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Fertilization failure and gamete health: Is there a link?

Snehil Budhwar[1], Vertika Singh[1], Priyanka Verma[1], Kiran Singh[1]

[1]Department of Molecular and Human Genetics, Banaras Hindu University, Varanasi, Uttar Pradesh, India

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1. ABSTRACT

Fertilization is a hallmark event of sexual reproduction marked by the fusion of male and female gamete to form zygote. It is a highly complex, yet a robust process that is intricately regulated by various signalling molecules. A healthy fertilization is determined by the quality of zygote which is contingent on the health of egg and sperm. The relationship between infertility and gametic health can be reciprocal. On one hand gametogenesis has to be dynamic and unremitting to sustain the reproductive health, while on the other hand it has to be error free for proper embryonic development. Complex cellular interactions make gametogenesis highly vulnerable to extrinsic as well as intrinsic intrusions. Molecular disparities during these phases may result in complete fertilization failure. Present review provides an overview of the regulation of gametogenesis, determinants of healthy gamete, players at fertilization window and what may go wrong during the development of zygote to embryo leading to implantation failure. We have outlined different 'windows' of vulnerability during gametogenesis supported by evidences affecting the fertility potential of both the partners.

2. OVERVIEW OF GAMETOGENESIS AND FERTILITY

The fusion of haploid spermatozoon and oocyte is the culminating event in mammalian fertilization. It enables the creation of a new, genetically distinct diploid organism. Sexual reproduction in mammals starts during gametogenesis, a process of formation of gamete from germ cells. Formation of male gamete spermatozoa and female gamete egg is known as spermatogenesis and oogenesis respectively. The formation of a genetically and functionally competent gamete is essential for normal fertilization and early embryonic development. In both the sexes, gametogenesis follows a series of mitotic and meiotic divisions to give rise to male and female gamete with haploid genome. In males, each spermatocyte undergoes meiosis and produces four haploid spermatids that differentiate into functional sperms. In contrast, primary oocyte in female produces four cells, out of which only one remains functional. Sperm and ova fertilize to form a zygote which develops into embryo and ultimately into a genetically and phenotypically distinct individual. The two key aspects of gametogenesis include (i) the expression of genes that transform the canonical mitotic cell division program into the specialized meiotic division pattern and (ii) a morphogenesis program that produces gametes (2). To decipher the probable causes of fertilization failure, it is imperative to understand the origin and formation of male and female gametes as well as molecular mechanism underlying fusion of gametes and further embryonic development. A multitude of reports and evidences have highlighted the critical determinants of healthy gametogenesis and embryonic development whose alteration may result in poor fertility outcomes. The need of the hour is to understand the mechanism of gamete development and fertilization followed by systematic investigation of the alterations which results in reproductive incompetence. This will certainly provide a roadmap to decipher the molecular targets and therapeutic interventions for treatment of infertility.

3. REGULATION OF OOGENESIS AND SPERMATOGENESIS

Oogenesis and spermatogenesis include multistep events of mitotic and meiotic divisions to finally produce ovum and sperm respectively, which differ characteristically in several aspects. Ovum contains all the indispensable components to initiate a life. The complex cytoplasm of egg is rich in enzymes, mRNAs, organelles etc. which regulates various metabolic events of fertilization and embryonic development, however sperm, contains a motile nucleus and is devoid of cytoplasm.

The mechanism of oogenesis varies drastically across different species. Some species such as sea urchins and frogs routinely produce thousands of eggs during their lifetime while some species like humans produces very few eggs in their lifetime. In humans, diploid cells in the ovary i.e. oogonium undergoes rapid cell division initially during embryonic stage till seventh month of gestation period to produce millions of primordial germ cells (PGCs) known as primary oocytes which are enclosed in a follicle inside ovary. Out of these million cells, only few primordial cells enter meiosis and remain halted in diplotene stage till puberty. In humans, first half of meiosis occurs in embryo which after resumption at puberty, gets completed in adult (3). During follicular growth, primary oocyte undergoes 500 folds increase in the volume along with an enormous increase in number of follicular granulosa cell, which surrounds the growing oocyte. One-third of known early miscarriage cases are due to chromosomal anomalies, which arise due to errors in the formation of eggs (4).

A significant progress has been made in the interpretation of factors regulating the development as well as maturation of gametes. Advancement in molecular biology and *in-vitro* culture techniques has enabled us to study the influence of these diverse factors on the process of folliculogenesis, oocyte growth and maturation. The regulation of events occurring before the birth of an individual has always been a matter of fascination and discussion. Errors occurring during gametogenesis, fertilization and early embryogenesis can have a great impact on fertility.

4. MOLECULAR PLAYERS OF OOGENESIS AND REPRODUCTIVE HEALTH

4.1. Regulation at early stages of oogenesis

Female fertility is determined by the ability of oocyte to undergo meiosis, successful fertilization and healthy embryonic development (5). Folliculogenesis starts during the second trimester of fetal development in humans. Human follicle development entails intra-ovarian and endocrine interactions that provide intrafollicular microenvironment for developing healthy oocyte. Lack of coordination between developing oocyte and the surrounding somatic cells results in poor developmental competence of the oocyte that may lead to infertility related issues (6,7). Apoptosis is a crucial strategy involved in follicular atresia. Many pro-apoptotic and anti-apoptotic proteins such as BAX, BAD and BCL2 regulate the process of germ cell death. A recent study showed that deficiency of Bax gene in mice resulted in more primordial follicles within their ovarian reserve that is very much needed for oocyte to undergo fertilization(7).

Majority of ovarian primordial follicles remain in quiescent phase as a reservoir of germ cells. A balance in dormancy and activation is controlled by coordinated actions of activators/suppressors

in close association with surrounding somatic cells and intra-oocyte interactions (8). Primordial follicles are under inhibitory control. They get release by this inhibition either by depletion of inhibitory factors or increase in stimulatory factors (9). Primordial follicles after activation get converted to primary, secondary and antral follicles. Depending on the stimulation of gonadotropins, these antral follicles may or may not reach till ovulation. Maximum antral follicles directly from the dormant stage or during folliculogenesis undergo atresia. Multiple growth factors and signalling pathways have been identified to regulate the activation of primordial follicles (10). A nerve growth factor (NGF) neurotropin and its tyrosine receptor kinase (NTRK1) are essential for activation of primordial follicles (11). The regulation of oocyte survival depends on other neurotropins like brain derived neurotrophic factor (BDNF) and neurotropin 4 (NT4) (12). Recently it has been shown that PTEN/PI3K signalling is also involved in primordial follicle activation. Absence of PTEN, a negative regulator of PI3K in oocytes result in increased phosphorylation of another components of the pathway like AKT and FOXO3a (Forkhead Box 03). The inactivation of AKT and FOXO3a leads to follicle activation (13). A recent study showed premature activation of primordial follicles in mouse ovaries, deficient in FOXO3a (14). Simultaneously, tuberous sclerosis complex I and mammalian target of rapamycin complex (mTORC1) are responsible for the arrest of primordial follicles. These two signalling pathways synergistically regulate the resting and activation of primordial follicles. Reports says that Forkhead boxL2 gene (*Foxl2*) is expressed in pre-granulosa cells but decreases gradually in granulosa cells of pre-antral follicles, focussing its role in quiescence of primordial follicles (15).

A positive effect of Anti Mullerian hormone (AMH), a member of TGFβ superfamily is reported in initiation of growth of primordial follicle. Null mutations in downstream intracellular signalling molecule of TGFβ ligand like SMAD lead to an arrest of primordial follicle to antral follicle transition (16). The conversion of primordial follicles to primary follicles depends on the early expression of oocyte originated transcription factors, Sohlh1 and Nobox. A deficiency of Nobox expression downregulates oocyte specific transcripts such as Mos, Oct4, Rfpl4, Fgf8, Dnmt1o, Gdf9, Bmp15, Zar1 and H100 showing an essential regulatory role of Nobox gene in follicular maturation(17).

4.2. Regulation of Oocyte maturation

Meiotic competence is the ability of oocyte to resume meiosis and get mature while developmental competence refers to the capacity of the oocyte to get fertilize and develop into a healthy embryo. It relates with cytoplasmic maturity of the developing oocyte. Cyclic adenosine monophosphate (cAMP) produced within the oocyte is crucial for tethering oocytes under meiotic arrest (18). The influx of cAMP within the oocyte occurs via gap junctions between cumulus cells and oocyte. cAMP is also endogenously produced by G-protein coupled receptor 3 and 12 activation (19, 20). The inflow of cyclic guanosine monophosphate (cGMP) via gap junction checks the activation of oocyte cAMP-phosphodiesterase enzyme that degrades intracellular cAMP and by-passes meiotic resumption (21). Recent studies showed a close association between developing oocyte and surrounding cumulus cells. The interactions mediated by gap junction communication and oocyte secreted paracrine factors are crucial determining factors in regulation of follicle growth and differentiation. Gap junctions allow the passage of different types of molecules (amino acids, pyruvate) from cumulus cells to the oocyte (22). Oocyte promotes the expression of NPR2 (natriuretic peptide receptor 2) on the cumulus cells. On activation, NPR2 stimulates the production of cGMP which inhibits PDE3A activity within the oocyte. Recently, *in vitro* experiments clearly revealed the role of GDF9, BMP15, FGF8 and estradiol in the regulation of NPR2 expression and activity (23). Thus oocyte meiotic maturation involves a cascade of events that initiates with LH surge and ends in the extrusion of first polar body. LH surge is followed by expansion of cumulus cells which initiates with the accumulation of hyaluronic acid within the cumulus cells in response to gonadotropins. LH surge further induces epidermal growth factor (EGF) like peptides that act through protein kinase A (PKA) and culminates in the elevated expression of transcripts like Has2, Ptx3 (Pentraxin3) and Tnfaip6 (tumor necrosis factor-induced protein-6) in the cumulus cells necessary for cumulus expansion, an event necessary for ovulation (24, 25). The release of developmentally competent oocytes from ovarian follicle at appropriate timing is under tight regulation. An ovulatory stimulus sensed by the somatic cells of follicle guides the resumption of meiosis, release of oocyte from the ovary as well as structural reorganization of the follicles (26). Successful ovulation is controlled by various endocrine, paracrine, immune and metabolic signals from the surrounding follicles and oocytes itself (27) as is shown in Figure 1.

5. HORMONAL PLAYERS OF OOGENESIS

5.1. Autocrine regulators

Many of the studies from past two decades have demonstrated that maturation of the cumulus-oocyte complexes (COC) is regulated locally by gonadotropins. However additionally, an autocrine regulatory system and paracrine mode of cross-talk between cumulus cells and oocyte play an indispensable role in sustaining the fertility potential of a female (28). The maturation of the COC is triggered by an autocrine secretion of epidermal growth factor

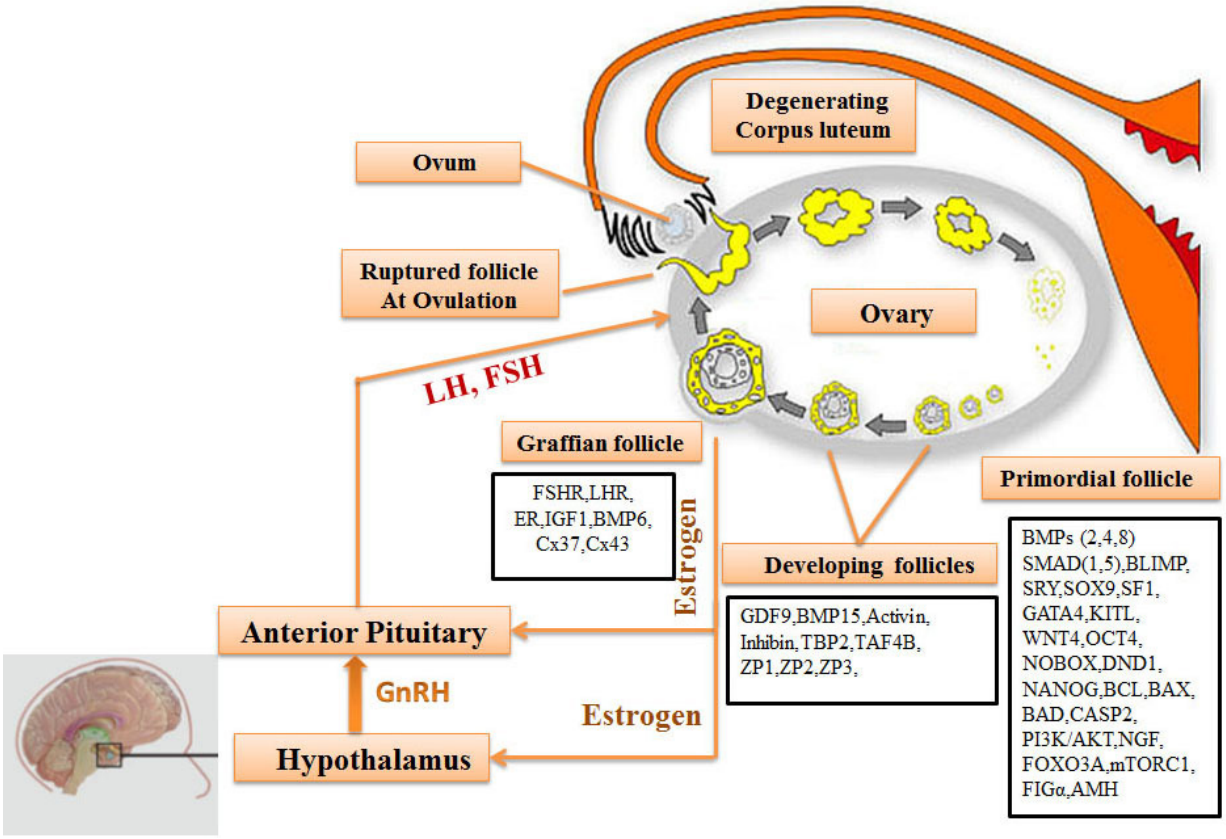

Figure 1. Genetic dissection of female fertility pathways in human. Every woman has a pool of resting oocytes in the form of primordial follicle. Various transcriptional regulators are involved in the recruitment and maintenance of follicles. Once these follicles are depleted, a woman cannot conceive naturally. Further development of follicles is gonadotropin dependent followed by ovulation.

(EGF)-like factors in the cumulus cells. These EGF-like factors stimulate their own synthesis, cleavage and release. Cleavage of EGF-like factors is sustained by members of disintegrin and metalloproteinase (ADAM) family thus, allowing the activation the EGF receptor (EGFR) in the cumulus cells (29). EGFR signalling stimulates oocyte maturation and synthesis of several proteins required for cumulus expansion such as, prostaglandin-endoperoxide synthase 2 (PTGS2), hyaluronan synthase 2 (HAS2), pentraxin 3 (PTX3) and tumor necrosis factor-stimulated gene 6 protein (TSG6) (30). The disruption of EGFR signalling has been shown to cause disruption of meiotic resumption in mice, thus highlighting its significance in oocyte nuclear maturation (31).

5.2. Paracrine regulators

Earlier studies reported that early preantral follicles are independent of hormonal regulation, although follicle stimulating hormone receptors are seen on granulosa cells, both in mouse and humans (32). Later on, data reported the stringent regulation of preantral follicles by FSH and other local intraovarian factors. Local intraovarian paracrine factors released from oocytes, theca cells and granulosa cells like Granulosa cell derived natriuretic factor drives the growth of preantral and antral follicles (33). TGFβ superfamily are pleiotropic cytokines and versatile regulators of numerous biological functions in metazoans. They play crucial role during cellular growth and tissue morphogenesis. Prospective functions of TGFβ in reproduction involve regulation of secondary sexual development, spermatogenesis, ovarian function, immunological regulation of pregnancy, embryonic implantation and placental development (34). Cell and tissue specific conditional knock-out studies have provided platform for understanding the *in vivo* functions of TGFβ superfamily signalling in reproduction and fertility. The TGFβ superfamily consists of more than 40 proteins discovered till date which share 30–80% sequence homology.

The most characterized modules of the TGFβ signalling pathway includes ligands, receptors, and SMAD transducers (SMA and MAD (mother against decapentaplegic-related proteins). Ligands of TGFβ includes activins/inhibins (activins A, AB, B, inhibins A, B), bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), anti-Müllerian hormone (AMH) also known as Mullerian-inhibiting substance, and nodal growth differentiation factor (35,36). TGFβ

ligands bind to their receptors and interact with SMADs to activate gene transcription. TGFB1R (TGFβ receptor 1) is a major Type 1 receptor of TGFβ ligands. The role of TGFβ signalling has been extensively studied in oogenesis and female reproduction. From the very early stages of oogenesis, two well-known members of TGFβ superfamily, GDF9 and BMP15 act as a positive regulator in the transition of preantral to antral stage. These two molecules act in a hormone independent manner and regulate granulosa cell proliferation. A clear participation of androgens and oocyte secreted factors has also been indicated in various studies. Another two members of TGFβ superfamily, activin and inhibin have an opposing effect in follicle development. Though activin is expressed in majority of the tissues, circulatory Inhibin is chiefly secreted from ovary and testis (37). Inhibin A and Inhibin B are produced during various stages of follicular development (38). Both inhibin A and inhibin B acts on pituitary to suppress FSH secretion, without causing any affect to the LH secretion. The principle site of inhibin synthesis in female is granulosa cells (39). In general, activin (Activin-A and Activin-B) are known to increase the follicle diameter as well as differentiation of granulosa cells however it seems to stimulate the follicular growth in the immature ovary and suppress the growth of surrounding follicles in adults (40). A large number of seminal studies have highlighted the potential of inhibin and activin in modulating the potential of the oocyte to undergo meiosis (41). Inhibin-A and Inhibin-B checks the active cell proliferation and ovarian tumor development (42, 43, 44). Another FSH suppressing protein, isolated from the ovarian follicular fluid is follistatin. This protein however, shows no homology with the activin/inhibin family (45,46). The actions of follistatin are reported to promote luteinization (47,48).

A group of investigators generated a conditional knockout (cKO) of TGFβ receptor 1 (Tgfbr1) in the female reproductive tract and found that Tgfbr1 cKO females show impaired embryonic development due to the formation of an oviductal diverticulum (36). Leiomyoma, commonly known as uterine fibroid, is a leading cause of fertility disorders and mortality in women (49). Among the three TGFβ isoforms (TGFBs1–3), TGFB3 has been shown to play a crucial role in development of leiomyoma by promoting cell growth and fibrogenesis (50). Deregulated TGFβ signalling is associated with Intrauterine growth restriction (IUGR), a complication of fetal growth in pregnancy. Serum levels of TGF β1 in the IUGR fetus are found to be lower than controls (51).

5.3. Endocrine regulators

The progression of folliculogenesis and ovulation is dependent on pituitary gland secreted gonadotropins, Follicle stimulating hormone (FSH) and Luteinizing hormone (LH). Principal actions of these hormones result in follicular maturation, steroidogenesis, granulosa cell luteinization and follicular rupture. These hormones act on the ovary to complete the maturation of follicles necessary before ovulation. Before a follicle ruptures and ovulates, it undergoes biochemical alterations like (i) increase in size, (ii) increase in LH/hCG receptors (iii) accumulation of cAMP (iv)increase in responsiveness from LH to FSH. Action of FSH and LH is mediated by binding with their respective receptors.

Knockout mouse models of FSH-R and LH-R showed the relevance of gonadotropin mediated signalling. Deficiency of FSH leads to infertility due to disruption of folliculogenesis and initiation of apoptosis (52, 53). Under the influence of gonadotropins, theca cells undergo active steroidogenesis producing androgens and estrogens. Acting through androgen receptor and estrogen receptor, steroids lead to granulosa cell proliferation and survival respectively (54). According to two-cell two- gonadotropin model, LH stimulation promotes theca cells to produce androgens and granulosa cells produce estrogen under the influence of FSH. Under the action of aromatase enzyme, estradiol is the predominant estrogen produced by pre ovulatory granulosa cells. It enhances the response of granulosa cells to the released gonadotropins. Members of Insulin growth factor (IGF) family (IGF1, IGF2) along with gonadotropins determine follicle selection and progression through different antral stages.

6. REGULATION OF SPERMATOGENESIS

Spermatogenesis is a process of male germ cell development which continues throughout the life of a male and underlies an orchestrated interaction between various metabolic pathways and factors. It starts with proliferation of spermatogonial stem cell and through a series of sequential divisions and differentiation gives rise to a mature sperm which fuses with egg to form a new individual. Thus, on one hand it has to be dynamic for regular production of gamete but has to ensure that it carries a healthy material for successful fertilization.

The anatomical compartmentalization of testicular tissue into the seminiferous tubule and the interstitium is achieved by peri tubular myoid cells which surrounds the seminiferous tubules. These compartments perform two essential functions of testis that is, the hormone production and germ cell development respectively. Though anatomically separated, both the compartments of testis are functionally connected.

Spermatogenesis starts with multiple rounds of mitotic spermatogonial stem cell (SSCs) division followed my meiotic division to produce primary spermatocyte. Primary spermatocyte divides to form

secondary spermatocyte which divides again to form round spermatids. The round spermatid then undergoes an extraordinary series of differentiating events that gives rise to a morphologically distinct mature sperm. This is during this phase when the sperm begins to form acrosomal and axonemal structures for successful fertilization and effective motility respectively. Further, it involves nuclear compaction and chromatin remodeling of the sperm head to form a microtubular structure known as manchette (55).

Sertoli cells also referred to as 'nurse cells' constitute the primary structural unit of seminiferous tubule. It nurtures the developing germ cells in the form of nutritional support by forming intimate cytoplasmic associations during various stages of germ cell development. Each Sertoli cell supports around 30–50 germ cells (56). Residing at the basement membrane these cells occupy around 17–20% of the volume of seminiferous epithelium of an adult male (57).

In males, Leydig cell is the chief cell present in interstitium responsible for testosterone production. Interstitial space also harbours some other cells such as mast cells, fibroblasts and macrophages. Under the stimulation of LH secreted by anterior pituitary and FSH secreted by Sertoli cells, Leydig cells produces testosterone that diffuses through the interstitium to drive the spematogenic wave.

From the past one decade, discoveries on the critical regulation of spermatogenesis have evidenced the significance of several molecular pathways that are crucially involved in testicular homeostasis. Most of these studies come from the experiments performed on genetically modified mice. The dynamic process of cell proliferation and differentiation is regulated by various endocrine, autocrine and paracrine factors and signalling molecules that commits a germ cell to either "differentiate or die" (58).

6.1. Endocrine performers at spermatogenesis

The endocrine regulation of spermatogenesis is largely defined and directed by hypothalamic-pituitary-gonadal (HPG) axis. The fundamental player of HPG axis include, the gonadotropin releasing hormone (GnRH), secreted by hypothalamus. A pulsatile release of GnRH regulate the secretion of FSH and LH. Abnormally low GnRH secretion in males may result in hypogonadotropic hypogonadism (HH) via decreased FSH and LH secretion (59). Inadequate functions of FSH and LH receptors and other signalling pathways of hormone synthesis due to genetic alterations may also result in abnormal spermatogenesis and male infertility.

Studies from past have highlighted a critical participation of testosterone in the maintenance of spermatogenic functions. FSH and testosterone are considered as master regulators of spermatogenesis. They perform their actions at multiple sites during spermatogenesis either alone or in concert. Testosterone performs its biological actions on spermatogenesis via androgen receptors (ARs) located on Sertoli cells (60). High testicular testosterone level and adequate expression of ARs on Sertoli cell is indispensable for male gonadal development (61). Various gene association studies on human have revealed that the loss of function mutation in FSHβ genes results in absence of sperm (azoospermia), and delayed virilisation in men. Recently, a peptide hormone known as INSL3 has come into picture. INSL3 is secreted by Leydig cells and act as a downstream effector of HPG axis. It safeguards the action of LH and FSH for proper reproductive functions (62).

Sertoli cells utilize unusual features of cellular metabolism by preferentially metabolizing glucose to lactate. However, the reason behind preferential transportation of lactate to germ cells is not well understood (63). Germ cells utilize lactate as main energy substrate. Lactate has been reported to provide anti-apoptotic effect to the germ cells. Sertoli cells produce lactate under the influence of FSH, insulin and IGF-1 (64, 65, 66).

6.2. Autocrine and paracrine regulation

A wide variety of cytokines and growth factors are involved in regulating the process of stem cell renewal during spermatogenesis. Interleukin-1 (IL-1) has been reported to play significant biological functions during spermatogenesis particularly during spermiogenesis (67,68). IL-1 expression is shown in various testicular cells such as Sertoli cells, Leydig cells, germ cells and macrophages. Human sperm produces bioactive IL-1 (69, 70, 71). IL-1 has been shown to effect Leydig cell proliferation and induction of acute inflammation in testicular circulation (72, 73, 74). It also plays a crucial role in germ cell development by regulating glucose metabolism pathway (75). Some other factors which regulate spermatogenesis include Leukemia inhibiting factor (LIF), which is involved in primordial germ cell proliferation and cell survival (76,77). Similarly, stem cell factor (SCF) and its receptor c-kit are shown to regulate the migration and proliferation of primordial spermatogonial cell population.

The potential role of TGFβ superfamily in spermatogenesis has been extensively studied. TGFβ signalling acts a mediator of cell-cell interaction during development of seminiferous epithelium at the time of puberty. All the three isoforms of TGFβ ligand, TGFB1–B3 are known to *in-vitro* regulate the gonocyte, pubertal spermatogonia, and spermatocyte cell numbers in rodents via apoptosis(78,79). TGFβ3

is the major isoform expressed in mature testicular tissues of rat and the targets of TGFβ signalling are shown to be present on Leydig cells and gonocytes (80,81). Recently a group of researchers developed a conditional knockout of TGFβ receptor type II in germ cells of mouse. Most of the knock-out animals died during fetal life, the surviving adults however showed a loss of spermatogonial stem/progenitor cells and sterility (82).

7. GAMETOGENESIS AND ENVIRONMENTAL INTERVENTIONS

The ability of humans to actively manipulate the environmental resources is setting enormous threats on reproductive fitness and survival of individuals and the species in long run. The effect of environment on gametogenesis and embryonic health has been widely studied. Environmental exposures are even shown to cause transgenerational long term effects. Numerous natural and synthetic compounds are reported to effect endocrine organs and impair human health. During the last decade, the plethora of synthetic chemicals developed has upraised significant trepidations with respect to their hostile effects on health (83).

Endocrine-disrupting compounds (EDCs) are synthetic or natural compounds that interfere with hormone-regulated cell signalling pathways and effects gene expression (84). These compounds interfere with endogenous endocrine actions (85). EDCs may act via nuclear receptors, non-nuclear steroid hormone receptors, non-steroid receptors, orphan receptors, enzymatic pathways of steroid biosynthesis and various other mechanisms which regulate the endocrine and reproductive functions. Most commonly found EDCs include diethylstilbestrol (DES), a synthetic estrogen as well as industrial or agricultural substances such as plasticizers or insecticides, industrial solvents/lubricants and their derivatives such as polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), plasticizers (phthalates), plastics (bisphenol A (BPA)), pesticides (methoxychlor, dichlorodiphenyltrichloroethane (DDT) and fungicides (vinclozolin) (86, 87, 88, 89, 90). Natural chemicals present in edible food products such as genistein and coumestrol also act as endocrine disruptors (83). EDCs affect the hormonal pathways through various mechanisms. Pubertal timing can be influenced by either prenatal or postnatal exposure to EDCs. EDCs exposure can disrupt early stages of the CNS development and sexual differentiation. Recent evidences have suggested that exposure to EDCs can adversely affect the future progeny of the exposed individuals (91, 92, 93). Kisspeptins are neuropeptides encoded by the KISS1 gene and are broadly documented as the fundamental activators of the HPG axis at the inception of puberty (94). In rats, the neonatal exposure to EDCs such as BPA and genestein has been shown to obstruct the kisspeptin synthesis (95, 96).

The reports on the effect of EDs on female reproductive development are few. Some reports suggest that a high serum concentration of BPA is associated with increased risk of infertility in women (97,98). Elevated levels of serum DDT during pregnancy may also result in embryonic lethality (99). Additionally, occupational exposure to pesticides and plastics may also contribute as a risk factor for female infertility (100). The clinical relevance of EDs are implicated in the development of multitude of pathogenic infertile phenotypes in women such as endometriosis, various uterine disorders and ovarian dysfunctions, such as polycystic ovary syndrome (PCOS) and premature ovarian failure (POF) (101). Furthermore, *in utero* exposure to DES in women raises an 80% higher risk of endometriosis development than unexposed women (102). ED may impede folliculogenesis, resulting in meiotic aberrations such as aneuploidies and multiple oocyte follicles or follicular atresia. ED are also involved in depletion of follicular reserves, resulting in POF (103). This syndrome occurs before the age of 40 years and affects around 1% of women (104).

A number of other lifestyle factors such as smoking, mobile phone usage, nutritional deficiencies may affect vital reproductive functions. A few studies have reported a decline in percentage of sperm cells and sperm motility in correlation with the frequency of mobile phones usage (105).

Consumption of a diet rich in carbohydrates, fiber, folate, and lycopene is correlated with enhanced semen quality. However, low consumption of proteins and fats are more beneficial for fertility. Furthermore, vitamin C has significant effects on semen quality (106). Obesity can also have significant effect on male and female infertility. Obese women present higher rate of recurrent and early miscarriage as compared to non-obese women (107). The association of obesity with erectile dysfunction is well documented (108). Psychological stress may also impair reproductive functions. Stress and depression are believed to decrease testosterone and LH pulses resulting in disruption of gonadal functions, which ultimately results in decline in normal sperm parameters (109, 110). Women who obtain support and counselling for increased anxiety and depression levels have increased chances of becoming pregnant (111). Cigarette smoking in men results in decrease in semen volume (112), decline in total sperm count, abnormal morphology, motility and poor fertilizing capacity (113, 114, 115). Chemicals present in cigarette smoke may impair the mechanism of oocyte pick-up and the transport of fertilized embryos which results in an increased incidence of ectopic

pregnancies, longer conception time and infertility in women (116). Soares *et al.* demonstrated that women with a smoking frequency of 0–10 cigarettes per day display a significantly higher pregnancy rate (52.2.%) than women who smoked 10 or more cigarettes per day (34.1.%), suggesting that cigarette smoking results in compromised uterine environment and lower pregnancy rate (117).

8. DETERMINANTS OF HEALTHY GAMETE: GOOD GUY OR BAD GUY

A drastic increase in female infertility rate has been observed from past decade. Fertility requires both sperm and egg to be potentially fit. Let's first discuss about male gamete. Sperm is a DNA filled bag with a tail behind. A sperm is considered healthy if it contains normal DNA and is able to efficiently transfer its content to the egg. Once the sperm delivers the DNA, the rest of the job is taken by the egg to accomplish. It provides the environment for perfect combination of the two DNA and further replication and division in an equal fashion.

From the last one decade, massive studies across the globe have highlighted the biological competence of sperm in fertilization and embryonic development. Sperm morphology, progressive motility and concentration are some of the potential and classically known determinants of sperm quality (118). However, the recent evidences report that increase in DNA fragmentation particularly during the clinical settings of assisted reproductive technology has attracted enormous attention. This established DNA fragmentation index (DFI) as an autonomous predictor of fertility. It has been estimated that a DFI above a threshold of 30 % results in complete pregnancy failure, either by natural conception or by an aid of assisted reproductive technologies (ART). DFI thus plays as a predictive indicator for male fertility *in vivo* (119). A sperm carrying damaged DNA is associated with high frequency of miscarriage and pregnancy loss (120, 121, 122). A damaged DNA may get incorporated into the embryonic genome that results in severe pathological consequences. It can also potentially be inherited through the germ line in several generations (120). Thus it becomes particularly essential to screen the sperm quality before proceeding for assisted reproductive technologies.

Women are born with a particular set of eggs whose numbers start depleting as they age. Each ovary depletes their egg at its own rate. The career of egg starts from puberty and continues till menopause. With each menstrual cycle, the conception rate drops. Poor egg quality is one of the main reason of infertility in women. It is not only the number of eggs that matter but also the quality of eggs produced that effects fertility. Egg quality refers to ability of the egg to get fertilize and develop into embryo. The quality of egg determines the embryo implantation rate. The quality of egg declines with the age of women and poses a threat to fertility. There are certain factors that are responsible for the assessment of poor egg quality. They are: i) Diminished ovarian reserve ii) Advanced maternal age. Ovarian reserve is the ability of a woman's ovaries to produce egg and giving birth to a baby (123). There are certain factors affecting ovarian reserve and age is one of them. The rate of infertility is universal and increases with age though the time is variable and may also occur in younger women. Two woman of the same age may have different probability of getting pregnant. In 20s or early 30s, a woman carries more good quality eggs but as she advances towards late 30s and 40s, quality and quantity both declines. Poor quality eggs are a major contributor towards infertility.

Ovarian reserve can also be affected at younger age. Genetic makeup of a woman and environmental factors like stress, smoking, alcohol consumption, cancer treatment or endometriosis may contribute to a decline in ovarian reserve of a woman. Ovarian reserve needs to be evaluated even in younger woman. There are few evaluation tests that are performed for assessing ovarian reserve of a woman who are suspected of being infertile and wish to go for Assisted Reproductive Technology like *in-vitro* fertilization. They include:

8.1. Day 3 FSH test

The evaluation begins with the test to measure the levels of hormone like FSH, LH and estradiol. They are generally measured at day 2, 3 and 4 of menstrual cycle. FSH level is most crucial among all. Brain establishes its connection with ovaries via FSH. When brain signals ovaries to mature and release an egg, the level of FSH rises, and once ovulation occurs, a signal is sent back to brain to stop the secretion of FSH, thus a feedback loop exists between brain and ovary. In case the communication between brain and ovary is disturbed, the level of FSH rises. Woman with abnormal FSH level at day 3 of the cycle are known to have poor ovarian reserve. They often face difficulty in conceiving and may lead to miscarriage if conception occurs.

8.2. Anti Mullerian hormone test (AMH)

A recent way to detect ovarian reserve is to check the level of AMH, a hormone secreted by cells residing within the developing follicle. The level of AMH in blood is a good indicator of ovarian reserve in a woman. AMH test tells us about fertile years of a woman and not about the quality of eggs she produces. The level of AMH does not vary with the cycle so the test can be done at any day of the month.

8.3. Clomiphene citrate challenge test (CCCT)

Ovulation inducing agent, clomiphene citrate (Clomid, serophene) is provided to woman undergoing CCCT for five days. FSH and estradiol levels are analysed both before and after treating with Clomid. Abnormal FSH level shows poor chances of conception and successful pregnancy (124).

8.4. Basal antral follicle count

A transvaginal ultrasound study to measure woman's ovarian reserve or remaining egg supply decides fertility potential. Antral follicle count predicts the number of mature follicles in the ovary. Along with woman's age and day 3 hormone levels, the basal antral follicle count is tested for women who are at risk of infertility and are planning to go for *in-vitro* fertilization.

8.5. Lifestyle and gamete quality

A poor diet and lifestyle can severely affect the fertility potential of an individual. Ovaries need a good blood supply of oxygen, nutrients and hormones to perform its function in an uncompromised manner. Blood supply is related to both quantity and quality of eggs within the ovary. As we know that potent oxidant increase with age and accumulates more with intake of highly cooked foods or with higher sugar consumption. In males, an imbalance of reactive oxygen species causes sperm DNA damage. In females, oxidation product accumulation correlates well with the decreased viability of granulosa cells and thus leading to poor egg and embryo quality. Studies reported that antioxidants act to reduce reactive oxygen species including superoxide anions, hydroxyl radicals and hydrogen peroxide (125). A better diet with more fruits, green vegetables, green tea rich in antioxidants are recommended for better egg quality. Dietary modifications have also been shown to improve ovulatory infertility disorder. Commonly reported supplements and dietary intake include vitamin C, D, E and folate. Studies show an increased rate of infertility in women, having Vitamin D deficiency. Women with higher Vitamin D level in their follicular fluid and serum showed good pregnancy result following IVF (126). The incidence of infertile men is also quite high having Vitamin D deficiency (127). Similarly Vitamin E deficiency is also related with negative reproductive outcomes in males and females affecting sperm motility in men and egg quality in women (128).

Folate is another important component that is needed for the synthesis of DNA, transfer RNA, methionine and cysteine required for rapid cell growth. DNA synthesis plays a crucial role in germ cell development; therefore folate is an obvious component to play its role in reproduction. Limited knowledge is available about its functional outcome in infertile couples. Studies from our lab have already established an association of folate and other derivatives with adverse pregnancy outcome like early miscarriage in North Indian population (129, 130). Folate supplementation during pre-conception period may improve various reproductive outcomes (131). Studies by Steegers-Theunissen *et al.*, Brouns *et al.*, Szymanski and Kazdepka-Zieminska, showed that women who received folic acid supplementation had better quality of eggs as well as good ovarian reserve (132, 133, 134). Folate has diverse role to play in different stages of female reproduction like oocyte maturation, implantation, placenta formation and embryo development. Because of its essential role in DNA synthesis and repair, folic acid present at preimplantation stage is essential for proper embryo development in mouse (135). Nutritional status of mother defines future fetal growth and development. It does not tell us that pregnant woman should 'eat for two', as various animal studies show that both maternal undernutrition and over-nutrition reduce placental-fetal blood flow and stunt fetal growth. Fetal growth is most vulnerable to maternal dietary deficiencies of nutrients (e.g., protein and micronutrients) during the peri-implantation period and the period of rapid placental development. Folate supplementation during pre-conception period may improve various reproductive outcomes.

Another important factor is obesity, especially in woman, as it interferes with the process of ovulation. Obese women are more prone to face poor pregnancy outcomes like stillbirth and miscarriage even when they are ovulating. Obesity in males is associated with erectile dysfunction and reduced libido (136). Obesity also results in deposition of fat in testes (137). Smoking is other environmental factor adversely effecting quality of the gamete. In females, natural fertility is greatly affected and rates of successful pregnancy with IVF decreased by 50%. Second hand smoking in infants leads to asthma, bronchitis, pneumonia and infant death syndrome. Active and passive smoking are harmful for both mother and infant. Several studies have highlighted the adverse effects of smoking on sperm quality. A recent study reported that smokers have significantly lower semen quality than non-smokers (138).

Alcohol and caffeine consumption in woman are known to have synergistic effect on infertility. Significant intake of caffeine reduces the chances of successful pregnancy with IVF. Similarly, in men, alcohol intake may lead to decreased libido and sexual potency (139). Alcohol abuse can also result in altered testosterone production and can result in testicular shrinkage (140). Finally, any discussion on lifestyle and fertility is incomplete without talking of stress, anxiety and depression. The effect of environment and lifestyle

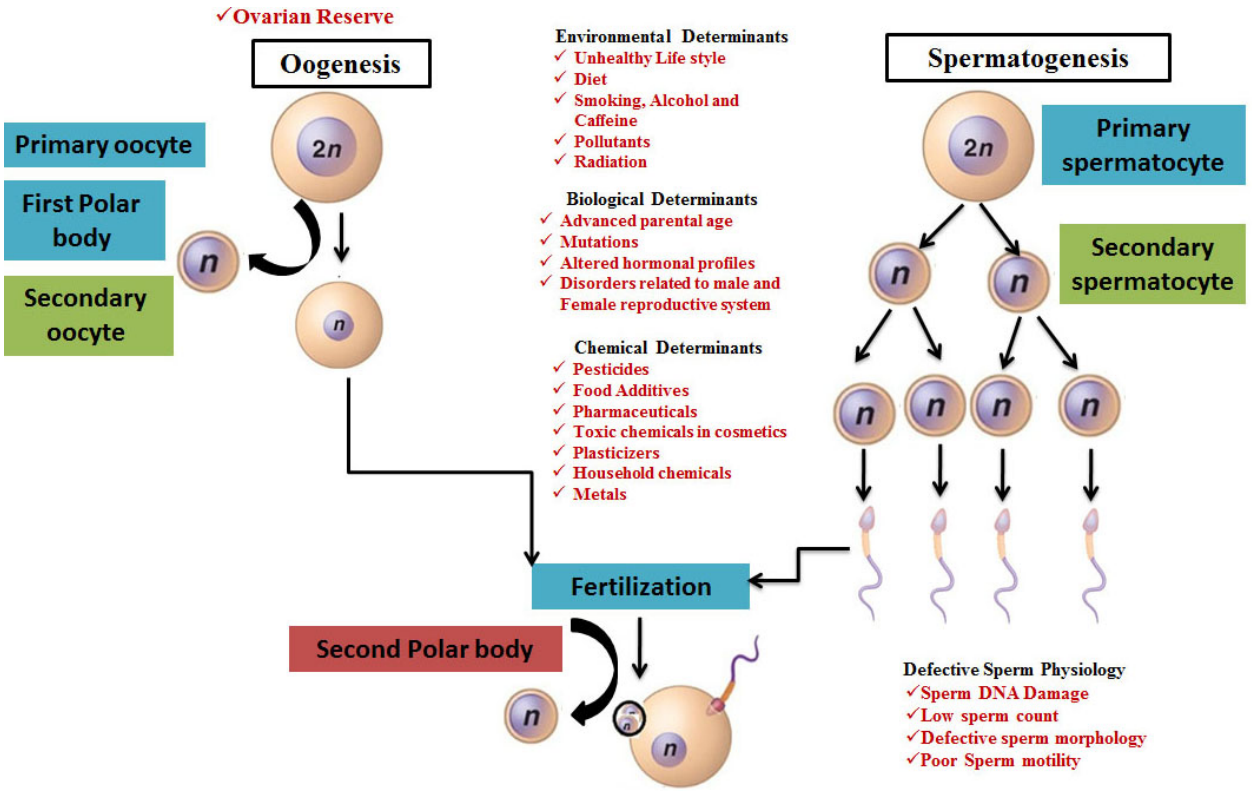

Figure 2. Various determinants of gametogenesis.

on gametes health is shown in Figure 2. A sharp decline in successful pregnancy rate involves stress as a major factor involved behind it. Stress reduction programs can play its role up to a certain extent but it depends on the couple to modify their lifestyle and choices and lessen the chances of failing pregnancy and adverse outcome. In conclusion, we can say that unlike genetic causes, environmental and lifestyle factors can be targeted for preventive measures.

9. GAMETE INTERSECTION: THE PLAYERS OF FERTILIZATION WINDOW

Mammalian gamete intersection is an intricate process of cell-cell interaction and signal transduction event that initiates with the maturation of the gametes in the oviduct and terminates with the formation of a zygote in receptive endometrium. Reproduction comprehends a series of different steps which has to be accomplished in a well-orchestrated manner to form the first cell of a new individual known as zygote. Post coitus, entry of sperm into the female reproductive tract culminates in various events like sperm capacitation, oocyte activation followed by binding and penetration into zona pellucida. These events are regulated by several variables which are secreted by oviduct. A failure of synchronization among these variables may lead to infertility related issues. Oviduct or fallopian tubes are the site for sperm capacitation, oocyte fertilization and early embryonic development. Functionally, oviduct plays important role in both hosting gametes and matching the optimal environment for maintenance of fertilization window.

Numerous evidences strongly support the fact that oviduct undergoes important modifications in numerous aspects, including its anatomy, histology and physiology of the mucosa. More precise information however, is needed about the genes expressed, proteins synthesized and secreted from the oviduct in different regions to clarify the key players of fertilization window. In this section, we focused on oviduct and its secretory proteins functions necessary for fertilization and implantation. We also discussed molecular determinants of sperm capacitation, acrosome reaction and cortical reaction during sperm-egg fusion.

Oviduct helps sperm to navigate toward the egg after being placed in the female reproductive tract. Oviduct also contributes in cryptic female choice, regulating the entry of only few sperm out of millions. The mechanisms of cryptic female choice are retrograde flow of sperms after coitus due to active contractions of female reproductive tract and selective phagocytic ingestion of sperms by neutrophils inside oviduct. Spermiation releases functionally incompetent and immotile sperms from the seminiferous tubules which are activated in epididymis producing motile sperm

Table 1. Key regulators for sperm motility, vitality, maintenance and oocyte preparation

S.No.	Proteins	Function	Reference
1	Glycodelin A and F	Inhibit capacitation, Inhibit Spermatozoa-zona pellucida binding	(165)
2	Glycodelin S	Suppresses albumin-induced cholesterol loss, Maintains the spermatozoa in an uncapacitated state	(166)
3	Lactoferrin	Inhibit gamete interaction and prevent polyspermy	(167)
4	Oviduct-specific glycoprotein (OVGP1)	Functional modification of the ZP, Promotes sperm capacitation, Increased embryo development	(168)
5	Glucose-regulated protein78 (Grp78/BiP)	Modulates sperm-zona pellucida binding	(169,170)
6	Acrosin (serine protease)	Activation of acrosome components, Secondary binding with the ZP, Hydrolysis of the ZP	(171)
7	SPESP1 (Sperm Equatorial Segment Protein 1)	Gamete interaction	(172)
8	TSSK6 (testis-specific serine kinase)	Actin polymerization	(173)
9	Calpains	Spectrin cleavage, Acrosomal Reaction, Gamete fusion	(174)
10	Human membrane cofactor protein (CD46)	Stabilization of the acrosomal membranes	(175)

with symmetrical flagellar beats and linear trajectory motion. Once they reach in the female reproductive tract, they interact with oviduct. Only few sperm reach the oviduct, they wait for several days until ovulation occurs. Oviduct microenvironment supports the viability of sperm population. Acidic vaginal pH is also helpful in sperm selection event by immobilizing the abnormal sperm. Oviductal fluid is a complex mixture of identified and unidentified components produced by oviductal epithelial cells and plasma transudate. Functionally, oviductal secretions are known to be involved in Zona pellucida (ZP) maturation and ZP hardening during gamete intersection (prevention of polyspermy) and active sperm physiology (110). These molecular factors can be classified in different groups as: (i) growth factors and their receptors (ii) hormones and their receptors (iii) proteases and inhibitors (iv) antioxidants(v) defense agents (vaginal pH) (vi) glycosidases and glycosyltransferases (viii) chaperones and heat shock proteins (ix) cytokines and their receptors (x) glycosaminoglycans and proteoglycans (141). These factors are essential for the sperm's maturation and receptive endometrium preparation. Disturbance in oviductal fluid components may lead to defective sperm capacitation, acrosomal reaction and non-competent blastocyst development. Various proteins responsible for maintenance of sperm motility and vitality, oocyte and endometrium preparation are summarized in Table 1. Increasing knowledge about the biochemical nature and function of the oviduct fluid will allow us to develop better assisted reproductive technologies, embryo culture medium and condition to improve the low rate of blastocyst formation in humans.

Various reports suggest that oviduct provides a microenvironment for sperm capacitation. Capacitation involves morphological, biochemical and physiological changes in sperm that confers the sperm ability to gain hyperactivated motility, actin remodeling, initiation of acrosomal reaction (AR) and gamete fusion. Recent studies shows that there is increase in Ca2+, bicarbonate (HCO3−) concentration, intracellular pH, cyclic adenosine monophosphate (cAMP) levels, reactive oxygen concentration and Kinase/Proteases activity for acquisition of motility and fluidity in sperm membrane. However inhibition in phosphatases activity and shedding of proteins and cholesterol from the sperm plasma membrane are required (142).

After capacitation, sperm are sequentially released from the reservoirs and rapidly transported to the fertilization site where acrosomal reaction takes place (143). Several glycoproteins, carbohydrates and adhesion proteins regulate actin remodeling process during these events. A study demonstrated that focal adhesion complexes (β1-integrin, FAK, paxillin, vinculin, talin, and α-actinin linked with integrin), present in mammalian spermatozoa are essential for maintaining the integrity of the acrosome via actin polymerization as well as remodeling (144). Another study suggests that a network composed of Ezrin, RhoGDI1, RhoA, F-actin and membrane proteins influence the fluidity of the sperm membrane to promote capacitation (145). Hyperactivation is a change in flagellar beating of sperm that reduces interaction between sperm and epithelium, initiates acrosomal reaction in sperm and aids sperm penetration into the zona pellucida of secondary oocyte. A study demonstrated that sperm from male mice that are null mutants for CatSper1 or CatSper2 gene fails to get hyperactivated and penetrate the zona. This study suggested that CatSper1 or CatSper2 genes play important role in infertility related issues. Mice homologues PDC-109 protein have heparin binding ability and is probably involved in stabilizing sperm membranes, reducing membrane fluidity and cholesterol mobilization (146).

In humans, oocytes arrested in prophase of meiosis I contain zona pellucida (ZP) proteins and undergo posttranslational modifications during progression of the oocyte towards metaphase of the second meiotic division. Human ovum is enveloped by ZP, which is transparent, porous and is glycoprotein coated. Human Zona pellucida (ZP) contains ZP1, ZP2, ZP3 proteins ZP4 where ZP3, ZP1, ZP4 acts to activate the acrosome reaction and ZP2 act as secondary acceptor for capacitated spermatozoa. A study demonstrated that only ZP2 protein undergo N linked glycosylation modifications via the activity of proteases released by the cortical granules during meiotic maturation of human oocytes. Cortical granule released in maturing human oocytes is involved in zona resistance after sperm penetration (147). Above study suggest that the zona resistance to sperm penetration is stabilized by factors secreted by the cumulus cells, particularly during Metaphase I to Metaphase II transition (148, 149, 150). Recent study documented that Estrogen-dependent oviduct-specific glycoprotein (OVGP1) is involved in sperm-egg binding and zona penetration rates in human (168). Another study suggested that notable increase in progesterone, estradiol and luteinizing hormone in the female reproductive tract must favor sperm-egg encounter and fusion (151).

Cortical reaction, also known as cortical granule exocytosis (CGE) is a calcium- regulated event. Cortical granules contain proteinases, ovoperoxidase, N-Acetylglucosaminidase, hydrolase that harden the zona pellucida (152, 153). Cortical reaction is mediated by activation of the inositol phosphate (PIP2) signalling cascade. The sperm-egg fusion, facilitated by G-protein's activation, might activate the generation of two important second messengers, IP3 and diacylglycerol (DAG). IP3 induces Ca^{+2} release from endoplasmic reticulum and the latter activates PKC, thus leading to the membrane fusion of the oocyte and CGs. Sperm-egg fusion and cortical reaction lead to changes that includes polyspermy prevention, resumption of the cell cycle and initiation of the embryonic mitotic divisions. In mammals, sperm enters the surface of the egg almost tangentially and fuses with numerous plasma membranes. Sperm nucleus undergoes chromatin decondensation and reconstruction by coalescing vesicles. Sperm nuclear DNA is bound by basic proteins called protamines, which are tightly compacted through disulfide bonds. In the egg cytoplasm, glutathione reduces these disulfide bonds and permits the uncoiling of the sperm chromatin. The mammalian male pronucleus enlarges and parallely the oocyte nucleus completes its meiotic II maturation. The centrosome associated with the male pronucleus produces its asters and interacts with the female pronucleus. Each pronucleus migrates toward the other and the two nuclear envelopes break down. However, instead of producing a common zygote nucleus, the sperm-derived and egg-derived chromosomes condense separately. At prometaphase, chromosomes from the sperm and egg intermix on the metaphase equator and a mitotic spindle initiates the first mitotic division for completion of zygote journey (154).

10. FROM ZYGOTE TO EMBRYO: WHAT MAY GO WRONG DURING IMPLANTATION WINDOW?

For successful accomplishment of zygote to embryo journey, fusion of healthy sperm and an egg (ovum) is the pre-requisite event, failure of which may lead to defective implantation. Mouse and human preimplantation embryo development is orchestrated by a series of mitotic divisions and cellular differentiation that leads to the formation of a multicellular embryo. A zygote undergoes several cleavage events, passing through various embryonic stages, including 2-, 4-, and 8-cell stage followed by compaction (morula formation), cavitation (blastocyst formation), zona hatching and finally gets implanted into the uterine wall.

Implantation of a blastocyst is a refined multistep process which is strongly regulated by numerous endometrial factors. During implantation, several biochemical changes take place, like cell to cell contact between functional blastocyst and receptive endometrium, blastocyst invasion and endometrial remodeling. Receptive endometrium is rich in numerous factors such as growth factors, hormones, cytokines, chemokines, proteases, anti-proteases and other unknown factors that have a potential to modulate blastocyst activity. Embryo-endometrial crosstalk disturbances underlie unexplained infertility, recurrent implantation failure and early miscarriage related issues; enhanced knowledge of this crosstalk, specifically at initial fetal-maternal crosstalk may disclose new targets to resolve these pregnancy related issues.

11. EMBRYO IMPLANTATION: KEY REGULATORY MOLECULES

Embryo implantation is a regulated and intricate event which requires a functional blastocyst and receptive endometrium for successful establishment of pregnancy. The key events demonstrated are: 1. Blastocyst orientation 2. Apposition (shedding of ZP) 3. Blastocyst adherence to the endometrial surface 4. Finally, blastocyst gets implanted into the receptive endometrium followed by invasion into the stroma. Receptive endometrium secretes numerous instructional signals and nutritional factors for the development of blastocyst (Table 2). In this section, we have summarized the role of these factors in endometrial receptivity and implantation.

Table 2. Key determinants of embryo implantation

S.No.	Proteins	Function	References
1	Selectins	Leukocyte transendothelial trafficking or Leukocyte rolling, Blastocyst apposition	(176,177,178)
2	Integrins	Cell–matrix and cell–cell adhesion	(161)
3	Immunoglobulins	Cell–cell adhesion, Transendothelial migration of leukocytes	(161)
4	MUC1	Negatively regulates embryo implantation	(161)
5	Cytokines (LIF, IL-6, IL-1, HMGB1)	Create pro-inflammatory microenvironment for receptive endometrium preparation	(161)
6	Prostaglandins	Vasoactive factors, Play an important role in ovulation, fertilization and parturition	(179)
7	Galectins	Supports blastocysts attachment on Luminal Epithelium	(180)
8	Heparansulfate proteoglycan (HSPG)	Initiating the implantation adhesion cascade	(181)

11.1. Uterine secretions

Uterine secretions are believed to play important roles in blastocyst/conceptus survival and implantation, uterine receptivity, stromal cell decidualization and protection of semiallogenic fetus from maternal immune attack in mammals. Another important function of uterine fluid includes its defensive activity against invading pathogens, sperm migration, and lubrication of endometrium. Uterine gland secretions include amino acids, ions, carbohydrates (glucose), lipids, proteins (cytokines, enzymes, hormones, growth factors, proteases and their inhibitors, transporters, etc.) and other substances in the human uterus (155). A study suggest that uterine fluids have different types of inflammatory molecules such as Interleukin (IL)-1 beta, IL-6, IL-12, IL-18, tumor necrosis factor-alpha (TNF-alpha), macrophage migration inhibitory factor, monocyte chemotactic protein-1, interferon gamma inducible protein-10, vascular endothelial growth factor (VEGF) for receptive endometrium preparation (156). Another study conducted by Bhusane *et al* suggest that uterine fluids have seven differentially abundant proteins in the receptive endometrium such as alpha-2-macroglobulin, serum albumin, activin receptor type-2B, AAT, interalphatrypsin inhibitor family heavy chain-related protein (157). Endometrial factors have been suggested to explain implantation failure and poor reproductive potential of patients with polycystic ovary syndrome. Exogenous hormone stimulation produces an asynchrony in the secretion of uterine fluid proteins.

11.2. Adhesion molecules

Adhesion of the embryo in the receptive endometrium is a crucial step in implantation. The mother and embryo influences the expression of adhesion molecules that is necessary to establish a successful pregnancy. Endometrial adhesion molecules including cadherins, integrins and selectins are known to involve in large number of cellular processes like apposition, adhesion, migration, and invasion process of embryo.

Selectins, integrins, cadherins, trophinin and heparin-binding epidermal growth factor (HBEGF) play an essential role in implantation (apposition and adhesion). To understand the embryo-endometrial interaction, possible mechanism is described as: 1. integrin–extracellular matrix (ECM) molecule attachment 2. integrin-integrin with osteopontin ligand 3. integrin-selectin interaction 4. tastin/trophinin–tastin/trophinin attachment 5. cadherin-11 interaction coupled by calcium ion 6. lectin-glycan interaction 7. glycan–glycan attachment.

The glycoproteins also expressed in the luminal epithelium are supposed to act as a uterine barrier which inhibits the interaction between the trophoblasts and luminal epithelium at the time of attachment (158). Unmasking of these glycoproteins at the implantation site correlates with increased blastocyst adhesiveness to the uterus (159). For example, MUC1, a mucin-type glycoprotein, is integrally located in the apical plasma membrane of the luminal epithelium before implantation, however, its expression substantially down-regulates during the receptive period in a time dependent manner (160).

Fertilization window is narrow time period that is indispensable for functional blastocyst attachment. It is an ovarian steroid-dependent phenomenon that encompasses key elements essential for proper hatching, adhesion and attachment of embryo. In humans, the fertile window spans around three days i.e. from LH+7 to LH+11 (day 20 to day 24) (161). During fertile window, endometrium undergoes histological modifications such as pinopods appearance i.e. belb like protrusions found on the apical surface of the endometrial epithelium and acquire adhesion ligands to receive the functional embryo. Endometrial pinopods express leukemia inhibitory factor (LIF) and its receptor, progesterone and integrin αVβ3at mid secretory phase which is necessary for blastocyst

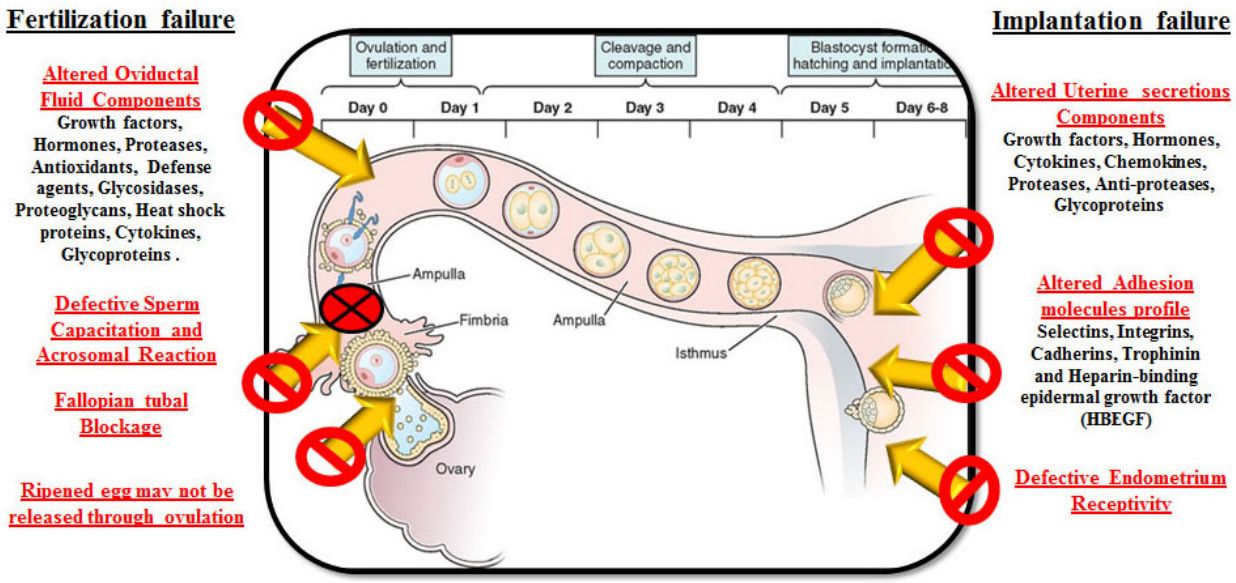

Figure 3. Factors affecting fertilization and implantation in humans.

attachment (162, 163, 164). Studies suggest that pinopods detection during the mid-secretory phase may be extremely useful for the endometrial receptivity assessment.

In a nut shell, oviduct and receptive endometrium has an active role in fertilization. If anything goes wrong at any stage of ovulation or fertilization, the whole process (from gametogenesis to fertilization) will be repeated again for successful conception. Like egg may not ripen; ripened egg may not be released through ovulation; ovulated egg may not come to intimate contact between adequately numerous, motile or healthy sperm, egg may not get fertilized, fertilized egg may not cleaved or not implanted properly, endometrium may not be receptive for healthy conceptus as shown in Figure 3. Numerous molecular mediators are involved in regulating these events like intrauterine cytokines, growth factors, adhesion molecules and hormones. Understanding the molecular mechanism related to fertilization window is the need of the hour which will open new methods for clinicians to treat infertility and recurrent implantation failure related issues and to develop new contraceptive approaches.

12. FUTURE DIRECTIONS

The formation of a genetically and functionally proficient gamete is indispensable for normal fertilization and early embryonic development. A multitude of signalling pathways, intricate series of interactions and molecular events are involved in sustaining the process of gamete formation and its way towards fertilization and development of a healthy embryo.

Despite enormous feats and advancement in the reproductive research arena, the frequency of infertility is still snowballing. The escalating incidences of infertility can be attributed to widespread etiologies that uncover several genetics, epigenetic and environmental factors culminating in poor gamete quality and fertilization failure. Though the Assisted reproductive technologies have provided some relief to the infertile couples, the quality of gametes and embryos still remain questionable. Investigations on the molecular mechanisms underlying the development of a poor quality gamete and fertilization failure are the need of the hour.

Implantation failure seems to be the bottleneck of the reproductive event. While a number of factors have been identified in the last one decade, substantial lacunae entail to understand the mechanism that manifests implantation failure. The definitive goal of reproductive research at this point must be directed towards deciphering the functional characteristics of a healthy gamete and to identify potential biomarkers that define the mechanisms underlying healthy embryonic development.

The knowledge, acquired from this line of research, will surely assist investigators to treat infertility and recurrent implantation failure related issues and will help in developing new approaches and specific therapeutics measures for the optimization of embryonic health.

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Send correspondence to: Kiran Singh, Department of Molecular and Human Genetics, Banaras Hindu University, Varanasi, U.P 221005, India, Tel: 0542 6702489, Fax: 91-542-6702499, E-mail: singhk4@rediffmail.com