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The Impact of Heavy Metals in Impairment of Spermatogenesis and Sperm Density in the Human

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Abstract

Spermatogenesis is the most important stage in male fertility. In the present study, many factors affecting spermatogenesis were evaluated in seminal plasma and homogenates of sperms from fertile and infertile males. The levels of some heavy metals, malondialdehyde (MDA) and reduced glutathione (GSH) as well as superoxide dismutase (SOD) activity were measured in both seminal plasma and homogenate supernatant of sperm from fertile and infertile males. Two hundred and sixteen human semen samples were collected and divided into five groups: group I (GI) included 70 semen samples as the control, and four infertile groups (GII - GV) according to WHO criteria. The levels of Fe2+, Cd2+ and MDA in seminal plasma and sperm homogenate supernatant of infertile groups were highly and significantly increased compared to their levels in control. However, GSH level and SOD activity in seminal plasma and sperm homogenate supernatant of infertile men were highly and significantly decreased compared to the control. In conclusion, the levels of heavy metals and oxidative stress were associated with human male spermatogenesis dysfunction and might be useful tools in predicting sperm density.

Keywords: Spermatogenesis, Heavy Metals, Superoxide Dismutase, Reduced Glutathione.

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Introduction

Reproductive health is a branch of life (Moeini, et al., 2015). Infertility affects 15% of couples, and in 30% of these couples, the cause of infertility has been associated with aberrations found in the male partner, termed as male infertility. Defective sperm function has been the most common cause of male infertility (Naz, 2017; Naser and Alhabbash, 2016). One of the factors that can potentially cause spermatogenesis dysfunction is the oxidative stress (Ashok et al., 2014; Eisa et al., 2016) .Oxidative stress is a condition associated with an increase in the rate of cellular damage induced by oxygen and oxygen-derived oxidants. These oxidative molecules have been commonly known as reactive oxygen species (ROS), which have been produced primarily by the physiological metabolism of O_2 in cells under aerobic conditions (Makker et al., 2009; Schieber and Chandel, 2014) . Normally a balance is maintained between the amount of ROS produced and that scavenged by a set of different antioxidants. The disturbance of this equilibrium leads to oxidative stress (Desai et al., 2010; Moon et al., 2012). Lipid peroxidation (LPO) of sperm membrane has been considered to be the key mechanism of ROS-induced sperm damage leading to loss of the germinating ability or infertility. Malondialdehyde (MDA) is an end product of LPO, and is considered as one of the important markers of oxidative stress. High levels of MDA have represented a high rate of cellular peroxidative damage (Makker et al., 2009). The protection against ROS is of critical importance, and can be provided by both enzymatic [e.g. superoxide dismutase (SOD)] and non-enzymatic [e.g. reduced glutathione (GSH)] antioxidants. GSH plays a central role in the defense against oxidative damage and toxins due to its ability to react directly with ROS by its free sulphydryl group (Eskiocak, et al., 2005; Valko et al., 2016). SOD is an important antioxidant enzyme that involves in the inhibition of sperm membrane LPO (Ighodaro and Akinloye, 2017).

It catalyzes the dis-mutation of the highly reactive superoxide anion radical (O_2^-) to form O_2 and H_2O_2 (Dorostghoal, et al., 2017; Halliwell and Gutteridge, 2015; Carocho and Ferreira, 2013). Another factor that may cause human infertility is the exposure to heavy metals either voluntarily through supplementation or involuntarily through the intake of

contaminated food and water or contact with contaminated soil, dust, or air. The sources of heavy metals that are released in the environment vary (Aljedani, 2017). Some of these metals get involved in the production of oxidants, and enhance the rate of oxidative stress leading to male infertility (Rui et al., 2017; Rodjana Chunhabundit, 2016).

The aim of this work was to study the role of heavy metals in oxidative stress production, and consequently their effects on human spermatogenesis. Additionally, this paper aimed at illustration of the role of seminal plasma in sperm protection.

Subjects and Methods

Semen collection:

Semen samples were collected by masturbation after 3 days of sexual abstinence. Sperm density was analyzed by a computer assisted sperm analyzer (CASA, Cell Soft 3000, Cryo Resources Co., U.S.A.). The samples with leukocyte counting more than 5/H.P.F were excluded. The semen samples were preserved at -180 °C until assay (Murawski, M., et al., 2007).

Study design:

Two hundred and sixteen human semen samples were classified into five groups based on WHO criteria (Rowe and Comhaire, 2000).
Group I (GI): 70 normozoospermic samples as control.
Group II (GII): 48 asthenozoospermic(A) samples.
Group III (GIII): 18 oligo-asthenozoospermic(OA) samples.
Group IV (GIV): 41 oligo-astheno-teratozoospermic(OAT) samples.
Group V (GV): 39 azoospermic(Azoo) samples.

Semen preparation

The semen samples were centrifuged at 5000 r.p.m. for 15 minutes to separate spermatozoa from seminal plasma. The supernatant seminal plasma was then carefully removed, and transferred to test tubes. The spermatozoa sediment was washed twice with physiological saline solution to remove the remained seminal plasma. The separated spermatozoa pellet was homogenized by a variable speed homogenizer for 5 minutes in ice bath using 2ml homogenizing buffer (pH 7.4) consisting of mannitol, sucrose and EDTA mixed together with tris-base and 0.1 % tritonX-100. The homogenate of the pellet was then centrifuged for 10 minutes at 5000 r.p.m. in a cooling centrifuge to discard debris, if any. The supernatant was then used for all the measurements (Dandekar et al., 2002).

Biochemical analysis

Protein content in the seminal plasma was determined by the Biuret reaction according to the method of Gornall et al. (1949). LPO was measured by the reaction of thiobarbituric acid with MDA according to the method of Storey (1997). GSH level was determined by the method of Beutler et al. (1963). SOD activity was assayed by the modified method of Dechatelet et al. (1974). Heavy metals were determined by Atomic Absorption Spectrophotometery (Rosner and Gorfien, 1968). Protein electrophoresis was carried out using polyacrylamide gel electrophoresis (Keith and John, 1994).

Statistical analysis

The statistical analysis was performed using Instate® program (version 2.03; from GraphPad software, U.S.A.). The correlations between parameters were assessed using the Microcal™ Origin® program`s coefficient (r- value) (version 0.6; from GraphPad software, U.S.A.). All parameters were expressed as mean ± standard deviation.

RESULTS

The levels of Fe and Cd in sperm homogenate of G_{II}-G_{IV} were highly increased compared to the corresponding controls. Also, the levels of Fe and Cd in seminal plasma of all infertile groups (G_{II}-G_V) were highly increased compared to the control **(Table 1)**. In seminal plasma, the levels of MDA in G_{II}-G_V were highly increased compared to the control. However, the levels of GSH and the activity of SOD in seminal plasma of G_{II}-G_V showed highly decreases when compared to the control **(Table 2)**.

The level of GSH in sperm homogenate supernatant of G_{II}-G_{IV} was highly decreased compared to the control. However, MDA level in sperm homogenate of G_{II}-G_{IV} was highly increased compared to the control. Also, the activity of SOD in sperm homogenate of G_{II} was lower than G_I **(Table 3)**.

Table 1: Mean values of Fe and Cd in spermatozoa homogenate supernatant and seminal plasma of groups I-V.

Parameter / *Group*	Spermatozoa homogenate Fe µg x 10^2/mg protein	Seminal plasma Fe µg/mg protein	Spermatozoa homogenate Cd µg x 10^2/mg protein	Seminal plasma Cd µg/mg protein
G_I(Control) M ± S.D. n.	1.7 ± 0.54 18	0.68 ± 0.13 17	0.021 ± 0.005 23	0.015 ± 0.005 16
G_{II}(A) M ± S.D. n.	2.6 ± 0.76** 9	0.95 ± 0.19** 11	0.061 ± 0.02** 12	0.065 ± 0.017** 11
G_{III}(OA) M ± S.D. n.	2.56 ± 0.72 ** 5	1 ± 0.17 ** 6	0.14 ± 0.03** 5	0.114 ± 0.018** 6

G_{IV}(OAT) M ± S.D. n.	3.78 ± 1.08** 18	1.36 ± 0.29** 15	0.29 ± 0.1** 16	0.072 ± 0.013** 17
G_{V}(Azoo) M ± S.D. n.		1.50 ± 0.39** 17		0.083 ± 0.028** 16

* significant ** highly significant - (n) number of cases

Table 2: Mean values of MDA and GSH and activity of SOD in seminal plasma of groups I-V.

Parameter / *Group*	**MDA** Mole x 10^{-7}/gm protein	**GSH** nMole/mg protein	**SOD** % inhibition
G_{I}(Control) M ± S.D. n.	0.55 ± 0. 19 36	97.8 ± 22.3 48	74.5 ± 11.61 47
G_{II}(A) M ± S.D. n.	1.9 ± 0.84** 44	68.1 ± 15.8 ** 33	65.74 ± 4.39** 30
G_{III}(OA) M ± S.D. n.	2.31 ± 0.72** 17	32.4 ± 11** 15	61.29 ± 4.16** 11
G_{IV}(OAT) M ± S.D. n.	3.64 ± 0.77 ** 21	31 ± 9.7 ** 30	44.2 ± 4.97 ** 17
G_{V}(Azoo) M ± S.D. n.	2.24 ± 0.66** 27	24.3 ± 6.7** 36-	59.28 ± 13.61** 26

Table 3: The mean values of MDA, GSH and SOD activity in sperm homogenate of groups I - IV.

Parameter / *Group*	**MDA** Mole x10^{-5}/gm protein	**GSH** nMole/mg protein	**SOD** % inhibition
G_{I}(Control) M ± S.D. n.	1.19 ± 0.44 32	20.06 ± 4.79 20	41.56 ± 8.8 39
G_{II}(A) M ± S.D. n.	2.51 ± 0.53** 30	16.51 ± 2.71 ** 18	37.42 ± 10.15* 25
G_{III}(OA) M ± S.D. n.	3.64 ± 0.87** 10	4.5 ± 1.2** 6	40.63 ± 7.03 16
G_{IV}(OAT) M ± S.D. n.	5.72 ± 1.37** 18	0.85 ± 0.25** 7	50.23 ±13.09** 32

In **Table 4**, no variation was observed in semen volume and seminal plasma protein of all groups, while, the sperm density of **G_{III}** and **G_{IV}** was highly and significantly decreased compared to **G_{I}**.

Electrophotogram of seminal plasma protein of different groups by PAGE was observed in **Figure 1**. The gel pro analysis of this electrophotogram can be seen in **Figures 2a,2b,2c and 2d**. In **Figure 2a,** comparing to group I (control) and group II a similarity has been demonestrated between the two groups. However, in **Figures 2b, 2c and 2d**, there are clear differences in gel pro-analysis of the control and groups III, IV aand V. In the other three groups, more bands were observed.

Table 4: Total semen volume (ml), sperm density (million/ml), and total protein in seminal plasma of groups I-V.

Parameter / *Group*	**Volume (ml)**	**Sperm density (million/ml)**	**T. Protein (g/100 ml seminal plasma)**
G_{I}(Control) M ± S.D. n.	3.1 ± 1.6 70	67.4 ± 44.1 70	5.71 ± 1.28 70
G_{II}(A) M ± S.D. n.	2.9 ± 1.3 48	64.4 ± 37.6 48	5.51 ± 1.25 48
G_{III}(OA) M ± S.D. n.	3.8 ± 2.2 18	10.6 ± 5.1*** 18	5.05 ± 1.54* 18

G_{IV}(OAT) M ± S.D. n.	3.7 ± 1.3 41	4.1 ± 3.5*** 41	5.44 ± 1.12 41
G_{V}(Azoo) M ± S.D. n.	3.4 ± 1.8 39	-	6.23 ± 1.51* 39

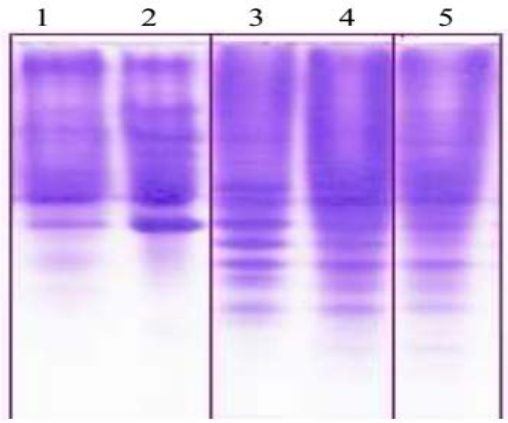

Figure 1: Electrophotogram of seminal plasma protein by PAGE. Lane 1 represents the control (G_I), lane 2 represents asthenozoospermic group (G_{II}), lane 3 represents oligo-asthenozoospermic group (G_{III}), lane 4 represents oligo-astheno-teratozoospermic group (G_{IV}) and lane 5 represents azoospermic group (G_V).

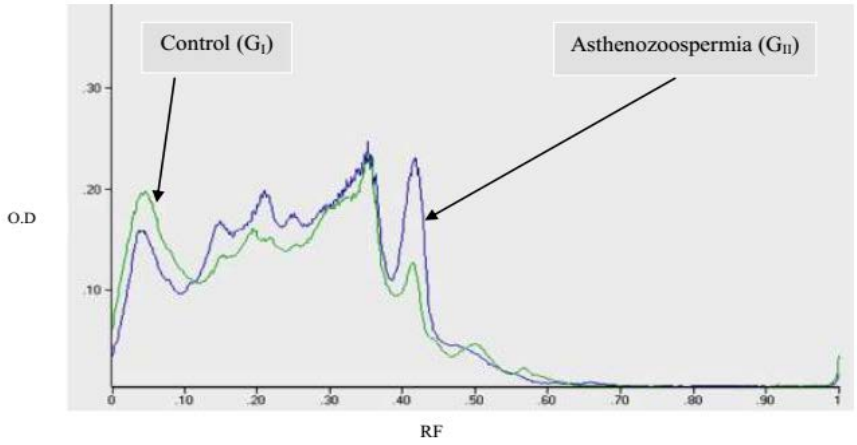

Figure 2a: Gel-pro analysis of different proteins in the seminal plasma of asthenozoospermic male (G_{II}) compared to the control.

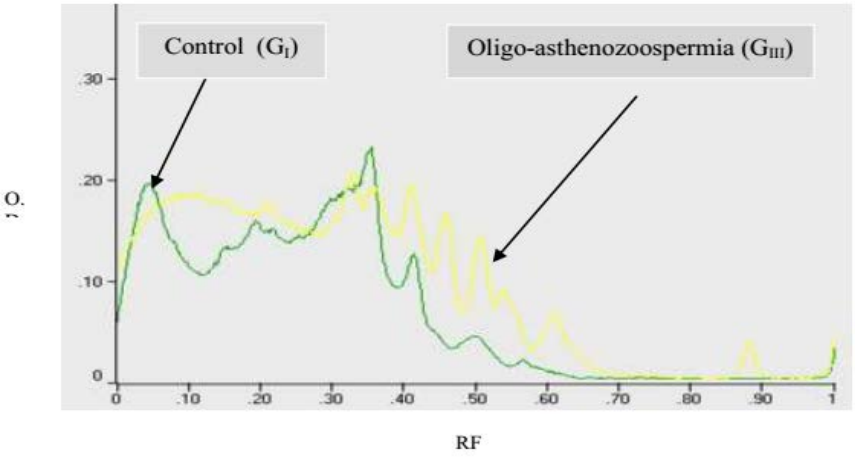

Figure 2b: Gel-pro analysis of different proteins in the seminal plasma of oligo-asthenozoospermic male (G_{III}) compared to the control.

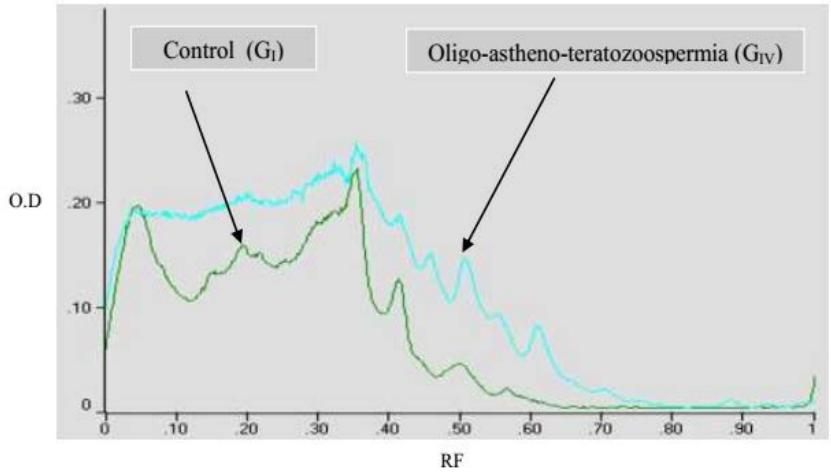

Figure 2c: Gel-pro analysis of different proteins in the seminal plasma of oligo-astheno-teratozoospermic male (G_{IV}) compared to the control.

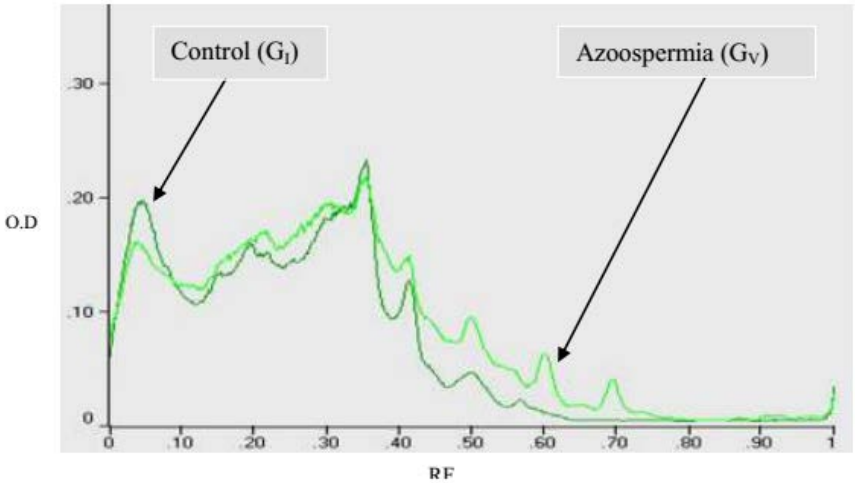

Figure 2d: Gel-pro analysis of different proteins in the seminal plasma of azoospermicmale (G_V) compared to the control.

In figure 3, there is a highly positive correlation between glutathione content and sperm density (A), while, malondialdehyde level and sperm density are highly and negatively correlated (B).

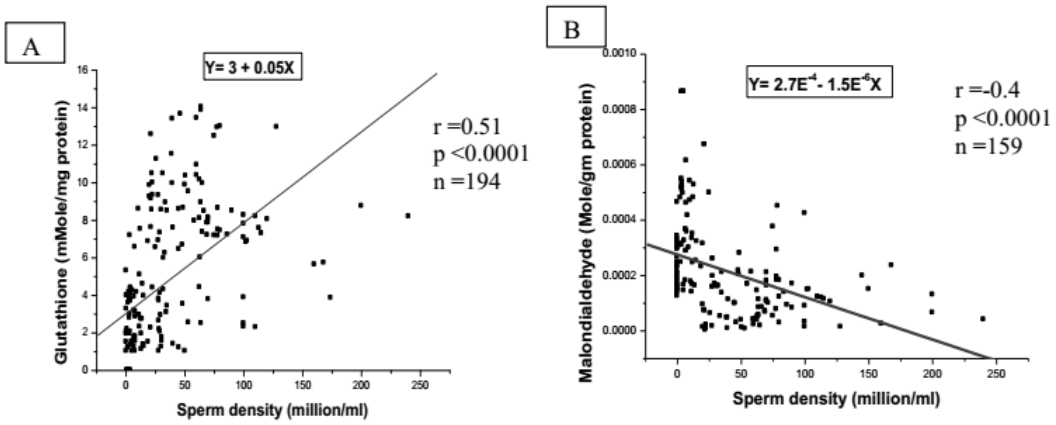

Figure 3: a- Correlation between glutathione content and sperm density. b- Correlation between malondialdehyde level and sperm density.

Discussion

The major causative factor of male infertility is the presence of oxidative stress which induces lipid peroxidation of sperm membrane (Ashok et al., 2014). The over load of many trace heavy metals' (discharged as environmental pollutants) impact increased the oxidative stress in the biological cells.

In this work, the obtained data illustrated that, the levels of Fe and Cd, were highly and significantly increased in sperm homogenate and seminal plasma of groups (GII - GV) compared to their corresponding controls. These increases were highly associated with the decrease in sperm density. Wirth and Mijal, (2010) and Aydemir et al. (2006) suggested that, Fe and Cd might have a strong toxic effect on spermatogenesis via producing excessive oxidants and inducing apoptosis, a process which can deplete the sperm concentration, and lead to male infertility. In addition, Demuynck et al. (2004) reported that, Pb and Cd were found to be responsible for different types of chromosomal aberrations.

An extensive study demonstrated that, phospholipids peroxidation causes sperm membrane damage. This damage led to the loss of the sperm quality and the membrane integrity (Tavilani et al., 2008). In the present study, high increases in the levels of MDA were observed in both seminal plasma and sperm homogenate of infertile males compared to the control. In azoospermic males (G_V), the seminal plasma level of MDA was higher than that of (G_I) (307.3 %). This result suggested that MDA levels in seminal plasma was not entirely derived from the abnormal spermatozoa but, at least in part, was originated from the existence of LPO in the accessory glands as seminal vesicles, prostate and epididymis indicating that such organelles were also exposed to oxidative damage in the infertile men, and subsequently spermatogenesis dysfunction. This suggestion was confirmed by the negative correlation between sperm density and MDA levels. The level of MDA in sperm homogenate was higher than its level in seminal plasma in all groups. This result illustrated the higher susceptibility of spermatozoa to peroxidative damage and suggested that, the spermatozoa might be the main source of oxidative stress in semen. This might be due to the presence of high density of mitochondria in their midpiece which might lead to the excessive oxygen radicals in cytoplasm with a simultaneous lack in their cytoplasmic antioxidant enzymes. This imbalance made the ability of spermatozoa antioxidants in scavenging the oxidants become limited, and increased the rate of oxidative stress. The present results of MDA were in agreement with the results of the other authors (Tavilani et al., 2008; Ben Abdallah et al., 2009; Huang et al., 2000).

In the present work, high decreases in the levels of GSH were found in both seminal plasma and sperm homogenate of infertile males when compared to the control. These results provided an evidence that the GSH seemed to play an important role in male fertility, sperm density and sperm quality, as was reported by the other investigators (Chaudhari and Singh, 2008; Agarwal and Sekhon, 2010), in addition to the positive correlation between GSH content and the sperm density. The present results indicated that, the seminal plasma might be the main source of GSH in semen, and also they emphasized on the role of seminal plasma in spermatozoa protection against the oxidative stress.

Murawski et al. (2007) showed that, SOD activity in seminal plasma of infertile males was significantly lowered compared to its activity in spermatozoa of normal men . The results of this study were in agreement with the results of Murawski et al.(2007), suggesting that the decrease in seminal plasma SOD activity might be one of the factors which are responsible for male infertility and low sperm density and quality. Moreover, the obtained results showed a highly significant elevation in SOD activity only in sperm homogenate supernatant of G_{IV} compared to the control. The males of this group might be at a greater risk of developing pathogenic levels of ROS, a phenomenon which can

cause the stimulation of SOD enzyme in spermatozoa to antagonist LPO (Shamsi et al., 2010). Based on the previous data, it can be suggested that SOD in spermatozoa seemed to be the most relevant enzyme in the protection of spermatozoa from LPO as was reported by Garrido et al. (2004). Also, gel pro analysis of the seminal plasma protein indicated the differences between proteins of normal group and the abnormal groups which might contain many abnormal proteins that might be produced as a result of the effect of the oxidative stress, affecting the semen quality. These abnormal proteins might affect the spermatogenesis and subsequently decrease the sperm density.

Finally, the high concentrations of Fe and Cd have been highly correlated with the increase in the oxidative stress and the decrease in the antioxidant in seminal plasma and sperm homogenates of the infertile groups which subsequently led to the strong toxic effect on spermatogenesis via producing excessive oxidants and inducing apoptosis.

Conclusion

The human male infertility was highly affected by the increase in heavy metals that impacted on the elevation of the oxidative stress in the semen, and were highly correlated with the decrease in the antioxidants. This disturbance in oxidants and antioxidants balance could lead to a strong toxic effect on spermatogenesis via producing excessive oxidants and male infertility. Also, these parameters can be used as tools in predicting semen quality.

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