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Review: A barnyard in the lab: prospect of generating animal germ cells for breeding and conservation

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ABSTRACT

In vitro gametogenesis (**IVG**) offers broad opportunities for gaining detailed new mechanistic knowledge of germ cell biology that will enable progress in the understanding of human infertility, as well as for applications in the conservation of endangered species and for accelerating genetic selection of livestock. The realisation of this potential depends on overcoming key technical challenges and of gaining more detailed knowledge of the ontogeny and developmental programme in different species. Important differences in the molecular mechanisms of germ cell determination and epigenetic reprogramming between mice and other animals have been elucidated in recent years. These must be carefully considered when developing IVG protocols, as cellular kinetics in mice may not accurately reflect mechanisms in other mammals. Similarly, diverse stem cell models with potential for germ cell differentiation may reflect alternative routes to successful IVG. In conclusion, the fidelity of the developmental programme recapitulated during IVG must be assessed against reference information from each species to ensure the production of healthy animals using these methods, as well as for developing genuine models of gametogenesis.

Implications

Considerable progress in our understanding of the developmental mechanisms of sperm and eggs has enabled researchers to completely recapitulate these processes in the laboratory resulting in the birth of viable offspring in the mouse. These promising findings suggest that it may be possible to develop similar methods for the generation of *in vitro* gametes in other species, which offers great opportunities for enhancing genetic selection in livestock, developing human fertility treatments and for the rescue of endangered species.

Introduction

Germ cells are the essential link between generations and critical for the continuity of the species. Since the turn of the 21st century, we have seen notable progress in our understanding of the molecular mechanisms of germ cell development, which has been fundamental for the progress in the development of methods for the generation of *in vitro* gametes from pluripotent stem cells (**PSCs**) (Saitou and Miyauchi, 2016). *In vitro* gametogenesis is a promising technology platform that could have multiple applications in medicine, agriculture and animal conservation (Hayashi et al., 2012b; Goszczynski et al., 2019). A key aspect of this technology is the availability of pluripotent stem cells from different species. Recent investigations have provided detailed information on the molecular programmes regulating pluripotency in different mammalian species which has contributed to the establishment of robust stem cell lines that can be used for the generation of *in vitro* gametes (Gao et al., 2019; Kinoshita et al., 2021b; Yu et al., 2021). However, the efficiency of germ cell differentiation remains very modest and further differentiation towards postmeiotic stages has not been achieved *in vitro*, except in mouse (Yoshino et al., 2021). Thus, a more detailed knowledge of the intricate molecular processes controlling gametogenesis will be paramount for the success in creating viable *in vitro* gametes in future.

In this review, we present a brief overview of the latest advances on in vivo and IVG, and we discuss some of the practical applications of this technology within livestock and for wildlife conservation. A key motivation for the development of this technology, especially in livestock, is the ability to select for desirable traits, either by *in vitro* gamete generation from specific animals or by direct genome manipulation. *In vitro* breeding is a term used to describe the use of *in vitro*-generated gametes as part of the technological pipeline for accelerating breeding programmes

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(Goszczynski et al., 2019). The challenge for the coming years will be to fully characterise the species-specific mechanisms regulating germ cell development; a comprehensive understanding of the genetic and epigenetic processes of germ cell development will enable the assessment of the quality of the gametes generated *in vitro*. A stringent set of parameters to assess the quality of the gametes produced *in vitro* will be particularly important to ensure that normal offspring is produced using these technologies.

Mammalian germ cell development

Primordial germ cells (**PGCs**) are the embryonic precursors of the mature germ cells. PGCs develop in two broad ways in vertebrates: one is via germ plasm inheritance, where maternal proteins and RNAs encoding germ cell determinants are directly passed from the egg after fertilisation (Johnson et al., 2003; Seydoux and Braun, 2006). This first mechanism is characteristic of many vertebrates such as teleost fish, frogs, birds (Johnson and Alberio, 2015). The other mechanism is via inductive signals, also known as epigenesis, where PGCs arise from a competent niche formed from a mesodermal cell type (Chatfield et al., 2014; Sasaki et al., 2016; Kojima et al., 2017). This mechanism is characteristic of axolotls, turtles and mammals.

In mammals, PGCs are induced very early in development, as one of the first cell types in the embryo, in response to signals. Soon after specification, they initiate their migration towards their final destination, the embryonic gonad. Here, they undergo extensive proliferation, differentiation and attain sexual dimorphism to form oogonia in females and gonocytes in males. Signals from the gonadal niche in females result in the differentiation to primary oocytes and the onset of meiotic prophase before birth. In males, gonocytes differentiate to prospermatogonia and spermatogonia stem cells (**SSCs**) and undergo mitotic arrest, halting development until puberty. In females, the primary oocytes surrounded by granulosa cells form primordial follicles, which pause development until hormonal stimulation at puberty triggers follicle growth, ovulation, and completion of meiosis upon fertilisation. In pubertal males, SSCs undergo mitotic expansion through spermatogenesis followed by spermiogenesis to form haploid spermatozoa after meiotic divisions. While this process is broadly similar among mammals, there are important species-specific mechanisms regulating these events, including differential transcriptional regulation, cellular dynamics, and cell migratory mechanisms. There are also important differences in the synchronicity of these events, reflecting developmental differences between animals.

The window for primordial germ cell specification

In mice, PGC specification involves bone morphogenetic protein 4 (**BMP4**), produced by the extraembryonic ectoderm, specifying a group of proximal epiblast cells that activate *Prdm14* and *Prdm1* becoming lineage-restricted precursors at around embryonic day 6.5 (**E6.5**) of development. By E7.75, a small niche of cells has expanded to around 40 cells and begun migration as a group (Molyneaux et al., 2001). These cells express the pluripotency markers *Oct-4*, *Sox2* and *Nanog*, as well as the germ cell marker *Nanos3*. These cells migrate through the gut to the genital ridge which they colonise and then begin mass DNA demethylation at day 9.5 (Yamaguchi et al., 2013).

In other mammals, which lack extraembryonic ectoderm, the source of BMP4 has been identified in other extraembryonic tissues, such as the amnion in monkeys and the extraembryonic mesoderm in the pig (Valdez Magana et al., 2014; Sasaki et al., 2016). Within this environment human, non-human primate, rabbit and pig PGCs are identified by the expression of *SOX17, PRDM1* and *TFAP2C* (Sasaki et al., 2016; Kobayashi et al., 2017; Kobayashi et al., 2021). A functional experiment using pig epiblasts determined the need of BMP4 for the induction of the PGC programme from competent epiblast (Kobayashi et al., 2017). In pig embryos, the window for PGC specification spans from E11.5, where PGCs appear in the posterior epiblast in a region expressing *TBXT* (also known as BRACHYURY), just before the onset of primitive streak formation. The number of PGCs, which are non-replicative during this stage, appears to be recruited from PGC competent mesoderm cells in the posterior end of the embryo (Kobayashi et al., 2017). Remarkably, the PGC cluster can still be observed at day 15, suggesting a window of at least 4 days when these cells can be induced in the pig (Kobayashi et al., 2017).

In monkeys, a few PGCs are first detected in the amnion, and later, this a cluster of cells is detected in the posterior end of the epiblast (Sasaki et al., 2016). The discrepancy on the origin of PGCs between primates and other large mammals appears confounded by the timing in which the amnion first appears (Kobayashi and Surani, 2018). In rabbits and pigs, the amnion forms after PGC specification, however in primates, the amnion delaminates from epiblast cells around the time of implantation, when PGC specification also occurs. As a result of this alternative origin, it has been proposed that in primates, PGCs have a dual origin: a founder population forming within the amnion and a cluster of PGCs emerging in the pregastrulation *TXBT* positive posterior epiblast of the embryo. It is thought that these two populations merge within the cluster in the posterior epiblast before PGC migration.

PGC competence in mammalian embryos is determined by WNT activity and is reflected in the expression of *EOMESODERMIN* and *TBXT* (Chen et al., 2017; Kojima et al., 2017). Thus, the capacity to form germ cells in the embryo depends on the capacitation of pluripotent cells by WNT to elicit the germ cell programme in response to BMP (Jo et al., 2022). In the pig embryo, this population of cells appears to persist for several days (~3.5 days), hence, PGCs are generated over a long period, in contrast to mice where the PGC pool is set by ~E8.5. *In vitro* experiments using human embryonic stem cells (**hESCs**) show that PGC differentiation potential is transient. In such conditions, after a brief exposure to mesoderm differentiation cues (WNT agonist CHIR99021, and Activin A), hESC differentiate within ~12 hrs into premesoderm cells that can respond to a cytokine cocktail driving cells towards primordial germ cell-like cells (**PGCLCs**). By 24 hrs, premesendoderm (**pre-Me**) cells progress towards mesoderm and the cells lose competence for PGC fate, and instead differentiate towards an endodermal or mesodermal fate. This indicates that current differentiation protocols cannot maintain the competent niche *in vitro* for more than a few hours. In the embryo, a population of cells known as germline competent mesoderm (Savage et al., 2021) is the source of PGCs in the pig between days 11.5 and 15 (Kobayashi et al., 2017), suggesting that specific signals in the posterior end of the embryo enable continuous specification of PGCs from these precursors.

Transcription factor network of primordial germ cell specification in non-rodent mammals

In the embryos of primates, rabbits and pigs, PGC specification is determined by the balanced expression of *SOX17*, *PRDM1* and *TFAP2C*. Soon after the activation of *SOX17*, the pluripotency gene *SOX2* is repressed and *NANOS3* is rapidly upregulated (Kobayashi et al., 2017; Kojima et al., 2017; Kobayashi et al., 2021).

Other regulators of the germline programme have recently been reported. *TFAP2A,* a classic amnion marker, is an early response gene following BMP stimulation of capacitated hESC, marking a

population of PGC-competent cells *in vitro* (Chen et al., 2019). A recent study provided further evidence demonstrating that *TFAP2A* plays a role in repressing *SOX2* as well as naïve pluripotency and neural genes during the establishment of the hPGC programme (Castillo-Venzor et al., 2022). Interestingly, *TFAP2A* is only transiently expressed and is downregulated after activation of *TFAP2C* and *SOX17*, suggesting it plays a key role in extinguishing some of the major players of the pluripotency network during PGC specification.

Other BMP4 targets identified in the capacitated hESC are *GATA2/3*, which when overexpressed with *SOX17* and *TFAP2C* can induce hPGCLCs that have properties equivalent to those induced by BMP4 (Kojima et al., 2017). Notably, *GATA2/3* are expressed in *Cynomolgus* monkey, rabbit and pig nascent PGCs, suggesting a conserved role of these factors in the germline programme across mammals.

In vitro generation of primordial germ cell-like cells from pluripotent stem cells across multiple species

Original approaches for the generation of mouse PGC, established nearly two decades ago, were based on the formation of cell aggregates called embryoid bodies (EBs). These EBs can give rise to PGCLCs at a variable efficiency (~5–40%) (Hubner et al., 2003). This variability led investigators to pursue alternative avenues. One such avenue was the use of a two-step mechanism whereby intermediate epiblast-like cells (**EpiLCs**) are first created by medium supplementation with Activin and fibroblast growth factor (**FGF**). This population, representing the mouse E6.0 epiblast, can be differentiated into PGCLC using BMP4, leukaemia inhibitory factor, epithelial growth factor and stem cell factor at high efficiency (Hayashi et al., 2011). It was shown later that BMP4 induces expression of *T (*or *Brachyury),* which in turn directly activates *Prdm1* and *Prdm14*, both of which are critical for mouse PGC specification (Aramaki et al., 2013).

Notably, mouse epiblast stem cells, equivalent to E6.5 epiblast, are not capable of efficient PGC differentiation (Brons et al., 2007; Tesar et al., 2007), indicating that the window for mPGC specification in vivo is after the establishment of the naïve state in the blastocyst and before the formation of the primed epiblast. This intermediate phase of mouse development has been recently characterised as the formative state (**FS**) of pluripotency (Smith, 2017; Kinoshita and Smith, 2018). Cells in the formative phase can be captured *in vitro*, they retain the potential to contribute to chimeras and importantly, they can produce PGCs. Indeed, *in vitro* differentiation potential of FS cells is very robust (Kinoshita et al., 2021a). Whether functionally equivalent formative cells exist in other species is still under investigation, although, human FS cells have been derived from blastocysts (Kinoshita et al., 2021a).

The developmental relationship between mFS cells and mEpiLC indicates that the former are equivalent to a more advanced epiblast (~E6) compared to mEpiLCs, which have a gene expression profile more akin to the E5.5 epiblast, displaying reduced Nanog levels and lack lineage priming. It is likely that the increased Nanog levels in FS cells are due to culture adaptation. mEpiLCs can contribute to fully functional male and female germ cells after transplantation into testes or the ovarian bursa, and give rise to fertile offspring. This demonstrates the equivalence between *in vitro*-generated PGCLC and natural PGCs (Hayashi et al., 2011; Hayashi et al., 2012a).

In humans, the *in vitro* generation of PGCLCs from PSC is essentially based on two starting conditions. One called 4i, in which a specific combination of inhibitors supports self-renewal of naïve hESC and can be induced either directly by BMP4 or after 48 hrs release from 4i, while maintained in bFGF, TGFb1 and 1% KnockOut Serum Replacement (**KSR**) for 2 days prior to PGC induction (Gafni et al., 2013; Irie et al., 2015). This strategy was further refined and simplified to promote a transition from the pluripotent state to pre-ME cells, in which cells are cultured with a WNT agonist (3 μM Glycogen synthase kinase 3βi) and high concentration of Activin A (100 ng/ml) for 12–16 hrs prior to PGC induction (Kobayashi et al., 2017). Under these conditions, ~40% PGCLCs can be induced in aggregates. Another method using a lower concentration of Activin A (50 ng/ml), a WNT agonist, plus supplementation with 15% KSR yields ~30% PGCLCs; however, in this case, the window for induction is 42–48 hrs (Sasaki et al., 2015). Cells produced under these conditions can also be expanded long-term, while maintaining PGCLC characteristics and retaining the potential for differentiation into oogonia/gonocytes (Murase et al., 2020). Key components of the carefully balanced medium include the use of forskolin, FGF and low levels of glucose. This is a major achievement that contrasts with the findings in the mouse, where mPGCLCs can only propagate for about one week and are very difficult to passage. It is possible that this capacity of long-term expansion in hPGCLCs reflects the natural capacity of these cells to expand in large numbers, reaching 7×10^6 by week 19 in human (Mamsen et al., 2011), whereas in the mouse, 25,000 oogonia/oocytes are counted by E13.5 (Kagiwada et al., 2013).

Another recent study demonstrated the long-term expansion of marmoset PGC-like cells from induced pluripotent stem cells (**iPSCs**) (Seita et al., 2023). The culture system relies on STO-feeder cells, low serum supplementation, forskolin and FGF2, similar to hPGCL cells. These cells are capable of differentiating (albeit inefficiently) into a more mature DAZL + DDX4 + population, demonstrating the scalability of this system. Adapting these conditions to PGCL cells from other species will be critical for accelerating the development of advanced methodologies for generating advanced developmental stages as serve as the foundation for the systematic enhancement of *in vitro* gametogenesis.

In domestic species, there has been far less success in the differentiation of stem cells towards PGCLCs, partly due to the lack of robust PSC lines. An early report using pig iPSC showed induction of PGCLCs using a similar cocktail of cytokines as used in mouse and human studies (Wang et al., 2016). These iPSCs were maintained under naïve conditions and required the transition through an induced mesoderm-like state for efficient PGCLC differentiation. After one week of *in vitro* differentiation, the cells were transplanted into the seminiferous tubules of busulfan-treated mouse testis, and 6 weeks later, they determined differentiation towards spermatogonial stem cells. A recent report in pigs showed variable fidelity in recapitulating the PGC programme *in vitro* depending on the culture conditions for maintaining iPSCs (Pieri et al., 2022). Thus, more work is needed to identify the best conditions for PGCLC induction from stem cells.

Attempts in cattle show a very inefficient induction of PGCLCs from iPSCs (Malaver-Ortega et al., 2016). An alternative method using expanded potential stem cells demonstrated the generation of pPGCLCs following a brief period of 12 hrs overexpression of *SOX17* and incubation in BMP4 containing medium (Gao et al., 2019). Although the cells activate multiple PGC markers, the proportion of PGCLCs was below 10%, suggesting that the transition from the expanded potential state to a PGCLCs requires further optimisation. Another type of embryonic stem cells derived from horse blastocysts, known as eqFTW stem cells, which require FGF, TGFB and a WNT agonist, can also be differentiated into PGCLCs (Yu et al., 2021). These cells however require feeder cells for culture, and therefore, it is unclear what other factors are

required for maintaining the pluripotent state. Nonetheless, the notable difference with other PGC differentiation protocols is that these cells can directly respond to BMP4, much like FS cells (Kinoshita et al., 2021b). The important difference with FS cells is that WNT is inhibited in FS cells compared to FTW cells. The extent to which feeder cells are modulating WNT signalling in FTW cells to confer PGC differentiation capacity requires further investigation.

In vitro gametogenesis

Differentiation to either male or female mature gametes *in vitro* (*in vitro* gametogenesis or IVG) has been the goal of many investigators for a long time, and great strides have been made recently across several animal models. Much of this work has been done in mouse models due to the ease of obtaining materials, both cultured stem cell lines and sex organs. A common approach to the generation of gametes using mouse material is to incubate *in vitro*-derived PGCLCs with either mouse testis or mouse ovarian cells (Hayashi et al., 2011; Hayashi and Saitou, 2013). This approach has also recently been reported in the rats (Oikawa et al., 2022). Oocytes generated in this manner can be fertilised with in vivo-produced sperm and implanted into mice to produce viable offspring. This procedure remains inefficient (3.5% of implanted two cell embryos produced viable pups) and there remain many differences in mitochondrial gene expression between *in vitro* oocytes and naturally ovulated eggs (Hikabe et al., 2016). Despite these difficulties, this landmark report represents a complete recapitulation of the female germ line *in vitro*. Since then, approaches to generate oocyte-like structures with competence for fertilisation were also generated directly from PSCs by overexpression of genes involved in oogenesis (Hamazaki et al., 2021). These oocyte-like structures did not undergo a complete reprogramming sequence, indicating that meiosis is decoupled from oocyte growth, but highlights the potential for generating developmentally competent ooplasm that could be used for understanding mechanisms of reprogramming and fertilisation.

Generation of male gametes has proved more challenging: whilst mouse spermatogonia can be generated as outlined above, these cells fail to undergo meiosis as they do in vivo. A paper by Zhou et al. used follicle-stimulating hormone, bovine pituitary extract and Testosterone after PGCLC/mouse testis cell mixing to produce haploid spermatid-like cells (Zhou et al., 2016). These mouse haploid spermatid-like cells can produce fertile offspring via Intracytoplasmic injection. Despite these promising results, the report lacks detailed analysis of the key intermediate events (Saitou and Miyauchi, 2016). As noted in other studies, *in vitro*-produced oocytes and spermatogonia produced *in vitro* exhibit multiple genetic and epigenetic anomalies (Ishikura et al., 2016), indicating that thorough analysis is critical to determine the appropriate developmental trajectories supported by the established cultured protocols.

Translating the protocols devised in the mouse to humans is not possible, because of the well-known physiological differences and the timing of gametogenesis. Nevertheless, a study that successfully achieved advanced stages of human gametogenesis showed the generation of oogonia and gonocytes 70 days after the aggregation of hPGCLCs with mouse ovarian somatic cells (Yamashiro et al., 2018). Further maturation has not been achieved, and a possible solution would be to use gonadal somatic cells from the same species to mature the germ cells. Generating these cells is a challenge, though was recently demonstrated in the mouse system (Yoshino et al., 2021).

In 2020, Hwang et al. reported the successful generation of human prospermatogonia-like cells from a population of hPGCLC, originally derived from human induced pluripotent stem cell (hiPSC). In brief, hPGCLCs were incubated with mouse somatic testis cells obtained from an E12.5 mouse embryo in floating culture (Hwang et al., 2020). Whilst there are problems with using iPSC to study fundamental developmental biology when compared to ESC, as iPSCs do not have a clear analogue *in vivo*, they carry advantages when thinking of applications, especially within the context of personalised healthcare and infertility treatment.

Future opportunities for *in vitro* gametogenesis and current challenges

Technological advances in recent years have generated great interest in the creation of gametes *in vitro*. The possibilities of producing such valuable cells offer opportunities for improving the genetic selection of commercial breeds of livestock, through a combination of techniques that employ a diversity of assisted reproductive technologies (Goszczynski et al., 2019) (Fig. 1). In time, the incorporation of gene editing within this platform will enable the rapid introduction of desired quantitative trait loci (QTLs) that will further enhance the phenotypes of the animals produced (Hu et al., 2016). These methods will also enable the generation of novel breeds, with enhanced adaptability to novel environments (e.g. increased heat tolerance).

Species conservation is also an area where assisted reproduction and IVG are set to contribute. IVG represents the best option for rescuing the last specimens of a given species such as the northern white rhinoceros, where recent work has demonstrated the ability to generate PGCLC from both ESC and iPSC (Hildebrandt et al., 2021; Hayashi et al., 2022). The prospect of generating robust iPSC technologies from biopsies of endangered species will enable the generation of gametes and offer the potential for sexual reproduction using laboratory-produced gametes.

Finally, IVG will also represent a valuable platform for the identification of genetic and epigenetic causes of infertility (in humans as well as in livestock species). Laboratory-grown gametes will also enable the improvement of assisted reproductive techniques and will be useful resource for investigations into the effects of environmental chemicals, endocrine disruptors, and other pollutants in the developmental potential of gametes and well as their effects on chromosomal abnormalities.

Before we can fully realise the full potential of IVG technology, there are technical aspects that must be improved. A better understanding of the cytokine requirements for robust induction and terminal differentiation of germ cells in defined culture conditions is of critical importance. We must also seek to fully elucidate the epigenetic landscape of early PGC differentiation and precisely delineate the sequence of these events and ensure a faithful recapitulation of the epigenetic resetting takes place in *in vitro* gametes.

In farm animals, more and more cell lines are becoming available for use, both in terms of species coverage and individual breeds, which affords the opportunity for the development of more robust PGC differentiation protocols. However, there is a need to further refine the establishment of stem cell lines with high competence for germ cell differentiation (Table 1). New understanding of the species-specific features of embryo development is becoming available through the use of advanced genomic technologies (e.g. temporally resolved single-cell RNAseq atlases). This information will serve as a basis for improving the conditions for stem cell culture and PGC induction. Broadening the knowledge of germ cell development in non-primate and non-rodent models, such as pig and rabbit, will have significant practical and theoretical impact in bridging the knowledge gap across mammalian species.

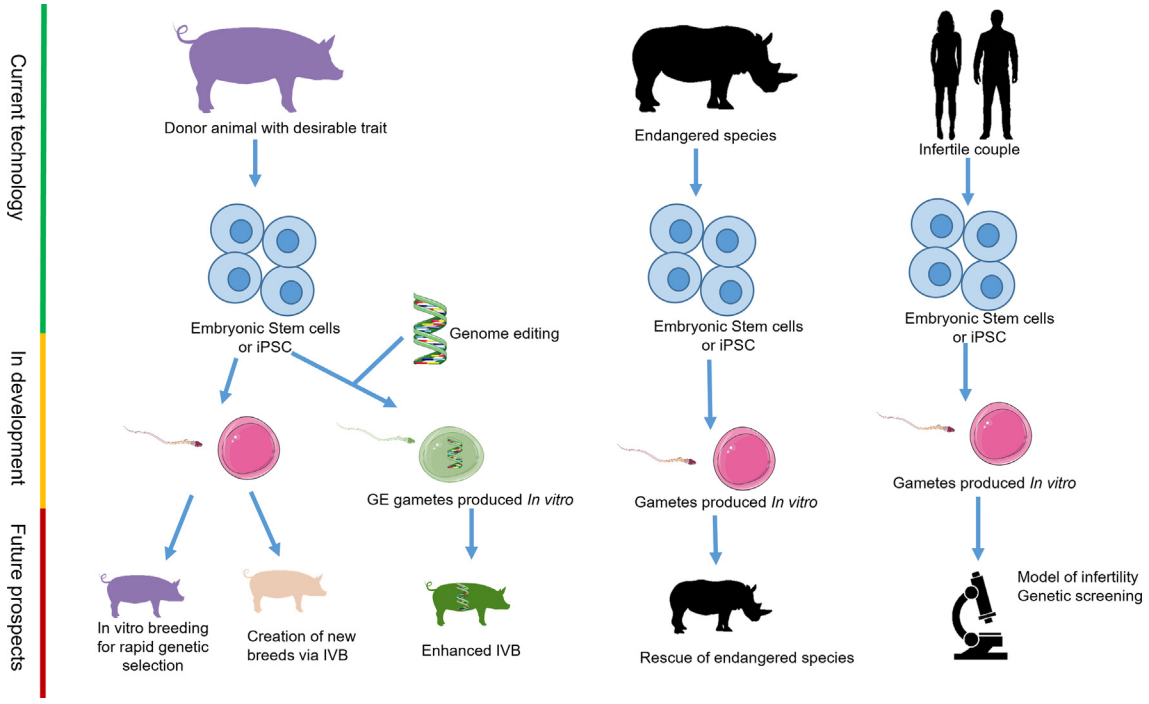

Fig. 1. Schematic of future applications of mammalian *in vitro* gamete production. From left to right: *In vitro* gametes will be used for more efficient production of offspring from elite animals with specific desirable traits. Genome editing of livestock for enhancement of specific trait using known QTLs will also be incorporated when the safety of these technologies is ascertained. Rescue or conservation of endangered species using a surrogate evolutionary-related species. Production of gametes from infertile couples for research purposes as a model of infertility and for improving assisted reproductive biotechnologies. GE: gene edited; iPSC: induced pluripotent stem cell; IVB: *in vitro* breeding; QTL: quantitative trait loci.

Table 1
Summary of comparative levels of *in vitro* gamete production between species.

Item	ESC lines establishment	PGCLC differentiation	Mature gamete production
Human/ Mouse	Technology is robust and efficient		Technology is inefficient, poorly understood, or uses chemically undefined methods (e.g. feeders)
Bovine/ Porcine/ Rabbit	Technology is robust but is restricted to a few cells lines and lacks widespread deployment	Technology is inefficient, and poorly understood.	Technology has yet to be achieved

ESC: embryonic stem cell; PGCLC: primordial germ cell-like cells.

Conclusion

For IVG to become a reality, numerous challenges need to be resolved. Whilst promising results in mice show that live offspring can be produced using this method, compromised development and reduced survival are reported in some of the offspring. It is essential to carry out comprehensive assessment of the molecular features of the animals generated using these methods to ensure that no abnormal genetic and epigenetic signatures are transferred across generations. It goes without saying that the potential for intergenerational inheritance makes the safety aspect of the technology of paramount importance to ensure that the fidelity of this information is not compromised by artefacts (epimutations) introduced during *in vitro* gametogenesis.

Ethics approval

Not applicable.

Data and model availability

No new datasets were created.

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Authors contributions

AS: conceptualisation, writing original draft and created the figure.
RA: conceptualisation, Writing, Review and Editing.

Declaration of interests

The authors declare no competing interests.

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Transparency Declaration

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References

Aramaki, S., Hayashi, K., Kurimoto, K., Ohta, H., Yabuta, Y., Iwanari, H., Mochizuki, Y., Hamakubo, T., Kato, Y., Shirahige, K., Saitou, M., 2013. A mesodermal factor, T, specifies mouse germ cell fate by directly activating germline determinants. Developmental Cell 27, 516–529.

Brons, I.G., Smithers, L.E., Trotter, M.W., Rugg-Gunn, P., Sun, B., de Sousa, C., 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.

Castillo-Venzor, A., Penfold, C.A., Morgan, M.D., Tang, W.W.C., Kobayashi, T., Wong, F.C.K., Bergmann, S., Slatery, E., Boroviak, T.E., Marioni, J.C., Surani, M.A., 2022. Origin and segregation of the human germline. bioRxiv, 2022.2007.2006.498671. doi: 10.1101/2022.07.06.498671. Retrieved on 14 February 2023.

Chatfield, J., O'Reilly, M.A., Bachvarova, R.F., Ferjentsik, Z., Redwood, C., Walmsley, M., Patient, R., Loose, M., Johnson, A.D., 2014. Stochastic specification of primordial germ cells from mesoderm precursors in axolotl embryos. Development 141, 2429–2440. https://doi.org/10.1242/dev.105346.

Chen, D., Liu, W., Lukianchikov, A., Hancock, G.V., Zimmerman, J., Lowe, M.G., Kim, R., Galic, Z., Irie, N., Surani, M.A., Jacobsen, S.E., Clark, A.T., 2017. Germline competency of human embryonic stem cells depends on eomesodermin. Biology of Reproduction 97, 850–861.

Chen, D., Sun, N., Hou, L., Kim, R., Faith, J., Aslanyan, M., Tao, Y., Zheng, Y., Fu, J., Liu, W., Kellis, M., Clark, A., 2019. Human Primordial Germ Cells Are Specified from Lineage-Primed Progenitors. Cell Reports 29 (4568–4582), e4565.

Gafni, O., Weinberger, L., Mansour, A.A., Manor, Y.S., Chomsky, E., Ben-Yosef, D., Kalma, Y., Viukov, S., Maza, I., Zviran, A., Rais, Y., Shipony, Z., Mukamel, Z., Krupalnik, V., Zerbib, M., Geula, S., Caspi, I., Schneir, D., Shwartz, T., Gilad, S., Amann-Zalcenstein, D., Benjamin, S., Amit, I., Tanay, A., Massarwa, R., Novershtern, N., Hanna, J.H., 2013. Derivation of novel human ground state naive pluripotent stem cells. Nature 504, 282–286.

Gao, X., Nowak-Imialek, M., Chen, X., Chen, D., Herrmann, D., Ruan, D., Chen, A.C.H., Eckersley-Maslin, M.A., Ahmad, S., Lee, Y.L., Kobayashi, T., Ryan, D., Zhong, J., Zhu, J., Wu, J., Lan, G., Petkov, S., Yang, J., Antunes, L., Campos, L.S., Fu, B., Wang, S., Yong, Y., Wang, X., Xue, S.G., Ge, L., Liu, Z., Huang, Y., Nie, T., Li, P., Wu, D., Pei, D., Zhang, Y., Lu, L., Yang, F., Kimber, S.J., Reik, W., Zou, X., Shang, Z., Lai, L., Surani, A., Tam, P.P.L., Ahmed, A., Yeung, W.S.B., Teichmann, S.A., Niemann, H., Liu, P., 2019. Establishment of porcine and human expanded potential stem cells. Nature Cell Biology 21, 687–699.

Goszczynski, D.E., Cheng, H., Demyda-Peyras, S., Medrano, J.F., Wu, J., Ross, P.J., 2019. In vitro breeding: application of embryonic stem cells to animal productiondagger. Biology of Reproduction 100, 885–895.

Hamazaki, N., Kyogoku, H., Araki, H., Miura, F., Horikawa, C., Hamada, N., Shimamoto, S., Hikabe, O., Nakashima, K., Kitajima, T.S., Ito, T., Leitch, H.G., Hayashi, K., 2021. Reconstitution of the oocyte transcriptional network with transcription factors. Nature 589, 264–269.

Hayashi, Y., Saitou, M., Yamanaka, S., 2012b. Germline development from human pluripotent stem cells toward disease modeling of infertility. Fertility and Sterility 97, 1250–1259.

Hayashi, K., Saitou, M., 2013. Generation of eggs from mouse embryonic stem cells and induced pluripotent stem cells. Nature Protocols 8, 1513–1524. https://doi.org/10.1038/nprot.2013.090.

Hayashi, K., Ohta, H., Kurimoto, K., Aramaki, S., Saitou, M., 2011. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell 146, 519–532.

Hayashi, K., Ogushi, S., Kurimoto, K., Shimamoto, S., Ohta, H., Saitou, M., 2012a. Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. Science 338, 971–975.

Hayashi, M., Zywitza, V., Naitou, Y., Hamazaki, N., Goeritz, F., Hermes, R., Holtze, S., Lazzari, G., Galli, C., Stejskal, J., Diecke, S., Hildebrandt, T.B., Hayashi, K., 2022. Robust induction of primordial germ cells of white rhinoceros on the brink of extinction. Science Advances 8, eabp9683.

Hikabe, O., Hamazaki, N., Nagamatsu, G., Obata, Y., Hirao, Y., Hamada, N., Shimamoto, S., Imamura, T., Nakashima, K., Saitou, M., Hayashi, K., 2016. Reconstitution in vitro of the entire cycle of the mouse female germ line. Nature 539, 299–303.

Hildebrandt, T.B., Hermes, R., Goeritz, F., Appeltant, R., Colleoni, S., de Mori, B., Diecke, S., Drukker, M., Galli, C., Hayashi, K., Lazzari, G., Loi, P., Payne, J., Renfree, M., Seet, S., Stejskal, J., Swegen, A., Williams, S.A., Zainuddin, Z.Z., Holtze, S., 2021. The ART of bringing extinction to a freeze - History and future of species conservation, exemplified by rhinos. Theriogenology 169, 76–88.

Hu, Z.L., Park, C.A., Reecy, J.M., 2016. Developmental progress and current status of the Animal QTLdb. Nucleic Acids Research 44, 827–833.

Hubner, K., Fuhrmann, G., Christenson, L.K., Kehler, J., Reinbold, R., De La Fuente, R., Wood, J., Strauss 3rd, J.F., Boiani, M., Scholer, H.R., 2003. Derivation of oocytes from mouse embryonic stem cells. Science 300, 1251–1256.

Hwang, Y.S., Suzuki, S., Seita, Y., Ito, J., Sakata, Y., Aso, H., Sato, K., Hermann, B.P., Sasaki, K., 2020. Reconstitution of prospermatogonial specification in vitro from human induced pluripotent stem cells. Nature Communications 11, 5656.

Irie, N., Weinberger, L., Tang, W.W., Kobayashi, T., Viukov, S., Manor, Y.S., Dietmann, S., Hanna, J.H., Surani, M.A., 2015. SOX17 is a critical specifier of human primordial germ cell fate. Cell 160, 253–268.

Ishikura, Y., Yabuta, Y., Ohta, H., Hayashi, K., Nakamura, T., Okamoto, I., Yamamoto, T., Kurimoto, K., Shirane, K., Sasaki, H., Saitou, M., 2016. In Vitro Derivation and Propagation of Spermatogonial Stem Cell Activity from Mouse Pluripotent Stem Cells. Cell Reports 17, 2789–2804.

Jo, K., Teague, S., Chen, B., Khan, H.A., Freeburne, E., Li, H., Li, B., Ran, R., Spence, J.R., Heemskerk, I., 2022. Efficient differentiation of human primordial germ cells through geometric control reveals a key role for Nodal signaling. Elife 11, e72811.

Johnson, A.D., Alberio, R., 2015. Primordial germ cells: the first cell lineage or the last cells standing? Development 142, 2730–2739.

Johnson, A.D., Crother, B., White, M.E., Patient, R., Bachvarova, R.F., Drum, M., Masi, T., 2003. Regulative germ cell specification in axolotl embryos: a primitive trait conserved in the mammalian lineage. Philosophical Transactions Royal Society of London B Biological Sciences 358, 1371–1379.

Kagiwada, S., Kurimoto, K., Hirota, T., Yamaji, M., Saitou, M., 2013. Replication-coupled passive DNA demethylation for the erasure of genome imprints in mice. EMBO Journal 32, 340–353.

Kinoshita, M., Kobayashi, T., Planells, B., Klisch, D., Spindlow, D., Masaki, H., Bornelov, S., Stirparo, G.G., Matsunari, H., Uchikura, A., Lamas-Toranzo, I., Nichols, J., Nakauchi, H., Nagashima, H., Alberio, R., Smith, A., 2021b. Pluripotent stem cells related to embryonic disc exhibit common self-renewal requirements in diverse livestock species. Development 148, dev199901.

Kinoshita, M., Smith, A., 2018. Pluripotency Deconstructed. Development Growth and Differentiation 60, 44–52.

Kinoshita, M., Barber, M., Mansfield, W., Cui, Y., Spindlow, D., Stirparo, G.G., Dietmann, S., Nichols, J., Smith, A., 2021a. Capture of Mouse and Human Stem Cells with Features of Formative Pluripotency. Cell Stem Cell 28, 2180.

Kobayashi, T., Castillo-Venzor, A., Penfold, C.A., Morgan, M., Mizuno, N., Tang, W.W.C., Osada, Y., Hirao, M., Yoshida, F., Sato, H., Nakauchi, H., Hirabayashi, M., Surani, M. A., 2021. Tracing the emergence of primordial germ cells from bilaminar disc rabbit embryos and pluripotent stem cells. Cell Reports 37, 109812.

Kobayashi, T., Surani, M.A., 2018. On the origin of the human germline. Development 145, dev.150433.

Kobayashi, T., Zhang, H., Tang, W.W.C., Irie, N., Withey, S., Klisch, D., Sybirna, A., Dietmann, S., Contreras, D.A., Webb, R., Allegrucci, C., Alberio, R., Surani, M.A., 2017. Principles of early human development and germ cell program from conserved model systems. Nature 546, 416–420.

Kojima, Y., Sasaki, K., Yokobayashi, S., Sakai, Y., Nakamura, T., Yabuta, Y., Nakaki, F., Nagaoka, S., Woltjen, K., Hotta, A., Yamamoto, T., Saitou, M., 2017. Evolutionarily Distinctive Transcriptional and Signaling Programs Drive Human Germ Cell Lineage Specification from Pluripotent Stem Cells. Cell Stem Cell 21, 517–532.

Malaver-Ortega, L.F., Sumer, H., Jain, K., Verma, P.J., 2016. Bone morphogenetic protein 4 and retinoic acid trigger bovine VASA homolog expression in differentiating bovine induced pluripotent stem cells. Molecular Reproduction and Development 83, 149–161.

Mamsen, L.S., Lutterodt, M.C., Andersen, E.W., Byskov, A.G., Andersen, C.Y., 2011. Germ cell numbers in human embryonic and fetal gonads during the first two trimesters of pregnancy: analysis of six published studies. Human Reproduction 26, 2140–2145.

Molyneaux, K.A., Stallock, J., Schaible, K., Wylie, C., 2001. Time-lapse analysis of living mouse germ cell migration. Developmental Biology 240, 488–498.

Murase, Y., Yabuta, Y., Ohta, H., Yamashiro, C., Nakamura, T., Yamamoto, T., Saitou, M., 2020. Long-term expansion with germline potential of human primordial germ cell-like cells in vitro. EMBO Journal 39, e104929.

Oikawa, M., Kobayashi, H., Sanbo, M., Mizuno, N., Iwatsuki, K., Takashima, T., Yamauchi, K., Yoshida, F., Yamamoto, T., Shinohara, T., Nakauchi, H., Kurimoto, K., Hirabayashi, M., Kobayashi, T., 2022. Functional primordial germ cell-like cells from pluripotent stem cells in rats. Science 376, 176–179.

Pieri, N.C.G., de Souza, A.F., Botigelli, R.C., Pessoa, L.V.F., Recchia, K., Machado, L.S., Gloria, M.H., de Castro, R.V.G., Leal, D.F., Fantinato Neto, P., Martins, S., Dos Santos Martins, D., Bressan, F.F., de Andrade, A.F.C., 2022. Porcine Primordial Germ Cell-Like Cells Generated from Induced Pluripotent Stem Cells Under Different Culture Conditions. Stem Cell Reviews and Reports 18, 1639–1656.

Saitou, M., Miyauchi, H., 2016. Gametogenesis from Pluripotent Stem Cells. Cell Stem Cell 18, 721–735.

Sasaki, K., Yokobayashi, S., Nakamura, T., Okamoto, I., Yabuta, Y., Kurimoto, K., Ohta, H., Moritoki, Y., Iwatani, C., Tsuchiya, H., Nakamura, S., Sekiguchi, K., Sakuma, T., Yamamoto, T., Mori, T., Woltjen, K., Nakagawa, M., Yamamoto, T., Takahashi, K., Yamanaka, S., Saitou, M., 2015. Robust In Vitro Induction of Human Germ Cell Fate from Pluripotent Stem Cells. Cell Stem Cell 17, 178–194.

Sasaki, K., Nakamura, T., Okamoto, I., Yabuta, Y., Iwatani, C., Tsuchiya, H., Seita, Y., Nakamura, S., Shiraki, N., Takakuwa, T., Yamamoto, T., Saitou, M., 2016. The Germ Cell Fate of Cynomolgus Monkeys Is Specified in the Nascent Amnion. Developmental Cell 39, 169–185.

Savage, A.M., Alberio, R., Johnson, A.D., 2021. Germline competent mesoderm: the substrate for vertebrate germline and somatic stem cells? Biology Open 10, bio.058890.
Seita, Y., Cheng, K., McCarrey, J.R., Yadu, N., Cheeseman, I.H., Bagwell, A., Ross, C.N., Santana Toro, I., Yen, L.H., Vargas, S., Navara, C.S., Hermann, B.P., Sasaki, K., 2023. Efficient generation of marmoset primordial germ cell-like cells using induced pluripotent stem cells. eLife 12, 82263.
Seydoux, G., Braun, R.E., 2006. Pathway to totipotency: lessons from germ cells. Cell 127, 891–904.
Smith, A., 2017. Formative pluripotency: the executive phase in a developmental continuum. Development 144, 365–373.
Tesar, P.J., Chenoweth, J.G., Brook, F.A., Davies, T.J., Evans, E.P., Mack, D.L., Gardner, R. L., McKay, R.D., 2007. New cell lines from mouse epiblast share defining features with human embryonic stem cells. Nature 448, 196–199.
Valdez Magana, G., Rodriguez, A., Zhang, H., Webb, R., Alberio, R., 2014. Paracrine effects of embryo-derived FGF4 and BMP4 during pig trophoblast elongation. Developmental Biology 387, 15–27.
Wang, H., Xiang, J., Zhang, W., Li, J., Wei, Q., Zhong, L., Ouyang, H., Han, J., 2016. Induction of Germ Cell-like Cells from Porcine Induced Pluripotent Stem Cells. Scientific Reports 6, 27256.
Yamaguchi, S., Hong, K., Liu, R., Inoue, A., Shen, L., Zhang, K., Zhang, Y., 2013. Dynamics of 5-methylcytosine and 5-hydroxymethylcytosine during germ cell reprogramming. Cell Research 23, 329–339.
Yamashiro, C., Sasaki, K., Yabuta, Y., Kojima, Y., Nakamura, T., Okamoto, I., Yokobayashi, S., Murase, Y., Ishikura, Y., Shirane, K., Sasaki, H., Yamamoto, T., Saitou, M., 2018. Generation of human oogonia from induced pluripotent stem cells in vitro. Science 362, 356–360.
Yoshino, T., Suzuki, T., Nagamatsu, G., Yabukami, H., Ikegaya, M., Kishima, M., Kita, H., Imamura, T., Nakashima, K., Nishinakamura, R., Tachibana, M., Inoue, M., Shima, Y., Morohashi, K.I., Hayashi, K., 2021. Generation of ovarian follicles from mouse pluripotent stem cells. Science 373, eabe0237.
Yu, L., Wei, Y., Sun, H.X., Mahdi, A.K., Pinzon Arteaga, C.A., Sakurai, M., Schmitz, D.A., Zheng, C., Ballard, E.D., Li, J., Tanaka, N., Kohara, A., Okamura, D., Mutto, A.A., Gu, Y., Ross, P.J., Wu, J., 2021. Derivation of Intermediate Pluripotent Stem Cells Amenable to Primordial Germ Cell Specification. Cell Stem Cell 28, 550–567.
Zhou, Q., Wang, M., Yuan, Y., Wang, X., Fu, R., Wan, H., Xie, M., Liu, M., Guo, X., Zheng, Y., Feng, G., Shi, Q., Zhao, X.Y., Sha, J., Zhou, Q., 2016. Complete Meiosis from Embryonic Stem Cell-Derived Germ Cells In Vitro. Cell Stem Cell 18, 330–340.