



Review Article

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Impact of gamete health on fertilization and embryo development: An overview

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ABSTRACT

A genetically and functionally proficient gamete is essential for normal fertilization and embryonic development. Any change in gamete health affects fertilization and subsequent events, including embryonic development, implantation, and successful pregnancy. This present review focuses on the role of gamete health on fertilization and embryo development. Several conventional and advanced methods are used to evaluate the morphology and functions of gametes. The abnormal spermatozoa adversely affect fertilization events, which results in reduced cleavage/blastocyst/implantation and pregnancy rate during assisted reproductive techniques. Poor oocyte quality is also one of the reasons for infertility, although the oocyte has an innate capacity to repair a certain amount of abnormality of both oocyte and spermatozoa. Therefore, oocyte health carries more responsibilities during fertilization events. The gamete, either spermatozoa or oocyte, should have optimum morphological and functional health to fertilize and develop a competent embryo successfully. Thus, it is of prime importance to consider the gamete health parameters while dealing with infertility.

KEYWORDS: Embryo; Fertilization; Gamete; Oocyte; Sperm**1. Introduction**

A gamete is a haploid cell that fuses with other haploid cells during the process of fertilization to carry the genetic material to progeny. The male gamete is produced by spermatogenesis and female gamete is produced by oogenesis[1]. Gamete health means by which normal fertilization and development of embryo occur, delineated by morphological and functional character (quality) of gametes. A genetically and functionally proficient gamete is required for normal fertilization and embryo development. The fertilization failure also results from molecular variations during gametogenesis[2] and alteration in gamete health[3]. The fertilization process comprises of three events: 1) sperm capacitation and acrosome reaction, 2) sperm-zona binding, oocyte activation, and

fusion of gamete membranes, 3) cortical reaction, pronuclei and zygote formation, and subsequent early embryonic development[4]. Gamete health is affected by various factors like genetics, age, heat stress, *in vitro* procedures, diseases, nutrition, and cryopreservation which alter their morphology and function[3,5–7] and can be assessed by various conventional and advanced methods[8–11].

2. Effect of spermatozoa health on fertilization and embryo development*2.1. Morphological abnormalities*

The quality of bull semen is generally predicted by evaluating sperm concentration, motility, live-dead percentage, morphological acceptance and other biochemical tests within semen samples. The head or tail abnormalities of sperm have been shown to cause a reduction in fertility[12]. The abnormal head morphology could interfere with motility and sperm binding to the three glycoproteins of zona pellucida[13]. The impaired sperm-oocyte binding will reduce fertility as spermatozoa could not pass through zona pellucida[14]. Decreased fertilization/cleavage/morula/blastocyst and pregnancy rates have been observed in morphological abnormal spermatozoa as compared to normal spermatozoa[14–17]. In an *in vitro* study, the mean number of sperm bound and penetrating the zona pellucida was lower ($P < 0.05$) in bull with pyriform spermatozoa (24.6 ± 1.2 ; 56.0%) than the control bull (46.6 ± 1.9 ;

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82.8%) having normal spermatozoa[14]. Further, cleavage and morula production rates were less in bulls with pyriform spermatozoa. Percentages of cleaved embryos, morulae and blastocysts produced were lower for the bulls with knobbed acrosomes; flattened (66.0%, 31.7% and 15.2 %), indented (54.1%, 28.3% and 10.3%) and deep indented (22.7%, 8.1% and 2.7%) acrosome than those of the control bull[15]. During cattle *in-vitro* fertilization (IVF) experiment, lower cleavage rate [(66.3±1.5)% vs. (85.5±1.7)%] and blastocyst rate [(13.5±1.6)% vs. (26.8±1.7)%] were observed in spermatozoa with various morphological abnormalities (decapitated spermatozoa, pyriform head, diadem defect/nuclear vacuole) than morphological normal spermatozoa[17]. The injection of morphologically abnormal spermatozoa resulted in a lower fertilization rate (60.7%) than injection of morphologically normal spermatozoa (71.7%) in the human intracytoplasmic sperm injection (ICSI) study[16]. The higher clinical pregnancy and live birth rates were obtained in patients with normal sperm morphology [32.6% and (14.9±2.84)%, respectively], than in those with abnormal sperm morphology [20.2% and (7.2±1.98)%, respectively]. The deficiency of phospholipase C zeta in a sperm impairs the Ca²⁺ dependent oocyte activation, leading to a fertilization failure[18].

2.2. Sperm DNA fragmentation/damage

The degree of sperm DNA fragmentation decides the fate of fertilized oocyte[19,20]. A sperm with a variable amount of DNA fragmentation may fertilize the oocyte, and zygote development begins, but damaged paternal chromatin can lead to early embryonic death or abortion at any stage of gestation[21]. Thus, DNA damage does not affect spermatozoon's ability to fertilize oocytes but negatively affects embryonic development[19]. Reactive oxygen species (ROS) attacks spermatozoa and leads to mitochondrial disruption and chromatin fragmentation, impairing sperm fertilizing capacity and embryo development[22,23]. Sperm DNA damage causes fertilization failure, poor embryo quality, implantation defects, spontaneous abortion, and congenital malformation, leading to infertility[3]. The damaged paternal DNA is cognizant for the apoptotic machinery of the embryo at the 4–8-cell stage, which blocks the mitosis after 2–3 embryo cleavages, and forms abnormal spindle and nuclear fragmentation, ultimately resulting in a developmental block prior to blastocyst formation[19]. Sperm DNA damage also causes epigenetic modifications such as DNA methylation, sperm histone modification, and chromatin remodeling, which alter the sperm physiology and suppress the embryonic gene expression at 4–8 cell stage embryo, which blocks further embryo development[3]. IVF experiments did not show much difference in fertilization rate between high and low DNA fragmentation/damage, but lower cleavage rate, blastocyst rate and pregnancy rate were observed with high DNA damage than low DNA damage and no DNA damaged spermatozoa[19,24–26]. Further, increased

miscarriage rate was also noted in high DNA damage group[20]. ICSI experiments involving higher DNA fragmentation/damage showed a lower pregnancy rate[27]. However, higher pregnancy rates were reported with high DNA damage than the low DNA damage and control group[25,26]. The human IVF trial resulted in lower blastocyst production with increasing terminal-deoxynucleotidyl transferase mediated nick end labeling (TUNEL) positive, *i.e.*, high DNA damage spermatozoa[28]. Younger and favorable female's (female age <35 years and anti-Müllerian hormone value ≥7.1 pmol/L) oocyte had more capacity to repair sperm DNA fragmentation during embryogenesis[19,20,29–32].

2.3. Sperm mitochondrial dysfunction

Sperm cryopreservation decreases the number of viable sperm and can affect the functions of surviving cells by impairing their motility, mitochondrial activity, chromatin integrity, and reproductive potential[33–35]. Exposure to cryoprotectants causes openings of mitochondrial permeability transition pore (mPTP) in response to intracellular Ca²⁺ increases, which trigger release of ROS and further Ca²⁺ release, loss of mitochondrial membrane potential, decreased adenosine triphosphate (ATP) content, and release of cytochrome C and pro-apoptotic factors[36,37] that culminate in DNA damage and apoptosis. Depending on the extent of mitochondrial damage, the sperm may die or survive cryopreservation. Oocytes fertilized by DNA damaged sperm could emerge into a compromised embryo[37]. The retrospective buffalo IVF study showed decreased cleavage rate (<40%) with spermatozoa having decreased mitochondrial membrane potential (4.47% vs. 8.53%), decreased forward progressive motility (38.74% vs. 51.65%), decreased plasmalemma integrity (31.86% vs. 45.47%) and decreased acrosome integrity (62.48% vs. 69.17%) as compared to the group having >40% cleavage rate[38].

2.4. Damaged sperm plasma membrane

Sperm membrane has particular importance, since it is involved in the metabolic exchanges of the cell with the surrounding medium. The process of capacitation, acrosome reaction and binding of the spermatozoa to the oocyte requires an active membrane. Mammalian fertilization means fusion of the sperm plasma membrane and oolemma, making sperm organelles direct access to the ooplasm, while the sperm plasma membrane itself remains outside. The integrity of the sperm plasma membrane is not necessary for successful fertilization in ICSI; therefore, fertilization and embryo development can be achieved by dead spermatozoa at an early stage of necrosis[39]. The fertilization rates (62.4% vs. 59.6%), blastocyst development (51.7% vs. 50.0%) and implantation rate (61.5% vs. 53.5%) did not differ significantly between plasma

membrane damaged and intact spermatozoa in ICSI trial because in ICSI, all sperm components are mechanically introduced into the oocyte cytoplasm[39,40]. The non significantly different pronuclear formation in the membrane-damaged sperm (40.0%–63.6%) and control groups (33.3%–52.9%) suggests that ICSI of membrane-damaged sperm does not influence pronuclear formation, which is a key point in fertilization, although sperm head decondensation could be affected[40].

2.5. Reduced sperm concentration and motility

The significantly reduced blastulation rate and embryo implantation are noted with oligozoospermia, which might be associated with elevated DNA fragmentation and poor chromatin quality. Reduced sperm motility affects the fertilization process due to the limited availability of spermatozoa. A negative correlation was observed between sperm motility and fertilization rate in the male having <5% sperm motility irrespective of concentration[41]. The group having motility <5% significantly resulted in lower fertilization rate than the control group having a motility $\geq 32\%$ (66.7% vs. 75.0%).

3. Effect of oocyte health on fertilization and embryo development

3.1. Dark zona pellucida

The morphology and structural appearance of zona pellucida can predict the oocyte and embryo quality. Thick or abnormal zona may not be directly linked to mechanical fertilization failure but may be a sign of general oocyte dysfunction[42]. The oocytes with abnormal zona pellucida transpire in decreased blastocysts, implantation, clinical pregnancy rates, and increased miscarriage rates[43]. The oocytes with a dark zona pellucida may have abnormal mitochondria and a large number of vacuoles which might affect the metabolic activity of the oocyte, fertilization rate and developmental competency of the embryos. Large vacuoles displace spindle position, which impairs embryonic genome activation at 4-8 cell stage embryos. The rates were significantly lower in the dark zona pellucida group compared to those in the control group of normal fertilization in IVF (59.4% vs. 76.3%), implantation (18.6% vs. 34.5%) and clinical pregnancy (25.0% vs. 53.3%) and live birth rates (20.0% vs. 42.7%)[43].

3.2. Cumulus cell's DNA damage

Cumulus cells originate from relatively undifferentiated granulosa cells. The granulosa cells are the primary cell type in the ovary that provides the physical support and microenvironment required for the developing oocyte. They are actively differentiating with several

distinct populations during folliculogenesis, from a primordial (squamous type) stage of development through ovulation (cuboidal type) to a luteal stage of development (hypertrophied lutein cells). The communication between cumulus cells and oocyte is bi-directional, and the role of the oocyte extends far beyond its functions in the transmission of genetic information and supply of raw materials to the early embryo. It also has a critical part to play in mammalian follicular control and the regulation of oogenesis, ovulation rate and fecundity[44–46]. The cumulus cells provide nutrition and protection to oocyte. The recent advancement in preservation of oocyte utilizing high concentration (16%–50%) of cryoprotective agents during vitrification affects both oocytes and cumulus cells viability. Ultimately, DNA damage occurs in cumulus cells and impairs fertilization and embryo development. The cumulus cells protect oocytes against zona pellucida hardening and cytoplasmic damage during vitrification-warming process, thereby preserving competence for fertilization. The comet tail length refers to the extent of DNA damage, and DNA integrity to the percentage of DNA in the comet head. Approximately <15 μm of comet tail length is related to undamaged DNA, and in damaged DNA it is >30 μm for porcine oocyte. The IVF, cleavage, and blastocyst rates increased significantly with the presence of cumulus cells than the denuded oocytes in the control groups, however, significantly decreased in the toxicity (cumulus oocyte complexes exposed to cryoprotective agents, 16% ethylene glycol plus dimethyl sulphoxide without vitrification) and vitrification groups having DNA damage in cumulus cells[47]. The addition of melatonin during *in vitro* maturation reduces the DNA damage of cumulus cells, but this effect does not influence the *in vitro* embryo development[48]. Copper increased blastocyst rates regardless of cumulus cells presence during *in vitro* maturation, highlighting the importance of this mineral in oocyte metabolism[49,50].

3.3. Abnormal polar body

The oocyte with either fragmented or large polar body represents cytoplasmic incompetence, and thereby impairs fertilization and embryonic development, hence resulting in lower implantation and increased abortion rate[51–53]. The abnormal embryo development includes higher grade of fragmentation and multinucleated blastomeres. The higher genetic or chromosomal abnormalities trigger aneuploidy lowering chances of implantation associated with multinucleation[53]. Retention of the second polar body during meiosis II is the most common cause of digynic triploid embryos but may less frequently result from the retention of the first polar body during meiosis I [54]. The 1st polar body shape can affect fertilization rate and embryo quality in ICSI cycles[55]. The relationship of the 1st polar body morphology to mature oocyte may be used as a prognostic factor to predict oocyte performance and pregnancy achievement after an ICSI treatment[56].

3.4. Oocyte vacuolization

Vacuoles in the oocyte form either due to uncontrollable endocytosis or *via* fusion of pre-existing vesicles produced by the smooth endoplasmic reticulum and golgi apparatus result into formation of fluid filled vacuoles. The large central vacuole in oocyte leads to lower pregnancy rates, higher miscarriage rates and greater obstetric problems[57]. Extremely large cytosolic vacuoles physically displace the metaphase II spindle from its usual polar position which impair activation of embryonic genome at 4-8 cell stage and thus might cause zygotic and embryonic arrest.

3.5. Oocyte mitochondrial dysfunction

In cryopreserved oocyte, exposure to cryoprotectants causes increased intracellular Ca^{2+} , further, propanediol and ethylene glycol vehiculate external Ca^{2+} , dimethyl sulfoxide triggers Ca^{2+} release from the endoplasmic reticulum. The premature Ca^{2+} rise causes partial cortical granule exocytosis and zona pellucida hardening and prolonged openings of mPTP trigger ROS, loss of mitochondrial membrane potential, decreased ATP content, and release of Cytochrome C as well as proapoptotic factors. Such events may culminate in DNA damage and apoptosis. The extent of mitochondrial injury in cryopreserved oocytes determines whether the oocyte dies or survives. Substantial mitochondrial damage might lead to altered Ca^{2+} and ROS signaling at fertilization, and compromised embryo development[37,58,59]. During meiosis I, if the oocyte has functional mitochondria, then chromosomes distribution occurred evenly, but for oocyte with dysfunctional mitochondria, the chromosome distribution occurred unevenly, which increased aneuploidy rate[60].

Oocyte mitochondrial dysfunction was observed in repeat breeder cows. In summer season, stress affects oocyte quality and alters estrous cycle which leads to repeat breeding. However, the mechanisms by which oocytes become less capable of supporting embryo development remain largely unknown. Decreased oocyte competence of repeat breeder cows during summer is associated with an altered gene expression profile and a decrease in mitochondrial DNA (mtDNA) copy number. The oocytes retrieved from repeat breeder cows during winter contained over eight times more mtDNA than those retrieved during summer. The expression of mitochondria-related (*NRF1*, *POLG*, *POLG2*, *PPARGC1A*, and *TFAM*) and apoptosis-related (*BAX* and *ITM2B*) genes in repeat breeder oocytes collected during summer was higher than winter and decreased oocyte maturation gene (*FGF10*, *GDF9*) leads to reduced blastocyst rate in summer season as compared to winter season in repeat breeder cows[61].

3.6. Amount of cumulus oocyte complexes

Cumulus cells provide nutrient to oocyte for their growth and

maturation as well as embryo development. Lack of cumulus cells/ removal of cumulus cells negatively affects oocyte maturation, fertilization and development[62]. The advanced atresia of oocyte with presence of granulations and less than five layers of cumulus cells, the absence of cumulus, or the presence of expanded cumulus with dark clumps reduced cleavage and blastocyst formation. The cumulus oocyte complex (COC) grade 3 and 4 harvested from freshly excised domestic cat ovaries revealed decreased cleavage rate and morula rate[63]. Sometimes, blood clots formation in COC during ovarian puncture lowers fertilization and blastocyst development because blood clots act as blocking barrier in spermatozoa-zona binding[64].

3.7. Abnormal perivitelline space

Perivitelline space abnormalities (large perivitelline space with or without granules) are among the most important extra cytoplasmic dysmorphisms of the oocyte, but the exact mechanism of these abnormalities is not understood yet[65]. Additionally, it is not clear if the abnormality is physiological or pathological in nature. Some perivitelline space granules are the remnants of coronal cell processes that usually withdraw as the oocyte undergoes resumption of meiosis[66]. Large perivitelline space increases oocyte degeneration[67], having lower fertilization rates[68]. Single abnormal perivitelline space abnormality as well as combined abnormality (large and granulated) show lower fertilization rate, blastocyst rate, implantation rate, pregnancy rate and higher miscarriage rate[69]. A large perivitelline space is one of the most significant factors associated with lower fertilization rate and pronuclear morphology[70]. The development rates of grade I, II, and III embryos were 68.5%, 23.8%, and 7.7%, respectively, in the 105 oocytes with perivitelline abnormalities (large perivitelline space with or without granules). The development rates of grade I, II, and III embryos were 82.1%, 17.9%, and 0%, respectively, in the 112 morphologically normal oocytes[71].

3.8. Lipid content

The high dose of fatty acids (palmitic acid, oleic acid, stearic acid) causes the lipotoxicity to the oocyte during *in vitro* maturation of oocytes. This ultimately causes oxidative stress as well as endoplasmic reticulum stress in COCs, and altered calcium regulation in oocyte resulting in mitochondrial dysfunction. It suppresses or inhibits carnitine palmitoyl transferases (*Cpt*) 1 gene which is required for beta oxidation in oocyte. Beta oxidation regulates lipid metabolism in oocyte and produces ATP for oocyte growth and further development. The altered beta oxidation affects resumption of meiosis and oocyte nuclear maturation, and ultimately impairs the fertilization process and further embryonic development[8].

3.9. Oocyte developmental competence

The developmental competence of oocytes was evaluated after separation and higher blastocyst rates were obtained from oocyte collected from lower outlet of microfluidic device (*i.e.* high quality oocyte)[9]. Microfluidic device has the same capability of oocyte separation as brilliant cresyl blue staining. Oocyte selection would largely affect the success of IVF since developmental competence of embryos highly depends on oocyte quality. Brilliant cresyl blue negative oocyte (developmentally less competent) shows lower blastocyst rate[72,73].

4. Conclusions and future prospects

The gamete health has supreme value and significant role in fertilization, embryo development and maintenance of pregnancy; hence, recent techniques should be incorporated over conventional methods for gamete evaluation. The gamete, either spermatozoa or oocyte, should be healthy both morphologically and functionally to achieve successful fertilization and development of competent embryo. The gamete health parameters should be considered while dealing with infertility, because abnormal gamete health is one of the major factors of infertility of both humans and animals. The single essential criteria for gamete health evaluation need to be identified. Studies are required in the future to prevent/minimize the adverse effects of poor gamete health on fertilization and pregnancy.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Authors' contributions

Chorya Jaypalsinh B prepared rough draft and literature search. Sutaria Tarunkumar V and Chaudhari Ravjibhai K conceived the concept, designed the study, carried out literature search, and made editing and revision of the manuscript. Chaudhari Chandrakant F did literature search and revision. All authors read and approved the final manuscript.

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