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Stem cells: A novel initiative to treat infertility

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Abstract
Advances in stem cell research have opened novel perspectives for regenerative and reproductive medicine. Several groups have reported *in vitro* differentiation of stem cells into germ cell lineages and some of them have been successful to obtain male and female gamete-like cells by using different methodologies. Foremost, *in vitro* germ cell differentiation depends on establishment of ideal culture system that should recapitulate *in vivo* niche of germ cells. Seemingly, this approach of producing artificial gametes provides a paradigm to study the molecular basis of germ line establishment and may create new avenue to treat reproductive disorders in the future. This present review briefly describes different assisted reproductive technologies (ART) that are currently being used to treat human infertility and summarizes current knowledge of derivation artificial gametes from pluripotent and multipotent stem cells and account different prospective of these *in vitro* differentiated cells in present and future reproductive biology.

Keywords: Infertility, stem cells, assisted reproductive technologies, artificial gametes, reproductive medicine

1. Introduction
Infertility is reproductive trauma or biological inability to conceive a child after >12 months of unprotected intercourse. Infertility is a heterogeneous condition associated with a number of pathophysiological factors, so, its etiology and pathogenesis is frequently undefined. So, often the treatment of both male and female infertility is not straight forward. Because of etiological heterogeneity, all etiological mechanisms causing these problems are still to reveal. Currently infertility is increasing rapidly in the world and utmost 75 million peoples of the world population are facing infertility problems including both male and female [1]. Many medicinal systems like ayurvedic, homeopathic, allopathic and alternative medicine claim to treat infertility [2]. However, there is no permanent solution to this problem is yet achieved. The administration of drugs, hormonal therapy and assisted reproductive technology (ART) are some modern remedial approaches followed to treat infertility. ART such as *in vitro* fertilization and embryo transfer (test tube babies), gamete intra-fallopian transfer, zygote intra-fallopian transfer, intra-cytoplasmic sperm injection (ICSI) are become routine practices for clinician.

Recent advance in stem cell technology further accelerate ART by producing *in vitro* sperm and ovum from pluripotent and multipotent stem cell lines [3]. This generation of human artificial gametes is of outstanding interest in the context of assisted reproductive medicine as they would offer a clinical solution for couples suffering with infertility and become attractive tools understand the complex mechanism of human gametogenesis as that is currently poorly studied due to the technical and ethical restrictions associated to obtain human samples [4]. However, derivation of gametes from stem cells in human is still at preliminary stage. This review briefly describes the major therapeutic strategies followed to treat infertility world-wide, further discuss production of germ cells from different sources of stem cells. Thus, *in vitro* derived (IVD) gametes could translate a dramatic improvement to treat infertility and other associated reproductive disorders.

2. Test tube babies
The first test tube baby "Loise Joy Brown" was born on 25th July, 1978 in United Kingdom [5]. Then exactly after 67 days the world second test tube baby, Kanupriya agrawal (Durga), was born on 3rd October, 1978 in Calcutta by Subhash Mukharzee [6]. The develop technique involves the following steps such as (i) monitoring the stimulation of woman's ovulatory process, (ii) removal of an ovum or ova (egg or eggs) from the woman's ovaries, (iii)

penetration of sperm to fertilize ova in medium *in vitro*, (iv) fertilized egg (zygote) is cultured for 2–6 days in a growth medium and is then implanted in the uterus with the intention of establishing a successful pregnancy. The incidence of heterotopic pregnancies is increased in recent years due to escalating use of new procedure "assisted reproductive technologies (ART)" [7] (Table 1). However, ART associated with the risks such as alternation of gene expression patterns of some developmentally regulated gene leading to the birth defects and genetic disorders that culminate with decrease intellectual, physiological, immunological potential of *in vitro* fertilized (IVF) babies [8].

3. Germline development

Germ cells develop in a specialized way, in specific niche, following complex developmental pathways depending upon involvement of several cell types in organism-specific context [9]. Germ cells function as a kind of "transgenerational stem cells", transmitting genetic information from one generation to the next [10]. This constitutes a very different cell population from somatic cells, with unique characteristics and displays a haploid chromosomal number after a delicate process of meiosis [11]. But, how do these cells acquire a different fate than the other cells of the individual? Does it possible to recapitulate the events using undifferentiated stem cells *in vitro*? However, detailed molecular mechanism underlying germline development in vertebrates especially of human is yet to be revealed. Germ cells originated from germline stem cells (GSCs), which are diploid in nature but capable of giving haploid gametes mature spermatocytes and ova by gametogenesis. The details of gametogenesis – and the accumulating knowledge of the mechanisms underlying it – have been described elsewhere in a number of excellent articles. The molecular mechanisms of germ cell development is well studied in murine models but yet the mechanisms of PGCs formation, commitment, migration, and gonad colonization still at a descriptive level in human. In humans and mammals in general, the germline originates from a founder pluripotent cell population called primordial germ cells (PGCs) that segregate from the somatic lineage during the early stages of embryogenesis. Just after their specification, PGCs proliferate and migrate through adjacent endoderm to the genital ridges. This migration occurs between E8.5 and E12.5 and the fourth and sixth week of pregnancy in mice and humans respectively. Once PGCs colonize genital ridges, they are termed gonocytes. The gonocytes involves proliferation and apoptosis before they differentiate into mature gametes within the gonads in response to external paracrine signals [12]. In the female gonad, germ cells generally enter meiosis and stay arrested in the first meiotic prophase during embryonic development around E13.5 in mice or week 12 in humans, whereas in males, spermatogonia arrest in mitosis and do not enter meiosis until puberty (Fig 1). The molecular cues BMP4 BMP2 and BMP8b, a subset of epiblast cells begins to express the Fragilis and Blimp1 gene, canonical WNT signaling (WNT 3) from the visceral endoderm situated adjacent to epiblast those help PGCs migration into the embryo towards the gonadal ridge and proliferate [13]. During migration these cells express other germ cell-related markers as Stella and c-kit and initiate colonization of the gonadal ridge. At this time, PGCs express the premeiotic markers Dahl and Vasa. The proteins SCP1, SCP2, and SCP3 are specific of meiotic germ cells, as well as the meiosis gene Dmc1. The post meiotic markers GDF9 and TEKT1 are specific for mature haploid gametes [11].

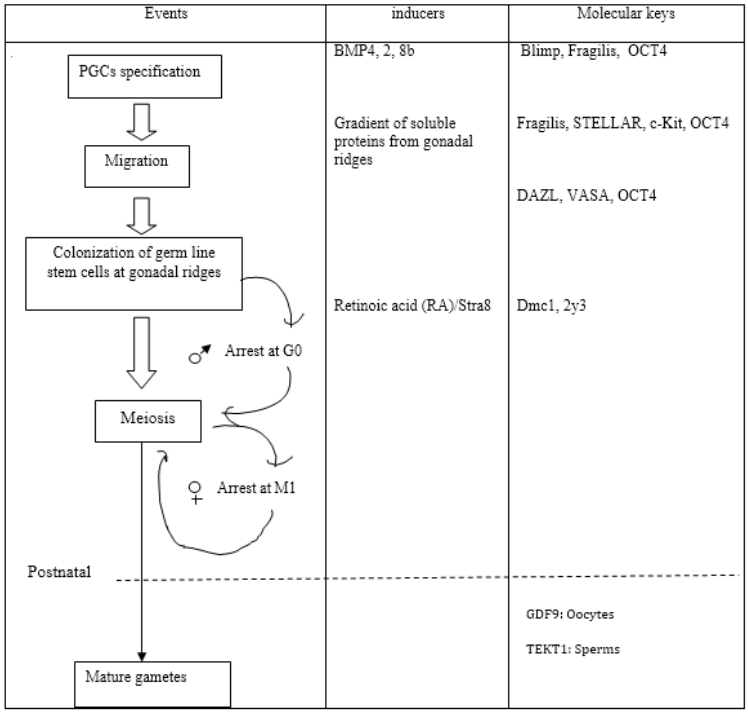

Fig 1: The germline development: Events and molecular cues.

4. Stem cells: past, present and future

Stem cells are unique cell population exist in multicellular organisms, responsible for embryogenesis and to maintain tissue homeostasis by specific cellular action self-renewal activity [14]. Another defined feature exhibited by stem cells is their ability to differentiate into at least one type of highly differentiated descendant. These cells can be derive, propagate and differentiate upon triggered by appropriate stimuli in provided *in vitro* condition [15]. Can therapeutic intervention of stem cells able to solve the problems of germinal cradles the "infertility? Embryonic stem cells (ESCs) which are derived from the inner cell mass of

blastocyst stage embryos [16, 17]. The *in vitro* cultured ESCs are ideal experimental model to understand vertebrate embryogenesis, neurogenesis, and identification of signaling pathways controlling differentiation [18]. Thus raises the possibility of 'designer' tissue and organ engineering by using stem cells.

Due to ethical controversies of using human embryo, ESCs derived from other vertebrate models like mice are taken as meticulous reference for human, as mice are genetically identical to human [19]. Nearly, all postnatal organs and tissues contain adult stem cells (ASCs) which are in quiescent stage; they divide upon activation of appropriate signals. The studies of past several years on plasticity of ASCs demonstrate that these are able to differentiate into other cell types at new locations, in addition to their usual progeny in their organ of residence [20]. Due to less ethical debate concerned around use of ASCs, these became pivotal tools to treat several diseases and disorders.

The most promising cell source of ASCs used for therapeutic translation are: hematopoietic stem cells (HSCs), MSCs (mesenchymal stem cells), tissue specific progenitor stem cells (TSPSCs), umbilical cord stem cells (UCSCs), bone marrow stem cells (BMSCs), and induced pluripotent stem cells (iPSCs) [21]. iPSCs are created by reprogramming of pluripotent genes in a differentiate cell, that are responsible for maintaining "stemness" of ESCs. Human iPSCs cells are initially generated from somatic cells by the ectopic expression of four transcription factor genes [octamer-binding transcription factor 4 (OCT4), SOX2, KLF4, and c-MYC] and are suppose be better alternatives of human ESCs for basic and applicative research [22]. Recent advancements in stem cell research open novel therapeutic approach to cure more complicated conditions of infertility by producing IVD gametes [23]. In addition IVD gametogenesis provides an accessible system for studying the specification, differentiation and development related processes such as meiosis, morphogenesis, and motility of sperm cells and growth of oocytes [24]. Recently, two main techniques adopted to produce gametes in *in vitro* condition such as germ cell differentiation from stem cells and somatic cell haploidization (nuclear transfer, cloning) [25].

5. Reproductive cells from stem cells

Spermatogenesis is a tightly regulated process that takes place in the seminiferous tubules within the testis. This process starts with spermatogonial stem cells (SSCs) that originate from PGCs and are responsible for continuous production of sperm from puberty onwards due to infinite self-renewal nature in the normospermic man. Similarly, oocyte "female gamete" generated from another source of multipotent stem cells the "ovarian stem cells (OSCs)" of female gonad. Differentiation of spermatocytes and oocytes in *in vitro* culture of stem cells occur upon trigger by appropriate inducers like BMP-4, retinoic acid (RA) and activin-A [26-28]. Successful results were achieved by exclusive studies conducted on murine ESCs to generate germ cells [29, 30]. In contrast, very few reports are available regarding differentiation of germ cells from hESCs [31-33].

PGCs and embryonic germ cells (EGCs) derived from post-natal stage of developing embryos share many of their properties with ES cells. ES cell lines are currently being used to study different stages of germ cell development. A number of culture systems have so far been developed to differentiate ES cells/iPSCs into the germ cell lineage in mice and humans [29-36]. The only group that reported about the birth of live offspring using ESC-derived gametes and analyzed the methylation status of several imprinted regions in the differentiated cells and observed that methylation patterns were not correctly profiled in pups [36]. It is noteworthy to mention that *in vitro*-derived germ cells and the progeny generated using the IVD gametes (spermtocytes) have abnormalities due to change in methylation pattern. The pups showed growth abnormalities (some of them were larger and others smaller), and most died during the first months of life. This further emphasized to study imprinting status of the derived gametes from stem cells (Fig. 2).

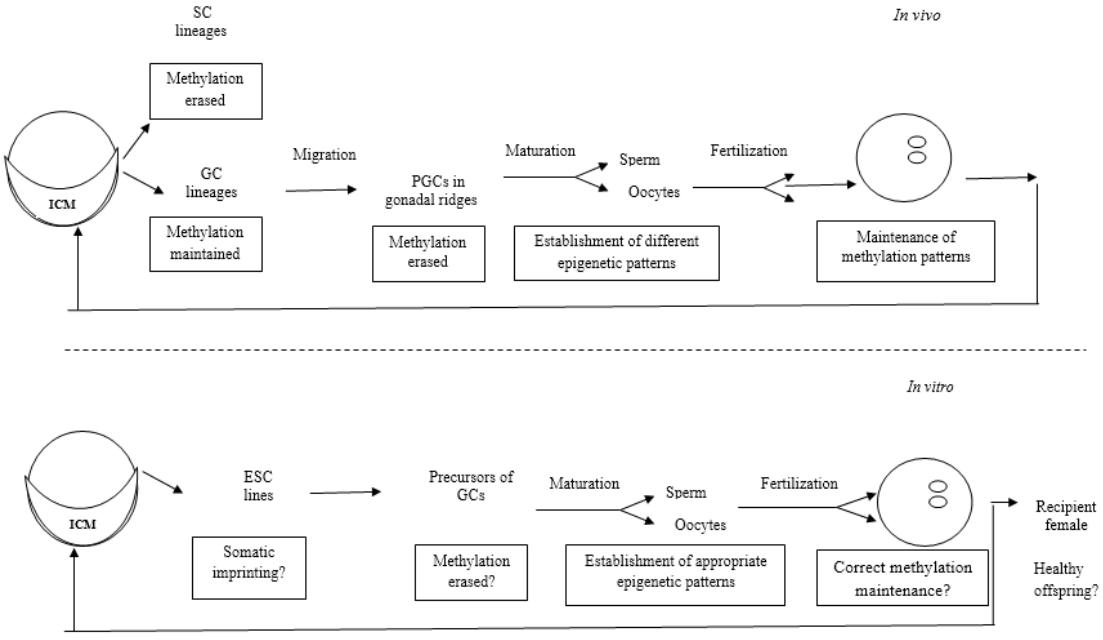

Fig 2: Genetic imprinting in the germinal lineage. After fertilization the parental epigenetic pattern is erased to recover totipotency. Then, the sequence of imprinting is modified during early embryo development; there is a demethylation process in the PGCs followed by a methylation recovery in more mature gametes depending on the sex. There is no evidence that the imprinting patterns are being properly established during production of in vitro gametes. This suggests that in vitro generated germ cells contain imprinting error

6. *In vitro* differentiation of germ cells from ESCs

Several studies follow different strategies to differentiate ESCs into PGCs and then to haploid germ cells. The reverse process also hold true that is adult SSCs, can dedifferentiate to ESCs [3]. Embryoid bodies (EBs) are three-dimensional structures formed by the aggregation of undifferentiated ES cells, in which different cell types from the three embryonic germ layers can be formed and also cells of the germ line lineage (Figure 3). Thus *in vitro* derived functional gametes may provide an accessible *in vitro* model system for studies of mammalian gametogenesis and a better option to treat infertility.

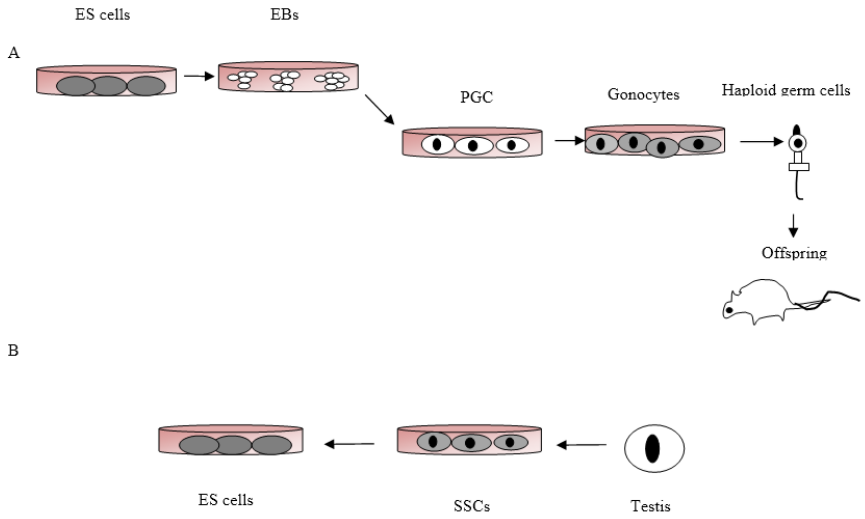

Fig 3: *In vitro* differentiation of ESC sinto gametes and back. (a) ESC scan differentiate into PGCs and haploid germ cells when these haploid cells injected into oocytes support embryo differentiation. (b) In the reverse process, testicular SSCs dedifferentiated into pluripotent ES-like stem cells *in vitro*. EBs: embryoid bodies.

Seminal studies propose the appearance of germ line stem cells (GSCs) spontaneously in ES cell cultures following the removal of pluripotency maintaining factors, such as feeder layer and leukaemia-inhibiting factor (LIF) from culture medium [11]. Other contemporary studies use exogenous supplements such as BMP4 and retinoic acid (RA) [34-36] that improves differentiation of GSCs and enhances their maintenance *in vitro*, but does not support their progress through meiosis. Human ESCs re-programmed into germline stem cells and then triggered to differentiate into sperm cells. The sperms derived *in vitro* were reported to be haploid and express some normal characteristics of mature human sperm including tail growth and motility [37]. *In vitro* derived spermatozoa must express male germ cell specific markers (Dazl, TSPY, Piwil2 and Stra8) [38]. Interestingly, Oct3/4, Sox2, Klf4, c-Myc, and Lin28 are all expressed by SSC-enriched germ cell population [39]. *In vitro* derived gametes must express the functional characteristics of *in vivo* produced gametes. However, the IVD sperm are far behind that truly replicates *in vivo* produced sperm of human. Exclusive studies report on gene expression profile of ICM, hES cells, PGCs and derived germ cells (Fig. 4).

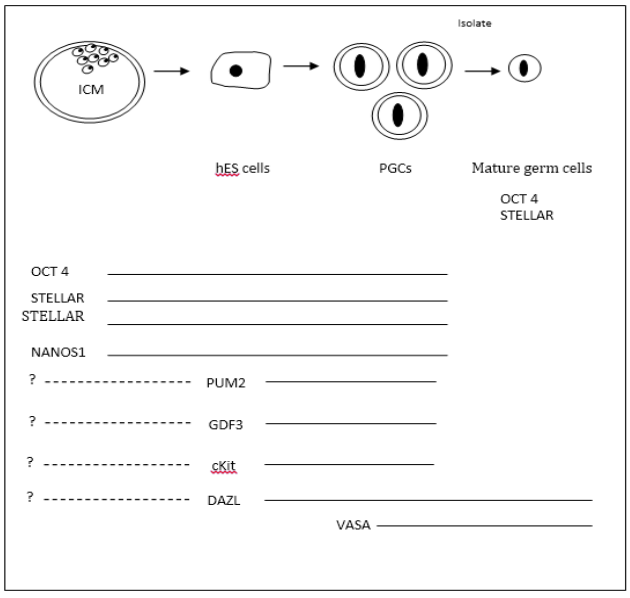

Fig 4: hESCs and PGCs express similar gene expression patterns: OCT4, NANOG, STELLAR and NANOS1 are expressed in ICM, hES cells. With the derivation of hES cells, these cells begin express DAZL, a gene not expressed normally by human ICM cells. DAZL continues to be expressed during germ cell formation to PGCs, and mature germ cells. Expression of VASA a germ

cell specific marker is restricted to PGCs and mature germ cells.

In vitro derived oocyte like cells should express oocyte specific markers, such as ZP3, growth differentiation factor 9 (GDF9) [40]. ZP3 is a glycoprotein of zona pellucida and GDF9, is a member of the transforming growth factor beta (TGF beta) superfamily. These factors secreted specifically by oocytes and involved in folliculogenesis. The ovary-like structures containing putative oocytes are differentiated from EBs by providing testicular cell culture conditioned medium [41]. These oocytes were surrounded by one to two layers of flattened cells but did not have a visible zona pellucida. The oocyte-specific markers Fig-alpha and ZP3 are also express by these ovarian structures. This experimental finding opens a novel perspective for infertile females those unable to produce functional oocytes. Appearance of putative oocytes was also evident in EBs co-cultured with ovarian granulose cells. The differentiated Oocyte-like cells also expressed the oocyte-specific genes Figalpha, GDF-9, and ZP1-3 but not any testis-specific genes [42]. This may be due to the fact that direct cell-to-cell contact between EBs and. granulosa cells act as inducing factor to differentiate into oocyte-like cells.

Cells within hESCs-derived EBs express markers for human germ cells including c-Kit, DAZL and VASA; as well as SSEA-1 and Tra-1-60 [31, 43-45]. The number of germ cells expressing VASA is increased when hESCs were cultured in presence of exogenous factors such as BMP-4, BMP-7 and BMP-8. However, did not support their progress into meiosis [44]. The study fail to characterize derive the cells enclosed within these follicular structures as oocytes [45]. However, published data suggest that the success rate of generation of gamete specific cells from ESC lines is very low and need experts and sophisticated instruments.

Recently, a 3D-co-culture system was introduced using calcium alginate encapsulation and testicular somatic cells to trigger differentiation efficiently into male germ like cells and haploid germ cells from hESCs-derived EBs [46]. But do *in vitro* derived gametes able to restore parental inheritance fully? Moreover, ethical issue surrounding uses of human embryos for derivation of ESCs draw attention of using adult stem cells to treat infertility issues. The *in vitro* generation of germ cells from somatic cells may provide a valuable model for identifying factors involved in germ cell formation and differentiation.

7. *In vitro* differentiation of germ cells from somatic stem cells

ASCs demonstrate that somatic stem cells have a more flexible potential than expected [47]. Exclusive studies on plasticity of adult stem cells suggest these are capable of differention across tissue specific lineage. ASCs are versatile effectors of therapeutic tissue regeneration and became pivotal elements for current clinical biology. Bone marrow (BM) is an efficient source of stem cells and reproducible system of *in vitro* culture for the expansion and differentiation and use critically in many therapeutic avenues. The trans-meiotic differentiation of germline from BM has potential clinical applications as it can be selected from autologous donor. An earlier study use adult BM stem cells for derivation of male germ cells by using Stra8-enhanced green fluoresence protein (EGFP) transgenic mouse line expressing EGFP specifically in male germ cells. It is evident from this experimental analysis that a small population stem cells reside within BM, able to trans-differentiate to male germ cell-like cells and also express known molecular markers of primordial germ cells, such as fragilis, stella, Rnf17, Mvh and Oct4; as well as molecular markers of spermatogonial stem cells and spermatogonia including Rbm, c-Kit, Tex18, Stra8, Piwil2, Dazl, Hsp90a, b1- and a6-integrin [48]. Another classic study also explains a small population of BM cells could able to trans- differentiate to male germ cell-like cells. These derived cells also express of early germ cell markers (Oct4, Fragilis, Stella and Vasa) and male germ cell specific markers (Dazl, TSPY, Piwil2 and Stra8) [49].

These preliminary observations provide strong evidence that human BM cells can differentiate to putative male germ cells and identified bone marrow as a potential source for *in vitro* sperm production. It is intriguing to notice that a clonal cell line at passage 75 derived from adult pancreatic stem cells (PSCs) from rat pancreas deviate from original properties and spontaneously differentiate to oocyte-like cells (OLCs). Thus derived OLCs also expressed meiosis-specific markers SCP3 and DMC1 [50]. Human amniotic epithelial cells (hAECs) when cultured in serum substitute supplements (SSS) medium, these triggered to differentiate into germ cells and oocyte specific cells. The gene and protein profile study of these cells revealed the expression the germ cell specific genes C-KIT, DAZL, VASA and ZP3 and at passage 5 large round cells, resembling oocytes, appeared. The cells express the germ cell specific marker DAZL, the oocyte specific markers GDF9 and ZP3 and the meiosis specific markers DMC1 and SCP3 at the protein level [51]. Cultured stem cells derived from human first-trimester umbilical cord (hFTUC) significantly differentiate to a subpopulation that had a morphological resemblance to primordial germ cells (PGCs) and cumulus-oocyte complex (COC)-like cells, and oocyte-like cells (OLCs). These cells functionally characterize by molecular markers specific for each differentiated cell types: PGC-like cells expressed specific markers indicative of germ cell formation such as (OCT4), stage-specific embryonic antigen 1 (SSEA1), B lymphocyte-induced maturation protein-1 (BLIMP1), PR domain containing 14 (PRDM14), transcription factor AP-2 gamma (TFAP2C), VASA, STELLA, deleted in azoospermia-like (DAZL) and interferon-induced trans-membrane protein 3 (IFITM3). The OLCs, which contained a single germinal vesicle, expressed oocyte-specific markers, such as synaptonemal complex protein 3 (SCP3), growth/differentiation factor-9 (GDF9), GDF9B and zona pellucida (ZP) 1, ZP2 and ZP3. The COC-like cells secreted estradiol, vascular endothelial growth factor and leukemia inhibitory factor. In humans, new oocytes can be derived from ovarian cortical mesenchymal cells [52]. However, it is clearly demonstrate that meiosis and neo-oogenesis are suspect to occur in GSCs of normal adult human ovaries. During early stages of female germ cell development, increased expression of Dmc1 and Scp3 and decreased expression of Oct3/4 and c-Kit are essential for meiotic entry *in vivo*. This observation rule-out the statement neo-oogenesis does not occur in adults [53]. This new theory indicates that somatic stem cell-derived oocytes may enter meiosis or support the development of offspring in cases of patients with allogenic BM transplant. Early meiotic-specific or oogenesis-associated mRNAs is SPO11 involved in the early steps of meiotic prophase [53]. PRDM9 is essential for progression through early meiotic prophase [54]. *In vitro* development of germ cells from various pluripotent and multipotent origin provides may provide a valuable model for identifying factors involved in germ cell formation, development and differentiation and better resources to understand etiology of infertility from grassroots level and

can be a promising agent for future fertility management (Table 2).

Recently, a novel population of very small embryonic-like stem cells (VSELs) has been identified in the adult mammalian gonads. These stem cells are more primitive to SSCs and are also implicated during postnatal ovarian neo-oogenesis and primordial follicle assembly. VSELs are pluripotent in nature and characterized by nuclear Oct-4A, cell surface SSEA-4, and other pluripotent markers like Nanog, Sox2, and TERT. VSELs are considered to be the progenitors of epiblast stem cells and possibly the primordial germ cells. These persist in adulthood and non-functional human gonads undergo asymmetric cell division to replenish the gonadal germ cells throughout life [57]. VSELs is not affected by oncotherapy in both mice and human gonads because of their quiescent nature whereas OSCs/SSCs and germ cells get destroyed since oncotherapy basically targets actively dividing cells [58]. These have definitive role during infertility, endometrial repair, superovulation, endometriosis, cancer and pathogenesis of various reproductive diseases like PCOS,

Well-characterized iPSCs may be a better alternative for obtaining sufficient numbers of autologous cells. hiPSCs could be successfully differentiated to post-meiotic cells without over-expression of germ line-related transcription factors [59]. Successful reprogramming of differentiated human somatic dermal fibroblast cells into a pluripotent state was carried out with four prime pluripotent factors: Oct3/4, Sox2, Klf4, and c-Myc [60]. Seminal studies have accounted differentiation of PGCs from iPSCs of mice and humans [61]. Yet another cohesive study reports efficient induction of PGC-like cells from mouse ESCs and iPSCs following a procedure that closely resembled with the developmental pathway of germ cells *in vivo*. In this procedure, ESCs and iPSCs were first induced into epiblast like cells (EpiLCs), which were subsequently differentiated to PGCLCs [62]. More recently a simplified strategy follows to differentiate male germ-like haploid cells from hiPSCs. The experiment was carried out culturing monolayers without bFGF for 3 weeks and down-regulation of pluripotent markers SSEA-4 and OCT4 at the end of this period. Under these circumstances, male germ-like haploid cells were obtained from hiPSCs [63]. Further, successful reproduction of viable cloned mice from inducible iPSCs through nuclear transfer approach was recorded [64]. The efficiency rate is similar to that of using ESCs derived via normal fertilization. Thus produced cloned mice are fertile and can produce second-generation offspring the implication of iPSCs cells to somatic cell nuclear transfer (SCNT) technique may pave new dimension to treat reproductive anomalies and disorders.

8. Conclusion

In vitro germ-cell-differentiation studies progress towards a stage where investigators are able to recapitulate germ-cell development *in vitro*. The focus of current and future studies should be develop new strategies that enable meiotic completion to avoid unwanted aneuploidies in *in vitro* fertilized embryos. Additionally determination and establishment of accurate epigenetic modifications and imprinting status of *in vitro* fertilized embryo provides reproductive hope and confine a normal progeny to humans lacking gametes. Further, there is a need to design protocol that assure appropriate quality and quantity of all reproductive tissues and *in vitro* produced gametes of human after cryopreservation. This will further facilitate to establish cryobanks of artificial gametes. This may be a relevant source to treat infertility and assist ART programs in future,

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Table 1: Assisted reproductive technology used to treat infertility.

Individuals	Condition	Name of ART	Procedure	Remarks	Success rate vs. age	Disadvantages
Male	Obstructive and non-obstructive azoospermia. Oligoasthenoteratozoospermia,	Intracytoplasmic sperm injection (ICSI), microsurgical epididymis sperm aspiration (MESA), testicular sperm extraction, (TESE)	Microinjection of a single spermatozoon into the ooplasm of a mature metaphase II oocyte.	First successful pregnancies were in the early 1990s.	35 % < 35 yr 29% 35 -37 yr 21% 38 – 39 yr 14% 40 – 42 yr 6% 43 – 44 yr 5% > 44 yr	Increase risk of chromosome aberrations in de novo sex and autosomal chromosome, increase imprinting disorders and costlier than IVF.
Female	Premature Ovarian Failure Treatment	*In vitro* fertilization and embryo transfer (IVF-ET)	Ovarian stimulation and allow the egg to develop. Efficient egg retrieval the eggs Fertilization of eggs in *in vitro* condition. Embryos are transfer to uterus for further development.	Donor egg fertilized in the laboratory, and implanted into the uterus of the woman who want to have a child or to other woman (surrogate mother).	32.2% < 35 27.7% 35–37 yr 20.8% 38–39 yr 13.6% 40–42 yr 5.0% 43–44 yr 1.9% ≥ 45	Birth defects, genetic disorders, multiple pregnancies.
	For all cause of infertility except women with infertility caused by tubal blockage or significant tubal damage, or an anatomic problem with the uterus, such as severe intrauterine adhesions.	Tubal embryo transfer (TET)		GIFT was developed in 1984 for women with unexplained infertility. Most accepted by certain religious and ethnic communities (in which fertilization inside the woman's body is the only type allowed). Involvement of most advance medical applications like ultra-sonographic guidance, and laparoscopy.	Live birth. 30%	IVF is less invasive than GIFT and ZIFT
		Gamete intrafallopian transfer (GIFT)	Eggs and sperm (gametes) are isolated and transferred directly into the fallopian Tubes by laparoscopy, where conception takes place.	The resultant cells transferred to fallopian tube rather than uterus. So improve pregnancy rate and increase embryo quality with less genetic defects.		
		Zygote intrafallopian transfer (ZIFT)	Fusion of IVF and GIFT in which embryos are transferred into fallopian tubes by Laparoscopy.			

Table 2: Strategies of germ cell differentiation from stem cells.

S.no.	Origin	Strategies of differentiation and	Differentiated cell types	Molecular characterization	Generation of offspring	References
Germ cell differentiation from embryonic stem cells (ES cells)						
1.	Mouse ESCs(mES cells)	Spontaneous differentiation in adherent culture.	Oocyte-like cells	Express (Gdf9, Sycp3, etc.), Production of estradiol.	No, Parthenogenic blastocyst (PB)	Húbner *et al*., 2003 [29]
2.	mES cells	Embryoid bodies (EBs) formation + growth factors produced from aggregated cells.	PGCs *in vitro*.	Express (Stella, Vasa, etc.), generate sperm *in vivo* after transplantation.	Not tested	Toyooka *et al*., 2003 [34]
3	mES cells	Addition of RA to EBs-derived cells.	PGCs, Spermatids	Express (Tex14, Acrosin, etc.), genomic imprint (H19, Igf2r), genome ploidy, Intracytoplasmic sperm injection (ICSI)).	No	Geijsen *et al*., 2004 [35]
4	mES cells	Spontaneous differentiation of EBs.	Oocyte-like cells (although TEKT1 Expression was observed).	Expression of (VASA, SYCP3, etc.)	Not tested	Clark *et al*., 2004 [31]
5	mES cells	EBs with conditioned media from testicular cell culture.	Immature oocyte-like cells.	Expression of (Figla, Zp3, etc.)	Not tested	Lacham-Kaplan *et al*., 2006 [41]
6	mES cells	Spontaneous differentiation in adherent culture and through EBs formation.	Ovarian follicles.	Estradiol production and Sycp3 expression.	Not tested	Novak *et al*., 2006 [55]
7	mES cells	Addition of Retinoic acid (RA) to spermatogonial stem cells derived EBs.	SSCs, sperm	Express (Stra8, Protamine1, etc.), genomic imprint (H19, Igf2r, etc.), genome ploidy, transplantation, ICSI	Yes	Nayernia *et al*., 2006a [36]
8	Human ESCs(h ES cells)	Spontaneous differentiation of EBs alongwith growth factor addition.	PGCs, spermatids	Express (VASA, ACROSIN, etc.), genomic imprint (H19, PEG1, etc.), Genome ploidy.	Not tested	Kee *et al*., 2009 [44]
9	h ES cells	Spontaneous differentiation in adherent culture and through EBs formation.	Oocyte-like cells	Express VASA	Not tested	Chen *et al*., 2007 [45]
Germ cell differentiation from somatic stem cells (SC cells)						
10.	Germ line stem cells present in ovarian surface epithelium (OSE) of human.	Culture medium with estrogenic stimulus.	Culture medium with estrogenic stimuli.	--	Not tested	Bukovsky *et al*., 2005 [52]
11.	SC cells in mouse bone marrow (BM).	RA added to cultured adherent cells from BM.	Spermatogonia-like cells	Express fragilis, stella, Rnf17, Mvh and Oct4 and spermatogonial cell markers Rbm, c-Kit, Tex18, Stra8, Piwil2, Dazl, Hsp90a, b1- and a6-integrin	Not tested	Nayernia *et al*., 2006b[48]
12.	SC cells in human BM.	Expression of early germ cell markers and male germ Cells specific markers.	Male germ cells	Express Oct4, Fragilis, Stella and Vasa) and male germ cell specific markers (Dazl, TSPY, Piwil2 and Stra8	Not tested	Drusenheimer *et al*., 2007[49]
13.	Human amniotic epithelial cells (hAECs)	hAECs were cultured in medium containing serum substitute supplement (SSS).	Germ cell specific lineages and Oocyte-like cells.	Expression of DAZL, GDF9 and ZP3 noticed.	Not tested	Evron *et al*., 2012 [51]
14.	hiPSCs	bFGF, ActA, BMP4 20% KSR, BMP4, LIF, ROCK inhibitor	Pre-migratory PGC like cells (PGCLCs)	OCT4, NANOG, SOX2, BLIMP1, STELLA, NANOS3, cKIT,	Not tested	Sugawa *et al*., 2015 [56]