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Effect of different concentrations of hydrogen peroxide on indigenous bull sperm motility

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Abstract
Spermatozoa are susceptible to oxidative stress due to their varying levels of polyunsaturated fatty acids (PUFAs) in the plasma membrane and oxidative stress affects sperm functions essential for fertilization. Hydrogen peroxide (H_2O_2), produced by dead or immature spermatozoa, is one of the major reasons for oxidative stress. In this study, we induced oxidative stress in Deoni bull spermatozoa by incubating them with different concentrations (10 µM, 25 µM, and 50 µM) of H_2O_2 at various time intervals (0 minutes, 30 minutes, and 60 minutes). Sperm motility was assessed at 30 and 60 minutes of incubation. At 30 minutes, the percentage of sperm motility in the control group was 60.8±1.3, while in concentrations of 25 µM and 50 µM, it decreased significantly to 45.8±1.5 and 27.7±2.2, respectively, indicating a notable decrease in sperm motility. After 60 minutes of incubation with concentrations of 25 µM and 50 µM, spermatozoa exhibited motility percentages of 26.5±1.9 and 14.0±1.1, respectively. It is inferred that sperm motility decreased significantly (p<0.01) with greater concentrations of H_2O_2 and longer incubation times.

Keywords: Deoni bull, sperm motility, oxidative stress, reactive oxygen species

Introduction
Bull subfertility is major cause of poor herd productivity and can be caused by a wide range of factors including management, stress and genetics which make spermatozoa vulnerable [1]. Among them oxidative stress is one of the factors responsible for a significant proportion of male infertility [2-3]. Oxidative stress (OS) is a condition when there is an increase in cellular damage promoted by oxygen and oxygen-derived free radicals known as ROS (Reactive oxygen species) [4]. In OS there is imbalance between ROS and antioxidant status of spermatozoa [5], while low level of antioxidant in cytoplasm and presence of PUFA in sperm membrane makes it vulnerable to oxidative stress [6]. ROS affects sperm by inducing protein modification, DNA damage and lipid peroxidation which causes loss of sperm viability, motility and ability to fertilize the ovum [7-9]. H_2O_2 is often associated with reduced sperm function due to oxidative stress [10]. Various researchers found deleterious effect of H_2O_2 on sperm motility [11-16]. Amino acid oxidase released from dead spermatozoa and sperm metabolism are two possible causes of H_2O_2 generation. Reports are available that at 50 µM concentration H_2O_2 affects buffalo bull sperm motility [13] but the effect of H_2O_2 on sperm motility in indigenous bulls has not been studied in detail. Therefore, this study was designed to investigate to study effect of different concentrations of H_2O_2 on Deoni (*Bos indicus*) bull sperm motility at different time intervals.

Materials and Methods
This study was conducted at the Theriogenology laboratory, Southern Regional Station of ICAR-National Dairy Research Institute, Bengaluru, India. This study was duly approved by the Institute Animal Ethics Committee (Approval number CCSEA/IAEC/LA/SRS-ICAR-NDRI-2023/No.07)

Sample preparation

Total of 6 ejaculates from Deoni bull were collected. Ejaculates were collected using an artificial vagina as per the standard procedure. Immediately after collection, each ejaculate was separately placed in a water bath set at 37 °C and assessed for routine semen quality parameters. All the ejaculates used in the study had mass activity >+3, a minimum 600 million/mL sperm concentration and ≥ 70% progressive motility. Sperm and seminal plasma were removed by centrifugation at 2000 rpm for 10 minutes.

Induction of oxidative stress

Each ejaculate was divided into 4 aliquots. Thirty percent stock of H_2O_2 (9.8 M) was diluted to 10µM, 25 µM and 50µM (final concentration). Sperm pellets were resuspended in sp-TALP (Tyrodes Albumin Lactate Pyruvate medium - 3.1 mM KCl, 100 mM NaCl, 0.29 mM NaH_2PO4, 25 mM $NaHCO_3$, 2.0 mM $CaCl_2$, 21.6 mM $C3H5NaO_3$ and 1.5 mM $MgCl_2$) containing 10µM, 25µM and 50µM concentration of H_2O_2 and incubated for 30 and 60 minutes at 37°C. For every concentration of H_2O_2 at least 6 aliquots of sperm sample were used. Sperm sample without H_2O_2 served as control.

Evaluation of sperm motility

After completion of respective incubation with H_2O_2 sperm motility was evaluated. A drop of semen was placed on a prewarmed grease-free glass slide and a coverslip was applied. The drop was allowed to spread uniformly under the cover slip (18 X 18 mm). Motility was evaluated under 20x magnification in a phase contrast microscope with a thermostatically controlled warm stage.

Statistical analysis

Effect of H_2O_2 on sperm motility during incubation with H_2O_2 were analysed and tabulated as mean with standard error. To examine significant differences in sperm motility between different levels of H_2O_2, minutes of incubation as well as the interaction between different level of H_2O_2 and minutes of incubation, two-way ANOVA test was used. Turkey post hoc test was used to assess the level of significance.

Results

The sperm motility before incubation was 77.3±1.1. The effect of different concentrations of H_2O_2 at different incubation level on sperm motility is given table 1. Sperm motility decreased with increasing concentration of H_2O_2 and incubation time. At 30 minutes of incubation, the percentage of sperm motility in control group was 60.8±1.3 while in 25 µM and 50µM concentration it was 45.8±1.5 and 27.7±2.2, respectively suggesting significant decrease in sperm motility. Following 60 minute of incubation with H_2O_2, semen sample having 25 µM concentration had 26.5±1.9% motility while the 50 µM group had 14.0±1.1 percent motility. In control group, at 60 minutes of incubation the sperm motility was 45.7±1.4%.

Discussion

Oxidative stress has long been implicated as a significant factor contributing to male subfertility and infertility [17]. Understanding the mechanisms by which oxidative stress affects sperm function holds immense potential for anthologists in developing effective therapies for individuals struggling with subfertility or infertility. Numerous studies have investigated the impact of hydrogen peroxide (H_2O_2) on sperm qualities, both in humans and animals, including motility [13-15], capacitation [18], sperm-oocyte interaction [19] and embryo development [20]. This study aims to provide information on the effect of different concentrations of H_2O_2 on indigenous bovine bull sperm motility.

Several previous studies have shown that exogenous H_2O_2 adversely affects progressive sperm motility [13, 14, 21], likely through its impact on polyunsaturated fatty acids (PUFAs) present in the sperm plasma membrane and the induction of series of chemical processes known as lipid peroxidation (LPO) processes [22]. By oxidizing sulphydryl groups or reducing the redox potential, H_2O_2 damages both plasma and mitochondrial membranes, which are crucial for maintaining sperm motility. It's noteworthy that our study utilized three different concentrations of H_2O_2, from lower (10µM) to higher (50 µM), to determine its effect on sperm motility. While bovine sperm require low levels of reactive oxygen species (ROS) for normal functions such as motility, capacitation and hyperactivation, while higher concentrations can be detrimental to sperm [5]. Nevertheless, studies indicate that low concentrations of H_2O_2 can activate defense systems and enhance human sperm motility [23].

Our observations revealed the most significant decrease in motility in semen samples incubated for 1 hour at a concentration of 50µM H_2O_2 (p<0.01). These findings are consistent with previous studies. For instance, Garg *et al.* (2009) [13], used different concentration (10-50 µM) of H_2O_2 at various incubation time (0 minute, 15 minutes, 30 minutes, 45 minutes and 60 minutes) in buffalo bull sperm and observed 15.0±2.0% motility in sample incubated in 50 µM H_2O_2 for 1 hr. This is in line with our study, in which we observed 14.0±1.1% motility in sperm incubated in 50 µM H_2O_2 for 1 hour. Similarly, O'Flaherty *et al.* (1999) [24] reported sperm motility in 25µM H_2O_2 and 50µM H_2O_2 group as 35±4% and 13±3%, respectively after 45 min of incubation against the motility of 55±6 in control spermatozoa sample in Holstein Friesian bulls. In our study, we observed 45.8±1.5% motility when incubated with 25 µM of H_2O_2 for 30 minutes. Maia *et al.* (2014) [15] reported that there was no progressive motility in frozen thawed ovine spermatozoa after induction of oxidative stress by incubating sperm with 100 µM of H_2O_2 for 30 minutes at 37 °C. Du Plessis *et al.* (2010) [14] reported that human sperm subjected to oxidative stress with 15 µM H_2O_2 for 1 hr caused decrease in progressive motility of spermatozoa. Collectively, the findings of the study underscore the detrimental effect of H_2O_2 on sperm motility and it is concluded that the detrimental effect of H_2O_2 on sperm motility increased with the dose of H_2O_2 and time of incubation.

Table 1: Mean sperm motility at different level of H_2O_2 and incubation time

Minutes of incubation	Control	10 µM	25 µM	50 µM
30	60.8 ± 1.3^{cB}	59.3 ± 1.4^{cB}	45.8 ± 1.5^{bB}	27.7 ± 2.2^{aB}
60	45.7 ± 1.4^{cA}	40.3 ± 1.2^{cA}	26.5 ± 1.9^{bA}	14.0 ± 1.1^{aA}

Values having different superscripts a, b, c and A, B varies significantly (p<0.01) between columns and rows, respectively.

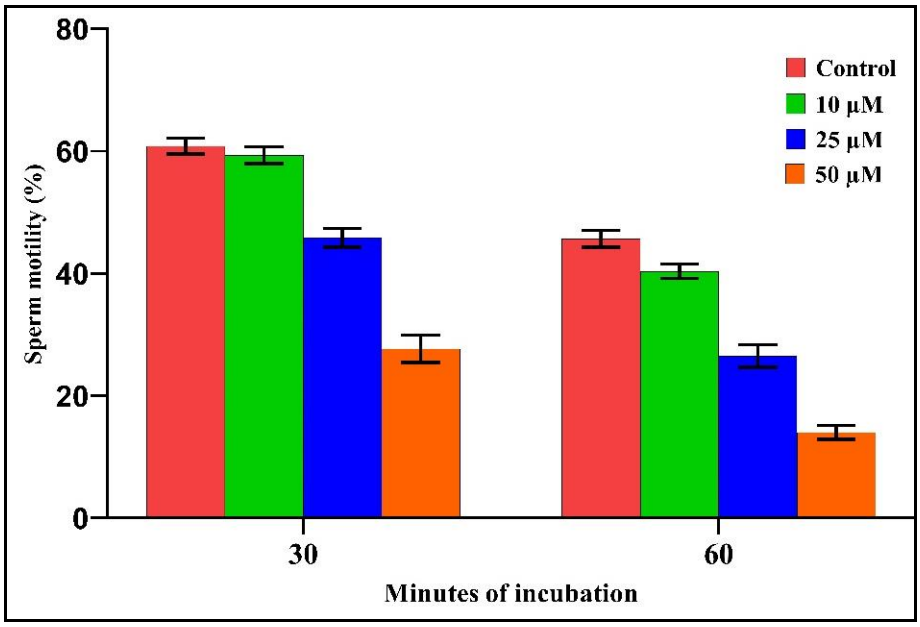

Fig 1: Mean sperm motility at different level of H_2O_2 and incubation time

Conclusion

In summary, our investigation delved into the impact of varying concentrations of hydrogen peroxide (H_2O_2) on indigenous bovine bull sperm motility. We observed a significant decrease in sperm motility with increasing H_2O_2 concentration and incubation time. Notably, the most pronounced decline occurred after one hour of incubation with 50µM H_2O_2. These findings align with previous research highlighting the deleterious effects of H_2O_2 on sperm motility across various species. Our study underscores the dose-dependent and time-dependent nature of H_2O_2's detrimental impact on sperm motility, shedding light on the mechanisms underlying male subfertility and infertility associated with oxidative stress. Further exploration of protective mechanisms against oxidative damage may offer potential therapeutic avenues for reproductive health.

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