Journal of Advanced Zoology
ISSN: 0253-7214
Volume 36 Issue 1 Year 2015 Page 35-40

in vitro Effects of Nicotine on Lipid Peroxidation and Motility in Cattle Bull Ejaculated Spermatozoa

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Abstract

Tobacco smoking, driven mainly by nicotine consumption, is a known environmental factor adversely affecting male reproductive health. This study investigates the in vitro impact of nicotine on lipid peroxidation and motility in cattle bull ejaculated spermatozoa. Nicotine exposure induced a dose-dependent increase in lipid peroxidation, as evidenced by elevated malondialdehyde (MDA) levels measured over a 120-minute period. Lipid peroxidation was measured by thiobarbituric acid-reactive substances (TBARS) and was shown to escalate significantly in nicotine-treated samples. Concurrently, sperm motility decreased significantly in nicotine-treated groups compared to controls, suggesting compromised sperm function. The findings highlight oxidative stress, mediated by reactive oxygen species, as the principal mechanism underlying nicotine-induced sperm damage. This research underscores the detrimental effects of nicotine on sperm quality through increased oxidative membrane damage and reduced motility, emphasizing the importance of targeting oxidative stress to preserve male fertility in cattle bulls.

Keywords: Nicotine, Mammalian Spermatozoa, cattle bull, Lipid Peroxidation, In Vitro, Antioxidants, Reproductive Health

1. Introduction

Smoking is one of the most prevalent voluntary actions that modifies susceptibility to a variety of diseases, including reproductive issues in men (Viczian, 1969). Historically, reproductive research has focused on female fertility; however, the increasing recognition of paternal factors in reproduction has shifted attention to male reproductive health (Stillman et al., 1986). Smoking is associated with a variety of detrimental effects on male fertility, including reduced sperm count, motility, and morphological abnormalities (Evans *et al*., 1981; Rodriguez et al., 1982).

Nicotine, a highly addictive alkaloid found in tobacco products, is well-known for its harmful effects on human health, including cardiovascular diseases, respiratory disorders, and cancer. However, nicotine's impact on male reproductive health, particularly spermatozoa, has garnered increasing attention in recent years. Nicotine and other tobacco-related chemicals are potent environmental toxicants that influence male fertility. Nicotine has been shown to disrupt sperm function in various mammals, including humans, rats, and rabbits. Nicotine exposure leads to increased oxidative stress, which adversely affects sperm motility, morphology, and DNA integrity (Saleh *et al.*, 2003).

In human studies, smoking has been linked to a reduction in sperm count and motility, with smokers demonstrating lower sperm quality compared to non-smokers (Benoff et al., 2000). Animal studies have confirmed that nicotine induces testicular atrophy, alters hormonal profiles, and impairs spermatogenesis, resulting in compromised fertility (Agarwal et al., 2004). Nicotine has been shown to affect various stages of

sperm development, motility, and morphology. In bovines, these disruptions can significantly impact fertility rates, making nicotine a crucial environmental toxin to consider in agricultural contexts.

Nicotine affects sperm through multiple pathways. The primary mechanism through which nicotine induces oxidative stress is the activation of ROS-producing enzymes and mitochondrial dysfunction. Studies have shown that nicotine increases mitochondrial ROS production, which contributes to lipid peroxidation and sperm dysfunction (Mukai et al., 2004). Additionally, nicotine interacts with nicotinic acetylcholine receptors (nAChRs) on the sperm plasma membrane. This interaction disrupts calcium ion influx, an essential process for sperm motility and capacitation. The disruption of calcium homeostasis can impair sperm function by inhibiting motility, preventing the acrosome reaction, and affecting the overall fertilizing potential of sperm (Yanagimachi, 1975). Nicotine also interferes with ATPase activity in spermatozoa, further exacerbating oxidative stress and impairing sperm function. ATPase activity is crucial for maintaining ion gradients across the sperm plasma membrane, and its inhibition by nicotine reduces sperm motility and capacitation (Agarwal et al., 2004).

Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) and antioxidant defences, is a primary mechanism through which nicotine damages spermatozoa. Spermatozoa are particularly vulnerable to oxidative stress due to their unique characteristics, including their high levels of polyunsaturated fatty acids, and these polyunsaturated fatty acids are readily oxidized by ROS. Lipid peroxidation, a key marker of oxidative damage, can disrupt sperm membrane integrity, leading to decreased motility and fertilization potential. a major end product of lipid peroxidation, MDA, is commonly used as a marker of oxidative damage in sperm cells (Alvarez & Storey, 1992). Increased levels of MDA in spermatozoa are correlated with decreased motility, viability, and fertilization capacity, indicating that lipid peroxidation is a critical factor in sperm quality (Agarwal et al., 2003). The resulting lipid peroxidation leads to disruption of the sperm membrane, affecting its fluidity, motility, and overall fertilization potential (Benoff et al., 2000). Elevated MDA levels in spermatozoa are associated with decreased sperm motility, reduced viability, and poor fertilization potential (Alvarez & Storey, 1992).

Among the mechanisms of damage, oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and antioxidant defence systems, plays a central role. Lipid peroxidation, the oxidative degradation of lipids, is a key consequence of ROS accumulation, resulting in cellular membrane damage. The concept of oxidative stress in sperm is grounded in the idea that reactive oxygen species (ROS), which are produced as by-products of normal metabolism, can accumulate in excessive amounts, leading to cellular damage. Given the central role of oxidative stress in sperm pathophysiology, this study investigates how nicotine, through its pro-oxidative effects, influences lipid peroxidation in mammalian spermatozoa.

This study aims to evaluate the impact of nicotine on lipid peroxidation in mammalian spermatozoa *in vitro*. In mammals, including humans and rodents, understanding how nicotine-induced oxidative stress affects sperm quality is critical, not only for male fertility but also for overall reproductive success. This study aims to evaluate the effects of nicotine on lipid peroxidation in mammalian spermatozoa and investigate the underlying molecular mechanisms involved in sperm dysfunction. Specifically, it will focus on changes in sperm motility, acrosome integrity, and membrane functionality following nicotine exposure at varying concentrations.

2. Materials and Methods

A. Sperm Samples

The cattle bull semen was collected at the Animal Breeding Complex, National Dairy Research Institute, Karnal. The semen samples, exhibiting +++ motility waves, were diluted (1:1, v/v) in a medium containing sodium citrate, citric acid, egg yolk, benzyl penicillin, and streptomycin. Chilled samples were transported in an ice bath to Chandigarh. The seminal plasma and diluting fluid were separated by centrifuging the liquefied samples at 300 × g for 10 minutes. The sperm pellet was washed in 0.2 M phosphate-buffered saline (PBS) and used for various experiments.

B. Drug Preparation

Nicotine (Sigma Chemical Co.) was dissolved in phosphate-buffered saline (PBS) to create stock solutions at concentrations of 0, 0.5, and 1.0 mM. Spermatozoa were incubated with these nicotine concentrations for various time periods (15, 30, 60, and 120 minutes) at 37°C. Control groups were treated with PBS alone.

C. Motility Test

To 1 ml of sperm sample (80 × 10^6 cells), 0.5 mM and 1 mM nicotine were added. Control samples received an equal amount of 0.2 M PBS. Incubation was carried out at 37°C, and at specified intervals, the number of motile and non-motile spermatozoa was counted under a low-power microscope (400x). The motility patterns were classified according to the WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction (2010). The categories used for classifying different patterns of motility have been designated as a, b, c, and d, and are defined as:

a - if the spermatozoon has a rapid and linear progressive motility.
b - if it has a slow or sluggish linear or non-linear movement.
c - -if it has a non-progressive motility.
d - if the spermatozoon is Immotile.

D. Lipid Peroxidation Measurement

Lipid peroxidation was quantified using the thiobarbituric acid (TBA) assay. TBA reactivity was used as an index of lipid peroxidation in nicotine-treated (incubated) sperm plasma membrane vesicles by reacting malonyl-dialdehyde (MDA) and other aldehyde by-products of peroxide decomposition with TBA (Maridomean et al,1979). Absorbance of the upper layer was read at 532 nm. Since MDA may not be the only species (adduct) detected in this assay, it was preferred to represent the data in terms of absorbance change at 532 nm, the approximate λ_{max} of the adduct produced.

E. Statistical Analysis

All the data were presented as mean ± SD. The comparison of control and nicotine-treated data (0.5 mM or 1 mM) was done using Student's t-test (Ipsen & Feigel, 1970).

3. Results

The present study aimed to investigate the effect of nicotine on sperm motility and the acrosome reaction in bull ejaculated spermatozoa. The various sperm function tests were performed on spermatozoal samples, while the biochemical assays were also conducted on the isolated plasma membrane fraction of cattle bull spermatozoa.

A. Spermatozoal motility:

Spermatozoal samples were incubated at 37 °C, and the sperm motility was assessed at an interval of 15 min for a total of 120 min. This was referred to as the normal rate of sperm motility (control). The samples incubated with 0.5 mM and 1 mM nicotine were assessed in the same manner as the others. A drastic effect was observed on sperm motility in cattle bull ejaculated spermatozoa following incubation with different concentrations of nicotine (Fig. 1). Both the pattern and per cent motility were altered after different time intervals of incubation. The pattern of sperm motility changed from a rapid and linear progression (category A) to slow or sluggish linear movement (category B) with 0.5 mM nicotine after 90 minutes in bull spermatozoal samples from cattle. With I mM nicotine, the pattern of sperm motility changed from a rapid and linear progression (category-a) to slow or sluggish linear movement (category-b) 75 min in bull ejaculated spermatozoa. After 105 min of incubation with 1 mM nicotine, the pattern of motility changed from slow or sluggish movement (category-b) to a non-progressive one (category-c), which finally led to sperm inactivation or immobilisation (category-d) after 120 min in bull ejaculated spermatozoa. The percentage of motile spermatozoa decreased significantly ($p < 0.001$) at all the time points in bull spermatozoal samples treated by different concentrations of nicotine, as revealed by Student's t-test (Tables 1).

Percentage of motile spermatozoa			
	Nicotine (mM)		
Time (min)	**Control**	**0.5**	**1**
0	90.76 ± 2.47	-------	-------
15	87.94 ±1.93	86.33 ±1.06	80.52 ± 2.21***
30	86.93 ± 1.80	85.55 ±3.66	75.41 ±0.49*
45	84.55 ± 1.19	83.69 ± 0.65	72.82 ± 2.14**
60	83.92 ±0.74	80.09 ± 0.75**	65.99 ± 3.15**
75	79.18 ± 1.01	73.24 ± 0.57**	58.03 ± 2.60*

90	73.57 ± 3.83	53.11 ± 2.84**	31.16 ± 1.38*
105	69.84 ± 1.39	38.91 ± 1.72*	13.88 ± 3.42*
120	66.35 ± 1.69	21.41 ± 1.64*	0

All data represented as mean ± SD; *p<0.001,**p<0.01,***p<0.05

Table 1: Effect of in vitro addition of different concentrations of nicotine on spermatozoal motility at different time intervals in ejaculated cattle bull spermatozoa

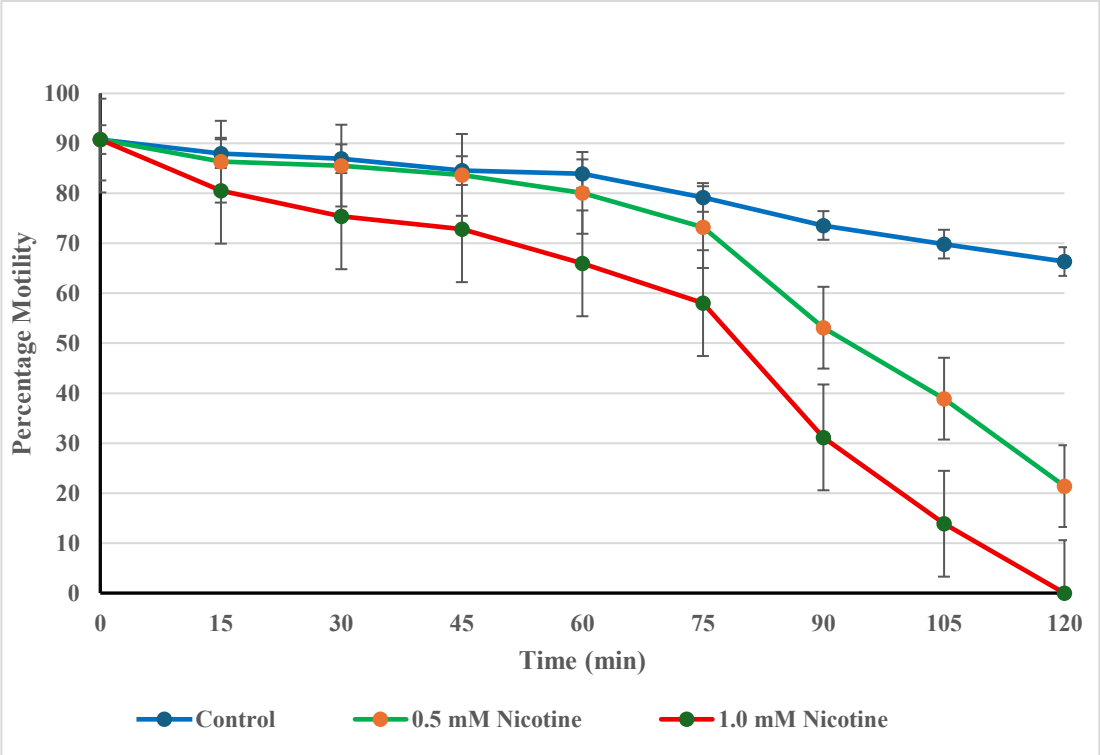

Figure 1: Effect of different concentrations of nicotine on spermatozoal motility at different time intervals in ejaculated cattle bull spermatozoa

Lipid Peroxidation

Nicotine treatment led to a dose-dependent increase in lipid peroxidation, as indicated by the elevated TBA reactivity. The extent of lipid peroxidation was significantly higher in nicotine-treated sperm samples compared to the control, confirming that nicotine induces oxidative damage to spermatozoal membranes. TBA reactive substances were measured up to 120 minutes in cattle bull sperm plasma membrane vesicles. MDA levels increased progressively with nicotine concentrations (Table 2). The initial value of MDA at time 0 in the control sample was low (0.16 ± 0.03). After 120 minutes, the 1.0 mM nicotine group exhibited the highest MDA levels (0.60 ± 0.027), compared to the control group (0.52 ± 0.03), reflecting the dose-dependent nature of lipid peroxidation (Figure 2).

Lipid Peroxidation in cattle bull ejaculated spermatozoa.			
		Nicotine (mM)	
Time (min)	**Control**	**0.5**	**1**
0	0.16 ± 0.03	0.18 ± 0.04	0.20± 0.025
30	0.33 ± 0.03	0.38 ± 0.02***	0.40 ± 0.026***
60	0.41 ± 0.05	0.45 ± 0.03	0.48 ± 0.03
90	0.46± 0.03	0.53 ± 0.026	0.55 ± 0.026***
120	0.52± 0.03	0.55 ± 0.04	0.6 ± 0.027***

All data represented as mean ± SD of absorbance at 532 nm; *p<0.001,**p<0.01,***p<0.05

Table 2: Effect of in vitro addition of different concentrations of nicotine on the extent of lipid peroxidation at different time intervals in ejaculated cattle bull spermatozoa

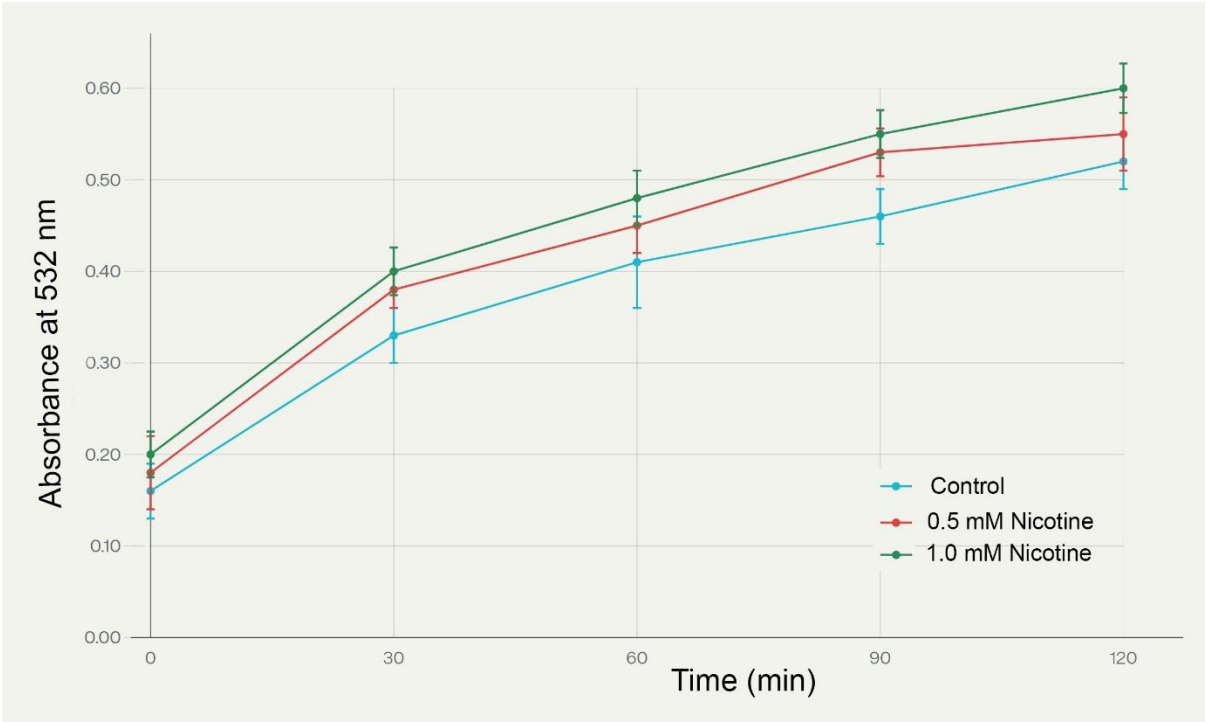

Figure 2: Effect of in vitro addition of different concentrations of nicotine on the extent of lipid peroxidation at different time intervals in ejaculated cattle bull spermatozoa

Discussion

The findings of this study confirm that nicotine induces oxidative stress in spermatozoa, leading to lipid peroxidation. The increase in lipid peroxidation suggests that nicotine disrupts sperm membrane integrity, which can impair sperm function and reduce fertility. The dose-dependent relationship observed in this study is consistent with previous research, which has demonstrated that higher levels of nicotine exposure result in greater oxidative stress (Zini et al., 1999).

Nicotine's effect on spermatozoa is likely mediated through its interaction with nicotinic acetylcholine receptors on the sperm membrane, which can lead to increased calcium influx and activation of oxidative processes (Guraya, 1987). This process generates reactive oxygen species, which initiate lipid peroxidation and damage cellular components. The findings from this study underscore the potential mechanism by which smoking contributes to male infertility.

This study provides compelling evidence that nicotine induces oxidative damage in spermatozoa, leading to impaired motility. These findings underscore the potential reproductive risks associated with smoking and nicotine exposure, highlighting the need for further studies on the long-term implications of nicotine-induced oxidative stress on male fertility.

Limitations and Future Research

While this study provides valuable insights into the effects of nicotine on spermatozoa, it is important to note that the *in vitro* nature of the study may not fully replicate *in vivo* conditions. Future research should investigate the long-term effects of nicotine exposure on sperm quality, as well as explore potential interventions to mitigate the oxidative damage caused by nicotine. Additionally, examining the effects of nicotine on sperm DNA integrity and fertilization capacity would provide further understanding of the impact of smoking on male fertility.

Conclusion

This study provides compelling evidence that nicotine exposure leads to oxidative stress in spermatozoa, resulting in lipid peroxidation, decreased motility, and impaired acrosome reaction. These findings contribute to our understanding of the detrimental effects of nicotine on male fertility and underscore the importance of

reducing nicotine exposure to preserve reproductive health. To explore the long-term effects of nicotine on fertility and potential therapeutic interventions to mitigate these effects, further studies are needed .

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