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Gametes from stem cells: Status and applications in animal reproduction

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Abstract

In vitro gamete differentiation could revolutionize animal production by decreasing generation intervals, increasing the number of gametes per animal and facilitating the dissemination of elite genetics. In addition, it could help to develop new strategies for the conservation of endangered species. The recent in vitro reconstitution of germ cell development in mice has inspired researchers to invest their best efforts into reproducing this achievement in livestock species. With this goal in mind, multiple differentiation approaches and cell sources have been evaluated. The degree of success in these evaluations varies according to the species and the stage of development studied, but, in general, partially positive results have been obtained. Evidence suggests that although functional gametes with true reproductive potential are still to be obtained, it is a matter of time before this goal is achieved.

KEYWORDS

embryo, gametogenesis, stem cells

1 | INTRODUCTION

Reproduction efficiency and genetic progress are important factors in animal production, with gamete production being a key aspect of the reproductive process. The recently successful in vitro generation of functional gametes from mouse stem cells (Hikabe et al., 2016; Li et al., 2019) presents new and promising avenues for reproductive medicine, as well as great potential for applications in livestock and endangered species reproduction.

In livestock production, increasing the number of gametes generated per animal and unit of time could radically change current breeding programmes, especially for females of non-ovulatory species. Scalable in vitro production of oocytes could be used for the generation of large numbers of in vitro-produced embryos to allow rapid and efficient dissemination of elite animal genetics by embryo transfer. Although the number of in vitro-generated spermatozoa would hardly compete with testicular production in livestock species, a sufficient amount could be produced to allow for the reproduction of unborn, young, castrated, infertile (in some cases) or dead animals through assisted reproductive technologies (ARTs) such as subsequent in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Also, recent strategies propose transplanting germinal stem cells into recipient testes that have been depleted of their own germ cells as an option to generate more spermatozoa from elite donors and to overcome the challenge of poor semen cryopreservation, which limits the use of artificial insemination (AI) in some species such as pigs (reviewed by Oatley, 2018). In vitro gametogenesis from genetically modified stem cells could also be used as an efficient approach for genetic engineering (Soto & Ross, 2016). In addition, the in vitro production of gametes could reduce generation intervals and lead to new schemes for genetic improvement, such as in vitro breeding (IVB) (Goszczynski et al., 2018). The essential idea of IVB is to recapitulate in vitro the cycle of meiotic recombination and fertilization, and to combine it with genomic selection in each cycle in order to provide directional genetic progress. This approach could be accomplished by isolation of embryonic stem cells from blastocysts, followed by genomic selection of the superior stem cells, in vitro generation of gametes, and subsequent IVF, resulting in a new generation of blastocyst-stage embryos with potential for a subsequent round of selection. Based on simulations performed by Goszczynski et al. (2018), this scheme could lead to genetic improvement 10 times faster than current breeding strategies, which are based on the intensive use of genomic selection and assisted reproductive technologies.

In vitro gamete generation could also play a critical role in the conservation of rare or endangered species. Perhaps the most important problem in captive breeding programmes is the limited number of individuals, which results in small gene pools and unsustainable populations. This is often accompanied by early death, inability to easily transport animals between facilities, and incompatibility between mates. In vitro-generated gametes could be easily stored and used for assisted reproduction, helping overcome some of these issues. In non-domestic species, semen is typically collected by electroejaculation. This method causes muscle damage and stress to the animals, requiring the use of anaesthesia, which can compromise the health of the animal and alter the quality of the ejaculate (Durrant, 2009). Semen collection can also be dangerous when working with wild carnivores. In these cases, in vitro spermatogenesis would constitute an alternative approach.

Although AI is the most common ART used in wild species, it is inefficient in species that produce few spermatozoa per ejaculate, such as felids (Swanson et al., 2003), and requires multiple ejaculates per insemination, especially when using frozen semen. In vitro spermatogenesis and the subsequent implementation of ICSI or IVF, which make more efficient use of spermatozoa, could solve these issues. A second limitation of AI is that it prevents the transmission of female genetics when females have a compromised uterine environment, which is common in zoo species kept in non-breeding conditions for prolonged periods, such as canids, elephants, white rhinoceros, Seba's bats, wildebeest, stingrays and some felid species (Penfold, Powell, Traylor-Holzer, & Asa, 2014). This problem could be overcome by using IVF or ICSI with donor oocytes from compromised females and transferring embryos into healthy females, although the number of oocytes isolated from endangered species is always a limiting factor (Herrick, 2019). The in vitro production of oocytes could constitute a fundamental tool to improve these processes and develop more efficient protocols.

In this manuscript, we review the current state of techniques with potential for in vitro production of gametes from stem cells, with a focus on domesticated mammalian livestock species when appropriate.

2 | STEM CELLS WITH POTENTIAL FOR GAMETE GENERATION

Stem cells are classified according to their origin and differentiation potential. Embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of pre-implantation embryos and can differentiate into any cell type of the body, including the germline (Evans & Kaufman, 1981). Thus, they are considered pluripotent stem cells (PSCs). This quality also applies to induced pluripotent stem cells (iPSCs), which are produced by de-differentiation of adult somatic cells by overexpression of pluripotency factors (Takahashi & Yamanaka, 2006). PSCs have a clear potential to generate germ cells as demonstrated by their capacity to contribute to germline chimeras after transplantation into blastocyst-stage embryos (Takahashi & Yamanaka, 2006). Recently, stem cells with enriched molecular signatures of blastomeres and developmental potency for all embryonic and extra-embryonic cell lineages, namely expanded potential stem cells (EPSCs), have been derived in mouse (Yang et al., 2017). These cells were derived from 8-cell blastomeres by inhibiting the critical molecular pathways that predispose their differentiation.

Most of the other existing types of stem cells have a more restricted potential and are typically derived from adult tissues. Adult stem cells are typically fated to differentiate into one or a few specialized cell types. Adult stem cells fated for germ cell development include spermatogonial stem cells (SSCs) (Tegelenbosch & de Rooij, 1993) and ovarian stem cells (OvSCs) (Johnson, Canning, Kaneko, Pru, & Tilly, 2004). In addition, other adult stem cells have shown some capacity for germline differentiation, including very small embryonic-like stem cells (VSELs) (Havens et al., 2014) and multipotent stromal cells (MSCs) (Cortes et al., 2013). Although foetal and adult sources of stem cells could provide gametes from valuable and well-characterized individuals, embryonic sources offer an outstandingly larger reduction in the generation interval.

3 | PLURIPOTENT STEM CELLS

Until recently, most of our knowledge about ESCs was derived exclusively from research in mice, rhesus monkeys and humans: species in which ESCs were firstly established (Evans & Kaufman, 1981; Thomson et al., 1998, 1995). However, after decades of only partially positive results due to a high tendency for spontaneous differentiation and cell death, efficient ESC derivation has finally been reported in cattle (Bogliotti et al., 2018). Bovine ESCs are cultured in TeSR1 base medium without TGFβ1, supplemented with basic fibroblast growth factor (bFGF) and WNT antagonist IWR1. Under these conditions, bovine ESCs are pluripotent, as shown by their capacity to form teratomas, and present long-term proliferative capacity with stable morphology, transcriptome, karyotype, population-doubling time and epigenetic characteristics.

In pigs, previous attempts to derive ESCs from ICMs and epiblasts in naïve or primed ESC culture conditions typically resulted in cells with limited capabilities for long-term survival and/or teratoma formation (reviewed by Ezashi, Yuan, & Roberts, 2016). However, the recently discovered enhanced pluripotency conditions were successfully optimized to support stable culture of pig blastocyst-derived EPSCs that express key pluripotency genes, are genetically stable, permit genome editing and differentiate to derivatives of the three germ layers in teratomas and chimeras (Gao et al., 2019).

Sheep ES-like cells with some signs of pluripotency and medium-term proliferation but no teratoma formation or chimera contribution have been reported (Zhao et al., 2011). In horse, attempts to derive ESCs have resulted in cells with either unproven or deficient potential for in vivo pluripotency (reviewed by Paterson, Kafarnik, & Guest, 2018). In small companion species, cat embryonic cells have shown very limited proliferation (reviewed by Paterson et al., 2018), while dog ESCs with high proliferative ability and proven potential for in vitro and in vivo pluripotency have been in use since 2009 (Vaags et al., 2009). While not all of the reports demonstrate all the features that define true ESCs, the number of partially positive results seems to indicate that ESCs from multiple species of domestic animals could possibly be established in the near future.

On the other hand, iPSCs are equivalent to ESCs in multiple aspects, including morphology, growth, gene expression, teratoma formation and contribution to germline chimeras. Lines of iPSCs have been reported for a number of livestock species, including cattle, pig, sheep, horse, goat, dog and some endangered species including drill monkeys, white rhinoceros, orangutan and snow leopard (Baird, Barsby, & Guest, 2015; Breton et al., 2013; Chu et al., 2015; Friedrich Ben-Nun et al., 2011; Fujishiro et al., 2013; Han et al., 2011; Hildebrandt et al., 2018; Nagy et al., 2011; Ramaswamy et al., 2015; Sartori et al., 2012). In general, reprogramming factors were delivered into embryonic or foetal fibroblasts by viral vectors, and pluripotency was determined by formation of teratomas. Of note, while human and mouse iPSCs can be weaned off of pluripotency factors, livestock iPSCs tend to require persistent expression of reprogramming factors in order to maintain pluripotency (Hall et al., 2012), indicating partial reprogramming or suboptimal culture conditions for maintaining the pluripotent state. This particular deficiency was exploited by Gao et al. (2019), to optimize EPSC conditions for pig cells. Using an inducible reprogramming system, they could discontinue the expression of the reprogramming factor and screen culture components for their capacity to maintain pluripotency in the absence of such expression generating reprogramming factor-independent pig iPSCs (Gao et al., 2019).

4 | SPERMATOGONIAL STEM CELLS

Sperm production in adult testes relies on the continuous activity of SSCs: unipotent stem cells that can be maintained throughout adult life. A remarkable feature of these cells is their ability to form colonies with continuous generation of sperm after isolation from one testis and transplantation into another one. Mouse SSCs can be cultured in vitro, resume sperm production and restore fertility in germline-depleted males upon transplantation into seminiferous tubules (Kanatsu-Shinohara et al., 2003; Nagano, Ryu, Brinster, Avarbock, & Brinster, 2003). Isolation and long-term culture of SSCs have been reported in rabbits, rats, hamsters and cattle, although their potential for in vivo spermatogenesis is yet to be proven (reviewed by Oatley, 2018). This is partly due to the lack of appropriate models for transplantation and low number of SSCs obtained (Zheng et al., 2014). Isolation of SSCs from testis has been reported using four techniques: differential plating on laminin- or Datura stramonium agglutinin (DSA)-coated flasks, Percoll gradient isolation, magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS) (Herrid, Davey, Hutton, Colditz, & Hill, 2009). In cattle, differential plating seems to produce better results (Herrid et al., 2009). Extended and efficient proliferation of SSC in culture has been achieved in mouse and human (Kanatsu-Shinohara et al., 2005; Sadri-Ardekani et al., 2009). In most livestock species, only short-term culture has been reported (reviewed by Sahare, Suyatno, & Imai, 2018), with better results in cattle (Oatley, Kaucher, Yang, Waqas, & Oatley, 2016) and pigs (Zhang et al., 2017).

5 | OVARIAN STEM CELLS

Within the last 15 years, a fair amount of research has been done to evaluate the existence and gametogenic potential of germline stem cells within the adult mammalian ovary. These cells have been referred to as oogonial stem cells (Johnson et al., 2004) or female germline stem cells (FGSCs) (Zou et al., 2009), collectively called here ovarian stem cells (and thus abbreviated as OvSCs). These cells are not pluripotent stem cells like ESC, but rather seem unipotent, similar to SSC. Evidence that OvSCs are not pluripotent comes from studies in which OvSC failed to form teratomas in nude mice after 4–8 weeks following subcutaneous (SC) injection of 1×10^5 cells (Zou et al., 2009) or after 6 weeks and 7 months following SC, intramuscular and testicular injection of 1×10^6 cells (Pacchiarotti et al., 2010).

Evidence pointing to the gametogenic potential of OvSCs in vivo was first demonstrated in 2009 when putative FGSCs were isolated from neonatal and adult mouse ovaries using magnetic-activated cell sorting (MACS) of cells labelled with an antibody against the C-terminus of Dead (Asp-Glu-Ala-Asp) box polypeptide 4 (DDX4). Isolated cells were cultured for over 68 (neonatal) and 25 (adult) passages and maintained morphological characteristics observed in freshly isolated cells, normal karyotype, high telomerase activity and expression of selected germ cell and stem cell markers at the transcrip and protein level. GFP-expressing FGSCs were injected into the ovaries of infertile mice, generating GFP^+ offspring at approximately 27% efficiency from both neonatal and adult FGSCs (Zou et al., 2009). These offspring were fertile and produced GFP^+ F2 litters. This first comprehensive report was followed by others describing generation of offspring after transplantation of FGSC in mice and rats, including transfection of FGSC followed by ovarian transplantation for production of transgenic mice (Zhang et al., 2011) and rats (Zhou et al., 2014).

OvSCs isolated from adult ovaries using an antibody against the C-terminus of the DDX4 (or mouse Vasa homolog, MVH) have been reported to form human and mouse oocytes in vitro (White et al., 2012). Isolation of OvSCs based on DDX4 detection received great criticism since DDX4 is known to be a cytoplasmic protein found in germ cells. Localization of DDX4 to the membrane of the putative OvSCs has yet to be confirmed, and reports have been published that demonstrate that no germline cells can be isolated using such method (Zarate-Garcia, Lane, Merriman, & Jones, 2016; Zhang et al., 2015). Despite criticisms, research groups worldwide continue to utilize and refine methods of isolation of OvSCs based on detection of DDX4, IFITM3 (Fragilis) (Zou, Hou, Sun, Xie, & Wu, 2011) and aldehyde dehydrogenase (ALDH1) (Clarkson et al., 2018). The latter report demonstrated that subpopulations of OvSCs exist in human ovaries that can be separated based on size and activity of ALDH1, a commonly used marker of viable stem cells. This report also showed that these different subpopulations express different splice variants of the DDX4 gene.

6 | OTHER ADULT STEM CELLS

VSELs represent an alternative type of stem cells that were initially isolated from bone marrow and later from multiple tissues (Suszynska, Ratajczak, & Ratajczak, 2016). VSELs are thought to derive from PGCs lost during their foetal migration to the gonad. These cells remain quiescent in vivo but can be cultured in vitro. Similar to PGCs, VSELs have the ability to differentiate into the three germ layers in vitro (Havens et al., 2014). Although their potential is still to be studied in livestock species, some evidence from mouse and human studies supports them as candidates for gamete generation with capacity to undergo oocyte-specific (Sriraman, Bhartiya, Anand, & Bhutda, 2015) and sperm-specific (Anand, Patel, & Bhartiya, 2015) differentiation.

MSCs can be easily isolated from multiple fetal and adult somatic tissues in different animal species, including bovine (Cortes et al., 2013), and propagated in culture. These cells have plasticity to differentiate into mesodermal tissue types. Given that PGCs derive from mesendoderm precursors (Kobayashi et al., 2017), the potential for MSC differentiation into gametes has been explored, with some preliminary positive results.

7 | IN VITRO DIFFERENTIATION OF GAMETES FROM PLURIPOTENT STEM CELLS

For in vitro gametogenesis, many of the developmental processes normally required for germ cell specification and differentiation must be recapitulated. Gamete precursors undergo a unique developmental process for chromosomal number reduction: meiosis, which is tightly controlled and regulated. Furthermore, germ cell precursors undergo extensive epigenetic reprogramming and chromatin rearrangement (Hajkova et al., 2002), required in part to ensure imprinting erasure and deposition of gamete-specific marks. Gametes also represent some of the most highly differentiated cells, with sperm being the smallest cell in the body and the egg among the largest ones. Despite the high complexity of the gametogenic process, in vitro differentiation of PSCs into gametes with potential for fertilization and full-term development has been successfully achieved in mice using ESCs and iPSCs (Hikabe et al., 2016; Li et al., 2019).

The first critical transition in gametogenesis is the formation of primordial germ cells (PGCs): the founder population of male and female gametes. Many strategies have been evaluated to generate PGCs from PSCs in vitro. The first reports included spontaneous differentiation into embryoid bodies (Geijsen et al., 2004) and stimulation of epiblast stem cells (EpiSCs) with BMPs, but the efficiency of germ cell derivation was low (Hayashi & Surani, 2009). This efficiency increased when PSCs were first differentiated into an intermediate state referred to as EpiSC-like cells (EpiLCs) and then to PGCs by stimulation with BMPs, leading to embryoid body formation (Hayashi, Ohta, Kurimoto, Aramaki, & Saitou, 2011).

In humans, PGCLCs have been generated through a similar two-step procedure: stem cells were converted into an intermediate type of mesodermal-like cells, which were then differentiated into PGCLCs by exposure to BMP4 (Irie et al., 2015; Kobayashi et al., 2017; Sasaki et al., 2015). Of note, the transcriptional profiles of the PGCLCs produced by both approaches were very similar to those of in vivo PGCs.

The differentiation approach used to generate PGCLCs from human EpiLCs served as template for the evaluation of a similar culture system in pigs using iPSCs. The PGCLC identity was supported by the expression of PGC (BLIMP1, PRDM14, STELLA) and pluripotency markers (OCT4, SOX2), an epigenetic status similar to in vivo PGCs, and transcriptome and gene ontology analyses that revealed a gamete production scheme (Wang et al., 2016). Also, the recently developed pig EPSCs produced PGCLCs in vitro (Gao et al., 2019).

The potential of in vitro-generated PGCs to undergo spermatogenesis and oogenesis in vitro has been studied in mice and a few livestock species through different assays. In mouse, spermatogonial stem cell-like cells (SSCLCs) have been derived from PGCLCs by co-culture with testicular somatic cells under retinoic acid (RA), BMP and activin A stimulation (Ishikura et al., 2016; Zhou et al., 2016). This procedure resulted in meiotic cells that gave rise to fertile offspring after injection into oocytes. Recently, a completely defined system has been used to generate PGCLCs that differentiated into SSCLCs capable of restoring fertility in mouse testis, generate offspring and generate haploid cells in vitro (Li et al., 2019).

In pigs, culture of PGCLCs under RA, glial cell line-derived neurotrophic factor and testosterone led to derivation of SSCLCs with evidence of in vitro meiosis. After xenotransplantation to immunodeficient mouse testis, cells with morphological characteristics of gametes were observed (Wang et al., 2016). Similar approaches have been investigated in cattle (Malaver-Ortega, Sumer, Jain, & Verma, 2016) and buffalo (Shah, Singla, Palta, Manik, & Chauhan, 2017).

The reconstitution of oogenesis from stem cells in mice has followed a similar co-culture strategy with ovarian foetal somatic cells (Hayashi et al., 2012), accompanied by the use of an estrogen receptor antagonist (ICI182780) that prevented the formation of follicles with abnormal external layers and multiple oocytes (Hikabe et al., 2016; Morohaku et al., 2016). This approach resulted in secondary follicles that, after in vitro growth and maturation, produced MII oocytes. After fertilization, these oocytes gave rise to seemingly normal fertile pups. Lastly, new ESC lines were derived from blastocysts generated using these oocytes. In humans, culture of in vitro-derived PGCLCs in xenogeneic reconstituted

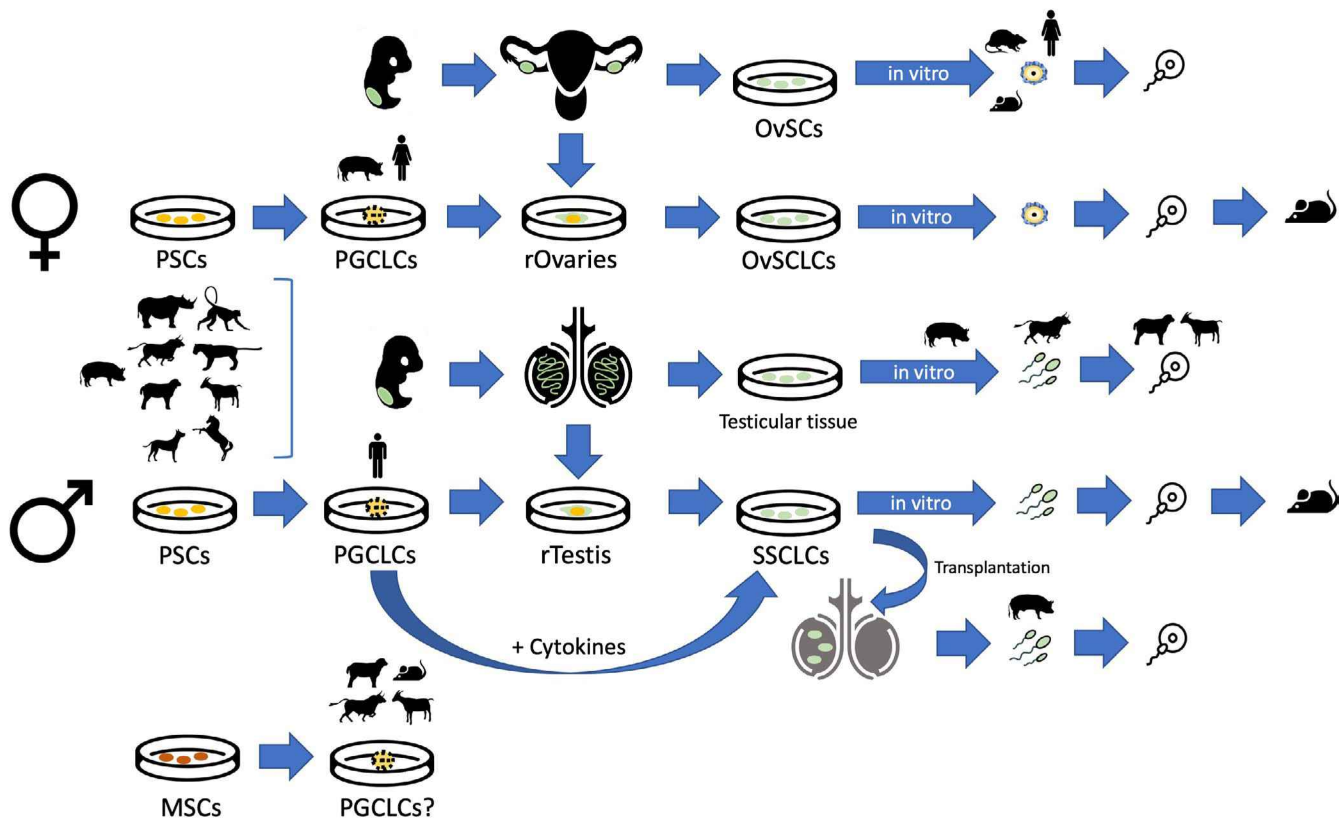

FIGURE 1 State of the science for in vitro gametogenesis in animal species. Symbols indicate the progress achieved in the different animal species to date

ovaries using foetal somatic mouse cells led to the generation of human oogonia-like cells (Yamashiro et al., 2018), representing a critical step towards in vitro gametogenesis in humans. The degree of success achieved in the different animal species up to date is summarized in Figure 1.

8 | IN VITRO SPERMATOGENESIS FROM ADULT SSCS

In general, in vitro spermatogenesis from resident SSCs has been investigated by culturing testicular tissues (Isoler-Alcaraz, Fernández-Pérez, Larriba, & Mazo, 2017; Nakamura et al., 2017). The successful induction of fertile sperm was reported for the first time in 2011 by culturing neonatal testis tissue under gas–liquid interphase with AlbuMAX, a purified albumin product (Sato et al., 2011). Since culture conditions must be optimized for each species, some groups are currently working on the chemical composition of the medium to allow for a better control and adjustment (Sanjo et al., 2018). The culture of testicular tissue has led to the in vitro generation of sperm in cattle (Kim et al., 2015). In sheep, sperm-like cells able to activate oocytes and produce blastocysts have been induced from testicular cells using melatonin (Deng et al., 2016), which has been shown to increase testosterone levels in testes and modify the morphology of spermatogenic cells (Tsantarliotou, Kokolis, & Smokovitis, 2008). Similar differentiation results have been obtained from goat testicular cells (Deng et al., 2017). In pigs, post-meiotic cells have been reported from cultures of testicular cells in α-MEM + KSR supplemented with RA, BMP4, activin A, follicle-stimulating hormone (FSH) and testosterone (Zhao et al., 2018). In a recent study, testicular graft-derived sperm were competent to fertilize rhesus macaque oocytes, leading to pre-implantation embryo development, pregnancy and the birth of a healthy female baby (Fayomi et al., 2019).

In humans, haploid spermatids with fertilizing and developmental capacity have been derived from SSCs isolated from cryptorchid patients after treatment with RA and stem cell factor (Yang et al., 2014).

9 | IN VITRO OOGENESIS FROM ADULT OVSCS

OvSCs have been reported to spontaneously differentiate, at low rates, into oocyte-like cells (large cells with or without a membrane resembling the zona pellucida) in culture. Oocyte-like cells express specific oocyte and meiotic markers including NOBOX, GDF9, ZP1-3, YBX2 and SCP3 (Pacchiarotti et al., 2010; Park, Woods, & Tilly, 2013; White et al., 2012). However, these cells are haploid based on analysis of DNA content (White et al., 2012) and chromosome copies (Silvestris et al., 2018), suggesting that the absence of the follicular environment, or perhaps other factors related to isolation and culture, may result in progression of meiosis beyond the physiological metaphase II arrest, likely rendering the resulting cell incapable of further development. Multistep culture systems have been designed to evaluate the gametogenic potential of OvSCs in vitro. In one study, rat OvSCs were cultured in a monolayer of mitomycin-treated granulosa cells in medium supplemented with BMP4 and RA, followed by addition of ovarian somatic cell suspension and reproductive hormones. This culture system resulted in development of round cells of 30–35 μm of diameter after 25 days (Zhou et al., 2014). A second study evaluated the in vitro differentiation potential of human OvSCs co-cultured with mouse granulosa cells in a 3-step system: in step 1, cells were cultured for 3 days in medium containing bFGF and RA; in step 2, cells were cultured for 6 days in medium containing bFGF, EGF, insulin, transferrin, PMSG and hCG; and in step 3, cells were cultured for 3 days in medium containing oestradiol, progesterone and human follicular fluid. This culture system resulted in development of large round cells resembling GV-stage oocytes and of diameter ranging from 40 to 100 μm (Ding et al., 2016). Most recently, Clarkson et al. (2018) observed development of putative follicular structures after culture of aggregates of DDX4-positive cells with human foetal ovarian tissue for 10 days. To our knowledge, this was the first controlled study to report development of putative follicular structures containing OvSCs. Despite these promising results, no attempt to fertilize oocyte-like cells has been reported in any study to date. Perhaps the first and critical step to obtain a viable oocyte would be to trigger the meiotic programme of putative OvSCs while providing an environment that resembles the foetal ovarian cortex in a cellular, architectural and humoral context to recapitulate follicle formation.

10 | IN VITRO GAMETOGENESIS FROM OTHER ADULT STEM CELLS

In vitro differentiation of VSELs into germline cells has been investigated in human and mouse. Male germ-like cells expressing GFRA, VASA and DAZL have been generated in mouse by culture of VSELs in Sertoli cell conditioned medium supplemented with FSH (Shaikh, Anand, Kapoor, Ganguly, & Bhartiya, 2017). On the other hand, oocyte-like structures have been generated in culture with FSH (Sriraman et al., 2015), RA and follicular fluid (Esmaeilian, Atalay, & Erdemli, 2017); however, their functional competence has not been demonstrated.

Given the ease of isolation and culture of MSCs, several groups have investigated their potential for germline differentiation. In mouse, transplantation of PGCLCs differentiated in vitro from MSCs into germline-depleted testes resulted in morphologically correct gametes, although their functionality was unproven (Nayernia et al., 2006). Expression of germ cell markers upon in vitro differentiation of MSCs isolated from different tissues of livestock species, including sheep (Ghasemzadeh-Hasankolaei, Sedighi-Gilani, & Eslaminejad, 2014), goat (Zhang et al., 2019) and cattle (Cortez et al., 2018), has been reported. In sheep, the use of RA and TGFβ1 as differentiation factors in MSC cultures from bone marrow has led to the generation of germ-like cells that were unable to differentiate after transplantation into testes (Ghasemzadeh-Hasankolaei, Eslaminejad, & Sedighi-Gilani, 2016). In cattle, MSCs isolated from foetal bone marrow have shown potential for generation of early germ cells under culture with BMP4, TGFβ1 and RA (Cortez et al., 2018). However, results were only partially positive since no expression was detected for VASA, STELLA, FRAGILIS, STRA8 or PIWIL2. A recent study has shown that co-culture with Sertoli cells from bull's testis might play an important role in the differentiation of bovine MSCs into germ cells (Segunda et al., 2019), upregulating the expression of DAZL, PIWIL2 and SCP3 compared to monocultures. In goats, male PGCLCs have been obtained by transfection of STRA8, BOULE and DAZL into bone MSCs (Zhang et al., 2019). After transfection, a small population of cells differentiated into PGCLCs with potential to enter meiosis. The PGC identity of these cells was supported by the expression of germ cell markers and decreased methylation at the H19 gene. While MSCs have shown some potential for in vitro germ cell differentiation, no mature gametes have yet been obtained from MSCs. Although the in vivo potential of MSCs to generate gametes has been suggested (Nayernia et al., 2006), further evidence will be required to establish these cell types as sources for in vitro gametogenesis in livestock species.

11 | CONCLUDING REMARKS

Multiple stem cell sources and methodologies have been evaluated for the in vitro differentiation of germ cells, but ESCs and iPSCs have undoubtedly been the most studied. In terms of genetic improvement acceleration, ESCs constitute the best candidate since they offer the largest reduction of generation intervals. Furthermore, these cells have already been deeply characterized, cultured and shown to differentiate into germ cells with developmental capacity. However, other sources of stem cells are still important since they could provide means to generate gametes destined for therapies or germplasm conservation. Besides, alternative stem cell sources could help to unveil and clarify the mechanisms operating within specific stages of germ cell development.

Although partially positive results have been reported for the in vitro generation of germ cells in a number of mammalian livestock species, currently mice remain the only species for which germ cell development has been fully reconstituted in vitro. It is expected that equivalent technologies will be developed for other animal species in the near future. It is only a matter of time until in vitro gametogenesis becomes accessible for use in reproductive medicine, endangered species conservation and genetic improvement of livestock.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORSHIP STATEMENT

All authors wrote the review paper.

DATA SHARING

Data sharing is not applicable to this article as no new data were created nor analyzed in this study.

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