

Review

Potential role of green tea catechins in the management of oxidative stress-associated infertility



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KEY MESSAGE

The outstanding antioxidant activity of green tea catechins demonstrated *in vitro* represents its great potential in improving fertility potential in human by alleviation of oxidative stress. Results from *in-vivo* studies are eagerly awaited.

ABSTRACT

Reactive oxygen species (ROS) are present in low concentrations in the genital tracts of males and females. Excessive ROS lead to oxidative stress, which damages DNA, lipids and proteins. Such molecular changes result in compromised vitality, increased morphological defects and decreased sperm motility in the male. In the female, oxidative stress interferes with oocyte maturation, and may inhibit *in-vitro* maturation of the oocyte. Recently, green tea supplementation has been reported to possess properties that may improve the quality of male and female gametes largely due to the ability of catechin polyphenols to quench ROS. Epigallocatechin-3-gallate (EGCG) is considered the most promising bioactive compound in green tea due to its strong antioxidant activity. The unique property of green tea catechins may potentially improve reproductive health and pose an important research area. We present a comprehensive overview on the effects and potential roles of green tea catechins on oxidative stress in male and female reproduction and fertility. In this review, possible mechanisms of action are highlighted to better understand the potential use of green tea catechins in the reduction of oxidative stress and its associated beneficial effects in the clinical setting.

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Introduction

Reactive oxygen species (ROS) are present in low concentrations in the genital tracts of both males and females under normal physiological circumstances [Guerin et al., 2001; Kwecien et al., 2014]. A delicate balance between reduction and oxidation is required for essential sperm functions including chromatin compaction in the maturation of spermatozoa during epididymal transit, which leads to capacitation, hyperactivation, acrosome reaction and sperm–oocyte fusion and, ultimately, successful fertilization [Agarwal and Said, 2004; Aitken et al., 1995; de Lamirande and Gagnon, 1993; Guthrie and Welch, 2012; Kothari et al., 2010; Wright et al., 2014]. ROS also appear to play a physiological role in oocyte maturation, ovarian steroidogenesis, folliculogenesis, ovulation, luteolysis, implantation, formation of the fluid-filled cavity and blastocyst development [Agarwal et al., 2006a, 2006b; Askoxylaki et al., 2013; Esfandiari et al., 2005; Siristatidis et al., 2015]. Interestingly, a certain amount of lipid peroxidation relevant to ROS in follicular fluid is necessary to establish pregnancy in human IVF [Agarwal et al., 2005].

Oxidative stress (OS) is a condition that occurs when there is an imbalance between ROS and a biological system's ability to readily detoxify the reactive intermediates or repair the resulting damage [Hampl et al., 2012; Saalu, 2010]. OS usually arises as a consequence of excessive ROS production *in vivo*. Over the years, infertility related to OS has become an area of great concern for clinicians and scientists because continuous formation of ROS throughout the life of an organism leads to cumulative damage, which impairs fertility via programmed deterioration. The deleterious consequences of OS include impairment of semen parameters [Agarwal et al., 2005; Bansal and Bilaspuri, 2010; Pasqualotto et al., 2000], poor fertilization, poor embryonic development [Jana et al., 2010; Oyawoye et al., 2003] and pregnancy loss [Cengiz et al., 2016; Daglar et al., 2016].

Green tea (GT) is a popular drink consumed daily by millions of people globally. Tea is an infusion or a boiling water extract prepared with the leaves of *Camellia sinensis* L. and water. Consumption of GT may prevent diseases because of its antiproliferative, antimutagenic, antioxidant, antibacterial, antiviral and chemopreventive properties [Li et al., 2014a; Rahmani et al., 2015; Schramm, 2013; Yang et al., 2009]. Studies suggest that consuming green tea extracts (GTEs) protects the cardiovascular system [Basu and Lucas, 2007], lowers blood glucose and cholesterol levels [Hara, 1994], and provides anti-inflammatory effects [Shapiro et al., 2009]. The beneficial effects of GT are attributed to the polyphenolic compounds, particularly the catechins, which make up 30% of the dry weight of GT leaves [Graham, 1992].

Oxidation of organic compounds is catalysed by metals [Sheldon and Kochi, 2016], and GT supplements are capable of acting as antioxidants both *in vivo* and *in vitro* by metal requisition due to the presence of catechin moieties in polyphenol molecules, which possess a high affinity for metal ions. The presence of GT supplements, therefore, may reduce the availability of metal ions acting as the catalyst in the oxidative reaction in a biological system. The findings support the use of GT in maintaining the reproductive health of the male and the female by alleviation of OS [Galleano et al., 2010; Perron and Brumaghim, 2009].

The current evidence in the literature, however, does not support the use of GT catechins as antioxidants in the clinical management of male and female infertility. Therefore, we review the possible beneficial effects of GT supplementation, particularly the catechins, on

reproductive health by summarizing the relevant data available from both human and animal experiments. A comprehensive overview of the effects and roles of GT in the management of male and female infertility will be provided. Furthermore, the possible mechanism of action of GT catechins and GTE will be highlighted in an attempt to understand the potential use of GT in the alleviation of OS and its associated effects on fertility in the clinical setting.

ROS and OS

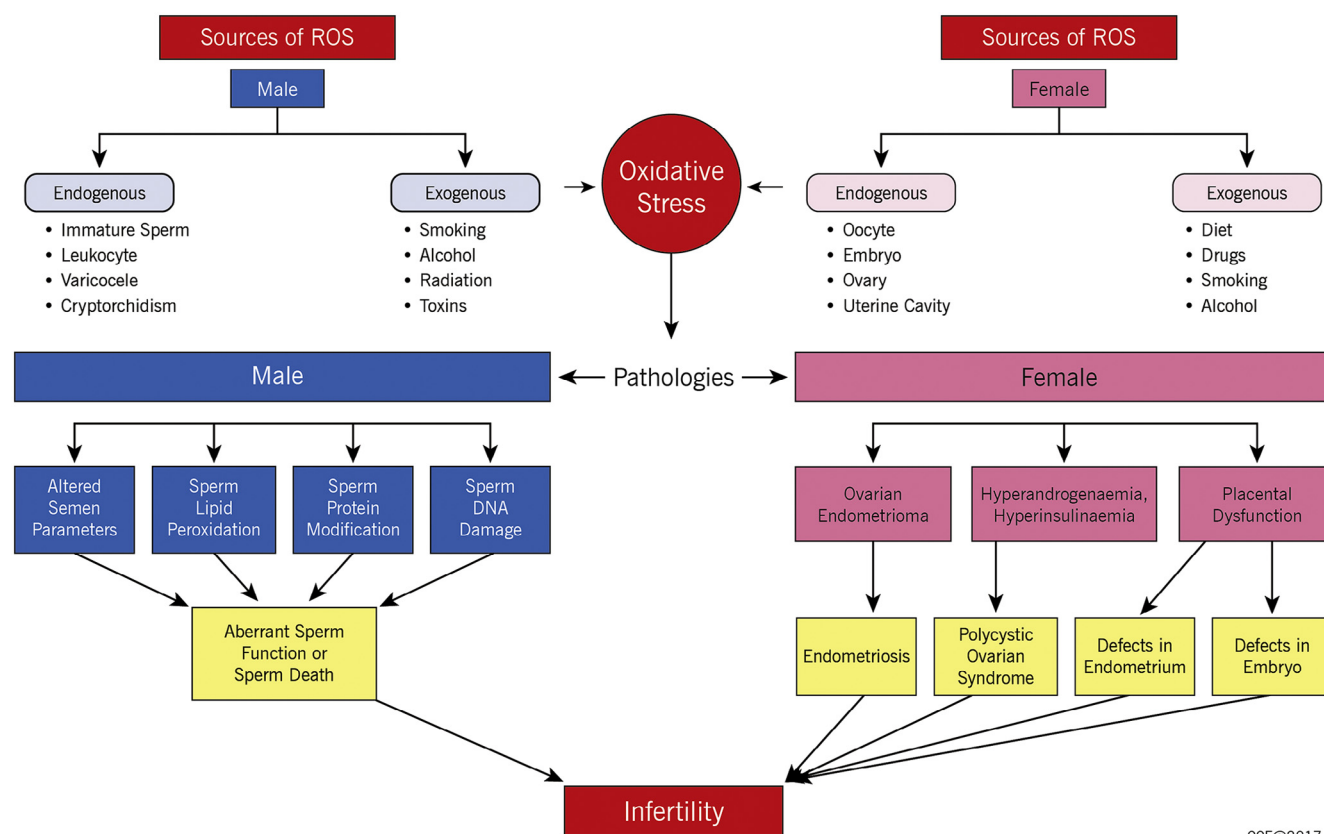
Reactive oxygen species (ROS) is a collective term used to describe oxygen radicals. Recent studies have shown that ROS, generated from various endogenous and exogenous sources, have a tremendous impact on reproduction and fertility [Figure 1]. Activated leukocytes are one of the important sources of ROS and adversely affect sperm motility, morphology and concentration. They also cause acrosomal damage, hyperactivation, DNA damage, and impair oocyte penetration [Aziz et al., 2004; Lackner et al., 2010; Pasqualotto et al., 2000].

Immature, defective, senescent and apoptotic sperm also produce high levels of ROS and contain docosahexaenoic acid, which is a target of ROS-induced lipoperoxidative damage [Williams and Ford, 2005]. OS is also associated with reduced total antioxidant capacity in seminal plasma of infertile men [Roychoudhury et al., 2016], which has a role in the aetiology of impaired sperm functions [Pahune et al., 2013].

ROS are involved in the initiation of apoptosis in antral follicles caused by several chemical and physical agents. It has a role in primordial and primary follicle death. Oxidative damage to lipids in the oocyte may cause persistently poor oocyte quality after early life exposure to several toxicants [Luderer, 2014]. Elevated levels of OS caused a decrease in oocyte numbers leading to a reduction in follicles, and these changes ultimately resulted in sub-fertility [Camlin et al., 2016]. Animal studies have further found that OS influences early embryo development [Blondin et al., 1997; de Castro et al., 2016; Harvey et al., 2002] and blocks the development of *in-vitro* two-cell embryos by modifying the key transcription factors, hence transforming gene expression, eventually resulting in female infertility [González-Fernández et al., 2016; Jana et al., 2010].

Green tea and its bioactive components

The processing of tea leaves modifies the chemical and physiological properties of black tea (fully fermented), oolong tea (semi-fermented) and GT (unfermented) [Beecher et al., 1999; Coimbra et al., 2006]. According to high-performance liquid chromatography data, GT leaves contain 26% fibres, 15% proteins, 2–7% lipids, and 5% vitamins and minerals. They also contain secondary metabolites such as pigments (1–2%), polyphenols (30–40%), of which at least 80% are flavonoids, and methylxanthines (3–4%) [Cabrera et al., 2003, 2006; Graham, 1992]. The composition varies and depends on factors such as geographical location (climate, soil), agricultural practices (fertilizers, deadheading) and the properties of the plant itself (variety, age of the leaf, and position of the leaf on the harvested shoot) [Cabrera et al., 2006]. Catechin polyphenols are believed to be the most important active component in GT. They are secondary metabolites possessing antioxidant activity, which is 20 times higher than that of vitamin C. Catechins have the ability to quench free radical species and chelate transition metals, which helps reduce OS levels [Hijazi



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Figure 1 – Sources of oxidative stress and their impact on reproduction and fertility. Various endogenous as well as exogenous sources of ROS and the association between OS and pathological conditions in male and female reproduction. In the male, it has been shown to impair sperm function and/or cause sperm death, whereas in the female it is related to endometriosis, PCOS and other defects in the endometrium as well as embryo.

et al., 2015). Cao et al. (2007) showed that a polyphenolic extract of GT at 1 or 2 g/kg diet can regulate gene expression in the glucose uptake and insulin signalling in rats fed a fructose-rich diet.

GTE is a common dietary herbal supplement manufactured into more than 100 different over-the-counter products (Patel et al., 2013). These products are marketed and generally used for weight reduction and maintenance of homeostasis. However, their use carries a risk of hepatotoxicity (Garcia-Cortes et al., 2016; Mazzanti et al., 2015; Patel et al., 2013). Acute impending liver failure was reported in an adolescent male using a weight-loss product containing GTE (Patel et al., 2013). Mazzanti and colleagues reported that GT may interact with other ingredients in the product and enhance the risk of liver damage (Mazzanti et al., 2015).

There are two major bioactive components of GT: polyphenols (also known as polyhydroxyphenols) and flavonoids (Awoniyi et al., 2012; Cabrera et al., 2006). GT also contains tannins and alkaloids, such as tristetruprolin, caffeine, theobromine and theophylline (Bokuchava and Skobeleva, 1980). There are four major polyphenols in GT that are classified as catechins: epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG) (Figure 2). Catechins are the main astringent components in GT that make up 30–40% of the water-soluble solids (Rahmani et al., 2015). Commonly available GT on the market contains 6.5–15.4 mg EC, 30.9–76.4 mg EGC, 3.6–15.9 mg ECG and 43.5–83.9 mg EGCG per 100 ml tea drinks (Henning et al., 2003). The principal GT catechins belong

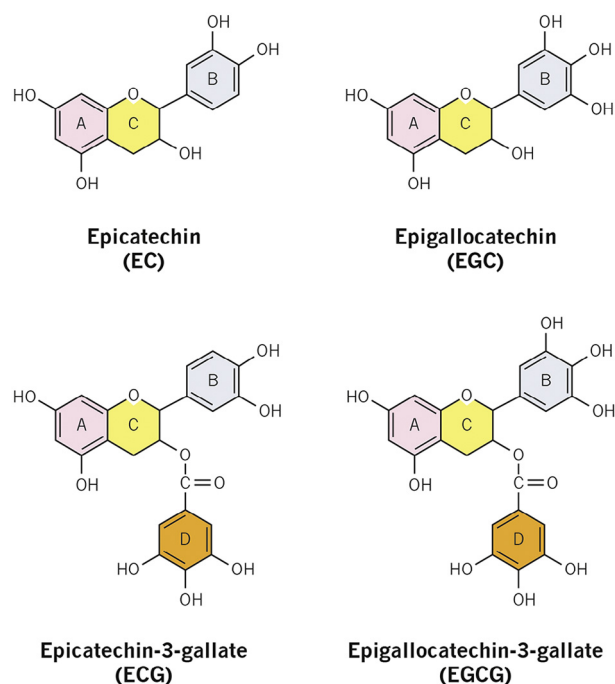
to the structural class of organic compounds with hydrocarbon rings. The hydroxyl molecules that are found at the 3, 5 and 7 positions further trigger the unique physical, chemical and biological properties characteristic of the class (Reygaert, 2014; Yang et al., 2009).

Epicatechin

EC is more formally known as (–)-epicatechin, pronounced 'minus epicatechin'. It represents approximately 6.4% of the total catechin content. EC possesses two benzene rings (called the A- and B-rings) and a dihydropyran heterocycle (the C-ring) with a hydroxyl group on carbon 3 (Awoniyi et al., 2012; Cabrera et al., 2006; Jung et al., 2003). When analysed in isolation, EC is a compound with high bioactivity. It is stable during gastric transit when taken orally but becomes glucuronidated and partially methylated in the small intestines – these processes continue in the liver, leaving only a small amount of native EC in the mesenteric circulation (Fraga and Oteiza, 2011).

Epigallocatechin

EGC is a flavan-3-ol, a type of chemical compound that comprises 19% of the total catechin content; the gallate residue is in an iso-



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Figure 2 – Principal catechin polyphenols in green tea. Most of the medicinal properties of green tea are associated with the four polyphenolic catechins: epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG). The antioxidant as well as the ROS scavenging properties of these catechins are attributed to the presence of phenolic hydroxyl groups on the B-ring in EC and EGC, and in the B- and D-rings of ECG and EGCG.

meric *trans* position. EGC possesses two epimers (one pair of stereoisomers) [Someya et al., 2002]. It has a pyrogallol structure with three hydroxyl groups at the C-3, C-4 and C-5 positions [Figure 2]. EGC is one of the GT polyphenols that has been shown to inhibit growth of breast cancer cell lines (MCF-7 and MDA-MB-231) [Vergote et al., 2002].

Epicatechin-3-gallate

ECG has a catechol structure on a B-ring that has two hydroxyl groups at the C-3 and C-4 positions. It represents approximately 13.6% of the total catechin content [Awoniyi et al., 2012]. In-vitro experiments have shown that ECG can reverse methicillin resistance in bacteria such as *Staphylococcus aureus* [Shiota et al., 1999]. This further supports the observation that the combined intake of tea extract containing ECG might enhance the effectiveness of methicillin treatment against some resistant bacteria *in vivo*. ECG also possesses potent antitumour activities [Chen et al., 2015].

Epigallocatechin-3-gallate

EGCG is the most abundant catechin in GT infusions and is considered one of the most active molecules known for its strong antioxidant properties [Kim et al., 2013]. EGCG can reduce OS and has been reported to play a role in reducing the incidence of cancer [Rahmani et al., 2015; Yang et al., 2009], osteoarthritis [Katiyar and Raman, 2011] and cardiovascular diseases [Basu and Lucas, 2007]. It also decreases the risk of type 2 diabetes mellitus by reducing insulin resistance [Kim, 2008; Park et al., 2014].

Low concentrations of EGCG have beneficial effects on cardiovascular and metabolic functions in normal physiology and in the pathophysiology of Parkinson's disease [Kim et al., 2014]. In-vitro and in-vivo studies using animal models and cell culture suggest that EGCG can prevent Parkinson's disease by decreasing OS and inflammatory reactions [Pan et al., 2003]. A pilot human study also supported these findings and suggest a long-term, higher dosage study with modified EGCG supplements to further clarify the possible therapeutic role in preventing Parkinson's disease [Chen, 2013].

Cia and coworkers suggested that EGCG may protect against retinal diseases associated with OS including age-related macular degeneration [Cia et al., 2011]. The compound may also have possible cardioprotective effects as it was capable of protecting human umbilical vein endothelial cells from particulate matter-induced OS injury by activating critical antioxidant pathways [Yang et al., 2015]. An in-vitro model of OS induced by ethanol showed that EGCG can prevent liver cell injury, which may prove potentially useful in patients with alcoholic liver disease [Oliva et al., 2011]. A number of in-vitro and animal model studies have also looked at its ability to prevent several chronic diseases, especially cardiovascular disease and cancer [Gupta et al., 2008]. EGCG has also received considerable attention as an antiangiogenic and antitumour agent with chemopreventive properties [Webb, 2000].

This compound is obtained by formal condensation of gallic acid with the [3R]-hydroxy group of EGC [Figure 2]. It represents approximately 59% of all catechins. EGCG can minimize the deleterious effects of ROS in a number of biological and pathological processes. As such, several pilot studies have demonstrated a potential association of GT catechins (mainly EGCG) in the modulation of oxidative damage in male and female infertility, as discussed later [Awoniyi et al., 2012; Ding et al., 2015; Sato et al., 2010; Spinaci et al., 2008; Wang et al., 2007; Wittayarat et al., 2013]. EGCG can chelate metals such as iron, copper, chromium and cadmium [Hyung et al., 2013; Mandel et al., 2008; Pirker et al., 2012]. It reduces Fe(III) and Cu(II) to Fe(II) and Cu(I), respectively. The production of free radicals from the Fenton reaction is thus inhibited.

Pharmacokinetics of GT catechins

Bioavailability (intestinal uptake) of GT catechins is as low as 1.68% [Warden et al., 2001], and the absorption takes place via passive diffusion [Konishi et al., 2003]. GT catechins, primarily EGCG, are inhibitors of intestinal sulfotransferase enzymes. These enzymes may metabolize other supplements or drugs [Tamura and Matsui, 2000], as well as exert anti-carcinogenic effects on other cells, such as colonic tumour cells via inhibiting the metabolism of some pro-carcinogens [Isozaki and Tamura, 2001]. Serum levels of GT catechins tend to be

at their peak around 2 h after oral consumption, although this can be reduced to around 1 h (Naumovski et al., 2015) by consuming them in a fasting state (Del Rio et al., 2010; Sang et al., 2008). EC and EGC exist as conjugated metabolites of either the sulfate or glucuronide (Del Rio et al., 2010) and hence are not detectable in the free form. The gallic acid moiety on ECG and EGCG prevents conjugation and keeps them in the free form. There is no impact of EC, ECG and EGC on the serum pharmacokinetics of EGCG (Henning et al., 2005). A dose-dependent increase is noted in circulating levels of EGCG with a tipping point at around 800 mg, where the amount in the blood increases more dramatically. Although EGCG can be tolerated up to 1600 mg as a single dosage, consumption of GT catechins at 800 mg has been found repeatedly safe in supplemental form (Chow et al., 2003, 2006; Nguyen et al., 2012; Ullmann et al., 2004). A body surface area reading that correlates with blood volume has demonstrated the maximum tolerable dose of EGCG at 4.2 g/m² once daily, or 1.0 g/m² thrice daily (Pisters et al., 2001). There is no report of clinical toxicity of EGCG in the literature. It is believed that the cellular concentrations of EGCG and other GT catechins are regulated, in part, by multidrug resistance associated proteins (Hong et al., 2003). Serum conjugates of EC and EGC are water soluble and hence they are excreted in the urine (Yang et al., 1998).

Cellular mechanism of action of GT catechins

To better understand the therapeutic effects of GT components in reproductive health, substantial research has been conducted to uncover the possible mechanisms of action at the cellular and molecular level. Although GT shows promising antioxidant activity, its potential beneficial effects in human reproduction have yet to be documented.

Oxidative mechanism

Oxidative addition is the addition of a substrate molecule to a transition metal complex. In this process, the metal centre is oxidized by the removal of two electrons, allowing it to take on different valencies as electrons are transferred to balance molecules for the detoxification process. Electrochemical experiments show that catechin oxidation mechanisms proceed in sequential steps and are pH dependent (Fang et al., 2003).

Antioxidative effect

The beneficial effects of EGCG most likely stem from its antioxidative activity (Bose et al., 2007). Electron paramagnetic resonance spectroscopy revealed that EGCG reacts with O₂^{•−}, leading to oxidation of the D-ring (Severino et al., 2009). Electron paramagnetic resonance has also shown that EGCG can scavenge HO[•] and O₂^{•−} (Shi et al., 2000). Hijazi and colleagues reported that GTEs consist predominantly of secondary metabolites and have antioxidant activity that is 20 times more powerful than that of vitamin C (Hijazi et al., 2015). GT catechins can act as antioxidants by requisitioning metal ions by the catechin moieties in polyphenol molecules (Perron and Brumaghim, 2009). The B-ring is the principal site of antioxidant reactions (Balentine et al., 1997; Valcic et al., 2000), and the antioxidant activity is further

augmented by the trihydroxyl structure on the B- and D-rings in EGCG. This polyphenolic structure allows electron delocalization, convening the ability to quench free radicals (Sang et al., 2002). The catechin–metal stability constant – a basic factor used to analyse the capacity of catechins for metal chelation – is similar to or even higher than the values of their widely used chelators for iron and copper (Galleano et al., 2010). Many polyphenol groups sharing the chemical characteristics of catechin could be used as chelating agents. Despite the large differences observed in the metal-chelating capacity of different catechins, they share similar molecular structures in certain aspects including the presence of 3'-4' hydroxyl groups in the B-ring, the presence of hydroxyl groups at positions 3 and 5, and the presence of the 4-oxo function. Therefore, flavonoid–metal complexes could favour the removal of the metal from the reaction milieu, thus divesting its catalytic activity (Perron and Brumaghim, 2009; Van Acker et al., 1996).

Pro-oxidative mechanism

Although many studies have documented the antioxidant properties of GT catechins, studies have also presented evidence of their pro-oxidant activity. Pro-oxidant activity is mainly caused by the metabolic conversion of catechins to pro-oxidant derivatives (Suh et al., 2010). Tea catechins are unstable and can generate ROS by undergoing auto-oxidation reactions in cell culture (Sang et al., 2005). When EGCG and EGC react with H₂O₂, the A-ring of both compounds becomes oxidized followed by decarboxylation to form two oxidation products of EGCG and one oxidation product of EGC. The reactions produce the hydroxyl radical in the presence of iron or copper (Fenton reaction) and exert a pro-oxidant effect (Zhu et al., 2000). Sang and colleagues established that the stability of EGCG depends on the concentration of EGCG, the pH of the system, the presence of oxygen, and the temperature of the incubation (Sang et al., 2005). Some studies have shown that production of ROS during auto-oxidation of tea catechins plays an important role in inducing their pro-apoptotic effects (Elbling et al., 2005; Nakagawa et al., 2004; Vittal et al., 2004; Yang et al., 2009).

Structural simulation predicted a strong affinity of EC, EGC, ECG and EGCG with lipid bilayers (Sirk et al., 2009). Nuclear magnetic resonance spectroscopy studies suggested that both ECG and EGCG can interact with phospholipid membranes (Uekusa et al., 2007). EGCG can interact with proteins and phospholipids in plasma membranes (Kim et al., 2004) and bind with albumins (Nozaki et al., 2009).

GT and male infertility

Association between ROS and male infertility is shown by several studies. Mahfouz et al. (2010) observed that in infertile men with various levels of OS, sperm motility was affected by seminal ROS. An association between high seminal ROS and sperm DNA fragmentation was also noted. Increased level of ROS was also reported in an asthenozoospermic man with repeated ICSI failure (Dada et al., 2011). It was suggested that high seminal ROS level with mitochondrial DNA mutations and higher sperm DNA fragmentation level results in decreased ATP production leading to sperm motility defects.

Sperm viability measures an intact plasma membrane, which is susceptible to disruption by ROS (Cocuzza et al., 2007). Supplementation of sperm storage media with tea extract and other supplements

has shown a dose-dependent increase in sperm viability [Lombardo et al., 2012], which may contribute to the success of IVF programmes, particularly in cases of idiopathic infertility. Comparatively lower concentrations of EGCG (2 μ M and 20 μ M) improved semen quality by increasing the motility, viability and known hallmarks of sperm capacitation in men through oestrogen receptors, whereas a higher concentration (60 μ M) exerted the opposite effect [De Amicis et al., 2012]. Supplementation with exogenous EGCG (50 mg/kg 3 days prior to start of intermittent irradiation treatment of 2 Gy for 24 days) protected against short-term germ cell loss and attenuated ionizing radiation-elicited testicular OS in mice. In addition, long-term EGCG use is believed to ameliorate ionizing radiation-induced blood-testis barrier permeability and suppress testicular steroidogenesis, thus exerting a stimulatory effect on the spermatogenic recovery and preventing germ cells from radiation-induced cell death [Ding et al., 2015]. A two-generation teratogenicity and reproductive toxicity study to evaluate the safety of EGCG revealed no adverse effect in rats fed 1200, 3600 or 12,000 ppm EGCG, although the highest dose was found to reduce the growth rate of offspring and slightly increase pup loss [Isbrucker et al., 2006].

Animal models have been used in a number of studies to evaluate the impact of GTE in improving sperm quality. These are expected to have potential benefit in the management of male infertility, particularly in cases of idiopathic infertility. Awoniyi and coworkers compared the modulation of OS by GTEs in rat sperm after 10 weeks of supplementation while OS was induced during the last 2 weeks. Male rats supplemented with fermented rooibos and green rooibos had a significantly higher sperm count and motility than rats supplemented with Chinese GT, rooibos and GT supplements. Rooibos extracts are believed to offer protection against induced oxidative damage by increasing the antioxidant defence mechanisms and thereby improving sperm quality and function [Awoniyi et al., 2012].

Abshenas and colleagues noted that GTE reversed the adverse effects of hyperthermia on semen parameters. Rat scrotum was submerged in a water bath (42°C) for 20 min (as opposed to 23°C in a control group), after which all the animals received GTE (500 and 750 mg/kg) administration orally for 49 consecutive days. Semen parameters (motility, concentration and hypo-osmotic swelling) declined in all the animals for the first 14 days. Subsequently, a partial recovery was observed at day 42 in the control animals, while a significant recovery occurred within 28 days in GTE administered groups [Abshenas et al., 2012].

Zanchi et al. assessed the effect of GT infusion (250 mg/kg for 14 days) in minimizing cyclophosphamide-induced damage in mice. Cyclophosphamide is an antineoplastic and immunosuppressive agent used in the treatment of various types of tumours, organ transplant rejection, as well as in the treatment of autoimmune diseases; it is known to be cytotoxic to the reproductive system. In this study, cyclophosphamide was intraperitoneally injected on day 14. The authors also used high-performance liquid chromatography to evaluate the catechin concentration in the reproductive system of the mice [Zanchi et al., 2015]. GT significantly reduced lipid peroxidation, protein carbonylation and DNA damage and restored glutathione peroxidase and glutathione S transferase activity in the mice testes, apart from considerably increasing the sperm concentration in the epididymis [Zanchi et al., 2015]. GTE was also shown to protect against damage induced by doxorubicin (anthracycline antitumour antibiotic used to treat leukaemia and other forms of cancer) in mice testes. Sperm density and motility were significantly higher in the GTE group (200 mg/kg administered orally) than in the controls (0.15 mg/kg of

doxorubicin twice weekly for 5 weeks co-administered intraperitoneally). Furthermore, histological analysis confirmed that germ cell damage was greatly attenuated and telomerase activity was significantly increased in the GTE group [Sato et al., 2010]. GTE can also attenuate acrylamide-induced testicular damage in albino rats, as serum testosterone level and testicular histopathology improved even after acrylamide exposure for 90 days [Yassa et al., 2014]. In another study, supplementing canine semen with GTE after long-term (4 weeks) storage at 5°C in Tris-egg yolk extender at 1 mg/ml was found to improve sperm viability [Wittayarat et al., 2013]. On the contrary, a study investigating the effect of GTE supplementation (2.5%, 5% and 10%) on the quality of cryopreserved boar semen reported no improvement in semen quality parameters, even though lipoxidation was reduced at the 5% concentration [Gale et al., 2015].

Despite the beneficial aspects of GT as mentioned above, possible negative effects of GT on male reproduction and fertility have been reported by other authors. At a relatively high dose (equivalent to five cups or more of tea per day for 26 days), GTE caused impairment of morphological and functional status of rat testis, including the inhibition of spermatogenesis [Chandra et al., 2011; Das and Karmakar, 2015].

The studies discussed in this section indicate a potential role of GT catechins in sperm storage media and/or sperm cryopreservation. GT may also confer protection against OS-inducing agents and OS-associated damage.

GT and female infertility

Every month, a cohort of oocytes begins to grow and enlarge in the ovary, but meiosis I resumes in only one of them – the dominant oocyte. Meiosis is initiated by an increase in ROS and inhibited by antioxidants. In contrast, the progression of meiosis II is promoted by antioxidants, signifying that there is an intricate relationship between ROS and antioxidants in the ovary [Behrman et al., 2001]. This relationship is exploited by clinicians in assisted reproductive techniques to overcome idiopathic infertility.

OS is one of the main factors responsible for the reduced competence and lower quality of in-vitro-produced bovine embryos [Wang et al., 2014]. The success rates of IVF depend on antioxidant supplementation to neutralize the effect of ROS on oocyte and embryo quality [Agarwal et al., 2008]. Vahedi and colleagues found that adding growth factors, oestradiol, gonadotrophins and antioxidants improves in-vitro maturation of oocytes in a bovine model [Vahedi et al., 2009].

Several researchers have identified the role GT plays in female reproduction and fertility. A significant improvement was noted in the blastocyst development rate and pregnancy rates in a bovine model after the addition of 15 μ M EGCG to the culture media. Their results demonstrated that GT catechins have two different actions: an antioxidant effect at a lower concentration (10 mg/ml) and a pro-oxidant effect at a higher concentration (25 mg/ml) [Wang et al., 2007]. In agreement with these observations, another study reported that EGCG supplementation to the culture media at a lower concentration (10 mg/ml) during IVF increase the fertilization rate whereas a higher EGCG concentration (25 mg/ml) decreases the percentage of fertilized porcine oocytes [Spinaci et al., 2008]. Yavari et al. (2010) also demonstrated that culture medium with higher concentrations of EGCG (10 and 50 μ M) is apparently harmful for in-vitro development of porcine parthenogenetic embryos. On the other hand, a lower con-

centration of EGCG (10 µg/ml) in the culture medium could improve the developmental competence of porcine oocytes. Supplementation of EGCG at a higher concentration (50 µg/ml) in in-vitro maturation medium marginally increased the porcine blastocyst development rate even though ROS levels were reduced, suggesting that higher concentrations of EGCG are not beneficial to oocytes (Li et al., 2014b). Animal studies were also used by other investigators to examine the impact of EGCG on oocytes and subsequent embryonic development. Roth and coworkers observed that EGCG, injected into female mice at a low concentration, improved embryo quality. They injected female mice with 0.4 ml EGCG (100 mg/kg body weight). When the animals were exposed to heat stress, blastocyst development competence increased, as denoted by an increased total cell number and percentage of embryos that underwent hatching (Roth et al., 2008). Adding a low concentration of GTE (0.3 mg/ml) as a source of antioxidant in maturation medium also increased the maturation rate of oocytes and improved morula and blastocyst formation rates in sheep (Barakat et al., 2014).

Endometriosis is an important cause of infertility and pelvic pain in females. Matsuzaki and Darcha treated endometrial tissue from patients aged 20–37 years undergoing laparoscopy for endometriosis and found that EGCG significantly inhibited cell proliferation and the migration and invasion of endometrial and endometriotic stromal cells (Matsuzaki and Darcha, 2014). A similar study reported that EGCG can inhibit cellular proliferation via inhibition of extracellular signal-regulated kinase (ERK) activation and induction of apoptosis through ROS generation in human tissue (Manohar et al., 2013). Others suggested that ROS generation plays a key role in the apoptosis induced by various anticancer agents (Jaganathan and Mandal, 2010; Simbula et al., 2007). EGCG was found to reduce the size of endometriotic implants by inhibiting cell proliferation and angiogenesis and inducing apoptosis in endometriotic implants in animal models (Laschke et al., 2008; Ricci et al., 2013; Xu et al., 2009, 2011).

Although EGCG is a strong antioxidant (Shi et al., 2000), it may retain significant pro-oxidant activity in certain cases (Azam et al., 2004). Copper-mediated oxidation of EGCG leads to the formation of polymerized polyphenols, which causes the induction of ROS. This is considered one of the mechanisms responsible for inducing apoptosis in endometrial carcinoma cells. When human endometrial cells were treated with EGCG (100, 125 and 150 µM) to examine its potential in inhibiting ERK activation, it reduced the expression of target genes involved in cell proliferation and cell survival (Azam et al., 2004). However, the significance of using EGCG to improve fertility in women with endometriosis is debated.

The potential use of GT catechins in assisted reproduction appears promising. Oocyte and embryo qualities may be improved with addition of GT catechins in the media. Attention to the concentration is of utmost importance because high concentrations of catechins may exert an opposite deleterious effect. The in-vivo use of GT catechins in female reproductive health is unclear. Although the possible benefit of GT catechins on female sub-fertility related to endometriosis is suggested by some authors, the bioavailability and effective dose of GT catechins and its use in sub-fertility associated with OS or other female factors are unclear.

Clinical use of GT

Tissue culture media 199 (TCM-199), which is used to preserve reproductive cells, contains catechins that occur in GT (EC, EGC, ECG

and EGCG) (Rozeboom, 2012). GTE supplementation with TCM-199 at 0.3 mg/ml improved the in-vitro maturation and embryo development of sheep cumulus oocyte complexes to blastocyst formation (Barakat et al., 2014). The use of GT components seems promising in the field of human assisted reproduction although its clinical use has yet to be reported. Further research is essential to determine the potential bioactivities and overall significance of GT in human fertility. Clinical use of GT products is only possible after the dosage, specificity, potency, feasibility and short- and long-term side effects of GTEs in humans have been adequately addressed. The opposing effects of GTEs at high and low concentrations in a biological system illustrate the complex interplay that occurs among different factors and the importance of precise delivery of GTEs at a certain concentration.

An association between high seminal ROS and sperm DNA fragmentation was noted (Mahfouz et al., 2010). High sperm DNA fragmentation level leads to decreased ATP production and may in turn decrease sperm motility. Such cases of infertility may qualify for oral supplementation with GT catechins, which seem suitable in patients diagnosed with idiopathic infertility.

GT may also exert some degree of protection to reproductive health, particularly in patients who are regularly exposed to gonadotoxin(s), as shown by animal studies. It is critical to realize that many of the studies discussed in this review included the effects of GT catechins in in-vitro culture systems whereas more research focused on the in-vivo effective dosage is warranted to elucidate the potential mechanisms of biological action and define clinically quantifiable fertility benefits. Definitive conclusions concerning the treatment of infertility using GT must arise from well-designed observational epidemiological studies and interventional trials.

Conclusions

GT supplementation has the potential to reduce OS levels and improve the quality of gametes in both males and females. At lower concentrations, GT catechins exhibit positive effects on sperm and oocyte parameters whereas they largely elicit an opposite action at comparatively higher concentrations. GT supplementation in males can significantly improve sperm parameters such as motility and viability. In females, significant improvement has been noted in oocyte and embryo quality as well as in fertilization and clinical pregnancy rates as suggested by animal studies (Figure 3). The EGCG has cell type- and environment-specific responses because the gene expression and signalling molecules are differentially regulated. Therefore, further investigations are necessary to understand how GT catechins (especially EGCG) act in reproductive cells *in vivo*. More clinical intervention trials are needed to elucidate the mechanism of action of GT in humans. Finally, a key question needs to be answered: how long does the effect of exogenous antioxidants last in reproductive cells and tissues after GTE are removed from the body? GT could be of considerable importance in the management of male and female infertility if it has long-term effects. An understanding of these pathways is important in developing treatment strategies against OS and infertility. More studies are warranted to fully reveal the molecular mechanisms behind the beneficial effects of GT on reproductive cells. The in-vivo effects are important for developing therapies for infertility, whereas the in-vitro

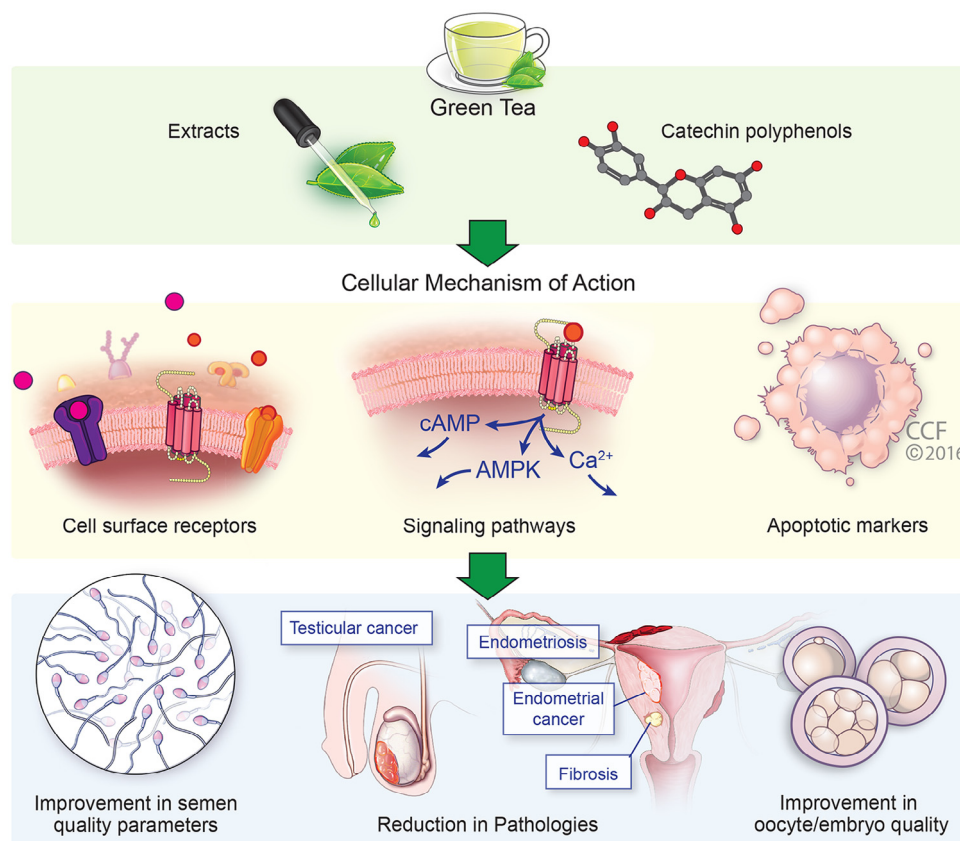


Figure 3 – Role of green tea extract and catechin polyphenols on reproductive functions and cellular mechanism of action. The figure indicates the possible cellular mechanism of action of green tea catechins via interactions with proteins and/or lipids in the plasma membrane, as well as apoptotic markers. It is postulated that through such interactions the green tea catechins are capable of improving semen, oocyte and embryo quality, thereby bringing about reduction in associated pathologies in the male and the female.

effects are relevant to sperm cryopreservation and assisted reproduction technologies. The use of GT catechins in assisted reproduction and sperm cryopreservation seems a promising area of development based on the current evidence. On the other hand, oral supplementation with GT catechins may serve as a trial for couples who suffer from idiopathic infertility. However, the dosing of GT catechins is of concern because high concentrations may lead to deleterious effects. Carefully designed dose–response studies in humans are required before GT catechins can be incorporated into the clinical management of infertile couples.

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