Recent Development in Embryo Technology in Pigs

- Review -

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ABSTRACT: Technologies on preimplantation porcine embryos have been developed quickly and significantly. Successful development of systems for culture of porcine zygotes to the blastocyst stage has made it possible to utilize follicular oocytes for in vitro production of embryos and thus stimulated research on various embryo technologies. Recent technological development of embryo cryopreservation, separation of X- and Y-bearing spermatozoa and non-surgical embryo transfer has also made it easy to utilize in vivo- and in vitro-produced embryos for artificial manipulation to produce clones and transgenic pigs. Further progress in overcoming various problems associated with each embryo technology will result in acceptable efficiency to utilize porcine embryos with a high or increased quality. Combining these technologies will accelerate further expansion of the swine industry not only for meat production but also for the production of therapeutic recombinant proteins and xenografts. (Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 6 : 966-975)

Key Words: Pig, Oocyte, IVM, IVF, Embryo Technology

INTRODUCTION

The final goal of embryo technologies in animal production is efficient utilizations of embryos with excellent quality for various purposes. Due to a short period of generation, multiple piglets per litter and managerial characteristics, pigs are good species to manipulate in the field of biotechnological industries. Therefore, porcine embryos should be good materials to introduce a new genetic inheritance into individuals or herds and to deal in international trade. So far, however, effective techniques to recover and transfer porcine embryos have been limited to surgical procedures, which have been developed in the 1960's (Hancock and Hovell, 1962; Day, 1979), due to the anatomical characteristics of sow's cervix and uterus. Furthermore, progress in porcine embryo technologies had been restricted within narrow limits because of delayed development of techniques for producing normal embryos in vitro, culturing embryos from 1- and 2-cell stages and freezing embryos. However, recent development of such techniques has freed and accelerated the progress to utilize porcine embryos for practical use and for various research purposes. This review summarizes recent advancements of porcine embryo technologies and discusses the possible future researches.

PRODUCTION OF EMBRYOS IN VITRO

Growth of oocytes in vitro

In a porcine ovary, relatively larger number of primordial follicles exits as compared with other species such as humans, cattle and mice (Gosden and Telfer, 1987). It seems to be quite difficult to support growth of porcine oocytes in vitro. However, the research on this subject has recently become more active and physiological requirements of growing oocytes are being characterized (Christmann et al., 1994; Lazzari et al., 1994; Petr et al., 1994a, b; Price et al., 1995). Oocytes derived from preantral follicles can grow, mature and fertilize in vitro (Hirao et al., 1994) but the success rate is still low. The developmental ability of this type of oocytes following in vitro fertilization (IVF) has not been clarified. Further research on utilization of such oocytes in porcine embryo technologies will be important to reduce the cost and effort to produce large number of embryos.

Maturation of oocytes in vitro

Fully grown oocytes with uniform ooplasm and surrounded by a compact cumulus cell mass, which were collected from antral follicles of slaughtered prepubertal gilts, have been generally used for in vitro maturation (IVM). Although a high incidence of nuclear maturation of such oocytes is achieved in vitro in the presence of gonadotropins, an abnormally low incidence of male pronuclear formation after IVF has been routinely observed (see Niwa, 1993; Mattioli, 1994; Nagai, 1994; Funahashi and Day, 1996). However, this problem has been currently overcome by modifications of conditions for IVM (see Day and Funahashi, 1996; Funahashi and Day, 1997). The incidence of male pronuclear formation and glutathione content in porcine oocytes increase when thiols such as cysteine (Yoshida et al., 1993a), cysteamine (Grupen et al., 1995) and beta-mercaptoethanol (Funahashi and Day, 1997) are added to maturation.

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media. The presence of cysteine in maturation medium is critical only between 42 and 48 h of culture both for glutathione synthesis and male pronuclear formation after sperm penetration in vitro (Sawai et al., 1997). Thus, the oxidative stress, especially during late stages of oocyte maturation may be involved in the failure of male pronuclear formation. On the other hand, the detrimental effects of an elevated NaCl concentration in maturation medium on histone H1 kinase activity (Funahashi et al., 1994c), microfilament organization (Funahashi et al., 1996) and glutathione content of oocytes have been demonstrated. Male pronuclear formation (Funahashi et al., 1994b) and subsequent embryonic development after IVF (Funahashi et al., 1994a) are also affected by the high NaCl concentration. The presence of organic osmolytes, such as taurine and sorbitol, in maturation media relieves the detrimental effects of a high concentration of NaCl (Funahashi et al., 1996).

**Fertilization of oocyte in vitro**

There are many reports describing successful IVF of porcine oocytes matured in vivo or in vitro. In most laboratories, freshly ejaculated spermatozoa are mainly used for porcine IVF. For repeated use of spermatozoa with the same characteristics for IVF, techniques for IVF by frozen-thawed (Nagai et al., 1988) and ejaculated (Wang et al., 1991; Abe and Day, 1997) spermatozoa have developed.

Although, successful sperm penetration has been achieved, a high incidence of polyspermic penetration is one of the persistent problems in porcine IVF (see Niwa, 1993; Day and Funahashi, 1996; Nagai, 1996; Funahashi and Day, 1997). In most IVF systems, a relatively high number of spermatozoa is added to fertilization medium to make sure of inducing sperm capacitation. However, an increased number of spermatozoa per oocyte is known to be associated with a high incidence of polyspermic penetration (Rath, 1992; Coy et al., 1993). This may be due to gradual induction of sperm capacitation and acrosome reaction. Since the rate of sperm capacitation and acrosome reaction appears to be affected by fertilization media (Mattioli et al., 1996), the media in which capacitation could be induced in most spermatozoa at the same time would reduce the incidence of polyspermy. Limited success in reducing the incidence of polyspermic penetration has been reported by using porcine oviductal secretion (Nagai and Moor, 1990; Kano et al., 1994; Kim et al., 1996) or porcine follicular fluid (Funahashi and Day, 1993). On the other hand, sperm penetration is not blocked even after electrical activation of oocytes matured in vitro (Funahashi et al., 1993, 1995) by which cortical granules are known to be released (Sun et al., 1992). Therefore, the timing of cortical granule reaction (Kim et al., 1996; Wang et al., 1997) may be an important factor for preventing polyspermy. Another possible means to reduce the incidence of polyspermic penetration in porcine IVF may be to control the number of capacitated spermatozoa reaching to the oocytes.

**DEVELOPMENT OF EMBRYOS IN VITRO**

**Culture systems**

One- and two-cell porcine embryos had been known to fail in the development in vitro beyond the 4-cell stage (see Davis, 1985; Reed et al., 1992; Petters and Wells, 1993). This developmental block has been overcome by culturing embryos in sheep or mouse oviducts (Krisher et al., 1989; Prather, 1991), in the amniotic fluid of developing chick embryos (Ocampo et al., 1994), in medium supplemented with oviductal fluid (Archibong et al., 1989), in conditioned media with homologous porcine uterine cells (Poulin et al., 1997) and in co-culture systems with oviductal epithelial cells (White et al., 1989; Choi et al., 1995) or porcine cumulus or trophoblastic cells (Nagai and Takahashi, 1992). In a simple culture medium (modified Whitten's medium), when 1- and 2-cell embryos are cultured in the atmosphere of 5% CO\(_2\), 5% O\(_2\) and 90% N\(_2\) at 39°C, more than 80% of them have developed to the morula or blastocyst stage, and after transfer of embryos developed to the 4- to 8-cell stage to the recipients, normal piglets were obtained (Beckmann and Day, 1993). Since Whitten's medium (Whitten and Biggers, 1968) contains relatively low concentration of NaCl (68.49 mM), an increased NaCl concentration likely impairs the development of porcine embryos at the 4-cell stage. This has been confirmed by Galvin et al. (1993) who found that higher proportion of 1- and 2-cell embryos developed to the morula and blastocyst stages in Whitten's medium containing 68 mM (53.4%) than 100 mM (0%) NaCl in 5% CO\(_2\) in air at 39°C. However, addition of 2 mM glutamine to the medium improved the early development of embryos in the presence of 100 mM, but not of 63 mM NaCl. The early development of embryos has also been supported in a simple medium supplemented with 5.55 mM glucose and 1 mM glutamine in the atmosphere of 5% CO\(_2\), 5% O\(_2\) and 90% N\(_2\) at 37°C (Petters et al., 1990). Modification of this medium adding 7 mM taurine and 5 mM hypotaurine, named NCSU-23, produced very high proportion (93%) of morulae and blastocysts even in the atmosphere of 5% CO\(_2\) in air at 37°C (Petters and Reed, 1991). It is known that each of glutamine, taurine and hypotaurine works as an organic osmolyte. Beneficial effects of organic osmolytes likely rescue porcine embryos from the detrimental effect of a high NaCl concentration.
Furthermore, successful in vitro development of early porcine embryos has also been reported using modified Tyrode’s solution supplemented with 10 mM N-2-hydroxyethyl-piperazone-N’-2-ethanesulfonic acid (HEPES) in air at 39°C (Hagen et al., 1991). Although it is not clarified why this medium supports the development, this finding may be important for commercial application of porcine embryo technologies because their culture system does not require supplementation of CO₂ during culture. However, higher developmental competence of 1- and 2-cell embryos from Sinclair miniature pigs is observed in Whitten’s medium in 5% CO₂ in air in HEPES buffered modified Tyrode’s solution in air (Prather et al., 1995).

Development to hatching blastocysts

Even in various successful culture systems, the incidence of porcine embryos to develop to the peri-hatching blastocyst stage appears to be very low (Dobrinsky et al., 1996). An increased incidence of hatching has been demonstrated when embryos were co-cultured with porcine endometrial monolayer (Allen and Wright, 1984) or bovine uterine fibroblast (Kuzan and Wright, 1982) and when they were cultured in conditioned media with homologous porcine uterine cells (Poulin et al., 1997). Recently, the development of porcine zygotes to the blastocyst stage in a defined culture medium, Beltsville Embryo Culture Medium (BECM-3), has been reported by Dobrinsky et al. (1996). They demonstrated that both the mean cell number of blastocysts and the incidence of hatched blastocysts are increased when the zygotes were cultured in BECM-3-based media in the absence of BSA fraction V; Furthermore, and that addition of fetal bovine serum to the medium by late Day 5 (at the late morula and blastocyst stages) increased the incidence (80%) of 2-cell embryos to develop to the hatched blastocyst stage. It has also been demonstrated that the incidence of hatching blastocysts is improved using BSA-free NCSU-23 medium which was supplemented with 20% fetal bovine serum when embryos were at the early morula stage, although supplementation of NCSU-23 with amino acids and insulin from 4 days after the start of culture enhanced only the development of embryos to the blastocyst stage (Koo et al., 1997a). Therefore, the presence of BSA in culture media beyond the morula stage seems to be detrimental for hatching of embryos and development of inner cell mass, but fetal bovine serum is stimulative.

Acquisition of developmental competence of IVM/IVF embryos

Although piglets from IVM/IVF oocytes have been produced after transfer them into the recipients (Mattioli et al., 1989; Yoshida et al., 1993b), the developmental competence of oocytes matured in vitro as determined by in vitro development to the blastocyst stage and the number of cells in a blastocyst after IVF is lower compared with those matured in vivo (Nagashima et al., 1996a). Culture conditions used for IVM of porcine oocytes affect their subsequent response to oocyte activation (Yamauchi et al., 1996). The ability of IVM/IVF porcine embryos to develop to the blastocyst stage in vitro has been improved by modification of maturation medium as reducing the concentration of NaCl (Funahashi et al., 1994a) or supplementing with cysteamine (Grupen et al., 1995) or organic osmolytes (Funahashi et al., 1996). Modification of various conditions between oocyte collection and germinal vesicle breakdown also improve embryonic development (Funahashi et al., 1997a, b). Reducing morphological variation in the germinal vesicles appears to enhance the developmental competence of porcine oocytes (Funahashi et al., 1997a). Exposure of oocyte-cumulus complexes to dibutyryl cyclic adenosine 3',5'-monophosphate (dbcAMP) for the first 20 h of culture for maturation does not affect the maturation of oocytes during a 44 h culture period and sperm penetration after IVF, but it does increase the homogeneity of oocyte nuclear maturation (Funahashi et al., 1997b). This treatment also improves embryonic development in vitro and, after surgical transfer of 2- and 4-cell embryos to the recipients, piglets have been produced with a high pregnancy rate and acceptable litter size (Funahashi et al., 1997b). The developmental competence of oocytes seems to be improved also by modification of IVM conditions after the germinal vesicle stage. The presence of a tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) from 20 to 44 h of culture for IVF enhances the competence of the oocytes to develop to the blastocyst stage without affecting factors associated with fertilization (Funahashi et al., 1997c). A culture system with treating oocytes with dbcAMP during the first 20 h of IVF and then with TIMP-1 from 20 to 44 h of IVF improves the ability of embryos to develop to the blastocyst stage to a more acceptable level (34%) (Funahashi et al., 1997c). A recent study has also demonstrated that co-culture of porcine oocyte-cumulus complexes with follicular shell pieces improved early embryonic development to the blastocyst stage after IVF (Abeyderra et al., 1996). In this IVF system, piglets were produced at a relatively high level of efficiency following surgical transfer of 2- to 4-cell embryos derived from IVF/IVF. The ability of IVM/IVF porcine embryos to develop to the blastocyst stage in simple media has also been improved.

TRANSFER OF EMBRYO

Successful embryo transfer techniques are expected
to reduce not only the high cost of shipping but also
the risk of disease transmission, which are always
involved in the case of transportation of animals or
herds. Although embryo transfer in pigs has been
accomplished with a high pregnancy rate (Polge,
1982), it is very restricted in surgical procedures
developed more than three decades ago (Hancock and
Hovell, 1962; Day, 1979). Practically, it is possible to
produce piglets by embryo transfer following the
international transportation of embryos (Niemann,
1989). However, the cost for surgical collection and
transplantation of porcine embryos is still high for
commercial application. Non-surgical embryo transfer
has been continuatively conducted since a limited
success at three decades ago (Polge and Day, 1968).
Recently, successful production of piglets following
non-surgical embryo transfer has been reported
(Reichenbach et al., 1993; Galvin et al., 1994). To
introduce embryos into the reproductive tract through
cervix, Reichenbach et al. (1993) have used a
disposable plastic spiral catheter, which is routinely
used for artificial insemination, and a bovine embryo
transfer cannula. Galvin et al. (1994) have used a
disposable insemination spirette with an attached 3-way
stopcock and embryos in 10-12 ml of culture medium
have been expelled into the uterus: however, the
pregnancy rates was low and litter size was also small
compared with those obtained in surgical transfer.
More recently, a set of instruments for non-surgical
transfer of porcine embryo has been developed to
improve both the farrowing rate and the average litter
size (Li et al., 1996). The development of these
non-surgical techniques for porcine embryo transfer
will result in the effective utilization of another
embryo technologies for practical use and scientific
research.

As a substitute for IVF and in vitro culture of
embryos, successful techniques for gamete intra-
fallopian transfer have been reported in pigs by Rath
et al. (1994) who transferred in vivo matured denuded
oocytes into the ampulla of the oviduct together with
preincubated spermatozoa and observed relatively high
rate (64%) of embryonic development to the morula/
blastocyst stages and obtained piglets.

CRYOPRESERVATION OF EMBRYOS

Storage techniques of porcine embryos are essential
not only for transporting them around the world but
also for holding them until embryo transfer to a
suitable recipient gilt. However, porcine embryos are
known to be very sensitive to the temperatures below
15°C (Polge, 1982). Subsequently, however, it was
reported that the embryos at the expanded and hatched
stages are more resistant to chilling (Nagashima et al.,
1988a). Then, the research on cryopreservation of
porcine embryos has rapidly progressed (see
Nagashima et al., 1994a). Generally, porcine peri-
hatching blastocysts are successfully frozen in a
phosphate buffered saline (PBS) supplemented with 1.5
M glycerol and 15-50% fetal bovine serum (see
Nagashima et al., 1994a). The birth of piglets from
frozen-thawed peri-hatching blastocysts has been
reported by several researchers (Hayashi et al., 1989;
Kashiwazaki et al., 1991; Fujino et al., 1993). The
freezability of porcine embryos seems to attain the
summit immediately after hatching, because hatched
blastocysts with a diameter of more than 300 μm can
not survive after freezing (Nagashima et al., 1992a).
Recently, it has been clearly demonstrated that piglets
can be produced from 2- to 4-cell embryos frozen-
thawed after removing the cytoplasmic lipid, showing
that the sensitivity of porcine embryos to low
temperatures is related to their cytoplasmic lipid
content (Nagashima et al., 1994b, 1995).

Vitrification of porcine embryos has also been
attempted. Expanded to hatched blastocysts can survive
after cryopreservation in vitrification solution at -196°C
(Yoshino et al., 1993; Dobrinsky and Johnson, 1994).
The embryos at the 2- to 4-cell stages are also
successfully cryopreserved by vitrification after removal
of cytoplasmic lipid (Nagashima et al., 1996b). In
these techniques, however, zona pellucida which is the
barrier to pathogens is removed after thawing in
expanded to hatched blastocysts or partially broken
before freezing in 2- to 4-cell stage embryos for
removing cytoplasmic lipid. This may introduce various
pathogens into the animals. Further improvement in the
techniques for freezing of porcine embryos with intact
zona pellucida should be needed for commercial use.

SEXING

Separation of X- and Y-bearing spermatozoa

Using flow cytometry, a method for sorting X- and
Y-bearing mammalian sperm cells has been developed
(Johnson, 1995; Johnson et al., 1996). It has been
reported that the sorted boar spermatozoa can penetrate
oocytes and viable embryos can be obtained after the
gamete intrafallopian transfer (Rath et al., 1994).
Recently, production of piglets preselected for sex
following IVF with X-bearing spermatozoa sorted by
flow cytometry has been reported by Rath et al.
(1997). In their study, purity of sorting was 92% and
83% for X- and Y-bearing spermatozoa, respectively.
After transfer of 92 IVF embryos produced from
X-sorted spermatozoa into 2 recipients, 6 and 4 female
piglets were delivered from each of the recipients.
Additionally, when 2 gilts were intratubally inseminated
with either X- or Y-sorted spermatozoa (2 × 10^5
cells per oviduct), 13 (85%) of 15 piglets produced
were of the predicted gender. Further application of
the sorting method for separating X- and Y-bearing boar spermatozoa is being expected for the production of sexed embryos.

Sexing of embryos

Sex determination of porcine embryos has been done by chromosomal analysis of bisected trophoderm cells from expanding blastocysts (Kato et al., 1987). The sex of porcine embryos has also been determined by using polymerase chain reaction (PCR; Pomp et al., 1995). Biopsied porcine 4-cell embryos are known to develop to the term after transfer of the embryos to the compacted morula to hatched blastocyst stages (Nakayama et al., 1997). Biopsied blastomeres of porcine embryos could be a good materials for chromosomal analysis or PCR analysis for sex determination. Although sexing of embryos would be of great advantage to various scientific researches, its commercial application to produce piglets of a peselected sex will require lowered cost as well as improved embryo transfer technology.

CLONING

It is possible to obtain piglets after transfer of bisected porcine embryos (Willadsen, 1982; Rorie et al., 1985). However, possibly due to less robust of porcine embryos, there are limited successful reports about production of identical twin piglets after transfer of bisected embryos (Polge, 1985; Nagashima et al., 1988b; Ash et al., 1989; Niemann and Reichelt, 1993). Recently, in vitro development of a blastomere derived from porcine embryos at the 4-cell and 8-cell stages to the blastocyst stage has been demonstrated (Niemann and Reichelt, 1993; Eckert et al., 1997).

Only one piglet has been produced after surgical transfer of reconstituted embryos which were produced by fusing a blastomere obtained from 4-cell embryo with an enucleated, activated meiotic metaphase II oocyte (Prather et al., 1989). Although a number of papers has discussed on the basic factors influencing the success of nuclear transplantation in pigs (Prather et al., 1992; Niemann and Reichelt, 1993; Parry and Prather, 1995), the developmental ability of reconstituted embryos to the blastocyst stage has been very low (Nagashima et al., 1992b). The reason for the low developmental ability of the reconstituted porcine embryos is still unknown. However, it has been reported that porcine oocytes which were matured in vitro and parthenogenetical activated have poor ability of development even in the culture conditions which can support early embryonic development (Prather et al., 1991; Funahashi et al., 1994a).

Improved parthenogenetic development of IVM porcine oocytes to the blastocyst stage has recently been reported in vivo and in vitro by the modifications of IVM systems and methods for artificial oocyte activation (Jolliff and Prather, 1997; Machaty et al., 1997). Recent progress in culture techniques for IVM and early embryonic development and in techniques for oocyte activation will contribute to the improvement of the developmental ability of porcine embryos reconstituted by nuclear transplantation.

TRANSGENIC PIGS

The first transgenic pigs which express human growth hormone transgenes have been reported more than a decade ago (Hammer et al., 1985). Recent progress in gene transfer in pigs has been reviewed elsewhere (Pursel et al., 1990, 1996; Niemann and Reichelt, 1993; Martin and Pinkert, 1994). To produce transgenic pigs, several hundred copies of DNA have been directly microinjected into the pronuclei of zygotes after visualization of the pronuclei by centrifugation. Nowadays, it is possible to utilize porcine zygotes produced in vitro for the study on the transfer of foreign DNA (Kubisch et al., 1995; Koo et al., 1997b). Further efforts to combine the recombinant DNA technologies with nuclear transplantation will be required to achieve a rapid progress to produce transgenic pigs. Furthermore, measures for public acceptance about the practical application of recombinant DNA technology and its safety are also required.

Improvement of production traits

Transgenic pigs bearing genes aimed at improving production traits have been produced for growth hormone, cSKI and insulin like growth factor-I (Pursel et al., 1996). A number of those recombinant DNA has contained the regulatory region of the metallothionein gene as a promoter and enhancer (Hammer et al., 1985; Vize et al., 1988; Pursel et al., 1989) since an advantage of utilizing the metallothionein promoter is that the level of gene expression can be increased by supplementation of food or water with zinc (Palmier et al., 1982). Marketing of transgenic pigs with elevated growth hormone would offer considerable potential value to both the producer and the consumer because those transgenic pigs gain weight more than 10% faster with higher feed efficiency and have much reduced carcass fat as compared to sibling controls (Pursel et al., 1996).

Utilization to xenotransplantation

Because of anatomical, physiological and immunological similarities of pigs to humans, recent progress in understanding the immunologic barriers to xenotransplantation (Lin and Platt, 1996) and a chronic lack of human tissues for transplantation, pigs are
being considered all the more as an important source for human organ replacement. A major barrier of human body to the transplantation of pig organ is the hyperacute rejection caused by pre-existing antibodies and complement. Development of transgenic pigs that do not express the alphaGal epitope and/or express a human complement inhibiting protein, such as decay-accelerating factor is expected to inhibit the hyperacute rejection by complement activation and consequently delay xenograft rejection (Cooper, 1996). The production of transgenic pigs expressing human decay-accelerating factor (Rosengard et al., 1995) and human complement-regulatory proteins, such as human CD59 (Byrne et al., 1997) and, as potential donors for clinical organ xenotransplantation has been reported. Recently, alpha1,2-fucosyltransferase transgenic pigs, which should have a reduced expression of Gal alpha1(3)Gal, have been suggested as a good source of xenotransplantation for human (Cohn et al., 1997).

Establishment of animal factory to produce therapeutic proteins

Transgenic pigs expressing recombinant human protein C in milk have also been produced (Morcol et al., 1994; Subramanian et al., 1996; VanCott et al., 1997). Establishment of a herd of transgenic pigs producing such therapeutic recombinant proteins would offer distinct advantages for treating genetic or other disease. At present, however, the efficiency of gene-injected embryos that develop to transgenic pigs seems to be very low (0.1 to 4.0%) due to a definitive problem, mosaicism (Pursel et al., 1990, 1996).

Establishment of embryonic stem cell lines and gene-targeting

Another method for manipulating genes of porcine embryos is to make chimera by microinjection of embryonic stem (ES) cells manipulated genetically. Using this technology, it would be possible to produce ‘knock-out’ pigs. Rapid progress is being made in attempts toward maintaining porcine EC cell pluripotency (see Wheeler, 1994). Although microinjection of inner cell mass (ICM) cells of porcine embryos into blastocoeals has resulted in chimerism in female germ line which was assessed by a progeny test (Onishi et al., 1994), definitive ES cell lines so far generated seem to be very difficult to contribute to the germ line of chimeric pigs. Recently, the achievement of chimerism and transgenesis in domestic pigs at a fetal stage by using an ES cell line has been reported (Notarianni et al., 1997). The recent experiments in which ICM cells were introduced into tetraploid 4-cell pig embryos suggest that it may be possible to create a fetus derived from ICM cells or potentially stem cells (Prather et al., 1996). More efforts are required to improve the efficiency to produce transgenic chimeric pigs.

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