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Research Article

Molecular-Genetic Mechanisms of Somatic Cell Reprogramming for Reproductive Biology Applications

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Abstract

The article examines a spectrum of molecular-genetic mechanisms that enable the reprogramming of differentiated somatic cells into gametes within the context of rapidly advancing in vitro gametogenesis (IVG) and its prospective applications in reproductive medicine and biodiversity conservation. The objective is to provide a comprehensive analysis of human-specific signaling pathways, transcriptional networks, and epigenetic modifications that determine the competence of pluripotent cells to enter the germline program, and to evaluate the performance of contemporary IVG protocols. The relevance of this topic stems from the fact that, despite successful implementation of the complete IVG cycle in mice, translation of analogous approaches to humans encounters fundamental divergences in the architecture of gene regulatory networks and unresolved epigenetic safety issues, particularly with respect to genomic imprinting and meiotic errors. The scientific novelty of this review lies in the integration of data from 2010–2025 with an emphasis on the human SOX17–BLIMP1–TFAP2C network, the temporal and dose-dependent interpretation of BMP/WNT signals, two-wave epigenetic reprogramming, and the critical role of metabolic tuning (N2B27, NAC, hypoxia) in sustaining germline competence. It is demonstrated that the principal limitation of IVG is not the mere derivation of pluripotent cells, but rather their positioning along a continuum of states (primed/naïve, iMeLC), the completeness of erasure of epigenetic memory, and the fidelity of meiotic progression; moreover, current 3D models of the gonadal niche (xrOvary, rOv) and synthetic gametogenesis strategies only partially mitigate these barriers and do not eliminate the risks of epigenetic insufficiency in the resulting gametes. The article is intended for researchers in human reproductive biology, regenerative medicine, stem cell biology, and reproductive tissue bioengineering.

Keywords: in vitro gametogenesis, induced pluripotent stem cells, primordial germ cells, SOX17, BLIMP1, TFAP2C, epigenetic reprogramming

1. Introduction

Reproductive biology is approaching a technological inflection point associated with the possibility of generating gametes de novo [1]. In vitro gametogenesis (IVG) comprises methods that reprogram differentiated somatic cells (e.g., skin fibroblasts or peripheral blood mononuclear cells) into a pluripotent state, followed by directed development along the germline trajectory to mature gametes [2]. Implementation of this technology opens unprecedented opportunities to treat absolute forms of infertility (absence of gonads, sequelae of oncotherapy), conserve endangered species, and fundamentally interrogate the early stages of human embryogenesis that have previously remained inaccessible to direct observation (the black box of implantation) [3].

A historical prerequisite for IVG development comprised two foundational discoveries: demonstration of reversibility of cellular differentiation via somatic cell nuclear transfer (SCNT), for which John Gurdon received the Nobel Prize, and the development of induced pluripotent stem cell (iPSC) technology by Shinya Yamanaka [4]. Whereas SCNT demonstrated the plasticity of the nucleus, iPSC technology provided an ethically acceptable, technically scalable source of autologous pluripotent cells for any

patient [5].

However, although the complete IVG cycle, from a somatic cell to the birth of fertile offspring, was successfully achieved in mice as early as 2016 (Hayashi and Saitou groups), replication of this success in humans has encountered unanticipated obstacles [6]. Molecular mechanisms governing germline specification have not been evolutionarily conserved between rodents and primates [7]. Direct extrapolation of murine protocols to human cells resulted in low efficiency or no induction of primordial germ cells (PGCs), the precursors of all gametes [8].

Over the past five years, the scientific community has concentrated efforts on deciphering the unique human gene regulatory network. It has been established that the key human PGC specifier is the transcription factor SOX17, which in mice functions as an endoderm marker and does not participate in early gametogenesis [9]. This discovery necessitated revising cultural strategies and developing new, human-specific protocols. In addition, epigenetic safety remains a critical barrier: the processes of global DNA demethylation and erasure of genomic imprinting, required to create a blank slate for the next generation, frequently proceed with errors under in vitro conditions [10].

The present work aims to provide a comprehensive analysis of the molecular-genetic mechanisms of somatic-to-gamete reprogramming, with an emphasis on human-specific signaling pathways, transcriptional networks, and epigenetic processes, and to evaluate the effectiveness of current biotechnological approaches to IVG implementation.

2. Materials and Methodology

For the preparation of the present analytical report, a systematic review methodology was applied, ensuring procedural reproducibility and reducing the risk of subjective selectivity in result interpretation. This format is oriented toward maximal yet controlled coverage of available publications, as well as comparability of conclusions derived from different study designs.

Sources were retrieved from the abstracting databases Scopus and Web of Science Core Collection, and the bibliographic databases PubMed/MEDLINE, in the period between January 2020 and April 2025, thus including the most recent methodological and conceptual advances in the area of interest. In parallel, foundational works from 2010–2019 were additionally considered as necessary for an accurate description of basic concepts and historically significant approaches, including pioneering studies by the Saitou and Surani groups.

Search queries were constructed using logical operators and combined key terms related to in vitro gametogenesis, somatic cell reprogramming, the derivation and characterization of primordial germ cells, and the analysis of molecular mechanisms, with explicit linkage to human studies. The following keyword combination was used: (in vitro gametogenesis OR IVG OR oogenesis in vitro OR spermatogenesis in vitro) AND (somatic cell reprogramming OR iPSC OR SCNT) AND (primordial germ cells OR PGCLC OR hPGCLC) AND (molecular mechanism OR signaling pathway OR gene regulatory network OR epigenetics) AND (human OR Homo sapiens).

Selection and subsequent analysis of publications were conducted based on predefined criteria constraining the source corpus to works relevant by data type, object, and subject of investigation. Included were original research articles containing experimental results and high-level systematic reviews. Study objects encompassed human cells, including hESC, hiPSC, and hPGCLC, as well as comparative studies juxtaposing human models with murine or primate systems. The analytical focus was on molecular mechanisms, including BMP/WNT signaling pathways, the roles of transcription factors SOX17/BLIMP1, and epigenetic mechanisms such as DNA methylation and histone modifications. An additional constraint was data quality, requiring quantitative efficiency metrics and reproducible measurements, including induction percentages, gene expression levels, and methylome analysis outputs.

Analytical processing of selected works included the extraction of data on culture media compositions, particularly applied cytokines and small molecules, as well as the temporal structure of protocols and key implementation stages. Experimental methods used to characterize cellular phenotypes (e.g. FACS, RNA-seq, bisulfite sequencing) were grouped independently, along with molecular targets the authors described as being essential for inducing or maintaining relevant states. Results from different groups were purposely compared, as these differences can arise from the experimental approach, criteria for defining a cell state, or measurement methods. Methodological dynamics over the past five years were also considered, as they largely determine the current level of reliability and interpretability of experimental data.

3. Results

3.1. Molecular Foundations of Pluripotency Induction and Germline Competence

The first and most fundamental step of IVG is the generation of pluripotent cells capable of differentiation into the germline. Only specific pluripotent cells in a particular position along the pluripotency continuum can generate fully viable gametes.

The two primary methods for somatic cell reprogramming are somatic cell nuclear transfer (SCNT) and transcription-factor-mediated induction of pluripotency (iPSC).

A somatic nucleus is introduced into an enucleated oocyte during SCNT. Added factors act immediately after the somatic nucleus fuses with the oocyte cytoplasm. These factors rapidly reset the nucleus's epigenetic state [11]. SCNT appears to more closely reset the natural DNA methylation of the embryonic stem cell (ESC) state than iPS cells [4]. SCNT does not inherit mutations in donor mitochondria, as the mitochondria belong only to the oocyte donor [12]. However, this method is inefficient and cannot be used at scale for ethical reasons.

It is based upon ectopic expression of the transcription factors OCT4, SOX2, KLF4 and c-MYC (OSKM). It represents the primary cell source for human IVG [13]. The major challenge of iPSCs is epigenetic memory, i.e., retention of residual DNA methylation marks at loci characteristic of the donor tissue (e.g., fibroblasts) [14]. These marks can distort downstream differentiation. Nevertheless, comparative analysis of isogenic lines generated by SCNT and iPSC methods has shown that, with rigorous clone selection, transcriptional and epigenetic differences between them are attenuated, supporting iPSCs as a valid model for IVG [15].

A key insight of the past decade is that hESCs and hiPSCs under standard culture conditions (FGF2/KSR media) occupy a so-called primed state corresponding to the post-implantation epiblast [3]. In this state, cells have already lost competence for efficient PGC formation.

To induce gametogenesis, cells must be transitioned into a naïve state corresponding to the pre-implantation epiblast. This is achieved using specialized media (e.g., 4i medium, which inhibits MEK, GSK3, p38, and JNK) or by brief induction of an incipient mesoderm-like state (iMeLC) [16]. It is specifically at the iMeLC stage that cells acquire the capacity to respond to BMP4 signals by activating the germline program.

3.2. Specification of Primordial Germ Cell-Like Cells (PGCLC): Signaling Pathways and Transcriptional Networks

Induction of PGC-like cells (PGCLCs) from pluripotent precursors is regarded as the central stage of in vitro gametogenesis (IVG) [17]. This transition is not spontaneous; it is determined by a conjunction of external signaling inputs and an internal gene regulatory network that specifies which expression programs are activated, sustained, or suppressed at distinct stages of differentiation.

PGC specification, both in vivo and in vitro, is initiated by the BMP (Bone Morphogenetic Protein) signaling cascade. Crucially, BMP action is not reducible to a simplistic higher-concentration, stronger-effect dependency. Experimental data indicate that BMP4 exhibits a pronounced temporal component, and a critical parameter is not only the signal magnitude but also its duration, i.e., a temporal form of morphogen effect [18]. In other words, the same molecule under different temporal regimes can launch fundamentally different cell-fate trajectories.

It has been shown that a short BMP4 pulse of less than 24 hours, in the presence of supportive factors such as LIF and SCF, induces both mesodermal and PGC-specific genes. In contrast, prolonged exposure exceeding 48 hours, particularly at high BMP4 doses, redirects differentiation toward amniotic or extraembryonic mesoderm [18]. Thus, the temporal signal profile is not a secondary protocol detail but a parameter that can alter the cell's biological interpretation of the signal.

In view of these regularities, optimized human protocols employ high BMP4 doses, on the order of 500 ng/mL, at the aggregation stage, while strictly restricting exposure to a defined time window [17]. This tuning strengthens the desired specification program while reducing the likelihood of cellular escape into alternative, off-target lineages that become dominant under excessive signal duration.

An additional necessary element is the WNT pathway, which can be activated by the ligand WNT3A or the small molecule CHIR99021. Its role is described as preparatory: WNT establishes a chromatin state that renders cells receptive to subsequent BMP4 signaling. Within this model, WNT primes enhancers of key genes, increasing their accessibility to transcription factors and thereby facilitating initiation of the target transcriptional program [19]. In IVG protocols, the iMeLC stage is induced precisely by a combination of Activin A, reflecting Nodal-pathway activation, and CHIR99021, reflecting WNT-pathway activation, underscoring the functional necessity of this combined signaling configuration [17].

Analysis of the 2020–2025 literature confirmed fundamental differences in the transcriptional networks underlying PGC specification between rodents and primates (including humans). Table 1 shows a comparative analysis of gene regulatory networks (GRNs) of PGC specification.

Table 1: Comparative analysis of gene regulatory networks (GRNs) of PGC specification

Network component	Mouse (*Mus musculus*)	Human (*Homo sapiens*)	Note
Master regulator	Blimp1 (*Prdm1*)	SOX17	Radical evolutionary difference
Initiating event	BMP4 → Blimp1	BMP4 → SOX17	In mouse, SOX17 is an endoderm marker
Secondary regulator	*Prdm14*	BLIMP1 (*PRDM1*)	In human, BLIMP1 is downstream of SOX17
Co-factor	Tfap2c (AP2γ)	TFAP2C (AP2γ)	Conserved network element
Role of SOX2	High expression, required	Repressed	SOX2 is not expressed in early hPGCLCs
Role of PRDM14	Critical for epigenetics	Low/variable expression	In humans, the role is likely performed by another factor
Surface markers	SSEA1, CD61	CD38, EpCAM, INTEGRINα6	CD38 is a primate-specific marker

The mechanism of action of the SOX17–BLIMP1–TFAP2C network in humans is considered below.

SOX17, Apex of the hierarchy. In response to BMP4, SOX17 expression is activated in competent cells (iMeLC). This factor, classically regarded as endodermal, functions in human IVG as a pioneer factor for the germline [9]. SOX17 binds to the BLIMP1 gene's enhancers and initiates its expression.

BLIMP1 (PRDM1), Somatic repressor. BLIMP1 acts as a transcriptional repressor. Its primary task is to suppress the expression of somatic lineage differentiation genes (mesoderm, neuroectoderm, trophoblast), such as HOX genes. This prevents diversion of cells into somatic tissues and canalizes development toward PGC identity [20]. In the absence of BLIMP1, SOX17-positive cells would differentiate into definitive endoderm (intestine/liver).

TFAP2C, Epigenetic modulator. TFAP2C is expressed in parallel and functions in a complex with SOX17 and BLIMP1. It recruits chromatin remodeling complexes, maintains the expression of pluripotency genes (OCT4, NANOG), and ensures epigenetic plasticity [21].

This unique interaction (SOX17 activates BLIMP1, which suppresses SOX17's endodermal signature, leaving only the PGC program) represents an elegant evolutionary acquisition in primates, distinguishing them from rodents [9].

3.3. Epigenetic Reprogramming: Memory Erasure and Preparation for the Future

The germline is regarded as the sole immortal lineage of the organism, insofar as it ensures transmission of genetic information across generations. To fulfill this function, germline cells must undergo total epigenetic renewal, effectively resetting epigenetic configurations accumulated during somatic development and thereby preparing the genome for the establishment of new, generation-relevant regulatory programs.

During migration of primordial germ cells (PGCs) into the gonads, as well as in hPGCLC culture, large-scale erasure of DNA methylation, primarily represented by 5-methylcytosine (5mC), is observed. Pronounced dynamics characterize this process. Methylation levels decline from approximately 70%, typical of somatic cells and iPSCs, to extraordinarily low levels of 4–8%, as observed in gonadal PGCs. This state is designated an epigenetic ground state [10], emphasizing that the phenomenon is not a local reconfiguration of individual loci but a global resetting of the genome's epigenetic memory.

According to the presented data, demethylation proceeds in two waves that differ both in biochemical nature and in the means by which the outcome is achieved. The first wave corresponds to passive demethylation and is mediated by replication-dependent dilution. During successive cell divisions, methyl marks are not restored on daughter DNA strands because activity of the maintenance methylation machinery, primarily UHRF1 and DNMT1, is suppressed, with DNMT1 serving as the maintenance DNA methyltransferase [10]. Under these conditions, each new replication reduces the fraction of methylated cytosines, and methylation decline arises from failure to replenish marks rather than from their direct removal.

In the second wave for active demethylation, TET-family enzymes TET1 and TET2 are upregulated, oxidizing 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). The modification of 5mC allows the removal of the modified base via base excision repair (BER) [10]. A transient wave of 5hmC formation in hPGCLC culture has been interpreted as evidence of this pathway's activation. At the same time, it is emphasized that complete methylation erasure under culture conditions is often difficult to achieve; i.e., the active component is present but does not invariably reach the same depth of zeroing as observed in vivo.

Even against the background of global demethylation, so-called resistant loci persist, i.e., genomic regions refractory to methyl-mark erasure. These include, in particular, young retrotransposons such as IAP in mice and LINE-1 in humans, as well as pericentromeric heterochromatin. Maintenance of methylation in these regions is interpreted as a protective mechanism preventing genomic instability under conditions in which much of the genome temporarily loses methyl constraints that ordinarily suppress mobile elements and excessive transcriptional activity [10].

In parallel with DNA demethylation, profound chromatin remodeling occurs, reflected in the dynamics of key histone modifications. In this context, epigenetic reprogramming should be viewed as a multi-component process in which the removal of DNA methylation marks is coupled with the redistribution of repressive and regulatory signals at the nucleosomal level. Such synchronization is critical because, upon loss of DNA methylation, an alternative mechanism is required to maintain transcriptional discipline, i.e., suppression of programs that must not be active during this period.

One early and well-characterized event is global loss of H3K9me2, a marker of repressive chromatin. In early PGCs, this mark is erased on a broad scale, consistent with an overall shift toward increased chromatin plasticity and relaxation of long-term somatic constraints [1]. Loss of H3K9me2 reflects not a local adjustment of discrete genes. Still, a systemic transition to a state in which prior repressive barriers are weakened, and chromatin becomes more accessible for subsequent stages of reprogramming.

In contrast to H3K9me2, H3K27me3 dynamics are of a different character. The level of this repressive mark, functionally linked to the Polycomb complex, transiently increases at a specific developmental stage, demonstrated for E8.5 in mice and corresponding approximately to weeks 4–5 in humans. This elevation is interpreted as a compensatory mechanism: under conditions of sharply reduced DNA methylation, H3K27me3 suppresses developmental genes and prevents premature or discordant activation of differentiation programs [1]. However, this peak does not represent a stable state. Subsequently, approximately by weeks 7–9, H3K27me3 levels also decline, indicating a shift in repression regimes as germ cells progress through later stages of specification and preparation for gametogenesis [1]. In hPGCLC culture, H3K27me3 dynamics are frequently disrupted, which may serve as a marker of incomplete reprogramming, i.e., a situation in which the cell fails to reproduce the correct sequence of epigenetic switches characteristic of in vivo development [21].

Within the broader epigenomic remodeling landscape, the most vulnerable and, in effect, limiting component of IVG is genomic imprinting. Imprinting refers to monoallelic gene expression dependent on parental origin, and the correctness of imprint erasure and subsequent establishment determines whether a future gamete is epigenetically adequate. At the PGC stage, imprints must be entirely erased, i.e., reset to a zeroed state. However, in hPGCLCs derived from iPSCs, incomplete erasure is frequently observed: several imprinted loci retain aberrant methylation. Commonly cited examples include H19, PEG1, KCNQ1OT1, and SNRPN [22], indicating a systemic rather than isolated difficulty in reproducing this stage under culture conditions.

An illustrative example of the clinical significance of correct imprint status is the H19/IGF2 (IC1) locus. Imprinting methylation errors have unidirectional effects on the phenotype: hypomethylation of the paternal allele causes Silver-Russell syndrome, a sporadic growth retardation disorder, while hypermethylation of the maternal allele causes Beckwith-Wiedemann syndrome, an overgrowth and predisposition to tumorigenesis syndrome [23]. This shows that, at least for imprinted regions, localized methylation errors can have predictable and medically essential consequences.

Following the erasure stage is imprint re-establishment, which occurs de novo at late stages of gametogenesis. In males, this is associated with meiotic prophase, whereas in females it is linked to oocyte growth. Establishment of imprints requires the precise and coordinated activity of DNMT3A and DNMT3L. In in vitro systems, this stage is currently reproduced with the lowest efficiency. It is regarded as a source of systemic risk: potential reproductive use of such gametes increases the likelihood of epigenetic diseases in offspring [24]. The epigenetic reprogramming process in Germline Development is shown in Figure 1.

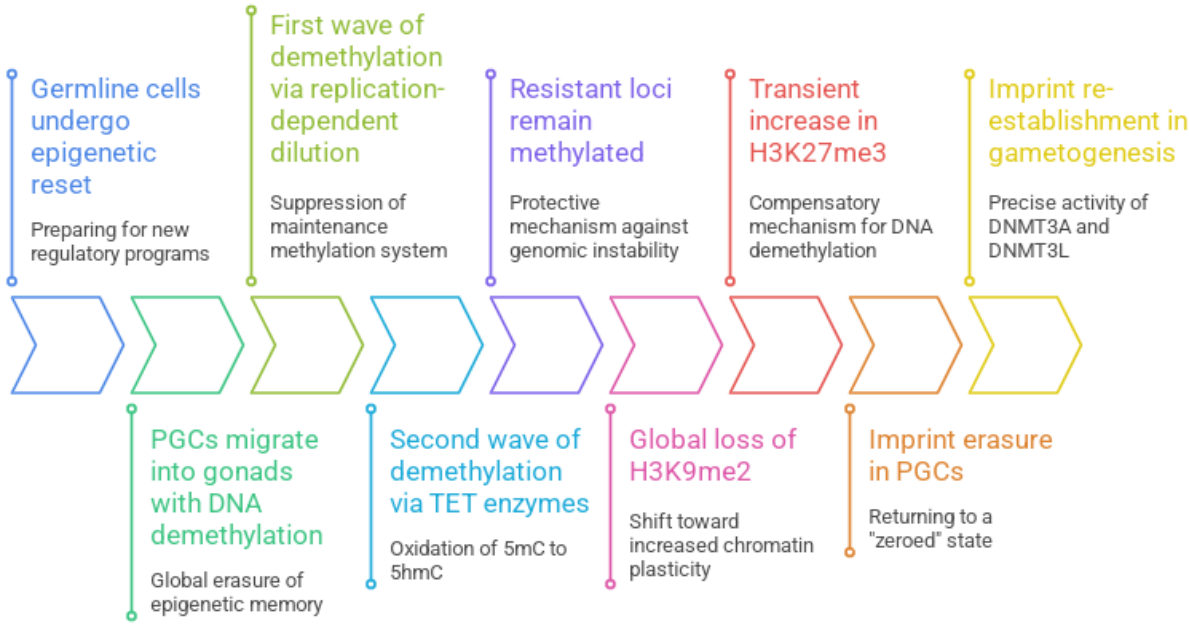

Figure 1: Epigenetic Reprogramming in Germline Development

3.4. Contemporary IVG Protocols: Methodological Evolution (2020–2025)

For an extended period, the efficiency of hPGCLC derivation ranged from 10% to 30% [17]. In recent years, protocols have undergone substantial optimization. Table 2 shows the evolution of hPGCLC induction protocols.

Table 2: Evolution of hPGCLC induction protocols

Parameter	1st-generation protocol (GK15)	2nd-generation protocol (N2B27)	Comment
Basal medium	GMEM + 15% KSR (serum replacement)	DMEM/F12 + N2 + B27 (chemically defined)	Switching to serum-free/chemically defined media improves reproducibility [25]
Inducers	BMP4, LIF, SCF, EGF	BMP4, LIF, SCF, EGF + antioxidants (NAC)	Adding NAC is critically important [25]
Format	Spheroids (floating aggregates)	Monolayer or aggregates	Monolayer is easier for screening; aggregates are better for differentiation [26]
Hypoxia level	Atmospheric O_2 (~20%)	Controlled hypoxia or mimicked via antioxidants	PGCs in vivo exist in hypoxic conditions [25]
Efficiency (yield)	~15–30% $EpCAM^+/INTEGRIN\alpha6^+$	1.5–2× increase (to ~50–60%)	Data from [25]
Mechanism of improvement	,	Reduced reactive oxygen species (ROS), protection from apoptosis, improved mitochondrial respiration	

Recent studies (2024–2025) have shown that hPGCLCs experience pronounced oxidative stress during culture. Supplementation of the medium with N-acetylcysteine (NAC) at 0.5–1 mM significantly increases cell survival [27]. The mechanism is linked to the expansion of the intracellular glutathione pool and mitochondrial protection, enabling cells to more effectively cope with the hyperoxia of culture conditions compared with the natural niche [25]. This indicates that metabolic reprogramming is no less consequential than transcriptional reprogramming.

Three-dimensional niche modeling in organoid formats is considered a key technological approach for inducing subsequent developmental stages beyond the PGCLC stage. At this phase, purely cell-autonomous programs are insufficient, as the typical germline differentiation trajectory is substantially determined by signals and the physicochemical microcontext created in vivo by gonadal somatic cells. Therefore, reproduction of later stages requires a system in which germ cells reside within a structured environment and receive a complex of supportive stimuli.

One such solution is xrOvary, a xenogeneic reconstructed ovary proposed by Yamashiro. In this approach, hPGCLCs are aggregated with ovarian somatic cells from mouse embryos at stage E12.5 [28]. In the described system, human cells exhibited further differentiation into oogonia and initiated meiosis; with prolonged culture, they reached the pachytene stage in approximately four months. Thus, xrOvary functions as an engineered gonadal niche model in which a murine somatic component provides signaling and structural conditions sufficient to advance the human germline through portions of the oogenesis and meiotic development program.

However, the use of a xenogeneic somatic environment predictably raises concerns about potential contamination and the extent to which an interspecies system accurately reproduces human-specific signaling regimes. Accordingly, the most recent studies describe the possibility of forming rOv, i.e., reconstructed ovaries on a fully human basis, using human fetal somatic cells [29]. This approach fundamentally reduces risks associated with xenogeneic components. It simultaneously implies more appropriate, species-specific signaling, since the somatic niche and germ cells belong to the same species and thus rely on more concordant molecular interactions.

3.5. Meiosis and Maturation: The Technological Bottleneck

Meiotic induction and its faithful completion remain the central, effectively decisive, unresolved task for human IVG. At this stage, system requirements increase sharply: it is necessary not only to initiate the program, but also to ensure precise spatiotemporal coordination of events that, under physiological conditions, are sustained by a complex gonadal niche and prolonged maturation periods. Any deviations directly translate into genomic instability and functional incompleteness in the resulting cells.

One of the most acute problems is aneuploidy. Human oocytes are intrinsically predisposed to chromosome segregation errors, and in vitro culture may exacerbate this vulnerability. It is reported that in vitro, aneuploidy frequency can exceed 30–50%, which is linked to defects in synaptonemal complex formation and spindle assembly [30]. These structures are critical for correct homologous chromosome synapsis and subsequent segregation; therefore, disturbances in their assembly or dynamics predictably lead to errors in chromosome distribution. Supporting evidence from murine IVG models indicates that the efficiency of producing live offspring remains exceptionally low, with meiotic errors as the primary limiting factor [31]. Thus, even when formal markers of meiotic program progression are present, the ultimate functional outcome may be strongly constrained by meiotic division quality.

A separate complexity is the block at prophase I. Under physiological conditions, human oocytes arrest at the diplotene stage, forming a prolonged dictyate arrest that may persist for years. Reproducing such extended arrest and subsequent synchronous reactivation in vitro is highly challenging, as it requires not only long-term maintenance of cell viability and stability, but also precise control of signals that удерживают the cell in arrest and subsequently advance it toward maturation. Descriptions of organoid and reconstructed systems indicate that a substantial fraction of in vitro-derived cells die or fail to develop beyond early prophase I, failing to reach a stable, functionally relevant state [28]. This indicates that current protocols more readily initiate meiotic events than carry them to a stage comparable to the natural oogenesis regime.

Against this backdrop, alternative approaches to bypass or mitigate differentiation blocks are being explored. It has been described that direct expression of meiotic factors such as DAZL, BOLL, and STRA8, or the use of transcription factor combinations including ZNF281, LHX8, and SOHLH1, can push cells through critical points and accelerate the derivation of oogonia-like cells denoted iOLCs [32]. This approach is often referred to as synthetic gametogenesis, as it relies on the deliberate programming of cell fate via specific regulators. At the same time, it is emphasized that the promise of such a solution does not obviate the need for stringent verification of epigenetic integrity of the resulting cells, because acceleration and forced progression of the developmental program increases the risk of deviation from natural epigenetic trajectories that usually form under prolonged maturation and complex tissue-level regulation.

4. Discussion

The reviewed data indicate that the decisive limitation of gamete derivation from somatic cells is not so much the attainment of pluripotency per se, but rather the quality of that state and its fitness for entry into the germline program. Somatic cell nuclear transfer demonstrates more complete epigenetic resetting, yet its practical value is diminished by low efficiency and application

constraints. Induced pluripotent cells remain the primary source of human models, but they primarily exhibit the problem of residual epigenetic memory that can bias differentiation trajectories. In this context, a controlled transition from the primed state to the naïve state, or passage through an intermediate state in which germline competence is established, becomes pivotal; without such tuning, downstream stages are reproduced нестабильно and with low yield.

A central conclusion with direct methodological implications is that human primordial germ cell specification relies on a regulatory hierarchy distinct from that of rodents; therefore, direct copying of mouse schemes predictably fails. In human systems, the верхний переключатель is SOX17, which initiates BLIMP1 expression and, together with TFAP2C, forms a network that simultaneously activates the germline program and blocks somatic alternatives. This architecture appears to be an acceptable compromise: SOX17 is inherently linked to endodermal contexts, and therefore a rigid repressive BLIMP1 loop is required to cut off aberrant programs and удержать the cell within a narrow corridor of germline identity. Hence, a practical protocol requirement follows: it is insufficient merely to deliver an inducing signal; conditions must be established such that the cell interprets the signal specifically as an instruction toward germline fate rather than as a stimulus toward amniotic or extraembryonic mesoderm.

The discussed works also converge on the notion that external signals act not as static switches, but as control regimes, where duration and sequence are more critical than dose alone. For the BMP cascade, a pronounced temporal dependence is observed: a brief exposure favors initiation of the desired program, whereas prolonged exposure drives cells onto off-target trajectories. WNT signaling, in turn, is not a redundant factor but a preparatory layer that alters chromatin accessibility of regulatory regions and increases responsiveness to subsequent input. Collectively, this explains why interlaboratory reproducibility is so sensitive to seemingly minor protocol details: in practice, not the details themselves. Still, the signal's meaning for the cell is changing.

The most stringent barrier to the technology's readiness for reproductive applications remains epigenetic safety. Even with proper induction of primordial germ cells, profound DNA demethylation and synchronized chromatin remodeling are required, and in culture, these processes are often incomplete or desynchronized. Genomic imprinting constitutes a particular vulnerability: incomplete erasure of marks at imprinted loci and difficulties in subsequent imprint establishment at later stages create predictable risks of developmental perturbations. It is also emphasized that improvements in yield may depend not only on transcriptional switches but also on metabolic conditions: attenuation of oxidative stress via antioxidant support increases survival and likely reduces the selective pressure that causes cells to deviate from the correct trajectory.

Finally, discussion inevitably converges on the bottleneck, meiosis and maturation, where formal markers of progression do not guarantee functional completeness. High aneuploidy rates and frequent arrests at prophase I indicate that current systems are more capable of initiating the program than delivering it to a state comparable to natural oogenesis or spermatogenesis. Consequently, increased interest in three-dimensional gonadal niche models and reconstructed ovarian systems appears logical: at late stages, the cell requires not only an internal regulatory circuit but also a prolonged, correctly organized tissue environment. Alternative strategies that directly express meiotic and oogenic regulators may accelerate traversal of critical points; however, they also increase the risk of divergence from natural epigenetic trajectories, thereby shifting the principal problem from efficiency to demonstrable safety.

5. Conclusion

The conducted review demonstrates that in vitro gametogenesis (IVG) in human reproductive biology should be conceptualized not as a linear switch from a somatic cell to a gamete, but as a multistep reconfiguration of cellular identity in which both the quality of initial pluripotency and the correctness of entry into the germline program are critical. The principal limiting factor is not the mere derivation of pluripotent cells (SCNT or iPSC), but their positioning along the continuum of states and their acquisition of germline competence, which requires a transition from the primed state to the naïve state or passage through iMeLC. Against this background, the central conceptual outcome of recent years is recognition of the evolutionary irreducibility of mouse and primate GRNs: in humans, PGC specification is determined by the SOX17→BLIMP1 architecture with TFAP2C participation, whereas attempts to directly extrapolate the rodent Blimp1–Prdm14–Tfap2c logic predictably yield low efficiency or failure of hPGCLC induction.

Critical analysis of protocols underscores that external signals function as control regimes rather than static dose stimuli: for BMP4, the temporal profile is decisive (a short window as a condition for удержание of the target trajectory and prevention of diversion into amnion/extraembryonic mesoderm), whereas WNT signaling is described as a priming layer that alters chromatin accessibility of regulatory elements and thereby determines whether BMP4 is interpreted as an instruction toward germline fate. Simultaneously, it is shown that technical optimization of the medium is biologically consequential: transition to chemically defined conditions (N2B27) and antioxidant support (NAC) address oxidative stress and mimic a hypoxic niche, increasing hPGCLC yield and indicating coupling of transcriptional and metabolic reprogramming. However, increased efficiency does not remove the most stringent barrier, epigenetic safety: global demethylation and coordinated chromatin dynamics in culture are often incomplete, and imprint erasure defects at imprinted loci (including H19 and KCNQ1OT1) create

a direct risk that future gametes will not attain the required zero epigenetic state.

Finally, the discussion inevitably focuses on the bottleneck of human IVG, meiosis, and maturation, where formal induction of the program is not equivalent to functional completeness: high aneuploidy rates and frequent prophase I arrests define the boundary of current reproducibility and highlight the necessity of a tissue microcontext generated in vivo by gonadal somatic cells. Within this logic, the rising interest in three-dimensional niche models and reconstructed ovarian systems (including xrOvary and fully human rOv) is not optional but methodologically necessary, since late-stage development requires long-term, structured, and species-specific signaling. Synthetic gametogenesis strategies with forced expression of meiotic/oogenic regulators may accelerate traversal of critical points, but simultaneously shift the primary criterion of progress from cell yield to demonstrable epigenetic and genomic correctness, without which clinical translation remains premature.

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