Abstract

Human reproductive health is an integral part of personal well-being that can markedly impact upon individuals, couples, families and the wider society. It is estimated that infertility affects 13 to 15% of the world’s population and with parents often deciding to delay planning for pregnancy until later in life, age-related concerns about the viability of female oocytes, in particular, are at the forefront of in vitro fertilisation (IVF) research. It is well regarded that approximately 30% of human infertility is a result of female-related issues; 30% to males, and 30% to a combination of male and female problems. In 10% of cases, there is no recognizable cause. A common underlying factor in both female and male fertility is an increase in reactive oxygen and nitrogen species (ROS/RNS), whilst analysis of the etiopathogenesis of pregnancy reveals that excessive levels of ROS (which breach endogenous antioxidant capacity) are an impellent factor affecting reproduction.

Molecular hydrogen (H₂) is emerging as a novel therapeutic gas. H₂ is an uncharged, non-polar, diatomic molecule with a low molecular weight (2.016 g/mol). Such characteristics make H₂ favourable for use in medical contexts as they allow the compound to diffuse through both cellular walls and phospholipid membranes including those that occur around organelles, the endoplasmic reticulum, mitochondrion and the nucleus. Hydrogen therapies such as oxyhydrogen inhalation and consumption of hydrogen-rich water, act as novel and non-toxic antioxidant and anti-inflammatory treatments, with both clinical and empirical research confidently suggesting such therapies may be beneficial to human health, reproduction and prosperity.

This mini-review focuses on the role of oxidative stress in conditions of the female and male reproductive systems and discusses the role of H₂ as a suitable antioxidant able to remediate the sequelae of poor reproductive health.

Keywords: Antioxidant; Anti-inflammatory; Infertility; Reproductive health; Molecular hydrogen.

Introduction

At physiological concentrations, reactive oxygen and nitrogen species (ROS/RNS) act as molecular mediators of cellular signal transduction pathways involved in the regulation of wider systemic processes involved in both female and male reproductive systems. To illustrate, the hypothalamic–pituitary–gonadal axis, responsible for the production of gonadotrophin hormones including oestrogen and testosterone can be disrupted by heightened ROS activity, and result in dysfunctional reproductive hormone signalling [4]. As raised levels of ROS, such as the highly reactive hydroxyl radical (•OH), are known to damage essential cellular structures and negatively impact energy-producing processes that occur within the mitochondria [5], it is unsurprising that individual reproductively, as well as...
the wider reproductive system, can be substantially affected by oxidative stress. Cellular accumulation of ROS/RNS impairs the function of energy dynamics, protein synthesis and activity, and can affect the structural integrity of the cytoskeleton and cellular membranes. If prolonged, such disturbances can negatively augment local tissue and wider systematic functions and inward the inflammatory response. Therefore, targeting underlying oxidative stress, inflammation, or both, is essential to consider when addressing mammalian reproductive dysfunction.

Since 2007 and the realisation that molecular hydrogen (H₂) is an effective antioxidant in biological systems, interest has been growing in the therapeutic value of this universal compound. A multitude of studies in this field demonstrate that H₂ administration can markedly reduce cellular oxidation through its influence on epigenetics [6], the redox environment [7] and signal modulation [8]. H₂ is Generally Regarded as Safe (GRAS) by the Food and Drug Agency (FDA) in the United States [9] and over 100 Worldwide clinical studies report no severe or long-lasting adverse effects of hydrogen treatments [10,11]. Research into the molecular effects of H₂ describes a notable reduction in harmful ROS/RNS levels, and diminished expression and release of pro-inflammatory chemokines (e.g., monocyte chemoattractant protein-1 (MCP-1)), and cytokines (e.g., TNF-α, IL-6) [12].

**Female reproduction**

In females, through remediation of excessive ROS/RNS, oxyhydrogen can protect against inflammatory reproductive autoimmunity and premature ovarian failure, substantial causes of infertility in women under 40 years old as both conditions are related to increased ROS/RNS levels and a hyperactive inflammatory response [13,14]. Disproportionate oxidation of cellular components is known to irreversibly damage the female oocyte by disrupting cellular membrane integrity, reducing DNA repair capacity and through the oxidation of essential proteins, actions that ultimately impair cellular functionality and the oocyte’s potential to become fertilised. Studies note that increased oxidation in this environment can lead to embryo fragmentation [15] as well as developmental abnormalities [1]. Oxidative stress is also regarded as a contributing factor to spontaneous and recurrent miscarriages through its interactions with hormonal signalling and endometrial structure and function [14], with oxidation of essential cellular components also having been demonstrated to affect embryo stability and transplant success in in vitro fertilisation (IVF) candidates [1]. To illustrate the wider impact of oxidative processes in the female reproductive system, scientific investigations into the impact of oxidative stress on female fertility [16,17] note that the reductive/oxidative (redox) status of the follicular fluid surrounding the oocyte within the ovarian cavity, is also correlated with the success of IVF implantation, with more than 30% of cells deemed unproductive in an increased oxidative environment [16]. Women experiencing fertility issues are often advised to increase their intake of digestible antioxidants such as Vitamins A and E, and whilst these can be effective, the cumulative effect of exogenous antioxidants can be detrimental long-term. Research into the effects of H₂ consumption reveals not only that H₂ upregulates reproductive hormone signalling, but also enhances natural cellular defences and reduces cell death through apoptosis in ovarian granulosa cells [18].

Endometriosis is a chronic inflammatory condition that, through extraneous implantation and growth of endometrial tissue on the exterior of the uterus, can be the direct cause of haemorrhage, infertility and debilitating pelvic and abdominal pain. Endometriosis affects approximately 10% of Women of reproductive age who can experience both physical and psychological symptoms ranging from gastrointestinal distress, fatigue and, or nausea, to anxiety, depression and withdrawal from daily routine [1]. Such variation in symptomology makes diagnosis and treatment of endometriosis challenging for physicians, and as yet, there is no cure, whilst treatments focus on the relief of individual symptoms through alternative and complementary therapies, or through hormonal and, or surgical interventions [2]. As such events can appreciably impact upon Women’s productivity, relationships and quality of life, it is imperative that a working solution that can improve reproductive health and overall well-being is found.

Pain is another symptom of endometriosis that is widely experienced and difficult to manage without the assistance of pharmaceuticals. On a molecular level pain can be caused by irreversible nitrosative damage to cellular structures by peroxynitrite (ONOO⁻), a reactive anion formed through a spontaneous reaction between nitric oxide (NO) and superoxide (O₂⁻). Peroxynitrite is known to enhance nociceptive communication and promote cytokine release, many of which can directly interact with transient receptor potential cation channel subfamily V member 1 (TRPV1) receptors [19], activating further chemokine (e.g., IL-8) and cytokine production (e.g., IL-6) via stimulation of intracellular MAPK cascades. Likewise, through its effect on intracellular signalling cascades, H₂ inhalation is shown to downregulate the expression of pro-inflammatory cytokines associated with the perception of pain, including pyrogens interleukin-1-beta (IL-1β) and interleukin-6 (IL-6), and TNF-α, a pro-inflammatory peptide which has an inducive role in acute inflammatory reactions and both chronic and systemic inflammation [20-22]. In laboratory models of endometriosis, inhalation of oxyhydrogen gas (33% O₂/66% H₂) was shown to significantly reduce explanted endometrial tissue, inhibit pro-inflammatory reproduction and upregulate expression of endogenous antioxidants including catalase, glutathione peroxidase and superoxide dismutase [5]. Importantly for female health, oxyhydrogen inhalation had no discernible effects on oestrogen cycling. Interestingly, the experiments used a nitric oxide and oxygen (NO/O₂) mixture as an antithetical balance and concluded that H₂ was determining antioxidant factor as no improvements were observed in the NO/O₂ control group [5]. Further empirical investigations reveal that H₂ can inhibit the growth of malignant endometrial cells by modifying the redox environment in favour of pyroptosis [18], thereby promoting the expeditious clearance of atypical endometrial cells.

**Male reproduction**

In males, elevated levels of ROS and inflammation are also known to affect the morphological alterations required for spermatozoa maturation, these include such processes as compaction of DNA and flagellar modification. In cases of male infertility, oxidative stress is known to negatively impact the fluidity of the plasma membrane, the production of ATP and affect the integrity of DNA in the nucleus of spermatozoa. Such oxidative conditions typically lead to diminished motility and a reduction in overall sperm count, two primary sources of male infertility [19]. As a result of its low molecular weight, H₂ is highly diffusible and is able to negotiate passage through the blood/brain, placental and testes barriers.

Up to 80% of infertile males present with a marked increase in ROS in the seminal fluid. This is an important factor as sper-
matozoa-producing Leydig cells are particularly susceptible to lipid peroxidation due to the high content of polyunsaturated fatty acids [20]. One way in which H₂ can improve aspects of male fertility is through an increase in spermatozoa motility linked to the protective effects of H₂ administration within the mitochondria, preserving ATP synthesis and mitochondrial membrane potential [21]. Although the primary mode of action by which H₂ exerts its effects is yet to be well understood, initial studies have identified that in cases of male infertility, H₂ is able to increase the antioxidant capacity and reduce the expression of pro-apoptotic proteins (e.g., Bak/ cleaved caspase 3), whilst concomitantly increasing testosterone levels in mammalian models of impaired reproducibility. An in-depth multiomics study conducted by Ma et al., [22] noted H₂ had a strong association with metabolic pathways, regulating the synthesis of betaine aldehyde, an intermediary in glycine, serine and threonine metabolism; [23] N6-(isopentenyl) adenosine, and anti-inflammatory agent; [24] and 3-(indole)propionic acid, an effective antioxidant [25].

Furthermore, ROS being responsible for modulating the cardinal processes of spermatogenesis including capacitation, hyperactivation, acrosome reaction and sperm–oocyte fusion (successful fertilisation), are able to influence numerous physiological processes downstream of spermatogenesis [4], affecting both sperm vitality and motility. In addition to the favourable antioxidant and anti-inflammatory effects of oxy-hydrogen inhalation, research focussing on dysregulated hormonal signalling shows that H₂, in particular, may enhance the production of testosterone via positive regulation of intrinsic signalling cascades including calcium (Ca²⁺), cyclic adenosine 3,5-monophosphate (cAMP) and subsequent mitogen-activated protein kinase (MAPK) cascades [21], MAPK signalling pathways are important within the reproductive system as they regulate a wide variety of processes including cellular apoptosis, differentiation, inflammation, proliferation and stress responses, under both normal and pathological conditions. Further studies report that H₂-inclusive therapies can also improve blood flow to the sexual organs, ensuring adequate oxygen and nutrient availability in all parts of the reproductive system [26,27]. To illustrate, a recent study on the effects of H₂ on sexual organ homeostasis describes H₂ as having propitious consequences on endocrine, gynaecological, genetic, neurological and psychological function, [27] improving many aspects associated with successful procreation.

**Conclusion**

In conclusion, human reproductive health is an integral part of personal well-being that can markedly impact individuals, couples, families and the wider society. Hydrogen therapies such as oxyhydrogen inhalation and consumption of hydrogen-rich water, act as novel and non-toxic antioxidant and anti-inflammatory treatments, with both clinical and empirical research confidently suggesting such therapies may be beneficial to human health, reproduction and prosperity.

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