blomberg et al., 2008; Hue et al., 2007). Ongoing studies in the cow aim at establishing whether trophoblast proliferation is kept constant through elongation, restricted to a ring of cells around the embryonic disc (by analogy with the mouse model) or spread out through the whole trophoblast as recently suggested on porcine tubular embryos (Blomberg et al., 2006). Even though elongation clearly depends on uterine secretions, as evidenced in sheep and cows using different physiological models

(Fléchon et al., 1986; Gray et al., 2001; Heyman et al., 1984), the molecular cascades downstream of these secretions wait for being deciphered *in vivo*.

The differences underlined here between ruminants and rodents, as well as the relatively easy access to early gastrulating embryos make the cow an interesting and likely alternative model for the study of trophoblast development and proliferation in mammals.

Trophoblast stem cells, a model for trophoblast growth and differentiation

Cell proliferation *in vivo* is supposedly sustained by a pool of stem cells able to self-renew and to give rise to rapidly proliferating progenitors. As for the mouse trophoblast, it is first formed at 3.5 dpc while the placenta is fully functional at 13.5 dpc. During this period of time, the tissue has to maintain a proliferating compartment in order to be able to provide all the trophoblastic cells of the placenta, such as: trophoblast giant cells, syncytiotrophoblasts, glycogen cells, spongiotrophoblasts (Simmons and Cross, 2005). In this regard it was not surprising that the team of Janet Rossant demonstrated in 1998 that trophoblast stem cells could be isolated from mouse blastocysts or extra-embryonic ectoderm of post-implantation embryos (Fig. 1A; Tanaka et al., 1998). They are unspecialized; they have the ability to self-renew indefinitely and *in vivo* to participate to the development of the placenta and thus were called trophoblast stem cells according to the NIH definition of a stem cell (http://stemcells.nih.gov/info/basics/basics2.asp). Recently, trophoblast stem (TS) cells have been shown to be able to rescue the placental defect of *Socs3*-deficient embryos (Takahashi et al., 2006). The mutant placenta displays an increased differentiation towards secondary trophoblast giant cells at the expense of the precursor population in the spongiotrophoblast. However, *Socs3*-deficient embryos are by themselves able to survive until E11 and thus start to form a placenta. It is yet not known whether TS cells could rescue an earlier defect affecting the formation of the extra-embryonic ectoderm. It means that the *in vivo* multipotency of TS cells, which is the ability to autonomously build a placenta, has not yet been demonstrated.

For their self-renewal and to keep them undifferentiated (see Fig. 3), TS cells are under the strict control of FGF4, heparin and medium conditioned by inactivated foetal fibroblasts (Tanaka et al., 1998). This conditioned medium can be replaced by ActivinA or TGF-β1 (Erlebacher et al., 2004). Under these conditions, they grow as flat epithelial colonies (Figs. 4A, B) and express many genes, the expression of which being essential for trophoblast growth and maintenance and TS derivation (see Table 1), such as: *Cdx2* (Fig. 4D), *Eomes*, *Esrrb* and *Fgfr2* (Tanaka et al., 1998). They also express *Sox2* and *Foxd3*. As other cells with rapid doubling time, nearly half of the cell population is in S-phase (46%, Fig. 4C and Rielland et al., in preparation). Fgf4-activated Erk2 signaling pathway (Fig. 4E) has been shown to inhibit differentiation and apoptosis in TS cells (Saba-El-Leil et al., 2003; Yang et al., 2006). By contrast, removal of FGF4 or Activin blocks self-renewal and leads to the down-regulation of *Cdx2*, *Esrrb* and *Eomes* (Erlebacher et al., 2004). Without both factors, TS cells readily differentiate and express markers of giant cells such as *Prl3d1* (Pl-1) and *Hand-1* (Riley et al., 1998; Scott et al., 2000), of spongiotrophoblasts such as *Tpbp2* (Lescisin et al., 1988) and *Ascl2*, and of syncytiotrophoblasts *Gcm1* (Hemberger et al., 2004; Hughes et al., 2004; Tanaka et al., 1998). The differentiated derivatives are apparently functionally equivalent to their *in vivo* counterparts: the giant cells display the migratory behaviour characteristic of invasive cells (Hemberger et al., 2004; Yan et al., 2001).

In ungulates, the huge elongation of the trophoblast also raises the issue of the existence of stem cells. There have been different trophoblastic cell lines established from goat, cow or pig embryos isolated before or after implantation. These cells can grow continuously in culture and are characterised as epithelial cells (cell polarity,

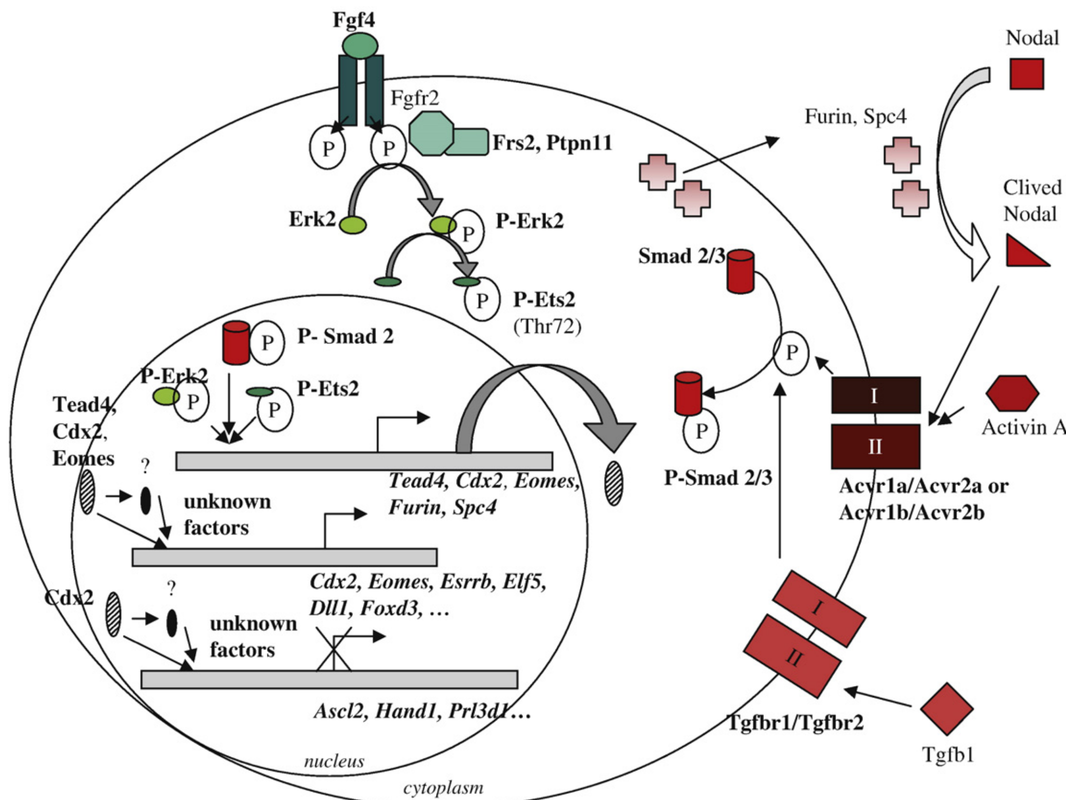


Fig. 3. Signal transduction controlling self-renewal in mouse TS cells. The link between the genes listed as transcriptionally regulated and the indicated transcription factors such as Cdx2, Eomes, P-Erk2, P-Smad2/3 … may be direct or indirect.

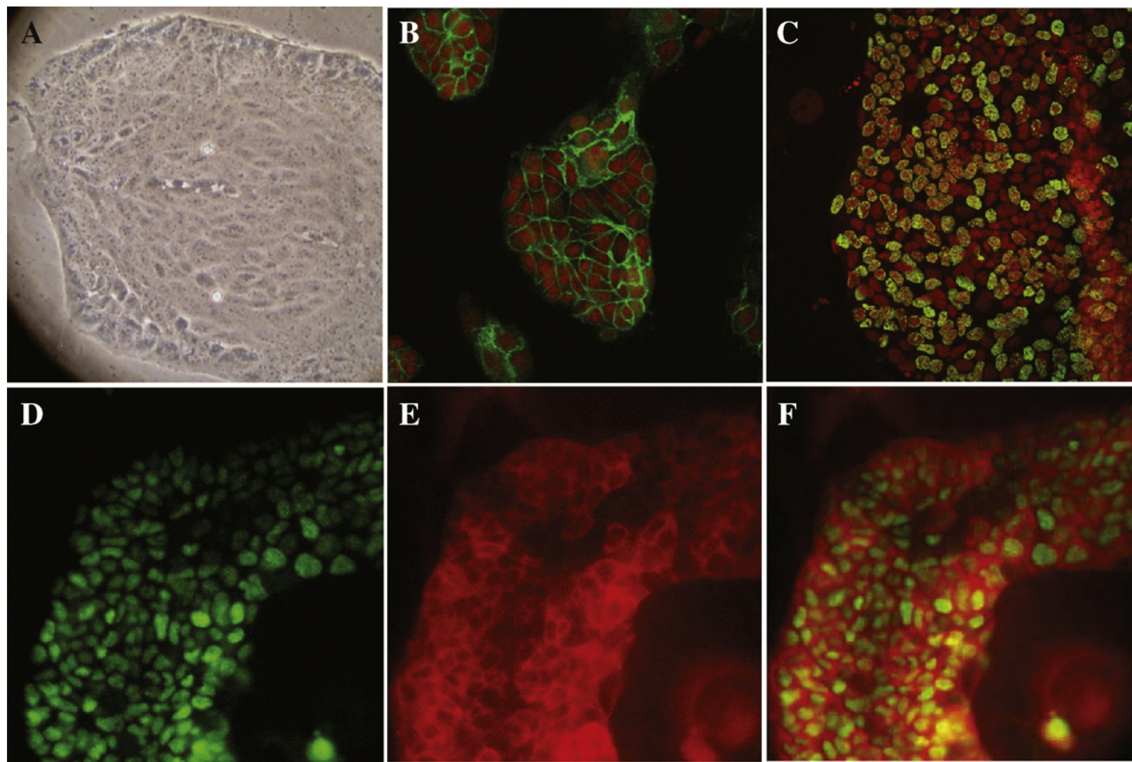


Fig. 4. Morphology and expression of typical markers of mouse TS cells. (A) Aspect of a TS cell colony under phase-contrast microscopy. (B) Expression of β-catenin showing that TS cells are epithelial cells. (C) High rate of proliferation: more than 60% of the cells are already BrdU positive after only 10 min of BrdU incorporation. (D–F) Double immuno-staining of Cdx2 (D) and P-Erk1/2 (E). All TS cells are positive for both markers (merge on panel F).

tight junctions or apical microvilli) with morphological or biochemical properties equivalent to *in vivo* trophoblast cells, such as secretion of IFN-τ or γ: caprine HTS-1: (Miyazaki et al., 2002); porcine TE1 and TB: (Fléchon et al., 1995; La Bonnardiere et al., 2002); bovine CT-1 and BT-1: (Shimada et al., 2001; Talbot et al., 2000). Trophoblast cell lines were also derived from sheep conceptuses at different stages prior to implantation (Liszewska E, submitted) or at one late elongating stage (Dunlap et al., 2006). The bovine BT-1 trophoblast cell line is the best characterised one. The cells grow in medium containing serum and fibroblast conditioned medium, but no added growth factors. In contrast to mouse TS cell culture requirements, such conditioned medium, although enhancing proliferation of BT-1 cells, is not strictly required for their self-renewal (Shimada et al., 2001). BT-1 cells express *Bmp4*, *Fgfr2* and *Oct-4*, but not *Eomes* (Hashizume et al., 2006; Ushizawa et al., 2005) and at the same time, markers of differentiated trophoblast cells such as placental lactogen or *PAG-1* (for Pregnancy-Associated Proteins). In ruminants, the only differentiated cell type derived from trophoblast cells is bi-nucleate cells (Cross et al., 2003). *In vivo*, prior to implantation, mono-nucleate trophoblast cells differentiate into bi-nucleate cells by acytokinesis and probable endoreplication (Klisch et al., 1999; Wathes and Wooding, 1980). As recently evidenced this process likely involves oncoproteins encoded by the Env proteins of endogenous retroviruses, expressed in trophoblast mono-nucleate cells once elongation has started (Dunlap et al., 2006). *In vitro*, the bovine trophoblast cell line BT1 spontaneously differentiates into bi-nucleate cells capable of *Prl3d1* expression when they are plated on collagen (Terada et al., 2002). So in this bovine cell line, there is an intrinsic balance (not dependent on exogenous factors) between proliferation and differentiation. Due to these characteristics such trophoblast cell lines are unlikely to be true stem cells. So far, they are more immortalised cells alike.

Nevertheless, if trophoblast proliferation in these species do not rely on TS cells, the question is even more puzzling: how, then, is this proliferation sustained? One possibility might be through the long lasting expression of epiblast specific genes to ensure these cells a durable undifferentiated state (Degrelle et al., 2005), another could be that cellular oncoproteins favour a fairly autonomous cell proliferation (enJSRV: Dunlap et al., 2006) and another that proliferation depends on uterine secretions. Sorting out such hypotheses awaits further studies.

In vivo localization of putative TS cells

It has been shown that mouse trophoblast stem (TS) cells could be derived from embryos until the 11-somite stage and that the proportion of cells with a TS cell potential (*id est*, able to form TS colonies when transplanted *in vitro*) increased until the first-somite stages (Uy et al., 2002).

All the genes listed in Table 1 are expressed *in vivo* in the trophoblast lineage of the blastocyst, in the Exe, and/or in the chorion. They delineate a micro-environment where cells with TS potential are supposed to be maintained (Guzman-Ayala et al., 2004) (Fig. 5). Most of these markers are co-expressed in the well delimitated region of the Exe proximal to the epiblast, such as *Cdx2*, P-Erk1/2, *Eomes*, *Esrrb*, and *Spc* proteases (Beck et al., 2002; Corson et al., 2003; Guzman-Ayala et al., 2004).

Strikingly, the region defined molecularly is partially in discrepancy with the detailed embryological analysis of the spatial localisation of putative TS progenitors made by Uy et al. (2002). They showed that cells able to give rise to TS cell colonies and then TS cell lines after transplantation *in vitro* are present in the entire Exe region except in the ectoplacental cone/Exe transition tissue. In addition, these TS progenitors do not account for more than 1% to 2% of the total Exe cells, whereas in the proximal Exe, all cells apparently express the same set of markers. *Fgfr2* is expressed in the entire Exe, but its downstream signaling pathway is probably activated only inside the proximal Exe where P-Erk1/2 has been detected (Corson et al., 2003). By contrast, the Nodal/Activin pathway is activated in the

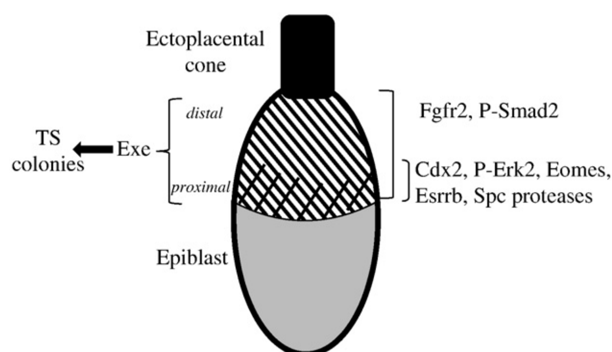


Fig. 5. *In vivo* spatial localization of factors known to be expressed in mouse TS cells (schematic drawing of the egg cylinder at 6.5 dpc).

whole Exe, as indicated by the detection of PSmad2 (Gao et al., 2003). However, experiments using Exe explants have shown that only one active signaling pathway is not sufficient to maintain expression of genes such as *Cdx2* and *Eomes* and to prevent differentiation (Guzman-Ayala et al., 2004). One possibility could be that among cells outside the proximal Exe could be some dormant progenitors of TS cells that would be stimulated to grow by the *in vitro* culture conditions. Alternatively, other important genes with a pattern of expression specifically restricted to proliferating TS cells may exist and not have been unravelled yet.

Stem cells *in vivo* are usually considered to reside into the niche where they self-renew very slowly, under the tight control of the supporting cells (Li and Xie, 2005; Moore and Lemischka, 2006). In this context, the extensive growth of the bovine trophoblast *in vivo* could be due to the rapid but finite proliferation of already committed progenitors. Alternatively, a phenomenon such as a community effect could play a role in the ruminant trophoblast growth so that this tissue would only grow as a whole, the signal to proliferate being transduced to a group of cells depending on how they respond to uterine secretions and interact with the underlying extra-embryonic endoderm which displays striking features during elongation (Fléchon, 2007). Such community effect has been shown to exist during morphogenesis or within a tumour (Gurdon, 1988; Jouanneau et al., 1994). In our opinion, a better characterisation of the bovine trophoblast *in vivo*, the trophoblast cell lines established *in vitro* as well as new attempts to derive bovine TS cells appear necessary to better understand (i) the nature of the biological processes underlying the trophoblast growth in ruminants and (ii) the differences in these processes between ruminants and rodents.

Abnormal development of the trophoblast following nuclear transfer

Reprogramming of the foreign chromatin occurs through still poorly known processes involving epigenetic and chromatin remodelling and eventually leads to the development of an embryo up to birth and adulthood. Although being able to give rise to healthy adults, nuclear transfer (NT) in mammals is a very inefficient process. We have previously shown that, although the epiblast apparently differentiates normally *in vivo*, half of the embryos at early post-implantation stages already exhibit morphological abnormalities that can be classified in a few recurrent types, including embryos with a rounded shape instead of being elongated and embryos with an enlarged Exe region, at the expense of the embryonic region (Jouneau et al., 2006). Inclusion of normal ES or ICM cells (chimeras) does not rescue the NT embryos, whereas tetraploid cells do, indicating that the trophoblast was the primary source of the defects. Later at foetal stages, the extra-embryonic region grows apparently without control, resulting in an oversized placenta in all surviving foetuses (Ono and Kono, 2006; Tanaka et al., 2001). Interestingly, such abnormal growth of the placenta has also been observed after NT in cattle (Constant et al., 2006). This study suggests that foetal abnormalities may be a consequence rather than a cause of the defect in placental growth and development.

Studies at blastocyst stage and early post-implantation mouse embryos have indicated that the trophoblast lineage is correctly specified, as suggested by the correct expression of *Cdx2* (Jouneau et al., 2006; Kishigami et al., 2006). TS cell derivation from NT embryos can be used as a cellular model to study the regulation of trophoblast proliferation and differentiation *in vitro*. Indeed, we have been able to establish TS cell lines from ES NT blastocysts with higher derivation efficiency than from fertilised blastocysts (Rielland et al., in preparation). It suggests that some epigenetic changes have occurred in the trophoblast of the NT blastocyst that confers the TS cells some modified features of proliferation. The re-methylation of the genome occurs at late blastocyst stage *in vivo* and this process ends up with the trophoblast being hypo-methylated compared to the epiblast (Monk et al., 1987; Santos et al., 2002). At least in the bovine, it has been shown that the trophoblast of NT blastocysts remains more methylated than control (Kang et al., 2003). Key genes for pluripotency maintenance in the epiblast such as *Oct-4* and *Nanog* are stably silenced by methylation in the trophoblast and can be re-activated in cells treated with demethylating agent (Hattori et al., 2004, 2007). Aberrant expression of some specific genes may account for the abnormal development of the trophoblast of clones (Degrelle et al., in preparation). Perturbation of the parental imprint has often been claimed as one of the cause of cloning defects (Rideout et al., 2001; Yang, 2007). It is true that many imprinted genes are expressed in the placenta and involved in its development. Genome-wide transcriptome analyses of placentas from cloned foetuses have revealed a set of abnormally expressed genes, imprinted (Humpherys et al., 2002; Singh et al., 2004), or not (Everts et al., 2008). However, Tanaka and colleagues, by using *in situ* hybridisation and northern blot couldn't show any obvious deregulation of the transcription of some imprinted genes in NT placentas (Tanaka et al., 2001). In addition, little is known about their expression and role in the trophoblast of the early embryo. Our opinion is that different genes may be involved in the abnormal phenotypes of NT trophoblast and that the use of trophoblast stem cells may help to unravel the epigenetic defects affecting development of clones.

Trophoblast cell lines from cloned blastocysts and controls have been derived from bovine blastocysts prior to elongation with no statistical difference in (i) the success rate of establishing them between clones and controls and (ii) the morphology, growth and maintenance of these lines (Talbot et al., 2007), the main difference residing in a reduced IFN-tau production (Talbot et al., 2008). Whether these data reinforce the differences between rodents and ruminants with regard to trophoblast development or the difference between long term trophoblast and TS cultures awaits further studies.

Conclusion and perspectives

The study of different kinds of stem cells derived from the embryo such as TS cells and ES cells and comparison between different species will provide information about how growth and stemness are controlled within an embryo. Molecular determinants of stemness are clearly different for TS and ES cells, exemplified by the opposite role of Cdx2 and Oct-4 in these cells, whereas some common gene expression may exist, such as for *Sox2* and *Foxd3*. Many studies have tried to bring to light a molecular portrait of a stem cell prototype, but it seems that stemness cannot be defined easily by a set of molecular determinants (Evsikov and Solter, 2003; Fortunel et al., 2003). Rather, the "stem state" (Zipori, 2004) may result from different combination of factors and signaling pathways interacting within the frame of specific chromatin conformation. By instance, ES cells have been shown to exhibit very peculiar local structures of chromatin that

reflect their molecular plasticity (Bernstein et al., 2006; Meshorer et al., 2006). Interestingly, the dependence of TS cells on both Fgf and Activin is similar to that of the epiblast stem cells, or EpiSC (Brons et al., 2007; Tesar et al., 2007) and also of human embryonic stem (ES) cells (Vallier et al., 2005). It is clear that the cell is able to read growth factor signaling differently depending of the interplay of downstream effectors. In TS and hES/EpiSc, although the molecular targets are different (*Cdx2* for TS and *Oct-4* for the other embryonic cells), both signaling pathways converge on inducing self-renewal and proliferation. The embryo itself provides the niche for EpiSc and TS cells: Fgf4 and Nodal are provided by the epiblast, later other Fgf such as Fgf8 and Fgf5 are produced by the epiblast and the primitive streak (Crossley et al., 1995; Hebert et al., 1991). After being processed by the proteases produced by the Exe, Nodal can signal back to the epiblast cells. For hES cells, it has been shown recently that they create their own niche as the surrounding differentiated cells produce IGF1, which is mitogenic for the stem cells (Bendall et al., 2007). In bovine, no true ES cells have been derived so far, despite many attempts in different labs (reviewed in Keefer et al., 2007), and even no TS nor TS-like cells. Crucial information is still missing, such as the signaling pathways active in bovine epiblast and trophoblast cells. The peculiar structure of the ungulate embryo, with its embryonic disc open to the uterine medium and the surrounding trophoblast elongating dramatically makes it unlikely that the niche can be completely provided by the embryo itself. Therefore, the source of growth factors that would control the maintenance of proliferation and stemness in both populations of cells might be looked for in the uterine environment. Moreover, the presence of *Oct-4* expression detected in the bovine trophoblast cells blurs the clear separation between ES and TS cells that has been depicted in the mouse. Further studies of the molecular determinants of the trophoblast and epiblast lineage in the bovine embryo will be necessary to clarify this paradox. Molecular comparisons between bovine trophoblast cell lines and mouse TS cells will also provide information about similarities and differences in the establishment and maintenance of trophoblast proliferation among mammals.

References

Arman, E., Haffner-Krausz, R., Chen, Y., Heath, J.K., Lonai, P., 1998. Targeted disruption of fibroblast growth factor (FGF) receptor 2 suggests a role for FGF signaling in pregastrulation mammalian development. Proc. Natl. Acad. Sci. U. S. A. 95, 5082–5087.

Avilion, A.A., 2003. Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev. 17, 126–140.

Beck, F., Erler, T., Russell, A., James, R., 1995. Expression of Cdx-2 in the mouse embryo and placenta: possible role in patterning of the extra-embryonic membranes. Dev. Dyn. 204, 219–227.

Beck, S., Le Good, J.A., Guzman, M., Ben Haim, N., Roy, K., Beermann, F., Constam, D.B., 2002. Extraembryonic proteases regulate Nodal signaling during gastrulation. Nat. Cell Biol. 4, 981–985.

Bendall, S.C., Stewart, M.H., Menendez, P., George, D., Vijayaragavan, K., Werbowetski-Ogilvie, T., Ramos-Mejia, V., Rouleau, A., Yang, J., Bosse, M., Lajoie, G., Bhatia, M., 2007. IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro. Nature 448, 1015–1021.

Ben-Haim, N., Lu, C., Guzman-Ayala, M., Pescatore, L., Mesnard, D., Bischofberger, M., Naef, F., Robertson, E.J., Constam, D.B., 2006. The nodal precursor acting via activin receptors induces mesoderm by maintaining a source of its convertases and BMP4. Dev. Cell 11, 313–323.

Bernstein, B.E., Mikkelsen, T.S., Xie, X., Kamal, M., Huebert, D.J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., Jaenish, R., Wagschal, A., Feil, R., Schreiber, S.L., Lander, E.S., 2006. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125, 315–326.

Betteridge, K.J., Fléchon, J.E., 1988. The anatomy and physiology of pre-attachment bovine embryos. Biol. Reprod. 74, 1007–1015.

Blomberg, L.A., Garrett, W.M., Guillomot, M., Miles, J.R., Sonstegard, T.S., Van Tassell, C.P., Zuelke, K.A., 2006. Transcriptome profiling of the tubular porcine conceptus identifies the differential regulation of growth and developmentally associated genes. Mol. Reprod. Dev. 73, 1491–1502.

Blomberg, L., Hashizume, K., Viebahn, C., 2008. Blastocyst elongation, trophoblastic differentiation, and embryonic pattern formation: focus on Mammalian Embryogenomics. Reproduction 135, 181–195.

Brennan, J., Lu, C.C., Norris, D.P., Rodriguez, T.A., Beddington, R.S., Robertson, E.J., 2001. Nodal signaling in the epiblast patterns the early mouse embryo. Nature 411, 965–969.

Brons, I.G.M., Smithers, L.E., Trotter, M.W.B., Rugg-Gunn, P., Sun, B., Chuva de Sousa Lopes, S.M., Howlett, S.K., Clarkson, A., Ahrlund-Richter, L., Pedersen, R.A., Vallier, L., 2007. Derivation of pluripotent epiblast stem cells from mammalian embryos. Nature 448, 191–195.

Carnegie, J.A., McCully, M.E., Robertson, H.A., 1985. The early development of the sheep trophoblast and the involvement of cell death. Am. J. Anat. 174, 471–488.

Chai, N., Patel, Y., Jacobson, K., McMahon, J., McMahon, A., Rappolee, D.A., 1998. FGF is an essential regulator of the fifth cell division in preimplantation mouse embryos. Dev. Biol. 198, 105–115.

Chang, M.C., 1952. Development of bovine blastocyst with a note on implantation. Anat. Rec. 113, 143–161.

Chang, H., Brown, C.W., Matzuk, M.M., 2002. Genetic analysis of the mammalian transforming growth factor-beta superfamily. Endocr. Rev. 23, 787–823.

Ciruna, B.G., Rossant, J., 1999. Expression of the T-box gene eomesodermin during early mouse development. Mech. Dev. 81, 199–203.

Constant, F., Guillomot, M., Heyman, Y., Vignon, X., Laigre, P., Servely, J.L., Renard, J.P., Chavatte-Palmer, P., 2006. Large offspring or large placenta syndrome? Morphometric analysis of late gestation bovine placentomes from somatic nuclear transfer pregnancies complicated by hydrallantois. Biol. Reprod. 75, 122–130.

Corson, L.B., Yamanaka, Y., Lai, K.M., Rossant, J., 2003. Spatial and temporal patterns of ERK signaling during mouse embryogenesis. Development 130, 4527–4537.

Cross, J.C., Baczyk, D., Dobric, N., Hemberger, M., Hughes, M., Simmons, D.G., Yamamoto, H., Kingdom, J.C., 2003. Genes, development and evolution of the placenta. Placenta 24, 123–130.

Crossley, P.H., Martin, G.R., Feijen, A., Goumans, M.J., van den Eijnden-van Raaij, A.J., 1995. The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. Development 121, 439–451.

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

Dietrich, J.E., Hiiragi, T., 2007. Stochastic patterning in the mouse pre-implantation embryo. Development 134, 4219–4231.

Donnison, M., Beaton, A., Davey, H.W., Broadhurst, R., L'Huillier, P., Pfeffer, P.L., 2005. Loss of the extraembryonic ectoderm in Elf5 mutants leads to defects in embryonic patterning. Development 132, 2299–2308.

Dunlap, K.A., Palmarini, M., Varela, M., Burghardt, R.C., Hayashi, K., Farmer, J.L., Spencer, T.E., 2006. Endogenous retroviruses regulate periimplantation placental growth and differentiation. Proc. Natl. Acad. Sci. U. S. A. 103, 14390–14395.

Eakin, G.S., Behringer, R., 2004. Gastrulation in other mammals and humans. In: Stern, C. (Ed.), "Gastrulation: from Cells to Embryo". Cold Spring Harbor Laboratory Press.

Erlebacher, A., Price, K.A., Glimcher, L.H., 2004. Maintenance of mouse trophoblast stem cell proliferation by TGF-beta/activin. Dev. Biol. 275, 158–169.

Everts, R.E., Chavatte-Palmer, P., Razzak, A., Hue, I., Green, C.A., Oliveira, R., Vignon, X., Rodriguez-Zas, S.L., Tian, X.C., Yang, X., Renard, J.P., Lewin, H.A., 2008. Aberrant gene expression patterns in placentomes are associated with phenotypically normal and abnormal cattle cloned by somatic cell nuclear transfer. Physiol. Genomics 33, 65–67.

Evsikov, A.V., Solter, D., 2003. Comment on "'Stemness': Transcriptional Profiling of Embryonic and Adult Stem Cells" and "A Stem Cell Molecular Signature" (II. Science 302, 393c.

Feijen, A., Goumans, M.J., van den Eijnden-van Raaij, A.J., 1994. Expression of activin subunits, activin receptors and follistatin in postimplantation mouse embryos suggests specific developmental functions for different activins. Development 120, 3621–3637.

Feldman, B., Poueymirou, W., Papaioannou, V.E., DeChiara, T.M., Goldfarb, M., 1995. Requirement of FGF-4 for postimplantation mouse development. Science 267, 246–249.

Fléchon, J.E., Guillomot, M., Charlier, M., Fléchon, B., Martal, J., 1986. Experimental studies on the elongation of the ewe blastocyst. Reprod. Nutr. Dev. 26, 1017–1024.

Fléchon, J.E., Laurie, S., Notarianni, E., 1995. Isolation and characterization of a feeder-dependent, porcine trophectoderm cell line obtained from a 9-day blastocyst. Placenta 16, 568–643.

Fortunel, N.O., Otu, H.H., Ng, H.-H., Chen, J., Mu, X., Chevassut, T., Li, X., Joseph, M., Bailey, C., Hatzfeld, J.A., Hatzfeld, A., Usta, F., Vega, V.B., Long, P.M., Libermann, T.A., Lim, B., 2003. Comment on "'Stemness': Transcriptional Profiling of Embryonic and Adult Stem Cells" and "A Stem Cell Molecular Signature" (I. Science 302, 393b.

Gao, S., McGarry, M., Priddle, H., Ferrier, T., Gasparrini, B., Fletcher, J., Harkness, L., De Sousa, P., McWhir, J., Wilmut, I., 2003. Effects of donor oocytes and culture conditions on development of cloned mice embryos. Mol. Reprod. Dev. 66, 126–133.

Giguere, V., Yang, N., Segui, P., Evans, R.M., 1988. Identification of a new class of steroid hormone receptors. Nature 331, 91–94.

Godfrey, K.M., 2002. The role of the placenta in fetal programming—a review. Placenta 23 Suppl A, S20–S27.

Goldin, S.N., Papaioannou, V.E., 2003. Paracrine action of FGF4 during periimplantation development maintains trophectoderm and primitive endoderm. Genesis 36, 40–47.

Goldman, D.C., Hackenmiller, R., Nakayama, T., Sopory, S., Wong, C., Kulessa, H., Christian, J.L., 2006. Mutation of an upstream cleavage site in the BMP4 prodomain leads to tissue-specific loss of activity. Development 133, 1933–1942.

Gotoh, N., Manova, K., Tanaka, S., Murohashi, M., Hadari, Y., Lee, A., Hamada, Y., Hiroe, T., Ito, M., Kurihara, T., Nakazato, H., Shibuya, M., Lax, I., Lacy, E., Schlessinger, J., 2005. The docking protein FRS2{alpha} is an essential component of multiple fibroblast growth factor responses during early mouse development. Mol. Cell Biol. 25, 4105–4116.

Gray, C.A., Taylor, K.M., Ramsey, W.S., Hill, J.R., Bazer, F.W., Bartol, F.F., Spencer, T.E., 2001. Endometrial glands are required for preimplantation conceptus elongation and survival. Biol. Reprod. 64, 1608–1613.

Greenstein, J.S., Murray, R.W., Foley, R.C., 1958. Observations on the morphogenesis and histochemistry of the bovine preattachment placenta between 16 and 33 days of gestation. Anat. Rec. 132, 321–341.

Gu, Z., Nomura, M., Simpson, B.B., Lei, H., Feijen, A., van den Eijnden-van Raaij, J., Donahoe, P.K., Li, E., 1998. The type I activin receptor ActRIB is required for egg cylinder organization and gastrulation in the mouse. Genes Dev. 12, 844–857.
Guillemot, F., Nagy, A., Auerbach, A., Rossant, J., Joyner, A.L., 1994. Essential role of Mash-2 in extraembryonic development. Nature 371, 333–336.
Guo, R.J., Suh, E.R., Lynch, J.P., 2004. The role of Cdx proteins in intestinal development and cancer. Cancer Biol. Ther. 3, 593–601.
Gurdon, J.B., 1988. A community effect in animal development. Nature 336, 772–774.
Guzman-Ayala, M., Ben-Haim, N., Beck, S., Constam, D.B., 2004. Nodal protein processing and fibroblast growth factor 4 synergize to maintain a trophoblast stem cell microenvironment. Proc. Natl. Acad. Sci. U. S. A. 101, 15656–15660.
Hanna, L.A., Foreman, R.K., Tarasenko, I.A., Kessler, D.S., Labosky, P.A., 2002. Requirement for Foxd3 in maintaining pluripotent cells of the early mouse embryo. Genes Dev. 16, 2650–2661.
Hashizume, K., Shimada, A., Nakano, H., Takahashi, T., 2006. Bovine trophoblast cell culture systems: a technique to culture bovine trophoblast cells without feeder cells. Methods Mol. Med. 121, 179–188.
Hattori, N., Nishino, K., Ko, Y.-g., Hattori, N., Ohgane, J., Tanaka, S., Shiota, K., 2004. Epigenetic control of mouse Oct-4 gene expression in embryonic stem cells and trophoblast stem cells. J. Biol. Chem. 279, 17063–17069.
Hattori, N., Imao, Y., Nishino, K., Ohgane, J., Yagi, S., Tanaka, S., Shiota, K., 2007. Epigenetic regulation of Nanog gene in embryonic stem and trophoblast stem cells. Genes Cells 12, 387–396.
Hebert, J.M., Boyle, M., Martin, G.R., 1991. mRNA localization studies suggest that murine FGF-5 plays a role in gastrulation. Development 112, 407–415.
Hemberger, M., Hughes, M., Cross, J.C., 2004. Trophoblast stem cells differentiate in vitro into invasive trophoblast giant cells. Dev. Biol. 271, 362–371.
Heyman, Y., Camous, S., Fevre, J., Meziou, W., Martal, J., 1984. Maintenance of the corpus luteum after uterine transfer of trophoblastic vesicles to cyclic cows and ewes. J. Reprod. Fertil. 70, 533–540.
Honda, H., Motosugi, N., Nagai, T., Tanemura, M., Hiiragi, T., 2008. Computer simulation of emerging asymmetry in the mouse blastocyst. Development 135, 1407–1414.
Hue, I., Degrelle, S.A., Campion, E., Renard, J.P., 2007. Gene expression in elongating and gastrulating embryos from ruminants. Soc. Reprod. Fertil. Suppl. 64, 365–377.
Hughes, M., Dobric, N., Scott, I.C., Su, L., Starovic, M., St-Pierre, B., Egan, S.E., Kingdom, J. C., Cross, J.C., 2004. The Hand1, Stra13 and Gcm1 transcription factors override FGF signaling to promote terminal differentiation of trophoblast stem cells. Dev. Biol. 271, 26–37.
Humpherys, D., Eggan, K., Akutsu, H., Friedman, A., Hochedlinger, K., Yanagimachi, R., Lander, E.S., Golub, T.R., Jaenisch, R., 2002. Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei. Proc. Natl. Acad. Sci. U. S. A. 99, 12889–12894.
James, D., Levine, A.J., Besser, D., Hemmati-Brivanlou, A., 2005. TGF{beta}/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. Development 132, 1273–1282.
Jouanneau, J., Moens, G., Bourgeois, Y., Poupon, M.F., Thiery, J.P., 1994. A minority of carcinoma cells producing acidic fibroblast growth factor induces a community effect for tumor progression. Proc. Natl. Acad. Sci. U. S. A. 91, 286–290.
Jouneau, A., Zhou, Q., Camus, A., Brochard, V., Maulny, L., Collignon, J., Renard, J.P., 2006. Developmental abnormalities of NT mouse embryos appear early after implantation. Development 133, 1597–1607.
Kang, Y.K., Yeo, S., Kim, S.H., Koo, D.B., Park, J.S., Wee, G., Han, J.S., Oh, K.B., Lee, K.K., Han, Y.M., 2003. Precise recapitulation of methylation change in early cloned embryos. Mol. Reprod. Dev. 66, 32–37.
Keefer, C.L., Pant, D., Blomberg, L., Talbot, N.C., 2007. Challenges and prospects for the establishment of embryonic stem cell lines of domesticated ungulates. Anim. Reprod. Sci. 98, 147–168.
Keenan, I.D., Sharrard, R.M., Isaacs, H.V., 2006. FGF signal transduction and the regulation of Cdx gene expression. Dev. Biol. 299, 478–488.
Kishigami, S., Hikichi, T., Van Thuan, N., Ohta, H., Wakayama, S., Bui, H.T., Mizutani, E., Wakayama, T., 2006. Normal specification of the extraembryonic lineage after somatic nuclear transfer. FEBS Lett. 580, 1801–1806.
Klisch, K., Pfarrer, C., Schuler, G., Hoffmann, B., Leiser, R., 1999. Tripolar acytokinetic mitosis and formation of feto-maternal syncytia in the bovine placentome: different modes of the generation of multinuclear cells. Anat. Embryol. Berl. 200, 229–237.
La Bonnardiere, C., Fléchon, J.E., Battegay, S., Fléchon, B., Degrouard, J., Lefevre, F., 2002. Polarized porcine trophoblastic cell lines spontaneously secrete interferon-gamma. Placenta 23, 716–726.
Lescisin, K.R., Varmuza, S., Rossant, J., 1988. Isolation and characterization of a novel trophoblast-specific cDNA in the mouse. Genes Dev. 2, 1639–1646.
Li, L., Xie, T., 2005. Stem cell niche: structure and function. Annu. Rev. Cell Dev. Biol. 21, 605–631.
Liu, P., 1999. Requirement for Wnt3 in vertebrate axis formation. 22, 361–365.
Liu, J., Puscheck, E.E., Wang, F., Trostinskaia, A., Barisic, D., Maniere, G., Wygle, D., Zhong, W., Rings, E.H., Rappolee, D.A., 2004. Serine-threonine kinases and transcription factors active in signal transduction are detected at high levels of phosphorylation during mitosis in preimplantation embryos and trophoblast stem cells. Reproduction 128, 643–654.
Luo, J., Sladek, R., Bader, J.A., Matthyssen, A., Rossant, J., Giguere, V., 1997. Placental abnormalities in mouse embryos lacking the orphan nuclear receptor ERR-beta. Nature 388, 778–782.
Ma, G.T., Soloveva, V., Tzeng, S.J., Lowe, L.A., Pfendler, K.C., Iannaccone, P.M., Kuehn, M.R., Linzer, D.I., 2001. Nodal regulates trophoblast differentiation and placental development. Dev. Biol. 236, 124–135.
Manova, K., Paynton, B.V., Bachvarova, R.F., 1992. Expression of activins and TGF beta 1 and beta 2 RNAs in early postimplantation mouse embryos and uterine decidua. Mech. Dev. 36, 141–152.
Meshorer, E., Yellajoshula, D., George, E., Scambler, P.J., Brown, D.T., Misteli, T., 2006. Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. Dev. Cell 10, 105–116.
Miyazaki, H., Imai, M., Hirayama, T., Saburi, S., Tanaka, M., Maruyama, M., Matsuo, C., Meguro, H., Nishibashi, K., Inoue, F., Djiane, J., Gertler, A., Tachi, S., Imakawa, K., Tachi, C., 2002. Establishment of feeder-independent cloned caprine trophoblast cell line which expresses placental lactogen and interferon tau. Placenta 23, 613–630.
Monk, M., Boubelik, M., Lehnert, S., 1987. Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. Development 99, 371–382.
Moore, K.A., Lemischka, I.R., 2006. Stem cells and their niches. Science 311, 1880–1885.
Nishioka, N., Yamamoto, S., Kiyonari, H., Sato, H., Sawada, A., Ota, M., Nakao, K., Sasaki, H., 2008. Tead4 is required for specification of trophectoderm in pre-implantation mouse embryos. Mech. Dev. 125, 270–283.
Niwa, H., Toyooka, Y., Shimosato, D., Strumpf, D., Takahashi, K., Yagi, R., Rossant, J., 2005. Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. Cell 123, 917–929.
Oh, S.P., Li, E., 1997. The signaling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. Genes Dev. 11, 1812–1826.
Ono, Y., Kono, T., 2006. Irreversible barrier to the reprogramming of donor cells in cloning with mouse embryos and embryonic stem cells. Biol. Reprod. 75, 210–216.
Papadaki, C., Alexiou, M., Cecena, G., Verykokakis, M., Bilitou, A., Cross, J.C., Oshima, R.G., Mavrothalassitis, G., 2007. Transcriptional repressor Erf determines extraembryonic ectoderm differentiation. Mol. Cell Biol. 27, 5201–5213.
Pettersson, K., Svensson, K., Mattsson, R., Carlsson, B., Ohlsson, R., Berkenstam, A., 1996. Expression of a novel member of estrogen response element-binding nuclear receptors is restricted to the early stages of chorion formation during mouse embryogenesis. Mech. Dev. 54, 211–223.
Ralston, A., Rossant, J., 2008. Cdx2 acts downstream of cell polarization to cell-autonomously promote trophectoderm fate in the early mouse embryo. Dev. Biol. 313, 614–629.
Rideout 3rd, W.M., Eggan, K., Jaenisch, R., 2001. Nuclear cloning and epigenetic reprogramming of the genome. Science 293, 1093–1098.
Riley, P., Anson-Cartwright, L., Cross, J.C., 1998. The Hand1 bHLH transcription factor is essential for placentation and cardiac morphogenesis. Nat. Genet. 18, 271–275.
Rings, E.H., Boudreau, F., Taylor, J.K., Moffett, J., Suh, E.R., Traber, P.G., 2001. Phosphorylation of the serine 60 residue within the Cdx2 activation domain mediates its transactivation capacity. Gastroenterology 121, 1437–1450.
Russ, A.P., Wattler, S., Colledge, W.H., Aparicio, S.A., Carlton, M.B., Pearce, J.J., Barton, S.C., Surani, M.A., Ryan, K., Nehls, M.C., Wilson, V., Evans, M.J., 2000. Eomesodermin is required for mouse trophoblast development and mesoderm formation. Nature 404, 95–99.
Saba-El-Leil, M.K., Vella, F.D., Vernay, B., Voisin, L., Chen, L., Labrecque, N., Ang, S.L., Meloche, S., 2003. An essential function of the mitogen-activated protein kinase Erk2 in mouse trophoblast development. EMBO Rep. 4, 964–968.
Santos, F., Hendrich, B., Reik, W., Dean, W., 2002. Dynamic reprogramming of DNA methylation in the early mouse embryo. Dev. Biol. 241, 172–182.
Scott, I.C., Anson-Cartwright, L., Riley, P., Reda, D., Cross, J.C., 2000. The HAND1 basic helix–loop–helix transcription factor regulates trophoblast differentiation via multiple mechanisms. Mol. Cell Biol. 20, 530–541.
Shimada, A., Nakano, H., Takahashi, T., Imai, K., Hashizume, K., 2001. Isolation and characterization of a bovine blastocyst-derived trophoblastic cell line, BT-1: development of a culture system in the absence of feeder cell. Placenta 22, 652–662.
Simmons, D.G., Cross, J.C., 2005. Determinants of trophoblast lineage and cell subtype specification in the mouse placenta. Dev. Biol. 284, 12–24.
Singh, U., Fohn, L.E., Wakayama, T., Ohgane, J., Steinhoff, C., Lipkowitz, B., Schulz, R., Orth, A., Ropers, H.H., Behringer, R.R., Tanaka, S., Shiota, K., Yanagimachi, R., Nuber, U.A., Fundele, R., 2004. Different molecular mechanisms underlie placental overgrowth phenotypes caused by interspecies hybridization, cloning, and Esx1 mutation. Dev. Dyn. 230, 149–164.
Smith, S.L., Everts, R.E., Tian, X.C., Du, F., Sung, L.-Y., Rodriguez-Zas, S.L., Jeong, B.-S., Renard, J.-P., Lewin, H.A., 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.
Soares, M.L., Haraguchi, S., Torres-Padilla, M.E., Kalmar, T., Carpenter, L., Bell, G., Morrison, A., Ring, C.J., Clarke, N.J., Glover, D.M., Zernicka-Goetz, M., 2005. Functional studies of signaling pathways in peri-implantation development of the mouse embryo by RNAi. BMC Dev. Biol. 5, 28.
Song, J., Oh, S.P., Schrewe, H., Nomura, M., Lei, H., Okano, M., Gridley, T., Li, E., 1999. The type II activin receptors are essential for egg cylinder growth, gastrulation, and rostral head development in mice. Dev. Biol. 213, 157–169.
Stroband, H.W., Taverne, N., vd Bogaard, M., 1984. The pig blastocyst: its ultrastructure and the uptake of protein macromolecules. Cell Tissue Res. 235, 347–356.
Strumpf, D., Mao, C.A., Yamanaka, Y., Ralston, A., Chawengsaksophak, K., Beck, F., Rossant, J., 2005. Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. Development 132, 2093–2102.
Takahashi, Y., Dominici, M., Swift, J., Nagy, C., Ihle, J.N., 2006. Trophoblast stem cells rescue placental defect in SOCS3-deficient mice. J. Biol. Chem. 281, 11444–11445.

Takaoka, K., Yamamoto, M., Shiratori, H., Meno, C., Rossant, J., Saijoh, Y., Hamada, H., 2006. The mouse embryo autonomously acquires anterior-posterior polarity at implantation. Dev. Cell 10, 451–459.

Talbot, N.C., Caperna, T.J., Edwards, J.L., Garrett, W., Wells, K.D., Ealy, A.D., 2000. Bovine blastocyst-derived trophectoderm and endoderm cell cultures: interferon tau and transferrin expression as respective in vitro markers. Biol. Reprod. 62, 235–247.

Talbot, N.C., Powell, A.M., Camp, M., Ealy, A.D., 2007. Establishment of a bovine blastocyst-derived cell line collection for the comparative analysis of embryos created in vivo and by in vitro fertilization, somatic cell nuclear transfer, or parthenogenetic activation. In Vitro Cell. Dev. Biol. Anim. 43, 59–71.

Talbot, N.C., Powell, A.M., Ocón, O.M., Caperna, T.J., Camp, M., Garrett, W.M., Ealy, A.D., 2008. Comparison of the interferon-tau expression from primary trophectoderm outgrowths derived from IVP, NT, and parthenogenote bovine blastocysts. Mol. Reprod. Dev. 75, 299–308.

Tanaka, S., Kunath, T., Hadjantonakis, A.K., Nagy, A., Rossant, J., 1998. Promotion of trophoblast stem cell proliferation by FGF4. Science 282, 2072–2075.

Tanaka, S., Oda, M., Toyoshima, Y., Wakayama, T., Tanaka, M., Yoshida, N., Hattori, N., Ohgane, J., Yanagimachi, R., Shiota, K., 2001. Placentomegaly in cloned mouse concepti caused by expansion of the spongiotrophoblast layer. Biol. Reprod. 65, 1813–1821.

Terada, N., Hamazaki, T., Oka, M., Hoki, M., Mastalerz, D.M., Nakano, Y., Meyer, E.M., Morel, L., Petersen, B.E., Scott, E.W., 2002. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 416, 542–545.

Tesar, P.J., Chenoweth, J.G., Brook, F.A., Davies, T.J., Evans, E.P., Mack, D.L., Gardner, R.L., McKay, R.D.G., 2007. New cell lines from mouse epiblast share defining features with human embryonic stem cells. Nature 448, 196–199.

Tompers, D.M., Foreman, R.K., Wang, Q., Kumanova, M., Labosky, P.A., 2005. Foxd3 is required in the trophoblast progenitor cell lineage of the mouse embryo. Dev. Biol. 285, 126–137.

Tremblay, G.B., Kunath, T., Bergeron, D., Lapointe, L., Champigny, C., Bader, J.A., Rossant, J., Giguere, V., 2001. Diethylstilbestrol regulates trophoblast stem cell differentiation as a ligand of orphan nuclear receptor ERR beta. Genes Dev. 15, 833–838.

Ushizawa, K., Takahashi, T., Kaneyama, K., Tokunaga, T., Tsunoda, Y., Hashizume, K., 2005. Gene expression profiles of bovine trophoblastic cell line (BT-1 analyzed by a custom cDNA microarray. J. Reprod. Dev. 51, 211–220.

Uy, G.D., Downs, K.M., Gardner, R.L., 2002. Inhibition of trophoblast stem cell potential in chorionic ectoderm coincides with occlusion of the ectoplacental cavity in the mouse. Development 129, 3913–3924.

Vallier, L., Alexander, M., Pedersen, R.A., 2005. Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. J. Cell Sci. 118, 4495–4509.

van Eijk, M.J.T., van Rooijen, M.A., Modina, S., Scesi, L., Folkers, G., van Tol, H.T.A., Bevers, M.M., Fisher, S.R., Lewin, H.A., Rakacolli, D., Galli, C., de Vaureix, C., Trounson, A.O., Mummery, C.L., Gandolfi, F., 1999. Molecular cloning, genetic mapping, and developmental expression of bovine POU5F1. Biol. Reprod. 60, 1093–1103.

Vandevoort, C.A., Thirkill, T.L., Douglas, G.C., 2007. Blastocyst-derived trophoblast stem cells from the rhesus monkey. Stem Cells Dev. 16, 779–788.

Varlet, I., Collignon, J., Robertson, E.J., 1997. Nodal expression in the primitive endoderm is required for specification of the anterior axis during mouse gastrulation. Development 124, 1033–1044.

Viebahn, C., 1999. The anterior margin of the mammalian gastrula: comparative and phylogenetic aspects of its role in axis formation and head induction. Curr. Top. Dev. Biol. 46, 63–103.

Wang, Z., Jaenisch, R., 2004. At most three ES cells contribute to the somatic lineages of chimeric mice and of mice produced by ES-tetraploid complementation. Dev. Biol. 275, 192–201.

Wang, Y., Wang, F., Sun, T., Trostinskaia, A., Wygle, D., Puscheck, E., Rappolee, D.A., 2004. Entire mitogen activated protein kinase (MAPK) pathway is present in preimplantation mouse embryos. Dev. Dyn. 231, 72–87.

Wathes, D.C., Wooding, F.B., 1980. An electron microscopic study of implantation in the cow. Am. J. Anat. 159, 285–306.

Weinstein, M., Yang, X., Li, C., Xu, X., Gotay, J., Deng, C.X., 1998. Failure of egg cylinder elongation and mesoderm induction in mouse embryos lacking the tumor suppressor smad2. Proc. Natl. Acad. Sci. U. S. A. 95, 9378–9383.

Wen, F., Tynan, J.A., Cecena, G., Williams, R., Múnera, J., Mavrothalassitis, G., Oshima, R.G., 2007. Ets2 is required for trophoblast stem cell self-renewal. Dev. Biol. 312, 284–299.

Winnier, G., Blessing, M., Labosky, P.A., Hogan, B.L., 1995. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes Dev. 9, 2105–2116.

Yagi, R., Kohn, M.J., Karavanova, I., Kaneko, K.J., Vullhorst, D., DePamphilis, M.L., Buonanno, A., 2007. Transcription factor TEAD4 specifies the trophectoderm lineage at the beginning of mammalian development. Development 134, 3827–3836.

Yamamoto, H., Flannery, M.L., Kupriyanov, S., Pearce, J., McKercher, S.R., Henkel, G.W., Maki, R.A., Werb, Z., Oshima, R.G., 1998. Defective trophoblast function in mice with a targeted mutation of Ets2. Genes Dev. 12, 1315–1326.

Yamanaka, Y., Ralston, A., Stephenson, R.O., Rossant, J., 2006. Cell and molecular regulation of the mouse blastocyst. Dev. Dyn. 235, 2301–2314.

Yan, J., Tanaka, S., Oda, M., Makino, T., Ohgane, J., Shiota, K., 2001. Retinoic acid promotes differentiation of trophoblast stem cells to a giant cell fate. Dev. Biol. 235, 422–432.

Yang, X., 2007. Nuclear reprogramming of cloned embryos and its implications for therapeutic cloning. Nat. Genet. 39, 295–302.

Yang, W., Klaman, L.D., Chen, B., Araki, T., Harada, H., Thomas, S.M., George, E.L., Neel, B.G., 2006. An Shp2/SFK/Ras/Erk signaling pathway controls trophoblast stem cell survival. Dev. Cell 10, 317–327.

Zipori, D., 2004. The nature of stem cells: state rather than entity. Nat. Rev. Genet. 5, 873–878.