Human and non-human primate female in vitro gametogenesis toward meiotic entry: a systematic review

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In vitro gametogenesis offers a powerful platform to explore the complexities of female germline development while bypassing ethical and technical barriers in human and non-human primate research. This systematic review examined 23 articles that reported meiotic entry from differentiated pluripotent stem cells or ex vivo–cultured fetal germ cells from humans, cynomolgus monkeys or marmosets and were published between 2009 and 2025. By comparing methodologies and outcomes, the review highlighted current progress and ongoing challenges in inducing meiotic progression in primates. Although complete oogenesis using in vitro gametogenesis has been successfully achieved in mice, extending this success to primates remains a major hurdle, with meiotic entry representing a key milestone toward realizing in vitro gametogenesis in humans. (Fertil Steril® 2025;124:6–21. ©2025 by American Society for Reproductive Medicine.)

Key Words: Human and non-human primate, meiosis, oogenesis, pluripotent stem cells, differentiation protocols

Deciphering the mechanisms of gamete development in human and non-human primates remains hindered by technical and ethical constraints as well as the limited availability of material. In vitro gametogenesis, using pluripotent stem cells (PSCs) as starting material for gamete differentiation, is a highly scalable and reproducible technology that would be adequate to investigate the mechanisms of germline development in different mammalian models. Oogenesis is best understood in mice as complete oogenesis has been achieved after culture of embryonic day (E)12.5 gonads (1). Moreover, mouse PSCs initially differentiated into primordial germ cell-like cells (PGCLCs) can mature to functional oocytes after a period of co-culture with E12.5 gonads followed by transplantation into the mice ovary (2), after an extended period of co-culture with E12.5 gonads (3). More recently, functional PSC-derived oocytes can also be achieved after an extended period of co-culture with mouse PSCs differentiated to ovarian somatic cell-like cells to reconstitute the ovarian environment (4).

In humans, female primordial germ cells (PGCs) are specified early during embryonic development (5) and migrate to the developing gonads (6), where they colonize the developing ovaries and differentiate into oogonia and later primary oocytes (7). In fetal cynomolgus monkey and human ovaries, mitotically active POU5F1+PDPN+SOX17+PGCs peripherally located tend to retain their molecular signature, whereas more internally located PGCs gradually transit into DDX4+STRA8+pre-meiotic oogonia in the confinement of ovarian cords, where they undergo meiotic entry followed by further compartmentalization into primordial follicles (8–11).

In mice, retinoic acid has been shown to trigger the expression of *Stra8* and *Rec8*, which activate the transcription of meiosis-associated genes (12, 13). However, the activation of *Stra8* is not solely dependent on retinoic acid, but it also requires the removal of repressive epigenetic modifications from the *Stra8* promoter, enabling its responsiveness to retinoic acid (14–16). In humans and non-human primates, the transcriptional profile of pre-meiotic germ cells revealed that they are also retinoic acid-responsive (17–20). Retinoic acid-responsive DDX4+STRA8+ oogonia enter meiosis, marked by the upregulation of SYCP1, SYCP3, HORMAD1, and H2AFX (21–23). These cells then progress throughout prophase I (leptotene, zygotene, pachytene, and diplotene/dictyate) and in response to

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signals secreted by the ovarian somatic niche and through the physical interaction with pre-granulosa cells in the ovarian cords, the germ cells compartmentalize further, whereby one oocyte is surrounded by granulosa cells, forming primordial follicles (7). In contrast to human and cynomolgus monkey ovaries, marmoset neonatal ovaries exhibit an immature histological appearance at birth (24).

Replicating the process from PGCs to primary oocytes in vitro in human and non-human primates remains currently challenging. Nevertheless, ongoing research has resulted in increasingly efficient differentiation protocols to generate nascent human PGCLCs (25–27), cynomolgus monkey PGCLCs (28), and common marmoset PGCLCs (29–32). This increased efficiency in PGCLCs differentiation provides a strong foundation for the upcoming focus on the optimization of further maturation steps. This review provides a comprehensive analysis of existing protocols and strategies used to drive the maturation of human, cynomolgus monkey, and common marmoset PGCLCs toward meiotic entry.

MATERIALS AND METHODS

Article collection

To identify research articles that were appropriate for this systematic review, a search was performed in the database Web of Science. Only articles published on or after January 1, 2009 were considered. This time frame was chosen because the first study reporting the achievement of meiotic germ cells from PSCs was published in 2009 (33). Preprints and early access papers were also included in this review, whereas review articles, book chapters, conference abstracts, and errata were excluded. Database searches were conducted between December 14, 2024 and January 5, 2025. Additional articles were identified through citation snowballing from the reference list of the initially selected articles.

Search strategy

The Web of Science database was searched for articles including the following combination of words in their title (TI) and abstract (AB): human, cynomolgus, marmoset, complete, differentiation, oogonia, meiosis, haploid, germ cell, and primordial.

Non-human primates:

(TI=("primordial germ cell*" OR "PGC*" OR "PGCLCs" OR "oogonia" OR "haploid gamete*" OR "meiotic progression" OR "oocyte" OR "differentiation") AND TI=("cynomolgus" OR "marmoset" OR "primates") AND TI=("induction" OR "reconstitution" OR "in vitro" OR "ex vivo") NOT TI=("mouse" OR "mice")) OR (AB=("primordial germ cell*" OR "PGC*" OR "PGCLCs" OR "oogonia" OR "haploid gamete*" OR "meiotic progression" OR "oocyte development") AND AB=("cynomolgus" OR "marmoset" OR "primates") AND AB=("reconstitution" OR "in vitro"))

Humans:

(TI=("primordial germ cell*" OR "PGC*" OR "PGCLCs" OR "oogonia" OR "haploid gamete*" OR "meiotic progression" OR "oocyte development" OR "germline differentiation") AND TI=("human*") AND TI=("induction" OR "reconstitution" OR "in vitro" OR "ex vivo") NOT TI=("mouse" OR "mice")) OR (AB=("primordial germ cell*" OR "PGC*" OR "PGCLCs" OR "oogonia" OR "haploid gamete*" OR "meiotic progression" OR "oocyte development" OR "germline differentiation") AND AB=("human") AND AB=("genetic induction" OR "reconstitution" OR "in vitro") NOT AB=("mouse" OR "mice" OR "rat" OR "fetus" OR "chicken" OR "canine" OR "rabbit" OR "bovine" OR "sheep" OR "porcine" OR "rhino"))

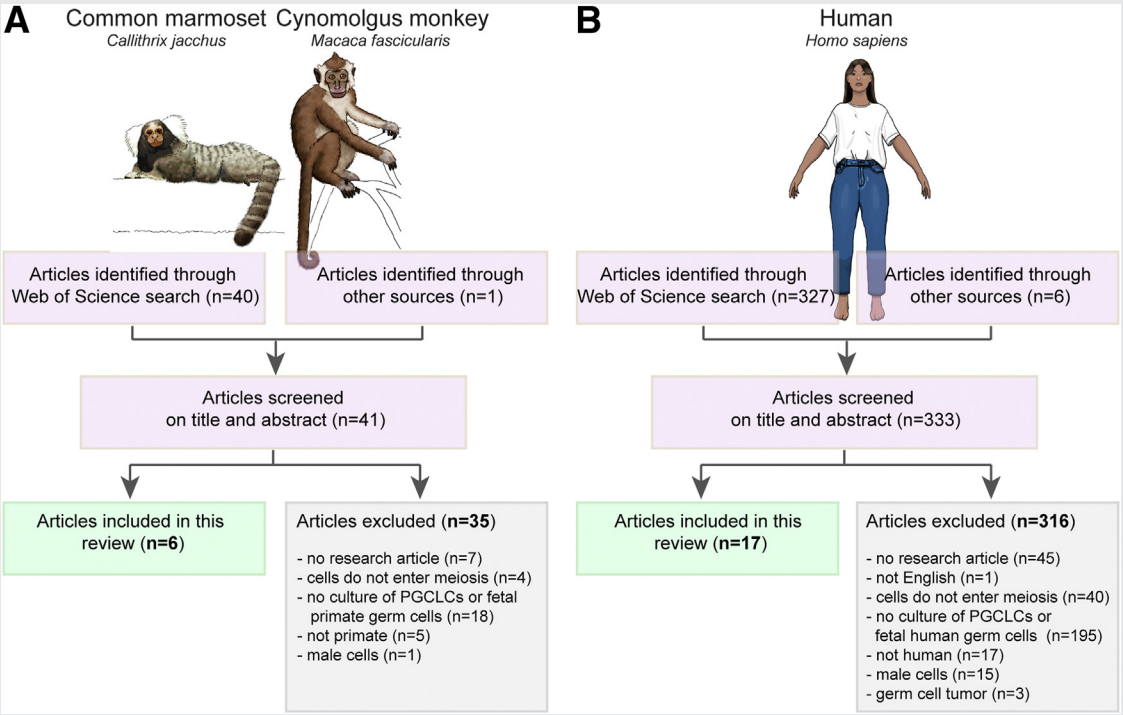
Inclusion and exclusion criteria

The identified articles based on the search strategy were screened further on the basis of inclusion and exclusion criteria (Fig. 1). Studies involving differentiation of PSCs or culture of germ cells through the addition of growth factors, cytokines, transcription factors, using overexpression of selected genes or using co-culture with primary material were included. Articles were excluded using the following criteria: not written in English, not research articles, not focused on human or non-human primates, related to germ cell tumors, articles only describing human PGCLC (hPGCLC) differentiation protocols, cells not showing expression of *DDX4*/DDX4 or *DAZL*/DAZL and cells not progressing into meiosis. Articles focused on recapitulating spermatogenesis and using only XY cells for experiments were also excluded.

Data analysis

Data from the articles reviewed here were collected and summarized in four tables (Tables 1 and 2; Supplemental Tables 1 and 2, available online) (11, 27, 28, 30, 32–49), reflecting the findings reported by the original investigators without additional analyses. The material and methods section of each article was examined to outline protocols, although results were assessed to understand the basis for the claimed conclusions. For articles with multiple protocol variations, only those yielding the most favorable outcomes were included. Protocol durations are described as "up to" when intermediate time points were reported. Table 1 and Supplemental Table 1 summarize protocols using PSCs and primary germ cells to obtain meiotic germ cells after culture. Table 2 and Supplemental Table 2 summarize differentiation efficiency on the basis of gene expression, immunofluorescence, ribonucleic acid (RNA) sequencing, and epigenetic changes observed after culture. Gene markers were categorized by developmental stage: mitotic PGCs or PGCLCs, pre-meiotic germ cells, meiotic germ cells, and primary oocyte. Additionally, the expression of other key genes involved in the differentiation and maturation of germ cells was also documented. To further assess the functionality of the cultured cells, we highlighted findings related to transcriptomic changes in the presence or absence of the ovarian somatic niche. The tables provided (Tables 1 and 2; Supplemental Tables 1 and 2) collectively synthesize the data, with more detailed information available in the original articles.

FIGURE 1



Study selection flow chart. (**A**) Flow chart used for non-human primates showing eligibility criteria for articles and the number of articles selected. (**B**) Flow chart used for humans showing eligibility criteria for articles and the number of articles selected. PGCLC = primordial germ cell-like cells.

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RESULTS

Article selection

Using the systematic search strategy outlined in the methods section, we identified 40 articles on non-human primates, common marmoset (MAR), and cynomolgus monkey (CYM). One additional article was identified through a PubMed search. Of the total number of articles on non-human primates (n = 41), 35 articles were excluded as they failed to meet the eligibility criteria and six articles were further analyzed (Fig. 1A). Moreover, using our systematic search strategy, we identified 327 articles on humans (HUM) and six additional articles were identified through other sources. Of the total number of articles on humans (n = 333), 316 articles were excluded because of failing eligibility criteria, and 17 articles were further analyzed (Fig. 1B). In total, 23 articles were analyzed and summarized in this review (Fig. 2).

Cell types and differentiation approaches

From the 23 selected articles, embryonic stem cells were used in 14 articles (1x MAR, 2x CYM, 11x HUM), and induced PSCs were used in 13 articles (1x MAR, 1x CYM, 11x HUM) as the starting cells to differentiate to PGCLCs (Table 1). The majority of articles (15 out of 23) used multiple embryonic stem cells or induced PSCs to generate PGCLCs (Supplemental Table 1). Additionally, three studies (3 out of 23 articles, 1x MAR, 1x CYM, 1x HUM) relied solely on the culture of primary fetal or neonatal ovarian cells to investigate meiotic entry and progression of female germ cells (Table 1).

Regarding the methodological approach used for PGCLC differentiation (19 out of 23 articles), 4 different culture platforms were used: 5 articles used a two-dimensional (2D) adherent culture (5x HUM), 1 article applied a semi-three-dimensional (3D) culture with basement membrane extract overlay (1x HUM) and nine articles generated PGCLCs using embryoid bodies (2x MAR, 3x CYM, 6x HUM) and five articles induced PGCLC fate through overexpression of transcription factors (1x MAR, 4x HUM) (Fig. 3, Supplemental Table 1). One article (1 out of 23 articles) used overexpression of transcription factors (1x HUM) to directly induce meiotic entry, skipping the PGCLC stage (Fig. 3, Table 1 and Supplemental 1) (49).

PGCLC differentiation: culture duration, growth factors, and medium composition

The differentiation of PGCLCs has been reported to take between 5 to 30 days (Supplemental Table 1). Various small molecules and growth factors were added to the culture medium to support PGCLC differentiation, with BMP4 being the most frequently used (15 out of 19 articles; 2× MAR, 2× CYM, 11× HUM). Other commonly included factors in the culture medium were leukemia inhibitory factor (LIF) (10 articles), stem cell factor (SCF) (9 articles), and epidermal

TABLE 1

Summary of culture conditions for meiotic induction.

General information				Methods		
Article ID (n = 23)	**Species**	**Cell type origin**	**Somatic niche**	**Maturation/meiotic entry approach**	**Culture medium composition formeiotic induction**	**Culture duration to reach the most advanced maturation state**
Fereydouni et al. (34), 2016	MAR	Germ cells	Neonatal **MAR** ovarian cells	Continued 2D adherent culture	DMEM/F12 + 10 % FBS + 10 μg/ml LIF	5 months
Shono et al. (32), 2023	MAR	cj_ESC	E12.5 **mouse** somatic ovarian cells	cj/m_rOvaries	**D6-D8**: GK15 + 1 μM RA + 10 μM Y-27632 **D9-D13**: αMEM + 2% FCS + 150 μM AA **D14-D90**: S10 + 10% FCS + 150 μM AA	cj/m_rOvaries cultured up to D90
Kurlovich et al. (30), 2024	MAR	cj_iPSC	None	N/A	N/A	N/A
Sakai et al. (28), 2020	CYM	cy_ESC cy_iPSC	None	N/A	N/A	N/A
Mizuta et al. (11), 2022	CYM	Germ cells	8WPF **CYM** ovarian cells	cy/cy_rOvaries xenotransplantation	**D0-D2:** αMEM + 10% FBS + 10 μM Y-27632 → xenotransplantation	cy/cy_rOvaries grafted in **mice** up to 21 weeks
				cy/cy_rOvaries	**D0-D2:** αMEM + 10% FBS + 10 μM Y-27632 **D2-W6:** αMEM + 10% FBS + 150 μM AA	cy/cy_rOvaries cultured up to 15 weeks
Gyobu-Motani et al. (35), 2023	CYM	cy_ESC	E13.5-E15.5 **mouse** somatic ovarian cells	cy/m_rOvaries	**D0-D2:** aRB27 + 10 μM Y-27632 + 1μM RA **D2-D49:** αMEM + 10% FBS + 150 μM AA **D49-D51:** αMEM + 10 μM Y-27632 **D51-D105:** αMEM + 10% FBS + 150 nM AA	cy/m_rOvaries cultured up to D49; second aggregation additional D56
Kee et al. (33), 2009	HUM	h_ESC	None	2D adherent differentiation + transcription factors overexpression	Overexpression of *DAZL* + *DAZ* + *BOULE*	7 days
Panula et al. (36), 2011	HUM	h_ESC h_iPSC	None	Continued 2D adherent differentiation	**D0-D14**: Knockout DMEM + 20% FBS + 50 ng/ml + 50 ng/ml BMP7 + 50 ng/ml BMP8b	14 days
Eguizabal et al. (37), 2011	HUM	h_ESC h_iPSC	None	Continued 2D adherent differentiation	**D42+2/3/4 weeks:** Knockout DMEM + 20% KRS + 8 ng/ml bFGF + 10 μM Forskolin + 1.000 U/ml hLIF + 1 μM R115866	3 weeks
Medrano et al. (38), 2012	HUM	h_ESC h_iPSC	None	2D adherent differentiation + transcription factors overexpression	**D0-D14**: DMEM/F12 + 20% FBS; Overexpression of *DDX4* and *DAZL*	14 days

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TABLE 1

Continued.

General information				Methods		
Article ID (n = 23)	**Species**	**Cell type origin**	**Somatic niche**	**Maturation/meiotic entry approach**	**Culture medium composition formeiotic induction**	**Culture duration to reach the most advanced maturation state**
Wongtrakoongate et al. (39), 2013	HUM	h_ESC	None	2D adherent differentiation + transcription factors overexpression	**D0-D7:** Knockout DMEM + 20% KSR + 4 ng/ml bFGF + 10 μM RA Overexpression of *DPPA3*	7 days
Xuemei et al. (40), 2013	HUM	h_ESC	None	Embryoid bodies	**D0-D7:** Knockout DMEM +20% FBS + 10 μM RA	5 days
Lin et al. (41), 2014	HUM	h_ESC	None	2D adherent differentiation	**D0-D30:** DMEM + 15% FBS + 100 ng/ml BMP4 + 50 ng/ml WNT3A	30 days
Jung et al. (42), 2017	HUM	h_ESC	None	Xenotransplantation	**D7-D8**: Knockout DMEM + 35 ng/ml GDF9:BMP15 heterodimer + 10 ng/ml EGF **D8-D13**: Knockout DMEM + 50 ng/ml GDF9 + 25 ng/ml BMP15 → **xenotransplantation**	60 days
Yamashiro et al. (43), 2018	HUM	h_iPSC	E12.5 **mouse** somatic ovary cells	h/m_rOvaries	**D0-D2:** GK15 + 10 μM Y-27632 **D2-D77:** αMEM + 10% FBS + 150 μM AA	h/m_rOvaries cultured up to 77 days
Abdyyev et al. (44), 2020	HUM	h_ESC h_iPSC	None	Embryoid bodies	**D10-D20**: DMEM/F12 + 3% FBS + 2 μM RA + 20 ng/ml hLIF + 100 ng/ml EGF + 10 ng/ml SCF	20 days
Yu et al. (45), 2021	HUM	h_ESC h_iPSC	Adult **HUM** granulosa cells	h/h_rOvaries xenotransplantation	xenotransplantation under kidney capsule in immune deficient **mice**	h/h_xrOvaries grafted up to 6 weeks
Yang et al. (46), 2022	HUM	h_iPSC	7-8WPF **HUM** ovarian cells	h/h_rOvaries xenotransplantation	**D0-D2**: GK15 + 10 μm Y-27632 **D2-D7**: αMEM +10 % FBS + 150 nM AA	h/h_xrOvaries grafted up to 4 weeks
Mizuta et al. (11), 2022	HUM	Germ cells	9WPF **HUM** ovarian cells	h/h_rOvaries	**D0-D2:** Advanced MEM + 10% FBS + 10 μM Y-27632 **D2-W14**: Advanced MEM + 10% FBS + 150 μM AA	Up to 14 weeks of in vitro culture

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TABLE 1

Continued.

General information				Methods		
Article ID (n = 23)	**Species**	**Cell type origin**	**Somatic niche**	**Maturation/meiotic entry approach**	**Culture medium composition formeiotic induction**	**Culture duration to reach the most advanced maturation state**
Arkoun et al. (47), 2022	HUM	h_iPSC	5.8-8WPF **HUM** somatic ovarian cells E12.5 **mouse** somatic ovarian cell	h/h_rOvaries h/m_rOvaries	**D4-D6**: DMEM/F12 + 15% KSR + 100 ng/ml BMP2 + 20ng/ml SCF + 1 μM RA **D6-D8:** DMEM/F12 + 15% KSR + 100 ng/ml BMP2 + 20ng/ml SCF +/- 1 μM RA	Up to 8 days
Yu et al. (48), 2023	HUM	h_ESC h_iPSCs	None	**D11-D15**: αMEM + 5% KSR + 5% bovine follicular fluid **D16-D25:** TCM 199 + 3 mg/mL BSA + 5 U/mL FSH + 10 U/mL hCG + 10 IU/mL pregnant mare serum gonadotropin + 0.23 mM pyruvic acid + 10 ng/mL EGF + 1% ITS	MEM + 10% FBS + 10 ng/ml LIF + 10 ng/ml EGF + 40 ng/ml GDNF + 10 ng/ml bFGF	Up to 25 days
Overeem et al. (27), 2023	HUM	h_iPSC	17WPF **HUM** ovarian cells	h/h_rOvaries in 3D substrate	**D1-D2**: aRB27 + 1x Revita Cell **D2-D17**: aRB27 + 100 ng/ml SCF + 5 μM Forskolin + 150 μM AA **D17-D25**: basal aRB27	30 days
Pierson Smela et al. (49), 2024	HUM	h_iPSC	None	2D adherent differentiation with transcription factors overexpression	**D0-D1**:mTeSR1 + 5 μM GSK3484862 + 1 μg/ml doxycycline + 10 μM Y-27632 **D1-D7**: APEL2 + 5 μM GSK3484862 + 1 μg/ml doxycycline **D7-D12**: APEL2 + 1 μg/ml doxycycline; Overexpression: *BCL2*, *HOXB5*, *BOLL*, *MEIOC*	12 days

Note: AA = ascorbic acid; ActA = Activin A; bFGF = basal fibroblast growth factor; BMEx = basement membrane extract; BSA = bovine serum albumin; cj = common marmoset; cy = cynomolgus monkey; CYM = cynomolgus monkey; D = day; E = embryonic day; EGF = epidermal growth factor; ESC = embryonic stem cells; FBS = fetal bovine serum; FCS = fetal calf serum; FSH = follicle-stimulating hormone; GDNF = glial cell line-derived neurotrophic factor; hCG = human chorionic gonadotropin; HUM = human; h = human; iPSC = induced pluripotent stem cells; ITS = insulin-transferrin-selenium; KSR = knockout serum replacement; LIF = leukemia inhibitory factor; MAR = common marmoset; N/A = not applicable; RA = retinoic acid; rOvaries = reconstituted ovaries; SCF = stem cell factor; 3D = three-dimensional; 2D = two-dimensional; W = weeks; WPF = weeks postfertilization; xrOvaries = xenotransplanted rOvaries.

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TABLE 2

Summary of methods and markers used for germ cell identification.

General information		Results				
			Markers of germ cell development			
Article (n = 23)	Species	Meiotic entry marker expression/maturation efficiency	Mitotic PGC(LCs)	Pre-meiotic	Meiotic	Primary oocyte
Fereydouni et al. (34), 2016	MAR	Meiotic entry detected by RNA-seq and qPCR	POU5F1 (qPCR, RNA, very low), PRDM14 (qPCR)	DDX4 (qPCR, RNA-seq), DAZL (qPCR)	MALE (RNA-seq), TEX12 (RNA-seq), SYCP2 (RNA-seq)	ZP3 (RNA-seq), SOHLH2 (RNA-seq), NOBOX (RNA-seq), FIGLA (RNA-seq)
Shono et al. (32), 2023	MAR	Meiotic entry detected by RNA-seq	SOX17 (FACS, RNA-seq), PRDM1 (FACS, RNA-seq), TFAP2C (FACS, RNA-seq), NANOS3 (RNA-seq)	DDX4 (IF, RNA-seq), DAZL (RNA-seq), PIWIL2 (RNA-seq)	SYCE3 (RNA-seq)	None
Kurlovich et al. (30), 2024	MAR	DAZL+ detected by RNA-seq	SOX17 (IF, RNA-seq), PRDM1 (IF, RNA-seq), TFAP2C (IF, RNA-seq), NANOG (IF), DPPA3 (RNA-seq), NANOS3 (RNA-seq), CXCR4 (FACS), ITGA6 (FACS), POU5F1 (RNA-seq), KIT (RNA-seq)	low expression DDX4 and DAZL (RNA-seq)	None	None
Sakai et al. (28), 2020	CYM	N/A	TFAP2C (IF), PDPN (IF), SOX17 (FACS)	DDX4 (IF)	None	None
Mizuta et al. (11), 2022	CYM	~40% SYCP3+ germ cells at W6 of in vitro culture and ~20% TP63+ and ZP3+ germ cells at W15 of in vitro culture ~60% SYCP3+ cy germ cells after xenotransplantation at W6 + ~20%; TP63+ and ~60% ZP3+ cy germ cells after transplantation at W15	TFAP2C (IF), POU5F1 (IF), NANOG (IF), PDPN (IF), Ki67 (IF), THY1 (RNA-seq), SOX17 (RNA-seq), TFCP2L1 (RNA-seq)	DDX4 (IF), ZGLP1 (IF), STRA8 (IF) DDX4 (IF), PBX1 (RNA-seq), CLGN (IF, RNA-seq)	PAX6 (RNA-seq), SYCP1 (IF), SYCP3 (IF), DMC1 (IF), H2AFX (IF)	ZP3 (IF), TP63 (IF), LMOD3 (RNA-seq)
Gyobu-Motani et al. (35), 2023	CYM	~22% thread-like SYCP3+ localization cyPGCLC derived cells at ag49+28; 5-13% SYCP3+ thread-like localization at ag49+56	POU5F1 (IF, RNA-seq), NANOG (RNA-seq), TFAP2C (RNA-seq), UTF1 (RNA-seq)	DDX4 (IF), ZGLP1 (IF, RNA-seq), STRA8 (IF, RNA-seq), REC8 (RNA-seq)	SYCP1 (IF), SYCP2 (RNA-seq) SYCP3 (IF, RNA-seq), DMC1 (IF, RNA-seq), SYCE1 (RNA-seq), HORMAD1 (RNA-seq), SPO11 (RNA-seq), PRDM9 (RNA-seq) H2AFX (IF), RPA2 (IF)	FIGLA (RNA-seq), NOBOX (RNA-seq), ZP3 (RNA-seq)

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TABLE 2

Continued.

General information			Results			
			Markers of germ cell development			
Article (n = 23)	Species	Meiotic entry marker expression/maturation efficiency	Mitotic PGC(LCs)	Pre-meiotic	Meiotic	Primary oocyte
Kee et al. (33), 2009	HUM	Up to 12% DDX4 cells at D7-14; after BOULE overexpression ~20% SYCP3+	PRDM1 (qPCR), DPPA3 (qPCR)	DDX4 (FACS, qPCR, WB), DAZL (FACS, qPCR, WB), 5mC (IF), DAZ (FACS, WB), BOULE (FACS)	SYCP3 (IF), H2AFX (IF)	None
Panula et al. (36), 2011	HUM	~5% DDX4+ cells	POU5F1 (qPCR), PRDM1 (qPCR), NANOG (qPCR)	DDX4 (IF, WB), DAZL (IF, WB)	SYCP3 (IF), DMC1 (qPCR)	None
Eguizabal et al. (37), 2011	HUM	SYCP3 detected with IF	SSEA1- (FACS)	DDX4 (IF, qPCR), STRA8 (PCR)	SYCP3 (IF), H2AFX (IF), CD9+/CD49f+ (IHC)	None
Medrano et al. (38), 2012	HUM	0.15% of all cells through meiosis → 6% thread-like SYCP3 staining	IFITM1 (qPCR), PRDM1A (qPCR), CKIT (qPCR)	DDX4 (IF, qPCR, FACS), DAZL (qPCR)	SYCP3 (IF, qPCR), MLH1 (qPCR), DMC1 (qPCR)	GDF9 (qPCR), ZP4 (qPCR)
Wongtrakoongate et al. (39), 2013	HUM	SYCP3 was detected with qPCR	DPPA3 (qPCR), POU5F1 (qPCR), NANOG (qPCR), SOX17 (qPCR)	DDX4 (qPCR)	SYCP3 (qPCR)	ZP1 (qPCR)
Xuemei et al. (40), 2013	HUM	6.9 fold increase of SYCP3+ cells	POU5F1 (qPCR)	DDX4 (qPCR)	SYCP3 (qPCR)	GDF9 (qPCR)
Lin et al. (41), 2014	HUM	SYCP3 was detected with qPCR and IF	POU5F1 (IF, qPCR), PRDM1 (IF, qPCR), DPPA3 (IF, qPCR), NANOS3 (IF, qPCR)	DDX4 (IF, qPCR)	SYCP3 (IF, qPCR)	None
Jung et al. (42), 2017	HUM	~20% SYCP3+ and ~30% PRDM9+H2AFX hPGCLCs derived germ cells at D7	POU5F1 (IF, qPCR, WB), NANOG (qPCR, WB), PRDM14 (qPCR)	DDX4 (IF, qPCR), DAZL (IF, qPCR, WB), BOULE (FACS)	SYCP3 (IF, qPCR), PRDM9 (IF), H2AFX (IF), MLH1 (IF)	ZP2 (IF), NOBOX (IF), SOHLH2 (qPCR)
Yamashiro et al. (43), 2018	HUM	0.5% DDX4+ hPGCLCs; meiotic entry detected with IF	EGFP reporter (FACS, IF), POU5F1 (IF, qPCR), SOX17 (IF, qPCR), TFAP2C (IF, qPCR), PRDM1 (qPCR), PDRM14 (qPCR), NANOS3 (qPCR), DPPA3 (qPCR),	DDX4 (IF, qPCR), DAZL (IF, qPCR), STRA8 (qPCR),	SYCP3 (IF, qPCR), REC8 (qPCR),	UHRF1 (qPCR)
Abdyyev et al. (44), 2020	HUM	~80% SSEA1+ cells; meiotic entry was detected with IF and qPCR	SOX17 (IF, qPCR), PRDM1 (IF), DPPA3 (IF), POU5F1 (IF), NANOG (qPCR)	DDX4 (IF, qPCR), DAZL (IF, qPCR), STRA8 (IF), PIWIL1 (qPCR), PIWIL4 (qPCR)	SYCP2 (qPCR), SYCP3 (IF, qPCR), REC8 (qPCR)	None
Yu et al. (45), 2021	HUM	10% hPGCLCs at D7	PRDM1 (WB)	DDX4 (IF, qPCR, WB), DAZL (WB)	SYCP3 (WB)	ZP3 (WB)
Yang et al. (46), 2022	HUM	~40% at D8 DDX4-GFP+ without feeder; SYCP3 was detected with qPCR and IF	SOX17 (qPCR), PRDM1 (qPCR)	DDX4 (qPCR), DAZL (qPCR)	SYCP3 (IF, qPCR), REC8 (qPCR)	None

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TABLE 2

Continued.

General information			Results			
			Markers of germ cell development			
Article (n = 23)	Species	Meiotic entry marker expression/maturation efficiency	Mitotic PGC(LCs)	Pre-meiotic	Meiotic	Primary oocyte
Mizuta et al. (11), 2022	HUM	~50% SYCP3+ germ cells at W7 of in vitro culture and ~3% TP63+ZP3+ germ cells at W14 of in vitro culture	POU5F1 (IF), Ki67 (IF)	DDX4 (IF), CLGN (IF, RNA-seq)	PAX6 (RNA-seq), SYCP1 (IF), SYCP3 (IF), DMC1 (IF), H2AFX (IF)	ZP3 (IF), TP63 (IF), LMOD3 (RNA-seq)
Arkoun et al. (47), 2022	HUM	Up to 13% DDX4+ cells at D6	SOX17 (qPCR), CD38 (qPCR), PRDM1 (RNA-seq) TFAP2C (IF, IHC, qPCR, RNA-seq), NANOG (RNA-seq), NANOS3 (qPCR), KIT (qPCR), PDPN (IF, IHC, qPCR, FACS), TET1 (RNA-seq), POU5F1 (IF, IHC, RNA-seq)	DDX4 (IF, IHC, qPCR), DAZL (qPCR)	MEIOC (qPCR), DMC1 (qPCR), SYCP3 (IF, qPCR)	None
Yu et al. (48), 2023	HUM	SYCP3 was detected with qPCR and IF	POU5F1 (IF, qPCR), PRDM14 (IF, qPCR), PRDM1 (qPCR), SOX17 (IF), DPPA3 (IF), CKIT (FACS), TFAP2C (qPCR)	DDX4 (IF)	SYCP3 (IF, qPCR)	NOBOX (IF), BMP15 (IF), ZP3 (IF, qPCR), ZP2 (IF, qPCR), CX37 (IF)
Overeem et al. (27), 2023	HUM	N/A	PRDM1 (IF, scRNA-seq), SOX17 (IF, scRNA-seq); PDPN (IF), SOX17 (IF), TFAP2C (IF), OCT4 (IF, scRNA-seq)	DDX4 (IF), DAZL (IF)	None	None
Pierson Smela et al. (49), 2024	HUM	~0.8% SYCP3+ cells	None	DDX4 (RNA-seq)	SYCP3 (IF, qPCR, RNA-seq), HORMAD1 (IF, qPCR), H2AFX (IF), TEX12 (qPCR), RAD51 (IF), REC8 (IF)	None

Note: ag = after culture day; CYM = cynomolgus monkey; D = day; FACS = fluorescence activated cell sorting; HUM = human; IF = immunofluorescence; IHC = immunohistochemistry; MAR = common marmoset; N/A = not applicable; qPCR = quantitative polymerase chain reaction; RNA-seq = RNA sequencing; W = weeks; WB = western blot analysis.

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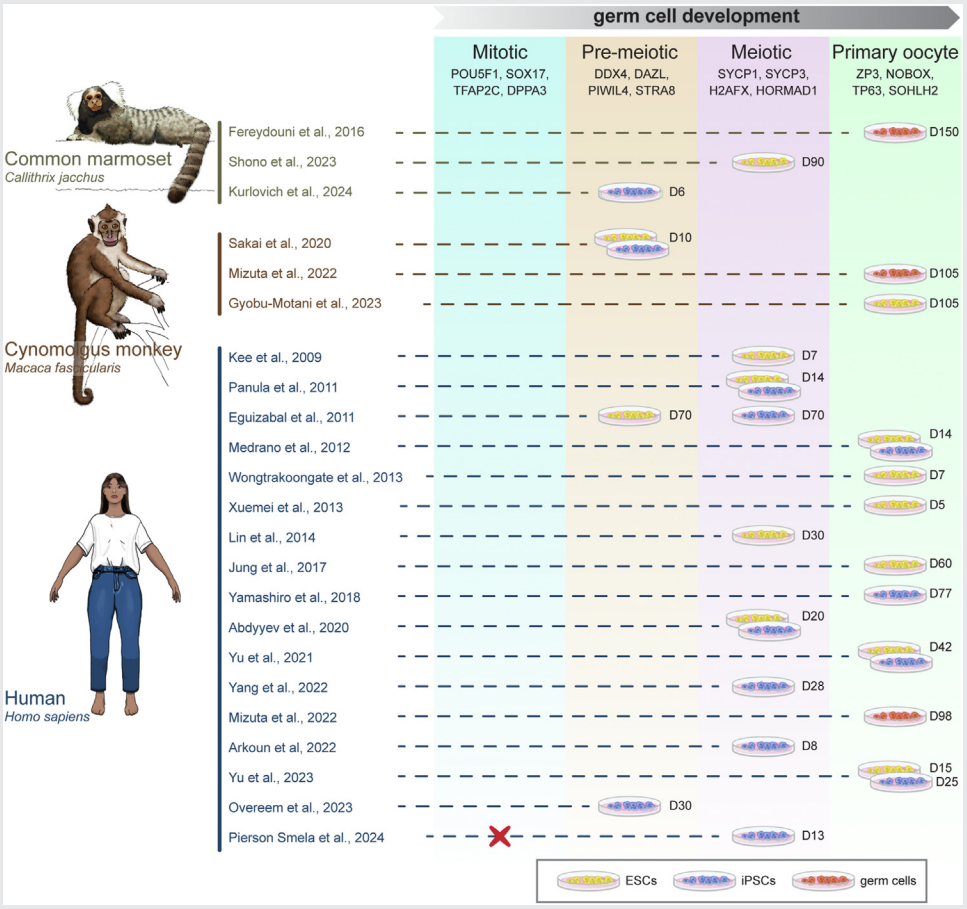
FIGURE 2



Summary of the analyzed studies regarding germ cell development in human and non-human primates. The germ cell development reported in the analyzed studies can be divided into four major stages (mitotic, pre-meiotic, meiotic and primary oocyte), each characterized by the expression of specific key genes. For each analyzed study, the cell type or origin used (embryonic stem cells [ESCs], induced pluripotent stem cells [iPSCs] or primary germ cells) as well as the time in culture in days (D) is depicted. *Red cross*: This stage of germ cell development was skipped in the culture protocol.

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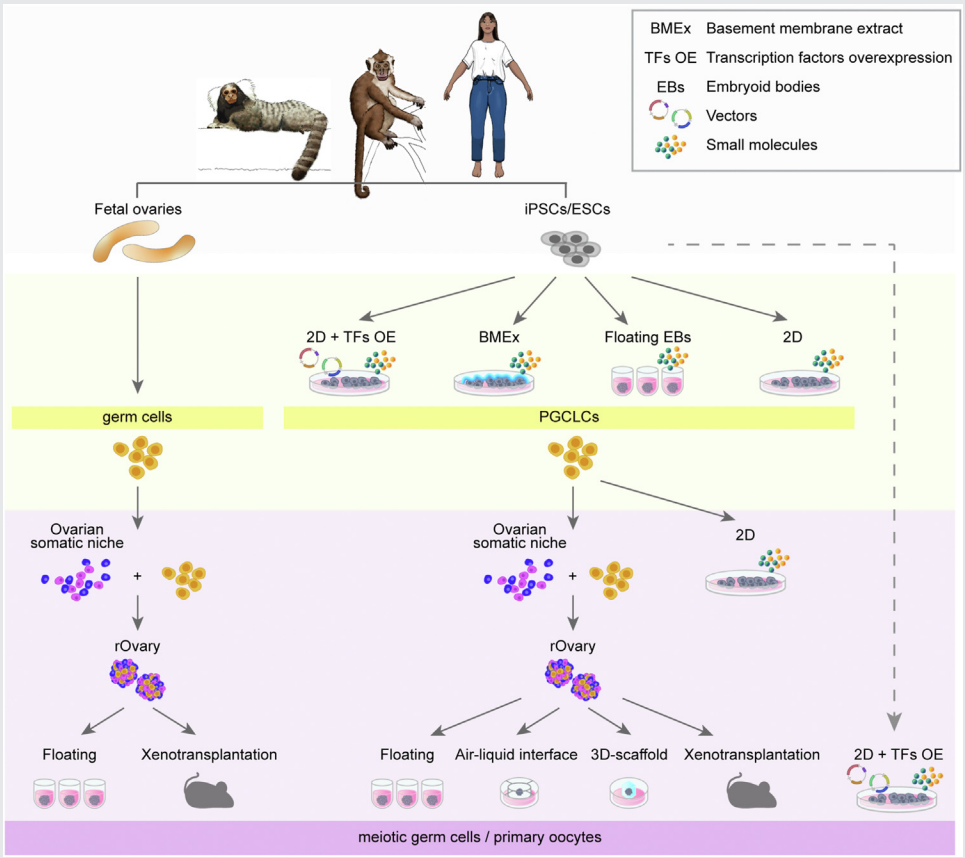
growth factor (EGF) (9 articles). Less frequently used factors included Activin A (3× HUM, 2× MAR), BMP2 (1× HUM), BMP7 (1× HUM), BMP8A (1× HUM), BMP8B (2× HUM), and WNT3A (1× HUM). Medium supplementation varied across articles, with 10%–20% fetal bovine serum (FBS) used in nine articles (9 out of 23 articles; 1× MAR, 1× CYM, 7× HUM) and 1%–20% knockout serum replacement (KSR) in nine articles (9 out of 23 articles; 1× MAR, 2× CYM, 6× HUM). Additionally, Y-27632 was incorporated into eight articles (8 out of 23 articles; 2× MAR, 2× CYM, 4× HUM) to enhance cell survival during differentiation.

The overexpression of transcription factors was used in five articles to successfully induce PGCLC fate (Supplemental Table 1), either independently or in combination with small molecules. The overexpression of *SOX17*, *TFAP2C*, and *PRDM1* was used in one MAR study (duration 8 days, with BMP4, LIF, SCF, and EGF) (32); the overexpression of *DPPA3* was used in one HUM study (duration 7 days, with basal fibroblast growth factor and retinoic acid) (39); the overexpression of *DDX4* and *DAZL* was used in one HUM study (duration 10 days, without small molecules) (38); and the overexpression of *DAZL* and *BOULE* in two human studies (duration 7 days, with BMPs) (33, 42).

Strategies to induce meiotic progression in non-human PGCLCs

To further advance PGCLCs (or primary germ cells) toward more mature germ cell stages, most protocols used either extended 2D adherent culture (7 out of 22 protocols; 1× MAR, 6× HUM) or used co-culture approaches using reconstituted ovaries (rOvaries), whereby PGCLCs (or primary germ cells) were co-cultured with gonadal tissue (9 out of 22 protocols; 1× MAR, 2× CYM, 6× HUM) (Fig. 3, Table 1). Different combinations of growth factors, small molecules, and other supplements were also used during this maturation step (Table 1).

FIGURE 3



Strategies used to differentiate pluripotent stem cells and primary germ cells into meiotic germ cells. Several studies analyzed here cultured germ cells from fetal ovarian tissue from human and non-human primates as reconstituted ovaries (rOvaries) followed by an extended culture period or xenotransplantation in mice. Other studies differentiated pluripotent stem cells (embryonic stem cells [ESCs] or induced pluripotent stem cells [iPSCs]) into primordial germ cell-like cells (PGCLCs) using different culture platforms: two-dimensional (2D) adherent culture, culture with basement membrane extract (BMEx), floating embryoid bodies (Floating EBs), and 2D adherent culture with overexpression of specific transcription factors (2D + TFs OE). Subsequently, the PGCLCs were matured in 2D culture or as rOvary and cultured floating, in an air-liquid interface, in a three-dimensional scaffold or using xenotransplantation in mice to promote further maturation. One strategy using 2D + TFs OE resulted in the direct induction of meiotic entry, skipping the PGCLC stage.

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In one article using MAR, cells from neonatal ovaries were cultured on a 2D setup on a mouse embryonic fibroblast feeder layer for 5 months, using culture medium supplemented only with FBS and LIF (34). In another article using MAR, rOvaries were generated by combining cjPGCLCs with mouse E12.5 female gonads and maintained in culture for up to 90 days (32). The medium in this study was supplemented with retinoic acid and ascorbic acid to promote meiotic entry.

In one article using CYM, embryoid bodies containing cyPGCLCs were cultured further to promote maturation (28). However, in two other articles using CYM, rOvaries were generated to promote maturation. In one article, rOvaries were generated by combining CYM primary germ cells with the respective ovarian cells at 8 weeks postfertilization (WPF) and cultured in air-liquid interface, in medium containing retinoic acid and ascorbic acid, for up to 15 weeks or transplanted under the kidney capsule of immunodeficient mice without retinoic acid/ ascorbic acid exposure (11). In a different article, CYM rOvaries were first generated by combining cyPGCLCs with mouse E12.5 female gonads, subjected to a retinoic acid pulse, and cultured for 49 days, followed by recombination with E15.5 female gonads and cultured for an additional 56 days (35).

Strategies to induce meiotic progression in HUM PGCLCs and primary germ cells

Several articles were reported to induce the maturation of HUM hPGCLCs (or primary germ cells) in rOvaries combined with either mouse (2 articles) (43, 47) or HUM (5 articles) fetal ovarian cells after a culture period between 8 days to 6 weeks (Table 1). One study generated rOvaries using solely primary cells from HUM 9WPF gonads (germ and somatic) and

cultured those for 14 weeks in the presence of FBS and ascorbic acid on air-liquid interface or floating (11). In two other studies, rOvaries were generated by combining hPGCLCs with HUM ovarian cells from either the first (47) or second trimester using 3D scaffold (27), with maturation reported to occur in 8 and 25 days respectively, using culture medium supplemented with FBS or KSR, ascorbic acid, SCF, BMP2, or forskolin. In two studies, rOvaries generated by combining hPGCLCs with HUM ovarian cells (7–8WPF and adult HUM granulosa cells, respectively) were transplanted into immunodeficient mice and harvested after 4 (46) to 6 weeks (45).

Interestingly, one single article using HUM reported xenotransplantation into mice of hPGCLCs after an initial period of 2D adherent differentiation of 13 days, which included overexpression of *DAZL* and *BOULE* (42). Finally, several articles using hPGCLC differentiation have reported further germ cell maturation by prolonging the culture period without including co-culture (9 articles) (Table 1). In three articles (3 out of 9 articles), embryoid bodies containing hPGCLCs were further cultured with FBS and retinoic acid for additional 5 (40) or 20 days (44) to promote maturation; or further cultured up to 25 days, in a medium with follicle-stimulating hormone, human chorionic gonadotropin, pregnant mare serum gonadotropin, EGF, and insulin-transferrin-selenium (48). Yu and colleagues reported that hPGCLCs differentiated in the presence of bovine follicular fluid become primed to reach an oocyte-like state, arrested at metaphase II with a polar body (48). In six articles (6 out of 9 articles), hPGCLCs were further matured in 2D adherent culture. In three of these articles, the culture medium was supplemented with FBS or KSR, retinoic acid or BMPs and hPGCLC culture was extended to 21 to 30 days (36, 37, 41); whereas in the other three articles overexpression of transcription factors, such as *DPPA3* (39), *DAZL* and *DDX4* (38) and *DAZL*, *DAZ* and *BOULE* (33) was used to extend culture for 7 to 12 days. Recently, an innovative study still in preprint has reported that overexpression of *BCL2*, *HOXB5*, *BOLL,* and *MEIOC* in human induced PSCs cultured in 2D adherent culture induced those cells to directly undergo meiotic entry, while skipping the hPGCLC stage (49) (Fig. 3, Table 1).

Assessment of maturation and meiotic progression

Next, we analyzed the results section of all 23 studies, focusing on the expression of mitotic, pre-meiotic, meiotic, and primary oocyte markers after germ cell maturation (Fig. 2, Table 2). All articles confirmed the expression of mitotic and pre-meiotic PGC(LC) markers in both human and non-human primate models, except one study that skipped the mitotic hPGCLC stage (49). From the articles on non-human primates reporting upregulation of DDX4 or DAZL in germ cells (6 articles), 3 reported the expression of both meiotic and primary oocyte markers (1× MAR, 2× CYM), whereas one study (1× MAR) detected only meiotic marker expression in cultured hPGCLCs. From the articles on HUM, 15 articles detected meiotic markers expression, whereas eight studies reported primary oocyte markers expression (Fig. 2, Table 2).

Marker expression was assessed at the protein level (immunofluorescence and western blot analysis), and at the RNA level (single-cell RNA sequencing, bulk RNA sequencing or quantitative polymerase chain reaction). Comparing RNA sequencing data from in vitro cultured PGCLCs with primary germ cells and PSCs provided deeper insights into cell states after culture (Supplemental Table 2). All 23 studies performed quantitative polymerase chain reaction, although single-cell RNA sequencing was conducted in eight studies (1× MAR, 1× CYM, 6× HUM), and bulk RNA sequencing was performed in five studies (2× MAR, 2× CYM, 1× HUM).

Notably, most studies performing RNA sequencing showed that differentiated PGCLCs and their further matured counterparts clustered separately. Furthermore, several articles (1× MAR, 2× CYM, 10× HUM) reported on the epigenetic profile (specific histone modifications or variants, DNA methylation, expression of long non-coding RNA such as *XIST*) or the expression of epigenetic modifying enzymes (*DNMT1*, *DNMT3A*, *DNMT3B*, *TET1*) in PGCLCs (or primary germ cells) analyzed through RNA sequencing, enzymatic methyl-sequencing, bisulfite sequencing, or immunofluorescence (Supplemental Table 2).

DISCUSSION

PGCLC induction strategies

The induction of PGCLCs from PSCs represents a critical step to achieve in vitro gametogenesis. Across the articles reviewed here, four strategies were used to generate PGCLCs from human and non-human primates: 2D adherent cultures, semi-3D culture, embryoid body formation, and transcription factor overexpression. Notably, 2D culture systems were predominantly applied in human studies, providing a controlled environment for differentiation, whereas embryoid body-based approaches were used in both human and non-human primate articles.

The inclusion of specific growth factors, such as BMP4, LIF, SCF, and EGF, played a significant role in directing differentiation to PGCLCs, with BMP4 being the master morphogen, known to be necessary to induce PGC fate in mice as well (50). One of the studies used WNT3A in the culture medium to differentiate PGCLCs (30), whereas other articles used the small molecule CHIR99021 as a WNT agonist. Additionally, retinoic acid was included in the culture medium to trigger meiotic induction in several articles because it has been shown that in mice retinoic acid is responsible for the activation of transcription of meiosis-associated genes in oogonia (12,13). Interestingly, the addition of retinoic acid was insufficient to trigger *STRA8* expression in hPGCLCs (47), in agreement with what has been reported in mice (16). The differentiation of hPGCLCs in the presence of bovine follicular fluid seemed to enhance the maturation potential the most, priming the hPGCLCs to display characteristics of metaphase II-arrested oocyte after exposure to follicle-

stimulating hormone, human chorionic gonadotropin, and pregnant mare serum gonadotropin (48).

Power of transcription factors overexpression

Overexpression of key transcription factors, such as *SOX17*, *TFAP2C*, *PRDM1*, *DPPA3*, *DDX4*, *DAZL*, and *BOULE*, has emerged as a potent strategy to induce PGCLC differentiation and enhance further maturation. These transcription factors are known to regulate aspects of germline specification (33, 51–54) and meiotic entry (55, 56), making their forced expression a powerful tool to drive PGCLC differentiation. From the articles reviewed here, several used transcription factor overexpression in HUM (33, 38, 39, 42) and in MAR (32) to boost hPGCLC differentiation. The efficiency of this approach was demonstrated by the rapid differentiation timelines, often requiring only 7 to 12 days. Importantly, some studies combined transcription factor overexpression with small molecules to further optimize differentiation conditions, highlighting the synergistic effects of molecular and genetic interventions in PGCLC induction. Most exciting and intriguing is the possibility to overexpress transcription factors, such as *BCL2*, *HOXB5*, *BOLL* and *MEIOC*, in hPSCs to promote meiotic entry directly, while skipping the hPGCLC stage (49). The potential of these meiotic-differentiated cells to mature further into oocyte-like cells remains to be demonstrated.

Advantages of using primary gonadal cells and xenotransplantation

Although PGCLCs provide a scalable and versatile approach to studying germline development, using germ cells from primary fetal and neonatal ovarian cells offer significant advantages, particularly in terms of physiological relevance. From the articles reviewed here, two have relied exclusively on primary cells to investigate culture conditions to recapitulate in vivo development using MAR (34) and using CYM and HUM (11). Unlike PGCLCs, germ cells isolated from ovaries retain epigenetic and transcriptional features of naturally occurring germ cells, thereby serving as valuable models for studying meiosis and epigenetic reprogramming.

The use of primary ovarian cells remains crucial to provide the necessary cellular niche, supplying species-specific cytokines and the native extracellular environment that endogenous (primate) germ cells need to mature in vivo. For this purpose, several studies have generated rOvaries combining cjPGCLCs with mouse gonadal cells (32), cyPGCLCs with either CYM gonadal cells (11) or mouse gonadal cells (35), and hPGCLCs with either HUM gonadal cells (11, 27, 45–47) or mouse gonadal cells (43, 47). However, as gametogenesis is a complex, long and multi-step process, the developmental stage of the primary somatic niche used will determine the specific stiffness and signals provided, and this may need to be adapted during extended culture for optimal maturation. This approach was used in CYM, where cyPGCLCs were first co-cultured with mouse E12.5 ovarian cells, followed by E15.5 ovarian cells (35). Moreover, several articles have explored the effects of xeno-transplanting of rOvaries into immunodeficient mice, not only stimulating the vascularization of the rOvaries, but also allowing the rOvaries to be exposed to the adult ovary cellular niche (when transplanted to the ovarian bursa). It remains important to determine in HUM whether primary germ cells and hPGCLCs need to be exposed sequentially to different somatic niches, corresponding to their specific developmental stage (early, mid, or late gestation), however the limited availability of HUM primary gonadal material poses a significant challenge.

Epigenetic roadblocks and species-specific transcriptional regulation

Despite advances in PGCLC maturation in culture, knowledge gaps on how to overcome existing epigenetic barriers and meiotic-associated checkpoints still remain. From the articles reviewed here, the expression dynamics of key maturation markers still revealed incomplete molecular signatures regarding meiotic and primary oocyte markers, indicating that the current differentiation protocols do not fully recapitulate the transcriptional landscape and developmental trajectory of germ cell counterparts in vivo.

A pronounced decrease in DNA methylation plays a key role in germ cell fate acquisition during PGCLC differentiation in mice and humans (11, 43, 57). However, the epigenetic analyses of maturing PGCLCs revealed defects in the expression of *DNMT1*, *DNMT3A*, *DNMT3B*, and *TET1*, which resulted in abnormal DNA methylation dynamics (43, 45), emphasizing the need to improve strategies that optimize culture conditions.

Finally, it is important to stress that although there are many similarities, there are also striking species-specific differences between primate PGCLCs (30). For example, in contrast to hPGCLCs, cjPGCLCs do not express *PRDM14*, but express high levels of *SOX2* (30). Hence species-specific transcription factors may play an important role in ensuring successful in vitro gametogenesis in different primate species. Comparative analyses underscore key differences in germ cell differentiation regulation across species, suggesting that tailored differentiation protocols for each model system will be necessary.

Meiotic entry in females vs. males

Although this review focuses on the differentiation of female PSCs toward oogenesis in vitro, there is a strong line of research investigating the differentiation of (human) male PSCs toward spermatogenesis in vitro (58–61). As expected, differentiation protocols into male and female hPGCLCs are similar, and the culture medium typically contains KSR, BMP4, SCF, EGF, LIF, and Y-27632. However, additional factors such as retinoic acid, glial cell line-derived neurotrophic factor, putrescine, testosterone, bovine pituitary extract, and IGF1 may aid in further male-specific differentiation, as reported in mice (62, 63).

In a parallel approach to induce oogenesis, to induce spermatogenesis, a co-culture step combining male hPGCLCs with testicular somatic cells is often included. For example,

reconstituted testes from human PGCLCs with E12.5 mouse testicular somatic cells, after culture in an air-liquid interface, have been shown to give rise to T1 prospermatogonia-like cells (59). The T1 prospermatogonia-like cells underwent global DNA demethylation, leading to transposable element activation. Genes related to piwi-interacting RNAs biogenesis and de novo DNA methylation were enriched, whereas ATAC-sequencing data showed chromatin accessibility changes, indicating epigenetic reprogramming during male hPGCLC maturation (59). Despite progress, achieving full epigenetic fidelity as well as completing meiosis and spermiogenesis remains a challenge, highlighting the need for refined human-derived testicular niches or organoid models to better mimic physiological conditions to support full spermatogenesis in vitro. Investigating sex-specific differences will contribute greatly to better understanding the complex mechanisms that regulate in vitro gametogenesis.

Limitations

The increased access to primary material and its use to determine the molecular signatures of the different cell types in the ovaries during development and adulthood has provided a much-needed robust benchmark to evaluate differentiation protocols in human and non-human primates. However, some degree of caution is necessary when comparing transcriptional profiles of (matured) PGCLCs to germ cell counterparts, as this may require batch correction that may ultimately attenuate differences. Additionally, immunofluorescence without proper positive and negative controls can lead to inconsistencies. Another limitation when performing differentiation of PSCs, is the fact that articles use a reduced number of cell lines and that different cell lines have distinct genomes and acquire different genetic mutations in culture (64). Moreover, PSCs derived in different laboratories under different culture conditions acquire distinctive epigenetic makeup and pluripotency state, resulting in different potential to differentiate (27, 65, 66). These differences can be caused with the use of different brands or even batches of the same reagent, resulting in difficulties in reproducing differentiation protocols.

Finally, the goal of a scoping review is to methodically examine the literature without merging data or conducting re-analyses. As a result, the conclusions drawn by the investigators in their original studies are incorporated into our review. This approach introduces the potential for discrepancies because of variations in experimental protocols and the different interpretations made by the investigators in their respective research.

CONCLUSION

Overall, the articles analyzed in this systematic review demonstrated that human and non-human primate PGCLCs can progress toward meiotic and post-meiotic stages with varying efficiency when appropriate differentiation strategies are applied. The efficiency of differentiation was influenced by the choice of culture medium composition and co-culture conditions, highlighting the importance of optimizing these parameters to achieve successful and efficient germ cell maturation during female in vitro gametogenesis.

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CRediT Authorship Contribution Statement

Sylwia M. Czukiewska: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Celine M. Roelse:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Susana M. Chuva de Sousa Lopes:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Conceptualization.

Declaration of Interests

S.M.C. has nothing to disclose. C.M.R. has nothing to disclose. S.M.C.d.S.L. has nothing to disclose.

SUPPLEMENTAL MATERIAL

Supplemental data for this article can be found online at https://doi.org/10.1016/j.fertnstert.2025.04.040.

REFERENCES

1. Morohaku K, Tanimoto R, Sasaki K, Kawahara-Miki R, Kono T, Hayashi K, et al. Complete in vitro generation of fertile oocytes from mouse primordial germ cells. Proc Natl Acad Sci U S A 2016;113:9021–6.
2. Hayashi K, Ogushi S, Kurimoto K, Shimamoto S, Ohta H, Saitou M. Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. Science 2012;338:971–5.
3. Hikabe O, Hamazaki N, Nagamatsu G, Obata Y, Hirao Y, Hamada N, et al. Reconstitution in vitro of the entire cycle of the mouse female germ line. Nature 2016;539:299–303.
4. Yoshino T, Suzuki T, Nagamatsu G, Yabukami H, Ikegaya M, Kishima M, et al. Generation of ovarian follicles from mouse pluripotent stem cells. Science 2021;373:eabe0237.
5. Tyser RCV, Mahammadov E, Nakanoh S, Vallier L, Scialdone A, Srinivas S. Single-cell transcriptomic characterization of a gastrulating human embryo. Nature 2021;600:285–9.
6. Gomes Fernandes M, Bialecka M, Salvatori DCF, Chuva de Sousa Lopes SM. Characterization of migratory primordial germ cells in the aorta-gonad-mesonephros of a 4.5-week-old human embryo: a toolbox to evaluate in vitro early gametogenesis. Mol Hum Reprod 2018;24:233–43.
7. Czukiewska SM, Fan X, Mulder AA, Van Der Helm T, Hillenius S, Van Der Meeren L, et al. Cell-cell interactions during the formation of primordial follicles in humans. Life Sci Alliance 2023;6:e202301926.
8. Anderson RA, Fulton N, Cowan G, Coutts S, Saunders PT. Conserved and divergent patterns of expression of DAZL, VASA and OCT4 in the germ cells of the human fetal ovary and testis. BMC Dev Biol 2007;7:136.
9. Heeren AM, He N, de Souza AF, Goercharn-Ramlal A, van Iperen L, Roost MS, et al. On the development of extragonadal and gonadal human germ cells. Biol Open 2016;5:185–94.
10. Heeren AM, van Iperen L, Klootwijk DB, de Melo Bernardo A, Roost MS, Gomes Fernandes MM, et al. Development of the follicular basement membrane during human gametogenesis and early folliculogenesis. BMC Dev Biol 2015;15:4.
11. Mizuta K, Katou Y, Nakakita B, Kishine A, Nosaka Y, Saito S, et al. Ex vivo reconstitution of fetal oocyte development in humans and cynomolgus monkeys. EMBO J 2022;41:e110815.

12. Koubova J, Hu YC, Bhattacharyya T, Soh YQ, Gill ME, Goodheart ML, et al. Retinoic acid activates two pathways required for meiosis in mice. PLoS Genet 2014;10:e1004541.
13. Zhang X, Gunewardena S, Wang N. Nutrient restriction synergizes with retinoic acid to induce mammalian meiotic initiation in vitro. Nat Commun 2021;12:1758.
14. Lundgaard Riis M, Jørgensen A. Deciphering sex-specific differentiation of human fetal gonads: insight from experimental models. Front Cell Dev Biol 2022;10:902082.
15. Spiller C, Bowles J. Instructing mouse germ cells to adopt a female fate. Sex Dev 2022;16:342–54.
16. Vernet N, Condrea D, Mayere C, Féret B, Klopfenstein M, Magnant W, et al. Meiosis occurs normally in the fetal ovary of mice lacking all retinoic acid receptors. Sci Adv 2020;6:eaaz1139.
17. Garcia-Alonso L, Lorenzi V, Mazzeo CI, Alves-Lopes JP, Roberts K, Sancho-Serra C, et al. Single-cell roadmap of human gonadal development. Nature 2022;607:540–7.
18. Li L, Dong J, Yan L, Yong J, Liu X, Hu Y, et al. Single-cell RNA-Seq analysis maps development of human germline cells and gonadal niche interactions. Cell Stem Cell 2017;20:858–73.e4.
19. Wamaitha SE, Rojas EJ, Monticolo F, Hsu FM, Sosa E, Mackie AM, et al. Defining the cell and molecular origins of the primate ovarian reserve. bioRxiv 2025;2025:2025.01.21.634052.
20. Zhao ZH, Li CY, Meng TG, Wang Y, Liu WB, Li A, et al. Single-cell RNA sequencing reveals regulation of fetal ovary development in the monkey (Macaca fascicularis). Cell Discov 2020;6:97.
21. de Vries FA, de Boer E, van den Bosch M, Baarends WM, Ooms M, Yuan L, et al. Mouse Sycp1 functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. Genes Dev 2005;19:1376–89.
22. Fan X, Moustakas I, Torrens-Juaneda V, Lei Q, Hamer G, Louwe LA, et al. Transcriptional progression during meiotic prophase I reveals sex-specific features and X chromosome dynamics in human fetal female germline. PLoS Genet 2021;17:e1009773.
23. Fukuda T, Daniel K, Wojtasz L, Toth A, Höög C. A novel mammalian HORMA domain-containing protein, HORMAD1, preferentially associates with unsynapsed meiotic chromosomes. Exp Cell Res 2010;316:158–71.
24. Fereydouni B, Drummer C, Aeckerle N, Schlatt S, Behr R. The neonatal marmoset monkey ovary is very primitive exhibiting many oogonia. Reproduction 2014;148:237–47.
25. Irie N, Weinberger L, Tang WW, Kobayashi T, Viukov S, Manor YS, et al. SOX17 is a critical specifier of human primordial germ cell fate. Cell 2015;160:253–68.
26. Murase Y, Yokogawa R, Yabuta Y, Nagano M, Katou Y, Mizuyama M, et al. In vitro reconstitution of epigenetic reprogramming in the human germ line. Nature 2024;631:170–8.
27. Overeem AW, Chang YW, Moustakas I, Roelse CM, Hillenius S, Helm TV, et al. Efficient and scalable generation of primordial germ cells in 2D culture using basement membrane extract overlay. Cell Rep Methods 2023;3:100488.
28. Sakai Y, Nakamura T, Okamoto I, Gyobu-Motani S, Ohta H, Yabuta Y, et al. Induction of the germ cell fate from pluripotent stem cells in cynomolgus monkeys. Biol Reprod 2020;102:620–38.
29. Kubiura-Ichimaru M, Penfold C, Kojima K, Dollet C, Yabukami H, Semi K, et al. mRNA-based generation of marmoset PGCLCs capable of differentiation into gonocyte-like cells. Stem Cell Reports 2023;18:1987–2002.
30. Kurlovich J, Rodriguez Polo I, Dovgusha O, Tereshchenko Y, Cruz CRV, Behr R, et al. Generation of marmoset primordial germ cell-like cells under chemically defined conditions. Life Sci Alliance 2024;7:e202302371.
31. Seita Y, Cheng K, McCarrey JR, Yadu N, Cheeseman IH, Bagwell A, et al. Efficient generation of marmoset primordial germ cell-like cells using induced pluripotent stem cells. Elife 2023;12:e82263.
32. Shono M, Kishimoto K, Hikabe O, Hayashi M, Semi K, Takashima Y, et al. Induction of primordial germ cell-like cells from common marmoset embryonic stem cells by inhibition of WNT and retinoic acid signaling. Sci Rep 2023;13:3186.
33. Kee K, Angeles VT, Flores M, Nguyen HN, Reijo Pera RA. Human DAZL, DAZ and BOULE genes modulate primordial germ-cell and haploid gamete formation. Nature 2009;462:222–5.
34. Fereydouni B, Salinas-Riester G, Heistermann M, Dressel R, Lewerich L, Drummer C, et al. Long-term oocyte-like cell development in cultures derived from neonatal marmoset monkey ovary. Stem Cells Int 2016;2016:2480298.
35. Gyobu-Motani S, Yabuta Y, Mizuta K, Katou Y, Okamoto I, Kawasaki M, et al. Induction of fetal meiotic oocytes from embryonic stem cells in cynomolgus monkeys. EMBO J 2023;42:e112962.
36. Panula S, Medrano JV, Kee K, Bergstrom R, Nguyen HN, Byers B, et al. Human germ cell differentiation from fetal- and adult-derived induced pluripotent stem cells. Hum Mol Genet 2011;20:752–62.
37. Eguizabal C, Montserrat N, Vassena R, Barragan M, Garreta E, Garcia-Quevedo L, et al. Complete meiosis from human induced pluripotent stem cells. Stem Cells 2011;29:1186–95.
38. Medrano JV, Ramathal C, Nguyen HN, Simon C, Reijo Pera RA. Divergent RNA-binding proteins, DAZL and VASA, induce meiotic progression in human germ cells derived in vitro. Stem Cells 2012;30:441–51.
39. Wongtrakoongate P, Jones M, Gokhale PJ, Andrews PW. STELLA facilitates differentiation of germ cell and endodermal lineages of human embryonic stem cells. PLoS One 2013;8:e56893.
40. Xuemei L, Jing Y, Bei X, Juan H, Xinling R, Qun L, et al. Retinoic acid improve germ cell differentiation from human embryonic stem cells. Iran J Reprod Med 2013;11:905–12.
41. Lin IY, Chiu FL, Yeang CH, Chen HF, Chuang CY, Yang SY, et al. Suppression of the SOX2 neural effector gene by PRDM1 promotes human germ cell fate in embryonic stem cells. Stem Cell Reports 2014;2:189–204.
42. Jung D, Xiong J, Ye M, Qin X, Li L, Cheng S, et al. In vitro differentiation of human embryonic stem cells into ovarian follicle-like cells. Nat Commun 2017;8:15680.
43. Yamashiro C, Sasaki K, Yabuta Y, Kojima Y, Nakamura T, Okamoto I, et al. Generation of human oogonia from induced pluripotent stem cells in vitro. Science 2018;362:356–60.
44. Abdyyev VK, Sant DW, Kiseleva EV, Spangenberg VE, Kolomiets OL, Andrade NS, et al. In vitro derived female hPGCLCs are unable to complete meiosis in embryoid bodies. Exp Cell Res 2020;397:112358.
45. Yu DCW, Wu FC, Wu CE, Chow LP, Ho HN, Chen HF. Human pluripotent stem cell-derived DDX4 and KRT-8 positive cells participate in ovarian follicle-like structure formation. iScience 2021;24:102003.
46. Yang S, Liu Z, Wu S, Zou L, Cao Y, Xu H, et al. Meiosis resumption in human primordial germ cells from induced pluripotent stem cells by in vitro activation and reconstruction of ovarian nests. Stem Cell Res Ther 2022;13:339.
47. Arkoun B, Moison P, Guerquin MJ, Messiaen S, Moison D, Tourpin S, et al. Sorting and manipulation of human PGC-LC using PDPN and hanging drop cultures. Cells 2022;11:3832.
48. Yu X, Wang N, Wang X, Ren H, Zhang Y, Zhang Y, et al. Oocyte arrested at metaphase II stage were derived from human pluripotent stem cells in vitro. Stem Cell Rev Rep 2023;19:1067–81.
49. Pierson Smela M, Adams J, Ma C, Breimann L, Widocki U, Shioda T, et al. Induction of meiosis from human pluripotent stem cells. bioRxiv 2024;2024:2024.05.31.596483.
50. Lawson KA, Dunn NR, Roelen BA, Zeinstra LM, Davis AM, Wright CV, et al. Bmp4 is required for the generation of primordial germ cells in the mouse embryo. Genes Dev 1999;13:424–36.
51. Chen HH, Welling M, Bloch DB, Muñoz J, Mientjes E, Chen X, et al. DAZL limits pluripotency, differentiation, and apoptosis in developing primordial germ cells. Stem Cell Reports 2014;3:892–904.
52. Kojima Y, Yamashiro C, Murase Y, Yabuta Y, Okamoto I, Iwatani C, et al. GATA transcription factors, SOX17 and TFAP2C, drive the human germ-cell specification program. Life Sci Alliance 2021;4:e202000974.
53. Rosario R, Crichton JH, Stewart HL, Childs AJ, Adams IR, Anderson RA. Dazl determines primordial follicle formation through the translational regulation of Tex14. FASEB J 2019;33:14221–33.
54. Sasaki K, Nakamura T, Okamoto I, Yabuta Y, Iwatani C, Tsuchiya H, et al. The germ cell fate of cynomolgus monkeys is specified in the nascent amnion. Dev Cell 2016;39:169–85.

55. Sekiné K, Furusawa T, Hatakeyama M. The boule gene is essential for spermatogenesis of haploid insect male. Dev Biol 2015;399:154–63.
56. VanGompel MJ, Xu EY. A novel requirement in mammalian spermatid differentiation for the DAZ-family protein Boule. Hum Mol Genet 2010;19:2360–9.
57. von Meyenn F, Berrens RV, Andrews S, Santos F, Collier AJ, Krueger F, et al. Comparative principles of DNA methylation reprogramming during human and mouse in vitro primordial germ cell specification. Dev Cell 2016;39:104–15.
58. Easley CA 4th, Phillips BT, McGuire MM, Barringer JM, Valli H, Hermann BP, et al. Direct differentiation of human pluripotent stem cells into haploid spermatogenic cells. Cell Rep 2012;2:440–6.
59. Hwang YS, Suzuki S, Seita Y, Ito J, Sakata Y, Aso H, et al. Reconstitution of prospermatogonial specification in vitro from human induced pluripotent stem cells. Nat Commun 2020;11:5656.
60. Yang S, Ping P, Ma M, Li P, Tian R, Yang H, et al. Generation of haploid spermatids with fertilization and development capacity from human spermatogonial stem cells of cryptorchid patients. Stem Cell Reports 2014;3:663–75.
61. Zhao Y, Ye S, Liang D, Wang P, Fu J, Ma Q, et al. In vitro modeling of human germ cell development using pluripotent stem cells. Stem Cell Reports 2018;10:509–23.
62. Lei Q, Zhang E, van Pelt AMM, Hamer G. Meiotic chromosome synapsis and XY-body formation in vitro. Front Endocrinol (Lausanne) 2021;12:761249.
63. Zhou Q, Wang M, Yuan Y, Wang X, Fu R, Wan H, et al. Complete meiosis from embryonic stem cell-derived germ cells in vitro. Cell Stem Cell 2016;18:330–40.
64. Weissbein U, Plotnik O, Vershkov D, Benvenisty N. Culture-induced recurrent epigenetic aberrations in human pluripotent stem cells. PLoS Genet 2017;13:e1006979.
65. Chang YW, Overeem AW, Roelse CM, Fan X, Freund C, Chuva de Sousa Lopes SM. Tissue of origin, but not XCI state, influences germ cell differentiation from human pluripotent stem cells. Cells 2021;10.
66. Chen D, Liu W, Lukianchikov A, Hancock GV, Zimmerman J, Lowe MG, et al. Germline competency of human embryonic stem cells depends on eomesodermin. Biol Reprod 2017;97:850–61.