# THE EFFECT OF INDOMETHACIN ON THE SPERM MOTILITY OF ADULT WISTAR RATS


## Abstract

The study aimed at assessing the effect of indomethacin on the male reproductive system, focusing on sperm quality. Twenty male rats were divided into four groups, with group one serving as the control group and the other three groups receiving different doses of indomethacin, (groups 2-4 receiving 1mg/kg, 2mg/kg and 4mg/kg respectively) for 21 days. After the period, sperm samples were collected from all the groups and analyzed to evaluate sperm quality. The sperm qualities analyzed include; sperm motility, semen viscosity, sperm morphology, sperm count, etc. the results of the study indicated that indomethacin had a negative impact on the sperm qualities of the rats, as it decreased sperm count and sperm motility , and had a detrimental effect on the overall sperm quality. This study provides important insights into the potential side effects of indomethacin on male fertility. Indomethacin is a Nonsteroidal Anti-inflammatory Drug (NSAID) commonly used to treat various conditions, including headaches, fever, and pain. While its therapeutic benefits are well established, its impact on male fertility has not been documented. The findings of this study suggest that men who use indomethacin regularly may be at a higher risk of experiencing negative impacts on their reproductive health, including reduced sperm quality.



## Keywords

Indomethacin, wistar rat, sperm motility, reproductive health


## Introduction

Indocid is a brand name for Indomethacin, a non-steroidal anti-inflammatory drug (NSAID) that is very effective in the treatment of pain, feverish conditions, and inflammation, like other NSAIDs it reduces pain and inflammation (Lucas 2016). Generally, non-steroidal anti-inflammatory drugs (NSAIDs) are a diverse group of drugs, frequently unrelated chemically, yet the majority are organic acids and known in the treatment of conditions, such as osteoarthritis, rheumatoid arthritis, different type of inflammation, gout, menstrual cramps, dental pain, and headache. However numerous adverse reactions have been linked to NSAIDs use which include nausea, vomiting, diarrhea, constipation, reduced appetite, rash, dizziness, headaches, sleepiness, and stomach ulcers (Lucas 2016 and Bagoji et al., 2014). Indomethacin is used mainly in the treatment of moderate pain since 1960 and it is frequently prescribed for the treatment of severe

gouty arthritis pain, it has also shown effectiveness in the treatment of several other painful disorders ((Nalamachu et al.,2014).

The chemical composition of indomethacin is 1- (p-chlorobenzoyl) 25-methoxy-2-methylindole-3-acetic acid, it is effective mainly because of the ability of indomethacin to inhibit prostaglandin production. Prostaglandins are important mediators of inflammation, fever, and pain and are predominantly generated by cyclooxygenase (COX) enzymes (Munjal and Allam 2020). The adverse effect of common NSAIDs like ibuprofen and Naproxen has been studied and patients are advised not to take NSAIDs on an empty stomach because of its ability to cause stomach ulcers. The adverse effect of indomethacin is one of the reasons why it is not generally prescribed clinically, like the way it works the adverse effects occur by non-selectively inhibiting the cyclooxygenase (COX1) and (COX2) enzymes, which prevent the synthesis of prostaglandins from arachidonic acid by the cyclooxygenase enzymes and the cyclooxygenase is necessary for physiological processes like the maintenance of gastrointestinal mucosa and vascular homeostasis (Sabeeh et al., 2014).

Though this drug is commonly used in treating various forms of pain and inflammation, indomethacin has been found to have potential adverse effects on reproductive health. Although previous studies have suggested a link between the use of indomethacin and decreased sperm quality, the relationship between the drug and specific aspects of sperm health such as sperm motility, count, and morphology, has not been well established (Bogaji et al., 2014). This lack of clarity regarding the specific impact of indomethacin on sperm quality is a major limitation in our understanding of the drug's potential effects on male fertility.

Given the widespread use of indomethacin and the increasing interest in understanding the impact of the medications on reproductive health, there is a pressing need for more information on the effect of indomethacin on sperm quality. The purpose of this study is to fill the gap by examining the effect of indomethacin on sperm quality in a controlled laboratory setting. The result of this study will help clarify the potential impact of indomethacin on sperm quality and male fertility

**Materials and Methods**

This research work was carried out in the Department of Animal and Environmental Biology animal house University Campus, University of Port Harcourt Choba, Port Harcourt Rivers State, Nigeria.

Indocid was bought from a local pharmacy at Choba Port Harcourt Rivers State Nigeria. The materials and specimens used in carrying out the experiment included Adult male rat, cages, Rat feed, drinkers, hand gloves, desiccator, dissecting board, chloroform, cotton, microscope, normal saline, stains (methylene blue, alcohol and strong carbon fischen), Neubauer counting chamber, weighing balance, pipette, plain bottle, surgical blade.

Twenty (20) rats were gotten from the Faculty of Basic Medical Sciences, Department of Pharmacology, University of Port Harcourt, and they were acclimated for 7 days. The 20 rats were placed into 4 groups. Their weight ranges from 180kg to 228kg. The animals were maintained under the standard condition of twelve hours' light/dark cycles at room temperature and fed with grower's mash feed and water.

Indocid was diluted serially into 1mg/kg (lowest dose), 2mg/kg, and 4mg/kg (highest dose). Group one (1) was used as the control group, while the other three (3) groups were treated with 1mg/kg, 2mg/kg and 4mg/kg of indocid respectively.

After 21 days of the administration, the semen was collected from the epididymis of the rats of each group, for microscopic examinations

The semen is usually hyper-viscous in nature, hence it was placed in a small plain bottle, and 2 drops of normal saline were added to separate the cells. A drop of the diluted semen was placed in a slide to check for sperm motility microscopically using the objective lens 10×.

A drop of dilute semen was taken and placed on a plain bottle, 19 drops of normal saline was added to achieve the 1:20 dilution. This dilution was charged on the counting chamber; after which it was placed on the moisturizer for 3 minutes to allow more separation of the cells. The counting chamber was then placed on the microscope and viewed using the objective lens of ×10. The cells were counted using the hand tally counter. The formula for calculating the sperm count, when 5 small squares within the large center square are counted is: Number of sperm counted x dilution factor/volume x 1000 = sperm/ml. Example: 50 sperm are counted in the five small squares. The dilution is 1:20.

Sperm/ml = 50 x 20/0.02 $mm^3$ x 1000 $mm^3$/ml = 50,000,000 sperm/ml.

If the dilution is 1:20, and the usual 5 small squares are counted, then the formula can be simplified as follow: Sperm/ml = Sperm counted x 1,000,000.

To check for defects in the sperm morphology (head , midpiece, and tail defects), a clean slide was placed on the microscope and a drop of the diluted semen was added, thereafter, a film was made out of it, and then it was flooded with alcohol and allowed to be air dried for 2 minutes. Strong carbon fischen was latter used to flood and further allowed to stand for 2-3 minutes before it was washed off with alcohol and then viewed under x100 oil immersion lens of the microscope.

**Results**

**Active sperm cells**

There were more active sperm cells in Group 1 (the control) (84%) compared to the treatment groups ( 62.75,53.25 and 63.00 % for the three treatment groups respectively). Group 3 with a dose of 2mg/kg had the lowest number of active sperm cells. The results showed that indomethacin affected the sperm cells because the control group had more active sperm cells than the administered groups (Figure 1)

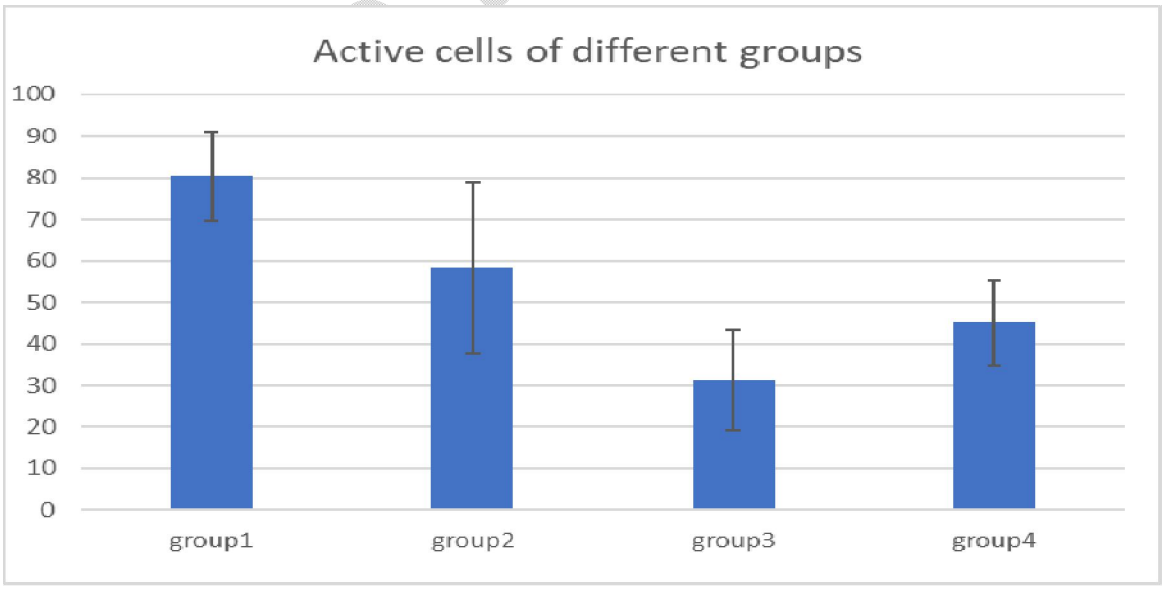


**Figure 1: The Active sperm cells across the different Groups**

**Sluggish Sperm Cells**

The treated groups had more sluggish sperm cells compared to the control, and Group 3 amongst the treated groups had the highest number of sluggish sperm cells. Indomethacin had a significant effect on the sperm cells motility because the treated groups have more sluggish sperm cells than the control. (Figure 2)

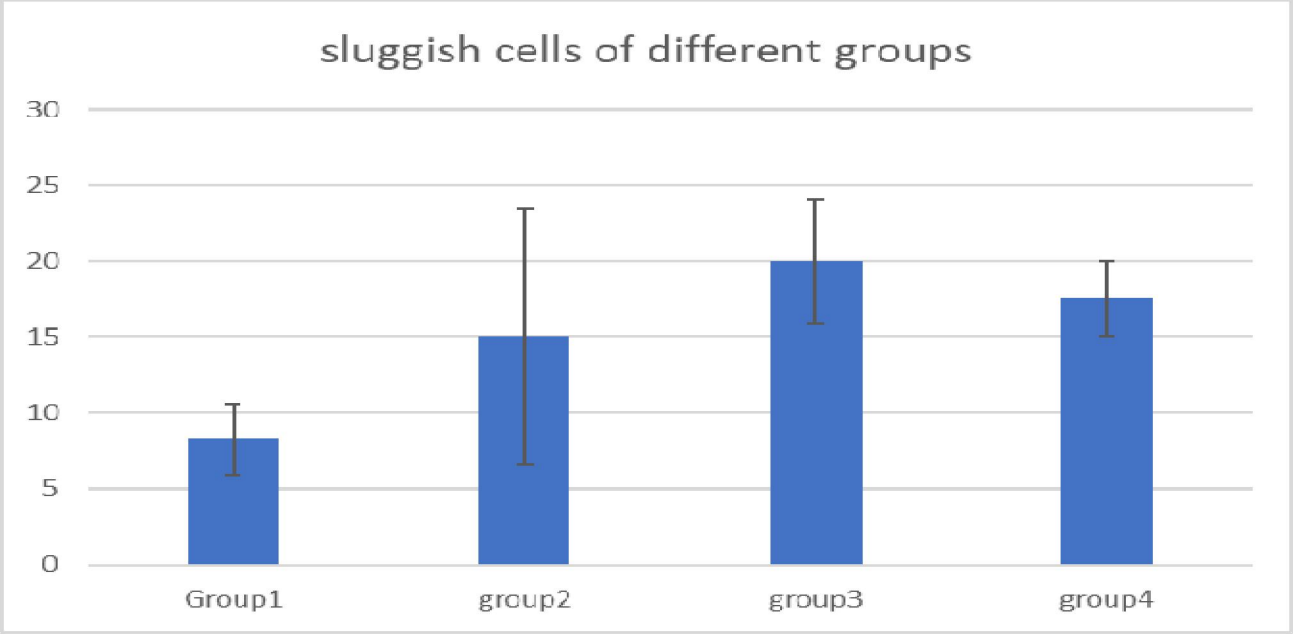


**Figure 2: The Sluggish cells across different group**

**Dead Sperm Cells**

The amount of dead sperm present in Group 3 was more than any in every group and there are more dead sperm cells in the groups where administration occurred compared to the control group (Group 1). the percentage dead cell were 11.25%, 26.25%, 48.75% and 30.00% for groups 1, 2 3 and 4 respectively.

The administration of indomethacin leads to the death of spermatozoa as the results showed that the treated groups had more dead sperm cells than the control. (Figure 3)

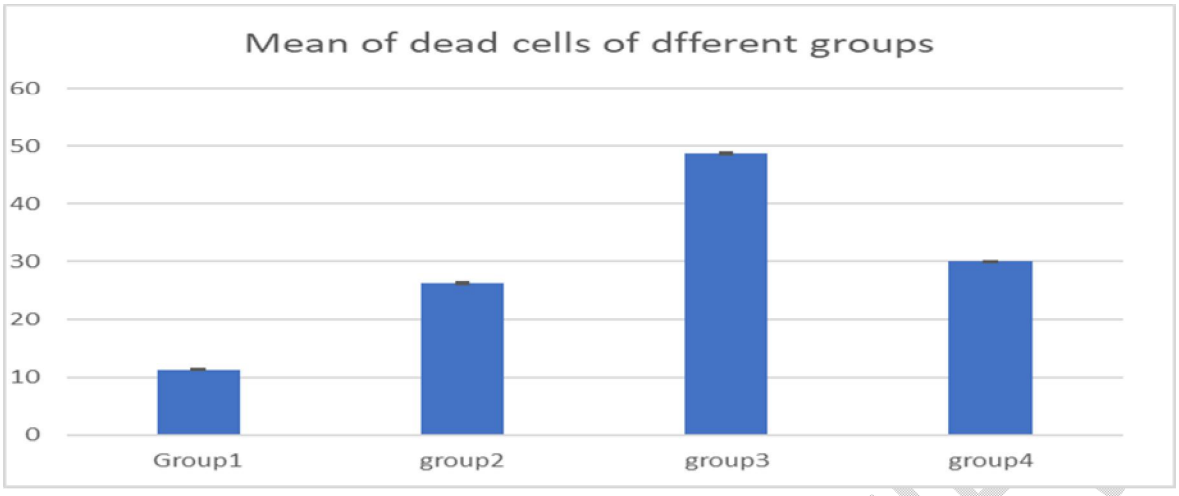


**Figure 3: The mean of dead Sperm cells across the different group**

**Percentage of Sperm Viability**

Group 1 (control) had highly viable sperm cells and very low non-viable sperm cells and when compared to the other groups, the control group has better sperm viability than any other groups. The control has more sperm viability, so indomethacin affects the viability and the administered groups had lower viability (Figure 4)

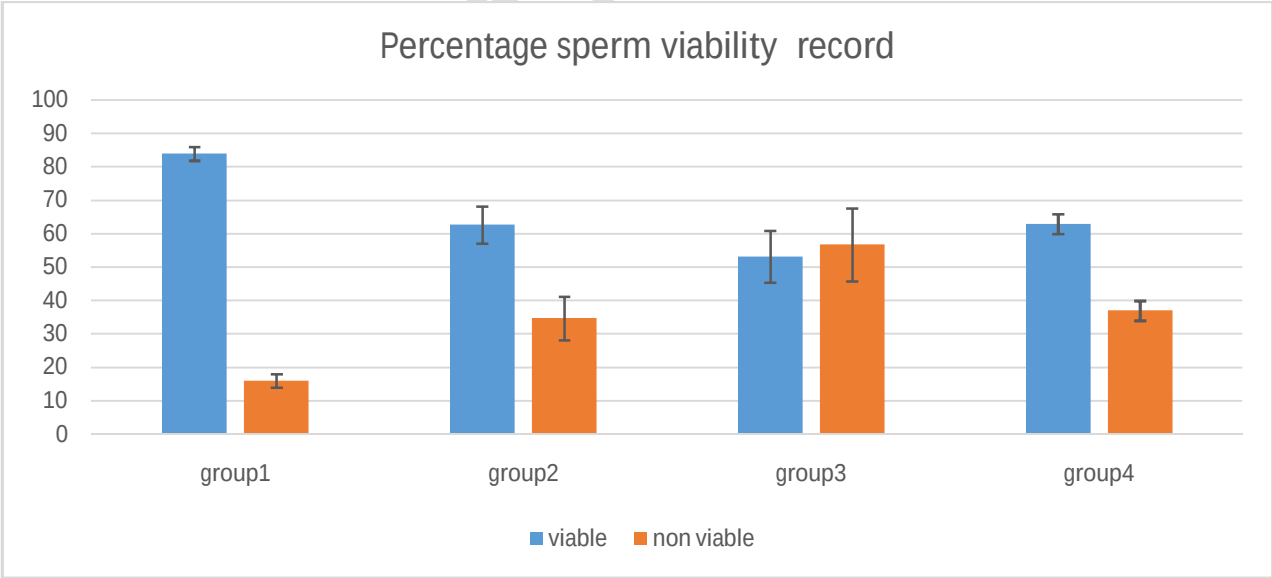


**Figure 4: The Sperm Viabilities across the different Groups**

**Viable vesus affected sperm cells ( non viable and dead)**

Comparism of the viable sperm cells and the nonviable and dead shows that group one (control) 74.55 % viable as against 25.25% non viable and dead combined ratio of 46.28% and 53.72%, 27.29%and 72.71%, and 44.1% and 55.9% for groups 2, 3 and 4 respectively.

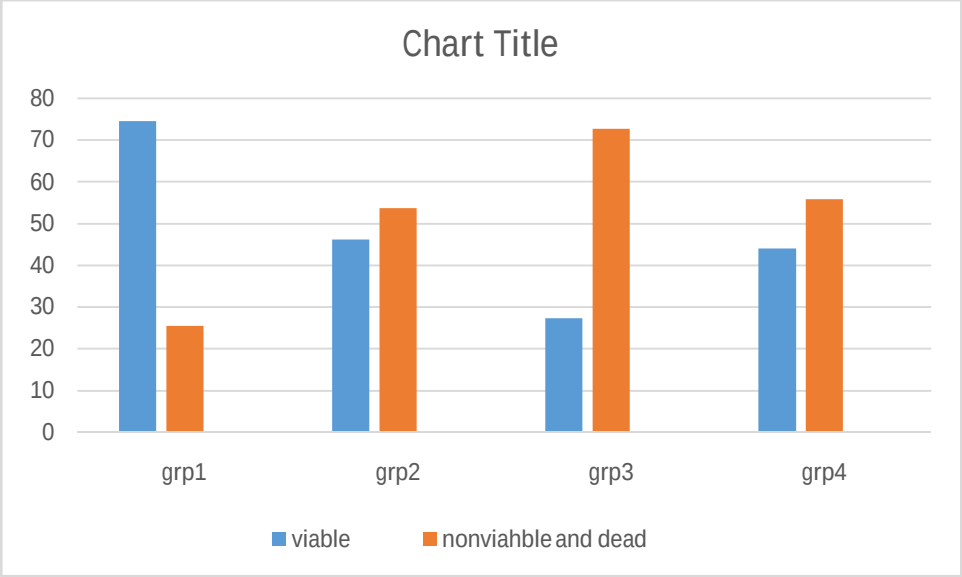


**Figure 5: comparison of viable cells with nonviable and dead cell ( affected cells)**

This implies that he indociddirectly affect the viability of the sperm and hence affecting reproductive health of rats which will also affect humans reproductive health.

**Discussion**

The study of the effects of indomethacin on sperm quality has generated significant interest in the scientific community due to the increasing number of couples facing infertility issues. The results of the study have shown that indomethacin administration has a detrimental effect on sperm quality. This is an important finding as it highlights the need for caution when using this medication in individuals of reproductive age.

The results of the study showed that the control group had a higher number of active sperm cells compared to the groups where indomethacin administration occurred. This finding is consistent with previous research that has found that nonsteroidal anti-inflammatory drugs (NSAIDs) can decrease sperm motility (Padrón et al, 1979) The decrease in active sperm cells may be due to the fact that indomethacin inhibits the production of prostaglandins, which are important for maintaining sperm motility.

However, the study also found out that the administration of indomethacin led to an increase in the number of sluggish sperm cells. This finding is also consistent with previous research that has shown that NSAIDs can negatively impact sperm function (Martini et al, 2008). The increase in sluggish sperm cells suggests that indomethacin may be affecting the energy metabolism of sperm cells, leading to a decrease in their ability to move efficiently.

The results also showed that the administered groups had a higher number of dead sperm cells compared to the control group. This finding is consistent with previous research that has shown that NSAIDs can increase oxidative stress and decrease sperm viability. The increased amount of dead sperm cells suggests that indomethacin may be affecting the antioxidant defense mechanisms of sperm cells, leading to an increase in oxidative stress and a decrease in sperm viability which correlated with Sabeeh et al., (2014) who investigated the histopathological changes in the male reproductive organs of rats treated with oral indomethacin, with the aim of understanding the potential negative effects of the drug on male reproductive health. The study found that rats treated with indomethacin exhibited a range of histopathological changes in their male reproductive organs, including the reduced thickness of seminiferous tubules, decreased numbers of spermatogenic cells, and increased numbers of degenerating cells. The study also reveals decreased weight of seminal vesicles in the treated group.

Finally, the study found that the control group had more viable sperm cells than the treated groups. This finding is also consistent with previous research that has shown that NSAIDs can decrease sperm viability (Bagoji et al, 2014). The decrease in sperm viability may be due to the fact that indomethacin affects the membrane integrity of sperm cells, leading to a decrease in their ability to survive (Ratnasooriya et al 1987). The results of this study provide further evidence that indomethacin administration can have a negative impact on sperm quality. This is an important finding as it highlights the need for caution when using this medication in individuals of reproductive age though may be effective for managing pain and inflammation. It is recommended that further research be conducted to better understand the mechanisms by which indomethacin affects sperm quality and to determine the optimal dose and duration of use that minimizes its negative impact on sperm quality.

**Conclusion**

The study has shown that indomethacin has a detrimental impact on sperm quality. The results showed that the control group had a higher number of active sperm cells compared to the group where indomethacin was administered. The administration of indomethacin led to an increase in the number of sluggish sperm cells and dead sperm cells and a decrease in the number of viable sperm cells. These findings highlight the need for caution when using this medication in individuals of reproductive age. The results of the study provide further evidence that indomethacin administration can negatively impact sperm quality.

**RECOMMENDATIONS**

Based on the results of the study, it is recommended that individuals of reproductive age should carefully consider the use of indomethacin. If possible, alternative pain management options should be considered and it is recommended that further research be conducted to better understand the mechanisms by which indomethacin affects sperm quality and to determine the optimal dose and duration of use that minimizes its negative impact on sperm quality.

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