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Quinestrol-induced Oxidative Stress: Implications for Vital Organ Function and Male Reproductive Health

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ABSTRACT: Quinestrol, a synthetic estrogen, has been shown to induce oxidative stress and alter biochemical functions in vital organs such as the liver and kidneys, in addition to its effects on reproductive tissues. This study evaluates the impact of quinestrol (2 mg/kg body weight) after 10 days of exposure on biochemical parameters, key antioxidative enzymes and lipid peroxidation levels in male albino rats. A total of 40 adult male rats were divided into two groups: control (n=20) and quinestrol-treated (n=20). Reduction in liver and kidney weights indicates organ atrophy and possible toxicity. Biochemical analysis revealed a significant decrease in total protein, total lipid, cholesterol and phospholipid levels in liver and kidney homogenates, suggesting metabolic disturbances and impaired organ function. Additionally activity of key antioxidant enzymes, including glutathione peroxidase, glutathione-S-transferase, catalase, glutathione reductase and superoxide dismutase decreased in all the reproductive organs, leading to a compromised antioxidant defense system. Increased lipid peroxidation confirmed heightened oxidative stress, which may contribute to reproductive toxicity, impaired spermatogenesis and reduced sperm quality. These findings emphasize the detrimental impact of quinestrol on vital organ integrity and male reproductive health, highlighting the need for further investigation into its toxicological effects.

Keywords: Antioxidant enzymes, biochemical functions, quinestrol, vital organs.

INTRODUCTION

Quinestrol, a synthetic estrogen analogue is commonly utilized in hormone replacement therapy and contraceptive formulations due to its prolonged bioavailability, a result of its accumulation in adipose tissues that enables sustained hormonal activity. Beyond its medical applications, quinestrol has been recognized for its effectiveness as a rodenticide, with studies demonstrating its significant antifertility effects in various rodent species, including Bandt's voles and lesser bandicoot rats (Zhang *et al.*, 2006; Zhao *et al.*, 2007). Despite its widespread use, the precise physiological mechanisms through which quinestrol mediates its effects, particularly in male rodents, remain an area of ongoing investigation. A comprehensive understanding of quinestrol's impact necessitates an exploration of the male reproductive system, which is governed by the hypothalamic-pituitary-gonadal axis (Chen *et al.*, 2021). This endocrine pathway regulates the secretion of key reproductive hormones, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both of which are essential for spermatogenesis and testosterone synthesis (Pun *et al.*, 2024). Disruptions to this axis-whether through exposure to exogenous estrogenic compounds like quinestrol or other environmental factors-can result in hormonal imbalances that negatively affect male fertility (Dhooge *et al.*, 2007). Moreover, quinestrol's impact extends beyond endocrine disruption, influencing reproductive organ morphology and function. Research has indicated that synthetic estrogens contribute to testicular atrophy and altered prostate gland function, both of which compromise reproductive capacity (Kumar *et al.*, 2004; Zhao *et al.*, 2007). Additionally, the effects of quinestrol are not confined to the reproductive system; studies suggest that excessive estrogen exposure may also impair the function of non-reproductive organs, such as the liver and kidneys. Notably, prolonged estrogenic activity has been associated with conditions such as nonalcoholic steatohepatitis, characterized by hepatic lipid accumulation and liver dysfunction (Zhao *et al.*, 2007). Biochemical markers serve as crucial indicators in assessing the systemic effects of quinestrol. Parameters such as serum testosterone levels, sperm count and motility provide critical insights into testicular function and reproductive potential. Additionally, estrogen-induced alterations in lipid metabolism including hypocholesterolemia-highlight the necessity for continuous biochemical monitoring (Agarwal *et al.*, 2008). Another pressing concern regarding quinestrol exposure is its potential to induce oxidative stress, a

state defined by an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms. Given their high metabolic rates and lipid-rich membranes, male germ cells are particularly vulnerable to oxidative damage, which can lead to lipid peroxidation, DNA fragmentation, and apoptosis (Mishra and Shaha 2005). Furthermore, oxidative stress may impair the activity of key antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which are essential for neutralizing ROS and maintaining cellular homeostasis (Chitra *et al.*, 2002).

Investigating the relationship between quinestrol exposure, oxidative stress and antioxidative enzyme activity is essential for elucidating the mechanisms underlying reproductive toxicity. The present study aims to assess the effects of quinestrol on vital organs, particularly the liver and kidney, while also examining antioxidant enzyme activity in the context of oxidative stress. By providing a comprehensive understanding of how synthetic estrogens disrupt male reproductive health, this research will contribute to the development of safer contraceptive strategies and inform broader discussions on the impact of endocrine disruptors on male fertility.

MATERIAL AND METHODS

Forty adult male albino rats (150-160 g) were obtained from LUVAS, Hisar, with ethical approval from IAEC. After a 15-day acclimatization, they were housed under standard conditions with controlled temperature, a 12-hour light/dark cycle and access to feed and water. The study was approved by IAEC, GADVASU (Protocol no. GADVASU/2022/IAEC/63/11) on January 13, 2022. Rats were randomly assigned to control (n=20) and treatment (n=20) groups, following CPCSEA guidelines. Rats were acclimated for 15 days before being divided into two groups (n=20): control and 2 mg/kg quinestrol. Quinestrol was administered orally for 10 days. After treatment, the rats were euthanized and vital organs (liver and kidney) along with reproductive organs (testis and epididymis) were excised. Excess tissue was removed for further processing and organ homogenates were prepared to analyze biochemical and antioxidant enzyme activity. Plasma malondialdehyde (MDA) levels were also measured as a marker of oxidative stress.

A. Observations noted

Body weight. The weight of vital organs (liver and kidney) was taken at end of treatment.

Biochemical Analysis

1. Total protein: Measured using the Lowry *et al.* (1951) method, with absorbance recorded at 520 nm. Protein concentration was expressed as mg/g wet tissue.

2. Total Lipid and Phospholipid Estimation: Lipids were extracted using the Folch *et al*. (1957) method with a chloroform-methanol mixture, while phospholipids were estimated using the Ames (1966) method via ashing and absorbance at 820 nm. Both were expressed as mg/g tissue.

3. Total Cholesterol Estimation: Measured in testicular tissue using the Chiamori and Henry (1959) method, with absorbance recorded at 560 nm and cholesterol content expressed as mg/g tissue.

Antioxidant Enzyme Activity Assays

4. SOD Activity: Determined by assessing the inhibition of pyrogallol auto-oxidation (Marklund & Marklund 1974) and expressed as units per mg protein.

5. Catalase (CAT) Activity: Measured based on the decomposition of hydrogen peroxide (Aebi, 1983) and expressed as micromoles of hydrogen peroxide decomposed per minute per mg protein.

6. GPx Activity: Evaluated by measuring the reduction of hydrogen peroxide by glutathione (Hafeman *et al.*, 1984), expressed as units per mg protein.

7. Glutathione Reductase (GR) Activity: Determined by monitoring the reduction of glutathione disulfide to glutathione using nicotinamide adenine dinucleotide phosphate (NADPH) (Carlberg & Mannervik 1985), expressed as micromoles of NADPH oxidized per minute per mg protein.

8. Glutathione-S-Transferase (GST) Activity: Assessed by measuring the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (Habig *et al.*, 1974), expressed as moles of conjugate produced per minute per mg protein.

Lipid Peroxidation (Malondialdehyde Levels)

1. MDA levels were measured using the Stocks and Dormandy (1971) method. Plasma samples were incubated with hydrogen peroxide and sodium azide, and absorbance was recorded at 532 nm. MDA concentration was expressed as nanomoles per 100 mg protein.

B. Statistical analysis

All values are expressed as mean±S.E. Statistical Package for Social Sciences (SPSS) version 16 for Windows was used to determine significant differences among the groups *i.e.*, control and group treated with 2 mg/kg b.wt. of quinestrol using t-test (independent), where i value of $p < 0.05$, the values are said to differ significantly.

RESULTS AND DISCUSSION

1. Effects of quinestrol on weight of vital organs. Table 1 illustrates significant reduction in liver and kidney weights in treated group as compared to control group. The liver weight in control group was 4.39 ± 0.13, whereas in treated group, it decreased to 3.25 ± 0.03. Similarly, the kidney weight in control group was 0.36 ± 0.01, while in treated group, it dropped to 0.29 ± 0.02. This suggests that the treatment administered to treated group had a measurable impact on organ weights.

2. Effects of quinestrol on biochemical parameters in liver. Table 2 illustrates the biochemical changes in tissue homogenate of liver following quinestrol exposure. Total protein levels significantly decreased from 32.19±1.49 mg/g in the control group to 27.60±0.79 mg/g in the treated group, indicating possible hepatic dysfunction or impaired protein synthesis. Similarly, total lipid levels showed a

significant reduction from 87.31±1.19 mg/g to 78.50±1.36 mg/g, suggesting alterations in lipid metabolism. Cholesterol levels also declined notably from 7.94±0.05 mg/g in controls to 5.95±0.06 mg/g in the treatment group, which could impact liver cell membrane integrity and overall metabolic processes. Additionally, phospholipid levels decreased from 12.39±0.13 mg/g to 10.29±0.09 mg/g further indicating disruptions in membrane stability and lipid homeostasis. These findings suggest that quinestrol exposure negatively affects liver biochemical composition, potentially impairing normal hepatic function and metabolic regulation.

3. Effects of quinestrol on biochemical parameters in kidney. Table 3 illustrates the biochemical changes in homogenate of kidney following quinestrol exposure. Total protein levels showed a significant decrease from 15.67±1.68 mg/g (control) to 14.74±0.90 mg/g (treatment). However, total lipid levels showed a slight decrease from 65.03±1.89 mg/g to 63.35±1.29 mg/g, suggesting minor metabolic disruptions. More notably, cholesterol levels significantly declined from 3.43±0.23 mg/g to 2.22±0.27 mg/g, which could compromise cell membrane integrity and function. Similarly, phospholipid levels dropped from 8.21±0.10 mg/g in the control group to 6.23±0.07 mg/g in the treated group, indicating possible damage to kidney cell membranes and altered lipid homeostasis. These findings suggest that while quinestrol exposure does not significantly impact kidney protein metabolism, it disrupts lipid composition, potentially affecting renal function and structural stability over time.

Antioxidant enzyme activity assays

4. SOD Activity. The SOD activity values in Table 4 indicate a significant decline in all reproductive organs of quinestrol-treated rats compared to the control group. In the testis, SOD activity decreased from 3.51±0.14 to 2.12±0.01 units/mg protein, reflecting a reduction in antioxidant defense. The caput epididymis showed a decrease from 1.73±0.01 to 1.40±0.02, while the corpus epididymis declined from 1.22±0.02 to 1.00±0.03, suggesting increased oxidative stress in these regions. The cauda epididymis exhibited a smaller reduction, from 2.32±0.03 to 1.95±0.01, indicating a relatively lesser impact in this region compared to other reproductive organs.

5. CAT Activity. The CAT activity values in Table 5 show a significant reduction in most reproductive organs of quinestrol-treated rats compared to the control group, indicating impaired antioxidant defense. In the testis, CAT activity decreased from 0.94±0.02 to 0.75±0.02 µmoles of H_2O_2 decomposed/min/mg protein, suggesting increased oxidative stress. The caput epididymis showed a decline from 1.3±0.02 to 1.07±0.01, while the corpus epididymis decreased from 1.57±0.02 to 1.34±0.02, reflecting a reduced ability to neutralize hydrogen peroxide. However, the cauda epididymis showed no change (5.08±0.02 in both groups), indicating that catalase activity in this region remained unaffected by quinestrol exposure.

6. GPx Activity. The GPx activity values in Table 6 show a significant decline in the reproductive organs of quinestrol-treated rats compared to control group. In the testis, GPx activity decreased from 1.73±0.02 to 1.27±0.03 µmoles of NADPH oxidized/min/mg protein, indicating oxidative stress. The caput epididymis also showed a reduction from 0.93±0.01 to 0.70±0.01, while the corpus epididymis exhibited a more pronounced drop from 2.13±0.02 to 1.02±0.01, suggesting a potential impact on sperm maturation. The cauda epididymis experienced a smaller decline, from 1.58±0.01 to 1.46±0.02, indicating that sperm storage function was less affected.

7. GR Activity. The GR activity values in Table 7 indicate a significant decline in quinestrol-treated rats compared to the control group, suggesting reduced antioxidant capacity. In the testis, GR activity decreased from 0.95±0.01 to 0.85±0.01 µmoles of NADPH oxidized/min/mg protein, indicating increased oxidative stress. The caput epididymis showed a slight decline from 0.75±0.01 to 0.70±0.01, while the corpus epididymis exhibited a more pronounced reduction from 2.11±0.01 to 1.93±0.02, reflecting impaired glutathione recycling. Similarly, the cauda epididymis showed a decrease from 1.41±0.02 to 1.21±0.02, further indicating weakened antioxidant defense mechanisms due to quinestrol exposure.

8. GST Activity. The GST activity values in Table 8 indicate a significant decline in quinestrol-treated rats compared to the control group, suggesting reduced antioxidant capacity. In the testis, activity dropped from 1.05±0.02 to 0.85±0.02. The caput epididymis showed a decline from 0.45±0.01 to 0.30±0.03, while the corpus epididymis decreased from 0.63±0.01 to 0.50±0.02. The cauda epididymis also exhibited a reduction from 0.82±0.02 to 0.63±0.01. These values indicate a significant reduction in GST activity across all measured reproductive organs.

9. Lipid peroxidation (malondialdehyde levels). After 10 days of quinestrol exposure, lipid peroxidation levels were measured. In the testis, the levels rose from 8.39 ± 0.02 in control group to 9.62 ± 0.02 in treated group. Similarly, in the caput, lipid peroxidation increased from 4.40 ± 0.03 to 5.61 ± 0.13, while in the corpus, it went up from 2.74 ± 0.03 to 3.13 ± 0.01. The cauda also exhibited an increased from 3.62 ± 0.02 to 4.93 ± 0.04. These significant increases suggest that quinestrol exposure enhances oxidative stress in the reproductive organs.

In the present study the weight of vital organs (liver and kidney) decreased as compared to control group. This is in accordance with findings by Gioia *et al.* (1978), who reported a reduction in liver weight in quinestrol-treated rats, and Su *et al.* (2017), who observed increases in both liver and kidney weights in gerbils. Given that organ weight is a sensitive indicator of drug toxicity, the reported decreases in liver and kidney weights raise concerns about a potential adverse impact of quinestrol on the general health of the male albino rats. The observed alterations suggest a possible disruption in normal physiological processes. Therefore, these findings indicate that quinestrol may have an adverse impact and necessitate further investigation to fully evaluate its toxicity and long-term effects. Several

studies suggest that OCPs, particularly those containing synthetic estrogens and progestogens, may influence renal and hepatic biochemical parameters, potentially leading to dysfunctions in homeostasis and metabolism. Ekhator *et al*. (2014) examined the impact of combined oral contraceptive pills (COCPs) containing levonorgestrel and ethinylestradiol on kidney function. Their findings indicated a significant increase in plasma creatinine, urea and potassium (K^+) levels, while sodium (Na^+) and chloride (Cl^-) levels decreased. These alterations suggest that COCPs could impair renal function, possibly through disruptions in electrolyte balance and nitrogenous waste excretion. This study highlights the necessity of further investigations into the long-term implications of OCP use on kidney health (Zhang *et al.,* 2006; Zhao *et al.,* 2007). Similarly, Oelkers (1996) reported that estrogens, particularly ethinylestradiol and mestranol, stimulate hepatic protein synthesis, leading to increased levels of angiotensinogen, which in turn influences the renin-angiotensin system (RAS) and may elevate blood pressure. This mechanism aligns with the observations of Kang *et al.* (2001), who demonstrated that oral contraceptive use could impact renal function through its effects on RAS, increasing the risk of hypertension and altering fluid balance (Zhang *et al.,* 2006; Zhao *et al.,* 2007). The liver is another major organ affected by OCP use. Ottosson (1984) observed that estrogens improve lipid profiles by increasing high-density lipoprotein (HDL) cholesterol, while progestogens such as levonorgestrel counteract these benefits by lowering sex hormone-binding globulin (SHBG) levels. Odinga *et al*. (2022) further investigated the hepatic effects of OCPs, reporting significant changes in biochemical markers such as total protein and albumin, which may indicate impaired liver function. The observed decrease in these proteins suggests an effect on protein synthesis, which could indirectly influence kidney filtration efficiency and osmotic balance. Additionally, increased bilirubin levels may suggest metabolic stress that could contribute to renal oxidative stress over prolonged use (Zhang *et al.,* 2006; Zhao *et al*., 2007). In a related study, Gioia *et al.* (1978) examined the effect of quinestrol on hepatic function in rats. Their findings indicated an increase in liver weight and alterations in bile flow, consistent with estrogenic effects on hepatic metabolism. However, there were no significant modifications in bilirubin concentration or hepatic histology, suggesting that the observed biochemical changes might not lead to overt hepatic damage. Research involving female albino rats demonstrated that quinestrol administration led to significant alterations in lipid metabolism, notably increasing lipid concentrations, while phospholipid levels remained unchanged. This suggests that quinestrol may influence lipid metabolism without affecting phospholipid content (Zhang *et al*., 2006; Zhao *et al.,* 2007). Collectively, these studies indicate that oral contraceptive use may influence kidney and liver function through multiple pathways, including alterations in biochemical markers, electrolyte balance, lipid metabolism, and blood pressure regulation. While estrogens may confer some benefits, their combination with synthetic progestogens may diminish these effects or even contribute to adverse outcomes (Zhang *et al.,* 2006; Zhao *et al.,* 2007). Quinestrol, a synthetic estrogen used for rodent population control, induces oxidative stress, leading to reproductive and hepatic toxicity. This occurs through the disruption of the balance between ROS production and the antioxidant defense system (Li *et al.,* 2014). Studies have shown that quinestrol exposure increases lipid peroxidation and MDA levels while reducing the activity of antioxidant enzymes like SOD, GSH-Px and total antioxidant capacity (Li *et al.,* 2014; Zhang *et al*., 2006; Zhao *et al.,* 2007). In the testes, quinestrol-induced oxidative stress results in decreased testicular weight and seminiferous tubular area, along with increased spermatogenic cell apoptosis (Li *et al.,* 2014). Histopathological changes, including germ cell depletion and vacuolization, have also been observed (Shen *et al.,* 2011). The severity of testicular damage is dose-dependent, with multi-dose treatments exacerbating the damage, although partial recovery can occur (Shen *et al.,* 2012). Quinestrol also affects hepatic metabolism, with exposure leading to a dose-dependent increase in liver weight and CYP3A4 enzyme activity, particularly in female rodents (Yujie *et al*., 2021). This suggests increased metabolism and sensitivity in females, while the kidneys do not exhibit significant dose-related effects (Yujie *et al*., 2021; Zhang *et al*., 2006; Zhao *et al.,* 2007). Furthermore, oxidative damage to spermatozoa can compromise sperm function and viability, reducing fertility (Rao *et al*., 2002). Elevated MDA levels contribute to sperm membrane damage, making sperm cells more vulnerable to oxidative stress, correlating with reduced sperm density and increased sperm abnormalities. The long-term effects of quinestrol on non-target species and ecosystems warrant further investigation, with concerns raised about potential irreversible damage from prolonged or repeated exposure (Shen *et al.,* 2012). Future studies should explore mitigation strategies, such as antioxidant supplementation, to counteract oxidative damage and preserve reproductive health (Zhang *et al.,* 2006; Zhao *et al*., 2007).

Table 1: Changes in weight of vital organs (g/100g b.wt.) of male albino rats.

Organs /Group	Control	Treatment
Liver	4.39 ± 0.13^a	3.25 ± 0.03^b
Kidney	0.36 ± 0.01^a	0.29 ± 0.02^b

Table 2: Biochemical parameters (mg/g wet weight) in liver homogenate of male albino rats.

Biochemical parameters/ Groups	Control	Treatment
Total protein	32.19±1.49[a]	27.60±0.79[b]
Total lipid	87.31±1.19[a]	78.50±1.36[b]
Cholesterol	7.94±0.05[a]	5.95±0.06[b]
Phospholipids	12.39±0.13[a]	10.29±0.09[b]

All values are expressed as mean±S.E.; n=6
Values with different alphabetical superscripts differ significantly Values are significant at 5% level of significance

Table 3: Biochemical parameters (mg/g wet weight) in kidney homogenate of male albino rats.

Biochemical parameters/Groups	Control	Treatment
Total protein	15.67±1.68[a]	14.74±0.90[b]
Total lipid	65.03±1.89[a]	63.35±1.29[b]
Cholesterol	3.43±0.23[a]	2.22±0.27[b]
Phospholipid	8.21±0.10[a]	6.23±0.07[b]

All values are expressed as mean±S.E.; n=6
Values with different alphabetical superscripts differ significantly Values are significant at 5% level of significance

Table 4: SOD activity (units/mg protein) in reproductive organs of male albino rats.

Organs/Group	Control	Treatment
Testis	3.51±0.14[a]	2.12±0.01[b]
Caput	1.73±0.01[a]	1.40±0.02[b]
Corpus	1.22±0.02[a]	1.00±0.03[b]
Cauda	2.32±0.03[a]	1.95±0.01[b]

All values are expressed as mean±S.E. ; n=6
Values with different alphabetical superscripts differ significantly
Values are significant at 5% level of significance

Table 5: CAT activity (µmoles of H_2O_2 decomposed/min./mg protein) in reproductive organs of male albino rats.

Organs/Group	Control	Treatment
Testis	0.94±0.02[a]	0.75±0.02[b]
Caput	1.3±0.02[a]	1.07±0.01[b]
Corpus	1.57±0.02[a]	1.34±0.02[b]
Cauda	5.08±0.02[a]	5.08±0.02[a]

All values are expressed as mean±S.E.; n=6
Values with different alphabetical superscripts differ significantly Values are significant at 5% level of significance

Table 6: GPx activity (µmoles of NADPH oxidized/min/mg protein) in reproductive organs of male albino rats.

Organs/Group	Control	Treatment
Testis	1.73±0.02[a]	1.27±0.03[b]
Caput	0.93±0.01[a]	0.70±0.01[b]
Corpus	2.13±0.02[a]	1.02±0.01[b]
Cauda	1.58±0.01[a]	1.46±0.02[b]

All values are expressed as mean±S.E.; n=6
Values with different alphabetical superscripts differ significantly Values are significant at 5% level of significance

Table 7: GR activity (µmoles of NADPH oxidized/min./mg protein) in reproductive organs of male albino rats.

Organs/Group	Control	Treatment
Testis	0.95±0.01[a]	0.85±0.01[b]
Caput	0.75±0.01[a]	0.70±0.01[b]
Corpus	2.11±0.01[a]	1.93±0.02[b]
Cauda	1.41±0.02[a]	1.21±0.02[b]

All values are expressed as mean±S.E. ; n=6
Values with different alphabetical superscripts differ significantly Values are significant at 5% level of significance

Table 8: GST activity (µmoles of GSH-CNDB conjugate formed/min./mg protein) in reproductive organs of male albino rats.

Organs /Group	Control	Treatment
Testis	1.05±0.02[a]	0.85±0.02[b]
Caput	0.45±0.01[a]	0.30±0.03[b]
Corpus	0.63±0.01[a]	0.50±0.02[b]
Cauda	0.82±0.02[a]	0.63±0.01[b]

All values are expressed as mean±S.E.; n=6
Values with different alphabetical superscripts differ significantly Values are significant at 5% level of significance

Table 9: Lipid peroxidation (nmol MDA produced/min./mg protein) in reproductive organs of male albino rats.

Organs/Group	Control	Treatment
Testis	8.39±0.02[a]	9.62±0.02[b]
Caput	4.40±0.03[a]	5.61±0.13[b]
Corpus	2.74±0.03[a]	3.13±0.01[b]
Cauda	3.62±0.02[a]	4.93±0.04[b]

All values are expressed as mean±S.E.; n=6.
Values with different alphabetical superscripts differ significantly.
Values are significant at 5% level of significance.

CONCLUSIONS

In short, oral contraceptives can affect kidney and liver function through multiple pathways, including altering biochemical markers and inducing oxidative stress, as seen with quinestrol if consumed in doses higher than the prescribed one. While some components may have benefits, combinations can lead to adverse effects which calls for further investigations to develop safer options in field of oral contraceptives.

FUTURE SCOPE

Future research should investigate the long-term effects of quinestrol on male reproductive health, focusing on chronic exposure and potential irreversible damage. Studies should also explore specific pathways of oxidative stress induction and test protective strategies like antioxidant supplementation. Further investigation into sperm DNA integrity, epigenetic modifications, and other endocrine systems is needed for a comprehensive understanding of quinestrol's risks and to develop effective mitigation strategies**.**

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Conflict of Interest. None.

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