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Short report

Title: The Effects of Molecular Hydrogen Therapies on Fertility.

Authors: Grace Russell and Alexander Nenov

G. Russell: BSC (Hons.) Biomedical Science, MSci Biomedical Science, PhD Researcher.

School of Applied Sciences,

University of the West of England

Coldharbour Lane,

Bristol, UK, BS16 1QY.

Email: Grace.russell@uwe.ac.uk

Tel: (+44) 0117 965 6261

A.Nenov: Deputy Managing Director,

Water Fuel Engineering,

Unit 3, Church Street

Wakefield, WF1 5QY

Email: Nenov.alexander@waterfuelengineering.com

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 (H_2) is emerging as a novel therapeutic gas. H_2 is an uncharged, non-polar, diatomic molecule with a low molecular weight (2.016 g/mol). Such characteristics make H_2 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 H_2 as a suitable antioxidant able to remediate the sequelae of poor reproductive health.

KEYWORDS

Antioxidant, Anti-inflammatory, Infertility, Reproductive Health, Molecular Hydrogen.

ABBREVIATIONS

Cyclic adenosine 3,5-monophosphate (cAMP), Food and Drug Agency (FDA), Generally regarded as safe (GRAS), Interleukins (IL-), *In vitro* fertilisation (IVF), Mitogen-activated protein kinase (MAPK), Monocyte chemoattractant protein-1 (MCP-1), Reactive oxygen species (ROS), Reactive nitrogen species (RNS), Transient receptor potential cation channel subfamily V member 1 (TrpV1), Tumour necrosis factor-alpha (TNF-α).

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 reproductive cells, 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 innervate 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 (H_2) 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 H_2 administration can markedly reduce cellular oxidation through its influence on epigenetics [6], the redox environment [7] and signal modulation. [8] H_2 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 H_2 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 accumulative effect of exogenous antioxidants can be detrimental long-term. Research into the effects of H_2 consumption reveals not only that H_2 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 ($O_2^{\cdot-}$). 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, H_2 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% O_2/66% H_2) was shown to significantly reduce explanted endometrial tissue, inhibit profuse cellular 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/O_2) mixture as an antithetical balance and concluded that H_2 was determining antioxidant factor as no improvements were observed in the NO/O_2 control group. [5] Further empirical investigations reveal that H_2 can inhibit the growth of malignant endometrial cells by modifying the redox environment in favour of pyroptosis[18], thereby promoting the expeditive 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, H_2 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 spermatozoa-producing Leydig cells are particularly susceptible to lipid peroxidation due to the high content of polyunsaturated fatty acids.[20] One way in which H_2 can improve aspects of male fertility is through an increase in spermatozoa motility linked to the protective effects of H_2 administration within the mitochondria, preserving ATP synthesis and mitochondrial membrane potential.[21] Although the primary mode of action by which H_2 exerts its effects is yet to be well understood, initial studies have identified that in cases of male infertility, H_2 is able to increase the antioxidant capacity and reduce the expression of pro-apoptotic proteins (*e.g.,* Bax/ cleaved caspase 3), whilst concomitantly increasing testosterone levels in mammalian models of impaired reproductivity. An in-depth multi-omics study conducted by Ma et al.,[22] noted H_2 had a strong association with metabolic pathways, regulating the synthesis of betaine aldehyde, an

intermediary in glycine, serine and threonine metabolism;[23] *N*6-(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 H_2, in particular, may enhance the production of testosterone via positive regulation of intrinsic signalling cascades including calcium (Ca^{2+}), 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 H_2-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 H_2 on sexual organ homeostasis describes H_2 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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