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The role of glutathione in mammalian gametes

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SUMMARY

The paper reviews a recent research on the role of glutathione (GSH) in the male and female germ cells as well as during the early stages of embryo development in mammals. In both the male and female gametes, GSH is involved in the protection of these cells against oxidative damage. Glutathione has been implicated in maintaining the meiotic spindle morphology of the oocyte. After fertilization, this thiol plays an active role in the formation of the male pronucleus, and has a beneficial effect on early embryogenesis to the blastocyst stage. GSH concentrations change within the oocytes during meiotic maturation and its synthesis is regulated by gonadotropins. Furthermore, GSH concentrations in the maturing spermatozoa gradually decline during spermatogenesis. This review also addresses the important role of cumulus cells in glutathione synthesis. *Reproductive Biology 2005 5(1): 5-17.*

Key words: glutathione, spermatozoa, oocyte, mammalian gametes, fertilization, embryo

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INTRODUCTION

Glutathione (GSH), a tripeptide thiol (γ-glutamylcysteinylglycine), is the major non-protein sulphydryl compound in mammalian cells that is known to have numerous biological functions. This thiol plays a prominent role in detoxification and antioxidation of exogenous and endogenous compounds, as well as maintaining the intracellular redox status. Glutathione is a natural reservoir of reducing power, which can be quickly used by the cells as a defense against oxidative stress.

The sulphydryl group (SH) of glutathione confers its protective action against oxidative damage. Glutathione exists in two forms: the reduced form (GSH) and the oxidized form (GSSG). The protective action of glutathione against reactive oxygen species (ROS) is facilitated by the interactions with its associated enzymes, such as glutathione peroxidase and glutathione reductase.

In animal tissues, glutathione peroxidase, a selenium-containing antioxidant enzyme, catalyses the reduction of hydrogen peroxide, (and lipid peroxides) in the presence of GSH, which is converted to GSSG. In turn, GSSG is reduced by glutathione reductase in the presence of nicotinamide adenine dinucleotide phosphate [NAD(P)H], which is generated mainly in the pentose phosphate pathway, as shown in the following equations:

$$2GSH + H_2O_2 \rightarrow GSSG + 2\ H_2O$$
$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

Glutathione is a widely distributed thiol in animal organisms, not only in somatic cells but also in the gametes. This paper presents substantial information from recent studies on glutathione concentrations in the mammalian germ cells, as well as its role in the process of oocyte maturation, fertilization and the pre-implantation stage of embryo development.

GLUTATHIONE IN SPERMATOZOA

In mammalian semen, the antioxidant defense capability consists of enzymatic and non-enzymatic systems, in which the latter is represented mainly

by glutathione. The basic function of GSH in mammalian semen is related to its interactions with other systems as a preventive mechanism against ROS. This scavenging function of GSH helps to counteract the effects of oxidative stress in sperm cells, which could result in lipid peroxidation of plasmalemma, irreversible loss of motility, leakage of intracellular enzymes and damage of the chromatin [3, 24]. Glutathione levels in mammalian spermatozoa (tab.1) and seminal plasma (tab. 2) showed marked species-specific differences. Different levels of GSH may also result from applying relatively diverse methods of quantitative analysis of this thiol in the semen.

Regardless of the applied method, the following observations were recorded. A relatively high GSH level occurs in mouse spermatozoa. In contrast, only trace or insignificant amounts of GSH were found in boar and rabbit spermatozoa (tab. 1). Furthermore, in boar and rabbit spermatozoa the activity of glutathione peroxidase and glutathione reductase is also low or undetectable [23]. Evidence has been shown that GSH is present in boar seminal plasma [29, 31].

In human spermatozoa, the glutathione cycle forms a basic support system (through the removal of H_2O_2) with superoxide dismutase (SOD). Studies have shown that H_2O_2 is the most toxic reactive oxygen species in human spermatozoa [4, 18]. In human semen, glutathione concentration in spermatozoa correlated with that in the seminal plasma [27].

Interestingly, that the glutathione concentration decreased by about 75% during bovine sperm maturation from the caput epididymis spermatozoa to ejaculated spermatozoa [2]. Moreover, SOD and the intracellular GSH are also two major antioxidants that occur in bovine spermatozoa. Considerable variations between individual bulls as regards GSH concentrations in spermatozoa have been reported. In a previous study, GSH concentrations in the spermatozoa of five breeding bulls ranged from 295 to 691 pmoles/mg protein [6].

The thiol antioxidant system, represented mainly by glutathione, dominates in stallion semen and occurs in large amounts in the seminal plasma. The GSH content in this fluid is over 10-fold higher than of boar seminal plasma (tab. 2). With respect to stallion spermatozoa, there is a lack of literature regarding GSH concentration in these cells.

Table 1. Glutathione concentrations in spermatozoa of different mammalian species including men

Species	Glutathione	References
Human	5.3 ± 2.2 nmol / 10^9 sp.* (0.53 ± 0.22 nmol//10^8 sp.)**	Li [23]
	6.7 ± 0.4 nmol /10^8 sp.	Alvarez & Storey [4]
	6.2 ± 0.6 nmol /10^8 sp.	Griveau et al. [18]
	3.49 ± 0.87 nmol /10^8 sp.	Ochsendorf et al. [27]
Boar	0.3 nmol /10^9 sp. (0.03 nmol /10^8 sp.) **	Li [23]
Bull	9 µg /10^9 sp. (2.93 nmol /10^8 sp.) **	Agrawal & Vanha-Perttula [2]
	566 ± 72 pmol / mg protein	Bilodeau et al. [6]
Dog	5.3 ± 1.1 nmol /10^9 sp. (0.53 ± 0.11 nmol /10^8 sp.)**	Li [23]
Hamster	30-40 nmol / mg protein	Den Boer et al. [12]
Mouse	90 nmol /10^8 sp.	Alvarez & Storey [4]
Rabbit	0.1 nmol /10^9 sp. (0.01 nmol /10^8 sp.)**	Li [23]
	< 0.1 nmol /10^8 sp.	Alvarez & Storey [4]
Ram	4.5 ± 1.4 nmol /10^9 sp. (0.45 ± 0.14 nmol /10^8 sp.)**	Li [23]

sp.*spermatozoa; ** values in parentheses were adjusted by the author

Evidence has been shown that the rapid decline in intracellular GSH concentrations, which occur during incubation of spermatozoa in aerobic conditions, is not associated with an increase in GSSG concentrations [6]. This phenomenon can be caused by this thiol reacting with other molecules

Table 2. Glutathione concentrations in seminal plasma of different mammalian species including men

Species	Glutathione	References
Human	0.5 – 2.0 μM	Li [23]
	0.19 ± 0.11 μM	Daunter et al. [13]
	0.2 – 1.4 μM	Ochsendorf et al. [27]
Boar	185.8 ± 46.7 μM 5.7 ± 1.4 mg / 100 cm^3	Strzezek et al. [29]
Bull	13 – 19 μM	Agrawal & Vanha-Perttula [2]
	17 ± 7 pmol / mg protein	Bilodeau et al. [6]
Stallion	77.27 ± 48.0 mg / 100 cm^3	Strzezek et al. [30]

and occurring as hidden GSH [5]. The fact that proteins bind with glutathione and form mixed disulphides (protein-S-S-glutathione) is well known. On the one hand, these disulphides can protect proteins against oxidative insult. On the other hand, the above-mentioned protein complexes with glutathione are regarded as a method of storing of low-molecular weight thiol in a cell. Another possible explanation for the decrease in intracellular GSH and GSSG during aerobic incubation could be the transport of these molecules out of the cell, across the cell membranes [33].

The effect of GSH supplementation to insemination medium on the fertilization dynamics and development of the mammalian embryo *in vitro* has been reported [7]. Accumulating evidence has shown that the addition of reduced glutathione, in adequate amounts, to insemination medium during *in vitro* fertilization (IVF) did not affect the rates of oocyte penetration by spermatozoa or formation of male pronucleus. However, the presence of GSH in the insemination medium has shown to increase the blastocyst production rates, but does not affect the blastocyst cell number. Furthemore, it has been shown that intracellular GSH concentrations in oocytes were not affected by

the presence of extracellular GSH, indicating that higher blastocyst production rates do not appear to be the result of increasing intracellular GSH levels within zygotes. Even though the precise mechanism of the positive effect of GSH on these discussed processes is still unclear, it can be suggested that the extracellular GSH prevents lipid peroxidation of cellular membranes of both gametes by removing excessive ROS in the insemination medium. This scavenging function of GSH might improve fertilization and subsequent embryo development [7]. Moreover, the effect of GSH supplementation to the insemination media during IVF depends on its concentration in the media employed, and may vary between individuals [7, 21].

GLUTATHIONE IN OOCYTES

Glutathione has been shown to play an important role in oocyte maturation. The process of oocyte cytoplasmic maturation involves numerous molecular events, including synthesis of biochemical compounds, protein phosphorylation and activation of particular metabolic pathways [14, 22]. These changes are a prerequisite for normal fertilization and embryo development. The synthesis of intracellular glutathione is a critical part of oocyte cytoplasmic maturation [14]. GSH function in oocytes is mainly related to antioxidative properties and protection against ROS toxic activity.

It has been suggested that the intracellular GSH concentrations of porcine oocytes at the end stage of *in vitro* maturation (IVM) reflected the degree of cytoplasmic maturation [15]. Many authors postulated that the measurements of GSH concentration in oocytes after IVM may be a valuable indicator of the oocyte cytoplasmic maturation [1, 9, 10, 16].

The glutathione concentrations in oocytes matured *in vitro* are not relatively different among several mammalian species (tab. 3). However, GSH concentrations in matured *in vivo* oocytes are much higher than those matured *in vitro*. In a previous study, GSH concentration in porcine oocytes matured *in vivo* averaged 36.26 pmol/oocyte [8]. A possible reasons for such differences may be that during IVM, the oocytes are exposed to higher oxygen concentrations and, consequently, to ROS, in comparison to oocytes

Table 3. Glutathione concentrations in pig, cow and hamster oocytes matured *in vitro*

Species	Glutathione (pmol / oocyte)	References
Pig	4.0 ± 0.8	Yoshida et al. [35]
	6.4 ± 1.8	Abeydeera et al. [1]
	9.82 ± 0.71 9.73 ± 0.81 7.89 ± 0.66	Brad et al. [8]
Cow	4.0 ± 0.9 4.5 ± 1.1 6.6 ± 0.6	Furnus et al. [16]
Hamster	3.24 ± 0.49	Zuelke et al. [37]

during *in vivo* maturation. This is because the oxygen concentration in the lumen of female reproductive tract is only about one-third of that occurring during IVM. In a recent study [8] it has been demonstrated that in oocytes during IVM, the mobilization of intracellular GSH to protect the cells against oxidative stress may result in a dramatic decline in its levels, which is lower than that observed in oocytes *in vivo* matured. Low intracellular GSH concentrations may be responsible, in part, for lower developmental competence of porcine oocytes during IVM [8]. Moreover, lower oxygen tension during IVM of bovine oocytes is accompanied by reduced H_2O_2 concentrations in these cells, which is beneficial for developmental competence, potentially due to decreased ROS [20].

Evidence has shown that GSH is active in oocyte function, including maintaining meiotic spindle morphology. GSH protects the spindle against oxidative damage and, consequently, ensures normal zygote formation [36]. In matured oocytes GSH plays an important role in male pronucleus formation after fertilization [7, 21, 32, 37]. GSH provides reducing power needed to initiate chromatin decondensation, prior to male pronucleus formation [15, 35]. Intracellular GSH improves the ability of the embryo to alleviate

the cytotoxic effects of hydrogen peroxide which can arrest its development *in vitro* at the two-cell stage in mice [26].

Glutathione concentration in oocytes increases during IVM, reaching a peak level at the metaphase II stage (MII). Generally, GSH concentration in ovulated MII oocytes is approximately twice as high as that in immature germinal vesicle stage oocytes (GV oocytes). For example, in hamster oocytes after induction of *in vivo* meiotic maturation with hCG (human chorionic gonadotropin), GSH concentration in subsequent developmental stages increases from 1.0 pmol/oocyte in the GV stage through 1.62 pmol/oocyte in the MI stage with an early meiotic spindle of up to approximately 2.0 pmol/oocyte in MII stage [37]. However, in zygotes and embryos at the early developmental stage, the GSH level drops rapidly. Less than 10% of the GSH concentration recorded in MII oocytes remains in the blastocyst stage of pre-implantation development of hamster embryos (fig. 1). The relatively high GSH concentrations in ovulated oocytes may serve as a potential reservoir of this antioxidant for pre-implantation stage of embryo development [10, 16]. Recent studies have confirmed that, under physiological condition, the high GSH levels in matured mouse and hamster oocytes, are essential for forming male pronucleus after fertilization and promoting the early embryo development [17, 37]. In conclusion, glutathione is a potentially highly relevant biochemical marker of the viability and developmental capacity of mammalian oocytes [37].

EFFECT OF LOW-MOLECULAR WEIGHT THIOL COMPOUNDS ON GLUTATHIONE SYNTHESIS IN OOCYTES

Glutathione synthesis in oocytes may be stimulated by different low-molecular weight thiol compounds such as sulphuric amino acids: cysteine and cystine, cysteamine or β-mercaptoethanol. This phenomenon has been observed in oocytes matured *in vitro* and fertilized *in vitro*. Induced GSH synthesis by the thiol compounds enhanced GSH concentration in the zygotes, which might improve embryo development to the blastocyst stage [10, 11]. For example, in an IVM media supplemented with 100 μM

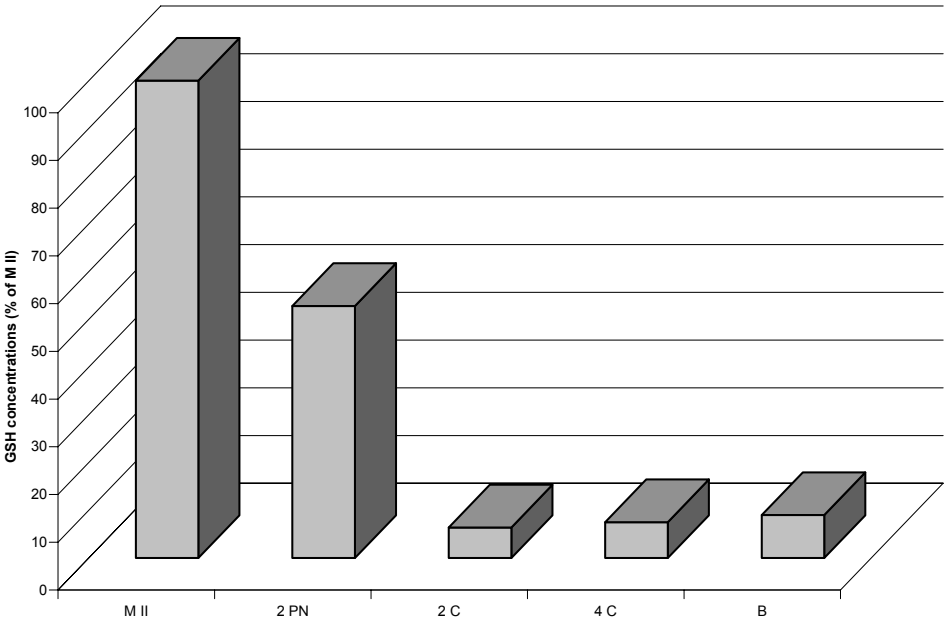

Fig. 1. Glutathione concentrations in matured hamster oocytes, zygotes and pre-implantation stages of embryos (modified, from Zuelke et al. 2003); MII – methaphase II, PN – pronucleus, C – cell embryo, B – blastocyst.

β-mercaptoethanol, 0.6 mM cysteine or 0.6 mM cystine, a significantly higher GSH concentration was observed in bovine oocytes during maturation compared with those unsupplemented. These thiol compounds have also a positive effect on the cleavage rates and the percentage of embryos reaching the morula and blastocyst stages [10]. In sheep oocytes, even though both cysteamine and β-mercaptoethanol exerted a stimulatory effect on GSH synthesis, only cysteamine had a positive effect on subsequent embryo development. An increase in intracellular GSH is concomitant with a decline in the peroxide levels in oocytes [11]. The effect of cysteine on enhanced glutathione synthesis is obvious. Accumulating evidence has shown that GSH is synthesized in the γ-glutamyl cycle, and its synthesis is dependent on the availability of amino acid precursors, such as cysteine. Cystine, on the other hand, is converted into cysteine, probably by the cumulus cells, and then incorporated into the glutathione synthesis [9]. In addition, the

other low-molecular weight thiol compounds, such as cysteamine or β-mercaptoethanol are able to reduce cystine into cysteine [10].

ROLE OF CUMULUS CELLS IN GLUTATHIONE SYNTHESIS IN OOCYTES

Cumulus cells surrounding oocytes are structurally and metabolically linked with the gametes and involved in the process of glutathione synthesis [25]. It has been shown that the cumulus cells play an important role in the intracellular GSH synthesis in the oocytes of cow [9], pig [34] and hamster [37]. Cumulus cells intensify the stimulating effect of low-molecular weight thiol compounds, such as cysteine or cystine on GSH synthesis in female gametes. Glutathione synthesis has been shown to occur simultaneously in both the oocytes and their enclosed cumulus cells, and during meiotic maturation these cell types have a similar profile of changes [16, 34, 37].

CONCLUSIONS

This review presents a comprehensive evaluation on the role of glutathione as an important low-molecular weight thiol antioxidant. Glutathione is a natural antioxidant present in both the male and female gametes, and its level varies widely. GSH has been confirmed to play an important role in maintaining the biological value of both male and female germ cells, and has been implicated in the fertilization process and early embryo development. There is evidence that intracellular GSH concentrations in oocyte may be a good biochemical marker to assess the degree of cytoplasmic and nuclear maturation in mammals.

The mechanisms that regulate glutathione level and oxidative stress during fertilization and early embryogenesis in mammals warrant further study [19, 28]. An understanding of the dynamics of GSH concentrations during oocyte maturation and embryo development may help to alleviate infertility and increase efficiency of embryo production.

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