Reactive oxygen species (ROS) are produced during the normal cellular metabolism and are essential in carrying out the cell functions. In semen, ROS are produced by spermatozoa and leukocytes. This chapter discusses the cellular and subcellular sources of ROS in semen and their regulation at the molecular level. Physiological levels of ROS are essential for normal sperm function; however, an imbalance between ROS and antioxidant levels causes oxidative stress (OS), which affects sperm function. The pathological effects of elevated levels of ROS on sperm parameters such as membrane function, motility, capacitation, acrosome reaction, and sperm–zona binding capacity are discussed in great details. Further, the OS-induced sperm apoptotic pathway explaining the key factors involved in the cell death has been deliberated. Finally, future directions and recommendations regarding the treatment of OS are discussed.
30.1 INTRODUCTION

Oxygen is essential to sustain life, but its harmful metabolites such as reactive oxygen species (ROS) pose a potential threat to cellular functions and cell survival. The abbreviation is used to describe several free radicals and highly reactive entities that are derived from the molecular oxygen. ROS are exceedingly reactive with one or more unpaired electrons. The production of oxygen-derived free radicals is the bane to every aerobic species. These are produced during the normal metabolism of the cells. Principal ROS agents involved in the biology of reproduction include hydrogen peroxide (H₂O₂), hydroxyl ion (OH•), superoxide anion (O₂⁻•), hydroxyl radical (·OH), and peroxide. Nitroxy radicals, on the other hand, which are derived from nitrogen, termed "reactive nitrogen species," such as nitric oxide (NO•) and peroxynitrite (ONOO•), also play an important role in reproduction (Agarwal et al., 2003; Maneesh and Jayalekshmi, 2006).

In semen, ROS are produced mainly by spermatozoa and leukocytes. Seminal plasma acts as an antioxidant reservoir and possesses strong antioxidant capacity. Indeed, a physiological balance exists between the production of ROS and antioxidants. Evidence supports the beneficial effects of physiological levels of ROS on the normal sperm function. However, an overproduction of ROS, which exceeds the antioxidant capacity of seminal plasma, results in oxidative stress (OS). This imbalance escalates the deleterious effects of ROS on spermatozoa function that leads to male infertility (Agarwal et al., 2008; Mahfouz et al., 2009). The primary objective of this chapter is to describe the production and molecular regulation of ROS in semen, to describe the impact of ROS on sperm function, and to shed light on OS-induced sperm apoptosis.

30.1.1 CELLULAR AND SUBCELLULAR ROS SOURCES IN SEMEN: FRIEND OR FOE?

Semen is an organic fluid that contains spermatozoa as its major constituent, along with secretions from the testes, epididymis, seminal vesicles, prostate, and to some extent the Cowper’s gland. In cases of male genital tract infection, leukocytes, macrophages, and sometimes Sertoli cells are also seen in seminal ejaculates (Fisher and Aitken, 1997). In semen, among all the key players, it is the spermatozoa, mainly the immature ones, and the leukocytes that contribute significantly to ROS production.

30.1.1.1 Spermatozoa-Mediated ROS Production

Immature spermatozoa with abnormal morphology particularly in the head and retained cytoplasm are substantial sources of ROS. Any abnormalities in cytoplasm extrusion during the differentiation phase of spermatogenesis deploy enzymes such as glucose-6-phosphate dehydrogenase (G6PD) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX2), which result in the production of ROS through NADPH formation (Aitken et al., 1997; Agarwal et al., 2014). There are two main ways of spermatozoa-mediated ROS production: (1) the NOX2 system in the spermatozoa plasma membrane and (2) the NADPH-dependent oxidoreductase (diaphorase) present in the sperm mitochondria (Aitken et al., 1992; Gavella and Lipovac, 1992). The production of ROS by spermatozoa is further related to the quality of semen. If the semen contains high number of immature spermatozoa, the rate of production of ROS is correspondingly higher. When different subpopulations of spermatozoa were separated by density gradients, ROS production was found to be highest in the immature population and lowest in mature and morphologically normal spermatozoa (Gil-Guzman et al., 2001). Studies have also shown that mature spermatozoa produce ROS during their normal metabolism. The generation of ROS by mature spermatozoa is considered as a normal physiological process, which is required for downstream spermatozoa function (Du Plessis et al., 2015). Increased ROS production has also been reported during the preparation of spermatozoa for assisted reproductive techniques (ARTs) such as the density gradient method when spermatozoa were exposed to repeated cycles of centrifugation. The length of time to which spermatozoa were exposed to centrifugation causes more ROS production compared to the force of centrifugation (Agarwal et al., 2003).
30.1.1.2 Leukocytes

The presence of leukocytes at concentrations of >10^6 mL\(^{-1}\) of semen is defined as leukocytospermia by the World Health Organization (WHO). Peroxidase-positive leukocytes, contributed mainly by the prostate and seminal vesicles, are the substantial generators of ROS in the semen. Myeloperoxidase staining is used to quantify their presence in the neat semen. Several controversial studies have reported the association of leukocytospermia, ROS production, and male infertility. Nonetheless, with the exception of a few reports, most studies indicate a strong relation between leukocytospermia and ROS production that lead to impaired sperm function (Wolff et al., 1990; Sharma et al., 2001; Mahfouz et al., 2009). Polymorphonuclear (PMN) leukocytes and macrophages are the main subpopulations of leukocytes in semen, which contribute 50%–60% and 20%–30% of ROS production, respectively (Wolff, 1995). ROS production depends on the activity of leukocytes because activated forms of cells generate 100-fold more oxygen species compared to nonactivated cells. Activation of leukocytes depends on several elements such as infection and inflammatory responses (Potts and Pasqualotto, 2003). These factors cause the activation of a cascade of reactions involved in NOX2 catalysis and monophosphate shunt in fighting against the invading antigens and infection. Once these responses are triggered, it leads to the production of free radicals (Babior, 1999). The PMN leukocytes, being the main subpopulation of white blood cells in the semen, contribute a larger share of ROS production. Nonetheless, once the macrophages are activated, it too causes a surge in ROS production (Ochsendorf, 1999). Direct cell-to-cell contact of leukocytes with spermatozoa or the liberation of cellular by-products also causes the production of ROS by spermatozoa themselves (Saleh et al., 2002). The subcellular components involved in ROS production include some cell organelles, different enzymes, and long-chain fatty acids (LCFA) (Figure 30.1).

**FIGURE 30.1** (See color insert.) Major cellular and subcellular components responsible for ROS production in semen. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved.)
30.1.2 Molecular Regulation of ROS Production

30.1.2.1 Activation of NADPH Oxidase

The respiratory burst occurs as a result of enhanced production of ROS by activated leukocytes due to the much higher consumption of oxygen by these cells. This process is catalyzed by the membrane-bound enzyme complex called NOX2 (Babior et al., 1973). Among several enzymes known for the production of different moieties of ROS, NOX2 is the most significant enzyme. The NOX2 complex of neutrophils and other immune responsive cells comprise membrane-linked cytochrome b$_{558}$ and several components of cytosol (p40$	ext{phox}$, P47$	ext{phox}$, and P67$	ext{phox}$). The functional activity of the NOX2 is regulated by G-protein Rac, which is part of a complex regulatory system (Figure 30.2) (Hancock et al., 2001; Bokoch and Diebold, 2002).

When immune cells (phagocytes and PMN) are in the resting state, a heterodimer consisting of two polypeptides, p22-phox and gp91-phox, two heme molecules, and one flavin adenine dinucleotide (FAD) group empowers the electron transmission from cytosolic NADPH to the molecular oxygen without the involvement of NOX2 activity (Hancock et al., 2001). The behavior of gp91-phox polypeptide as the H$^+$ ion channel results in charge compensation. Once a transmembrane stimulatory response is initiated, several cytosolic polypeptides (p40$	ext{phox}$, P47$	ext{phox}$, and p67$	ext{phox}$) assemble at the interior face of the cell membrane to form the fully activated NOX2 enzyme complex. The active NOX2 also requires the simultaneous translocation of Rac GTPase. The whole complex of membrane-associated heterodimer cytochrome b$_{558}$, cytosolic polypeptides, two heme groups, and one FAD allows the flow of electrons from NADPH to FAD and then to heme and finally to molecular oxygen. As a result of activation of the NOX2 and transfer of electrons, O$_2^-$ are produced by the reduction of molecular oxygen (Figure 30.2) (Hancock et al., 2001).

30.1.2.2 Signal Transduction in ROS Production

Intracellular production of ROS in concurrence with several antioxidant enzymes plays a pertinent role in switching enzymes on and off by a redox signaling mechanism that is similar to a cyclic adenosine monophosphate (cAMP) second messenger system (Hou et al., 1999). The two main ROS species that act through this signaling pathway are the O$_2^-$ and H$_2$O$_2$. Due to the very low steady-state levels of O$_2^-$, their spatial activity is very much limited. H$_2$O$_2$ reacts with thiolate anion (S-) to generate sulfenic acid, which undergoes ionization to produce sulfoxide (SO$_2^-$) (Forman and Torres, 2002). When a ligand binds with the tyrosine kinase receptor on the cell surface, the downstream signaling begins. This signaling activates the mitogenic-activated protein (MAP) kinase cascades. These cascades lead to the enormous generation of H$_2$O$_2$ from many other enzyme catalysts, along with NADPH oxidase (Park et al., 2006). H$_2$O$_2$ interacts with other pathways, especially SOS-Ras-Raf-ERK and PI3K/Akt in a dose-dependent mode. Even a small increase in H$_2$O$_2$ levels can

![FIGURE 30.2](See color insert.) Mechanistic activation of NADPH oxidase complex and production of superoxide anions. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved.)
cause tremendous reentry into the cell as occurs in the case of NOX1 expression. However, permanent elevation in cellular H$_2$O$_2$ levels causes cell cycle arrest and, eventually, apoptosis (Burch and Heintz, 2005).

Peroxiredoxins, a novel family of peroxidases, in conjunction with reducing equivalents delivered by thioredoxin, serve as the key regulators of H$_2$O$_2$ and mitogenic signaling. As the mitogenic signaling begins and the ROS production commences, these peroxiredoxins are activated and interacted with mitogenic signaling to control further signaling and to limit the effect of ROS-mediated activation on downstream mitogenic cascade targets (Choi et al., 2005).

### 30.1.3 Redox Signaling

Spermatogonia are nondividing cells and are transcriptionally inactive. However, it is important to understand the basic mechanism of cell division, as the division in terminal differentiated cells is controlled by oxidants (Latella et al., 2001). Cell cycle is an extremely ordered and sequential process that ensures the truthful replication of cellular genome and division into two daughter cells. The cell cycle consists of four active stages: that is, Gap 1 (G$_1$), Synthesis (S), Gap 2 (G$_2$), and Mitosis (M), and one quiescent state (G$_0$). In order to guarantee the faithful progression through the cell cycle, there are three checkpoints that play a strategic role. The first, the G$_1$ checkpoint safeguards cell nutrients, cell size, and growth factors required for DNA synthesis. The second, the G$_2$ checkpoint ensures that an appropriate cell size has been achieved and that DNA damage has been repaired before it confirms the correct entry of the cell into M phase. The third, the metaphase checkpoint or spindle assembly checkpoint controls the accurate attachment of spindles with chromosomes and ensures that the cell is ready for division (Lilly and Duronio, 2005).

In the cells that withdraw from the cell cycle and enter in G$_0$ state, their reentry into G$_1$ is regulated by oxidants. The expression of cyclin D1, the main protein required for reentry into the cell cycle, is promoted by the redox signaling pathway. The expression of this protein is further regulated and is considered as a marker for mitogenic stimulation (Burch and Heintz, 2005). The cyclin D/CDK complex phosphorylates the retinoblastoma (pRb) protein with a redox potential of around −207 mV at G$_1$ phase. At higher redox potentials, the pRb protein undergoes dephosphorylation and the cell cycle ceases. However, maximum ROS production occurs during the G$_2$/M phase of the cell cycle (Havens et al., 2006).

### 30.1.4 Cellular Glutathione Redox System and Spermatogenesis

In testes and epididymal tissue, cellular levels of glutathione (GSH) are very low. During sperm elongation, more than 90% of GSH is removed (Conrad et al., 2015). Such low GSH levels are required to allow the oxidative phases to shape the spermatozoa acrosome, nucleus, midpiece, and flagellum. Despite the low abundance, some of the glutathione peroxidase (Gpx) family members exercise an essential role in the maturation process of mammalian spermatozoa (Noblanc et al., 2011). Mammals express eight Gpxs; nonetheless, the presence of all these in spermatozoa has not been confirmed. Glutathione peroxidase 1 (Gpx1) is expressed in testes, Gpx2 in intestinal tissue, and Gpx3 and Gpx5 in epididymal tissue. Gpx4 has the most abundant expression including all essential compartments of the testes and in mature spermatozoa. Gpx6 is expressed in olfactory epithelium, while scanty information is available about the expression of Gpx7 and Gpx8, which are known as endoplasmic reticulum resident (ER resident) (Conrad et al., 2015).

Among the eight Gpxs, Gpx3, Gpx4, and Gpx5 have the more important role in sperm function. Gpx3 and Gpx5, being present throughout the epididymis and caput, respectively, protect the male gamete genome from deleterious effects of increased ROS. Although there is a lack of evidence of any phenotype in the absence of Gpx3, elevated levels of ROS were observed in spermatozoa and cauda epididymis epithelium in mice lacking Gpx5. When the offspring of these mice that were older than a year were allowed to breed, significant miscarriages and fetal developmental defects
were seen. These findings were in concurrence with the fact that Gpx5<sup>−/−</sup> sperm manifest higher DNA oxidation (Chabory et al., 2009).

Gpx4 is considered the ideal target for the testicular selenium and is rendered responsible for male infertility (Ursini et al., 1999). Gpx4 was identified as the most copious selenoprotein in the capsule of sperm mitochondria, which provides stability to the sperm midpiece. Gpx4 is the key enzyme involved in sperm capsule protein oxidation. The involvement of Gpx4 has also been confirmed with other capsular proteins, such as sperm mitochondria-associated cysteine-rich protein (SMCP). When the Gpx4 gene was disrupted, increased embryonic mortality was observed. Additionally, the role of Gpx4 has also been associated with the sperm fibrous sheath and the acrosome, and its disruption causes male infertility (Maiorino et al., 2005; Schneider et al., 2009).

In the previous sections of this chapter, the major players involved in the production of ROS, the regulation of ROS production at molecular levels, and the important redox system implicated in normal spermatogenesis have been discussed. In the subsequent sections, the impact of OS on functional sperm parameters is emphasized.

### 30.1.5 OS AND SPERM DYSFUNCTION

The conditions when the levels of oxidants exceed antioxidants are called OS. During the state of OS, the levels of peroxidation increase and pathological effects cause the impairment of cellular function. All integral cellular components, that is, proteins, nucleic acids, lipids, and carbohydrates, become critical targets of OS (Agarwal et al., 2003). OS-induced cellular damage depends on the type and amount of ROS involved, the duration of exposure to ROS and several other factors such as antioxidants capacity, neighboring environment, available oxygen and the temperature.

#### 30.1.5.1 Spermatozoa Membrane Lipid Peroxidation

The plasma membrane of spermatozoa is rich in polyunsaturated fatty acids (PUFAs). The fatty acids are highly susceptible to ROS attack, which leads to the activation of downstream chemical reactions. The oxidative deterioration of PUFAs is called the lipid peroxidation (LPO). There are two kinds of LPO: (1) enzymatic LPO, which depends on the NADPH and adenosine diphosphate (ADP), and (2) nonenzymatic LPO, which is independent of such enzymes. The most common effect of LPO on cellular function is the perturbation in the structure and function of cellular and organelle membranes. The intracellular ion transport, metabolite gradients, and receptor-mediated signal transduction operate aberrantly. The transcriptionally inactive state of the sperm cell makes it much more susceptible to LPO. Unlike other cells, spermatozoa cannot repair the damage caused by ROS due to the lack of essential cytoplasmic enzymes involved in the repair mechanism (Alvarez et al., 1987; Krausz et al., 1994; Agarwal et al., 2003; Aitken and Baker, 2013).

#### 30.1.5.2 Spermatozoa Motility

The impact of increased ROS on sperm motility has been clearly documented (de Lamirande and Gagnon, 1992; Agarwal et al., 1994; Armstrong et al., 1999; Desai et al., 2009). Increased ROS levels may hinder the normal function of the redox system that is required for sperm motility. As explained in previous sections, the systematic knockout of mitochondrial Gpx4 resulted in massive reduction in sperm motility and dramatic midpiece phenotypic abnormalities (Schneider et al., 2009). Another reason could be the intercellular diffusion of H₂O₂ across the plasma membrane that hinders the normal functions of certain enzymes, such as G6PD. The function of this enzyme is to control the efflux of glucose through the hexose monophosphate shunt, which eventually regulates intracellular NADPH. NOX2 system is the key player in ROS production (Aitken et al., 1997). Once G6PD activity is altered, NADPH becomes unavailable and the oxidized glutathione accumulates in the cells, which results in reduced levels of glutathione. Consequently, the antioxidant system of spermatozoa weakens, rendering the sperm membrane susceptible to LPO (Griveau et al., 1995).
Other speculation in reduced sperm motility is the decreased axonemal protein phosphorylation involved in sperm membrane fluidity (de Lamirande and Gagnon, 1995).

### 30.1.5.3 Spermatozoa Capacitation

Capacitation is the ability of spermatozoa to fertilize the oocyte, and to achieve this, it requires a series of morphological and metabolic changes of the sperms in the female reproductive tract. This process enables the spermatozoa to bind and penetrate the zona pellucida. Both biochemical and molecular events are involved in sperm capacitation; production of cAMP through the activation of adenylyl cyclase (AC), activation of calcium channels, production of ROS, efflux of cholesterol from the plasma membrane, increased intracellular pH, and activation of protein kinases (O'Flaherty, 2015). Sperm capacitation in mammals is an oxidative phenomenon. Although normal levels of ROS are a prerequisite for sperm capacitation, higher and sustained ROS levels, such as during OS, can lead to premature capacitation (O’Flaherty, 2015) or prevent spermatozoa from undergoing capacitation (Morielli and O’Flaherty, 2015). Since phosphorylation events are mandatory during sperm capacitation, any hindrance in these events may lead to failure in sperm capacitation. In infertile men, it was observed that spermatozoa were unable to undergo tyrosine phosphorylation (Buffone et al., 2005). When spermatozoa were treated with H$_2$O$_2$ at a concentration of 1 mM before undergoing capacitation, decreased tyrosine phosphorylation was noted. These findings propose a delayed sperm capacitation under OS conditions. On the other side, multifaceted redox changes of protein sulphhydryl (SH) occur during the events of sperm capacitation and the levels of SH are changed. During the initial 30–60 min of capacitation, the SH content of detergent-soluble proteins (Triton X-10) increases (de Lamirande and Gagnon, 2003), which on one hand is beneficial, but at the same time, it also creates a situation of OS and, ultimately, early capacitation. This is similar to sperm cryopreservation, where increased SH content of Triton-soluble proteins is known to cause OS (Cormier et al., 1997), leading to premature sperm capacitation. Unlike sperm motility, the effects of OS on sperm capacitation are scarcely reported and those reported remain controversial (Morielli and O’Flaherty, 2015). Furthermore, the detailed underlying molecular mechanism of sperm capacitation is yet to be elucidated. Likewise, exogenous ROS causes hyperactivation of spermatozoa but is prevented by ROS scavengers, that is, catalase, SOD, and NOX2. However, the exact mechanism with regard to the impact of ROS on sperm hyperactivation is not known.

### 30.1.5.4 Acrosome Reaction

Sperm acrosome is a cap-shaped structure derived from the Golgi apparatus. It develops over the anterior part of the sperm head. It contains several enzymes playing a crucial role in fertilization. The intact acrosome is important for fertilization, and if the acrosome reaction (release of enzymes) has occurred, spermatozoa are unable to fertilize the oocyte. ROS have a negative correlation with acrosome function. The higher the OS, the lower the percentage of spermatozoa with intact acrosomes. Currently, there is a lack of consensus as to which free radical or ROS are the major contributors in the regulation of acrosome function. Nonetheless, the significant stimulatory effect of H$_2$O$_2$ on spontaneous acrosome reaction was observed compared to controls. The effects were evident even at 0.01 mM concentration of H$_2$O$_2$. On the other hand, H$_2$O$_2$ showed inhibitory effects on acrosome reaction in spermatozoa treated with progesterone (Oehniger et al., 1995). Dose-dependent decrease in lysophosphatidylcholine-induced acrosome reaction was also observed in spermatozoa treated with H$_2$O$_2$ (Morielli and O’Flaherty, 2015). Likewise, in previous experiments, a significant increase was observed in the percentage of acrosome-reacted (loss of acrosome function) spermatozoa when treated with H$_2$O$_2$ compared to controls (Aitken et al., 1993).

### 30.1.5.5 Spermatozoa–Zona Pellucida Binding

Sperm–zona pellucida binding is a crucial step in achieving successful fertilization and eventually pregnancy. Physiological levels of ROS are required for the effective binding of spermatozoa to the zona pellucida. However, raised ROS levels have a deleterious effect on sperm–zona binding
and result in fertilization failure. A significant inhibitory effect of H$_2$O$_2$ on sperm–zona binding has been reported at doses 0.2 mM and above. Lower doses of H$_2$O$_2$, that is, 0.05 and 0.1 mM, did not reveal any negative effect, and this demonstrated that spermatozoa were bound to the zona pellucida in a similar fashion as that of the controls (Oehninger et al., 1995). This again reveals the importance of physiological levels of ROS in the normal sperm function. Whenever OS occurs as result of higher ROS, it gives a negative impact on sperm–zona binding. Studies have also shown a negative association between the levels of ROS and the decreased capacity of sperm–egg fusion (zona-free hamster egg) (Aitken et al., 1991). Similarly, a significant reduction in sperm–zona binding and penetration was observed in spermatozoa treated with xanthine oxidase and directly with H$_2$O$_2$ (0.2 mM) (Aitken et al., 1993).

30.1.6 OS and Sperm Apoptosis

Apoptosis is a genetically determined process of programmed cell death. Indeed, each spermatozoon is destined to undergo apoptosis except for a few, which achieves immortality through fertilization (Aitken and Baker, 2013). The majority of the ejaculated spermatozoa will undergo apoptosis during their strenuous effort to embrace the oocyte for fertilization. Therefore, apoptosis is not induced in spermatozoa, rather it results because of the spontaneous attempts the spermatozoa have to make to engage with the oocyte. In order to recognize the oocyte, inseminated spermatozoa will undergo a cascade of cellular events and then achieve capacitation in the female reproductive tract. During the course of these events, the numerous surface receptors present on the spermatozoa help in the recognition of the zona pellucida (Reid et al., 2011; Baker et al., 2012). The hyperactivity achieved through the capacitation of spermatozoa plays a key role in defining the spermatozoa with the greatest motility to successfully fertilize the oocyte.

The ROS generated during the sperm capacitation significantly deplete the cholesterol from the plasma membrane, thus increasing its fluidity, in order to facilitate the fertilization process (Aitken and Baker, 2013). However, the continuous production of ROS by spermatozoa eventually overcomes their limited defensive mechanisms and the cell undergoes OS, which in turn activates the intrinsic cascade of apoptosis (Aitken, 2011). Unlike somatic cells, apoptosis in spermatozoa is not triggered during the cell cycle checkpoints but is instead a programmed manifestation.

Human spermatozoa face different challenges compared to other mammalian species. Unlike mammals, humans do not exhibit a behavioral estrous pattern, which guides the synchronized insemination just before ovulation. Rather, human spermatozoa have to wait for the ovulation to occur, and the waiting may prolong for several days. A pertinent question then arises as to what prevents the spermatozoa from undergoing apoptosis while waiting for such a long time in the female reproductive tract? Indeed, several factors such as glucose regulation and hormones such as insulin or prolactin play a pro-survival role in protecting the spermatozoa from undergoing apoptosis. These factors play a significant anti-apoptotic role by activating the phosphatidylinositol 3-kinase (PI3 kinase), which produces phosphatidylinositol triphosphate (PIP3) (Pujianto et al., 2010; Aitken and Baker, 2013). However, the physiological functioning of these factors is hampered when there is OS due to persistent ROS production.

Sustained generation of ROS by sperm mitochondria is crucial in triggering sperm apoptosis. One of the most known reasons of continuous mitochondrial ROS production is LPO, an unavoidable consequence of OS, which causes interruption in the mitochondrial electron transport chain (mETC). Thus, the first sign of activation of intrinsic cascades in sperm apoptosis is the induction of OS, further synchronized by several other factors, that is, age, lifestyle exposure, smoking, varicocele, leukocytes, and environmental factors (Aitken and Baker, 2013).

Spermatozoa contain a high number of unsaturated fatty acids, which make these cells vulnerable to free radicals and consequently trigger increased LPO, thus placing the spermatozoa under immense OS. As a result of such stress, electrophilic lipid aldehydes such as 4-hydroxyxynonenal (4HNE) are produced by making adducts with susceptible proteins more commonly at lysine,
FIGURE 30.3 (See color insert.) Key players involved in oxidative stress and sperm apoptosis. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved.)

Histidine, and cysteine residues. Such adducts impair the mETC function leading to leakage of electrons and formation of oxides. ROS generation is considered a self-disseminating phenomenon, which once initiated cannot be broken or reversed and leads to cell death (Figure 30.3) (du Plessis et al., 2010).

30.2 CONCLUSION

A conclusive body of evidence supports the drastic effects of elevated ROS/OS on sperm function, which ultimately leads to male subfertility or infertility. Despite the fact that common conditions such as age, smoking, heat, pollution, toxins, diet, obesity lead to OS, little attention is given to the prevention of this cause by physicians specializing in infertility. In infertile couples where male has high ROS/OS, many clinicians treat with ART to achieve pregnancy. However, the impact of OS on the quality of sperm (sperm DNA) should not be neglected. Sperm with damaged DNA can fertilize the oocyte but cannot maintain the pregnancy and results in poor quality of embryos/blastocysts, which consequently end up in a miscarriage. Therefore, as a priority, OS should be addressed before attempting pregnancy to avoid financial and psychological distress. Though controversial, antioxidant therapy in men with OS has proven to be beneficial, and almost one-third of
men experiencing infertility are prescribing to this therapy. Besides nutritional changes, it is also imperative to modify the lifestyle of the couples to minimize the risk of OS. However, further large randomized controlled trials are needed in order to validate the best dose and combination of antioxidant supplements.

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