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Reproductive and Mortality Outcomes in Wistar Rats Following Oral Polio Vaccine Administration

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Abstract

Rumors surrounding the potential side effect of infertility caused by the oral polio vaccine (OPV) have led parents and caregivers in Northern Nigeria to reject the OPVs. Unfortunately, little attention has been given to confirming or refuting this concern, and limited research addresses this gap. OPVs were administered to Wistar rats in varying doses, and their reproductive health and mortality indices were evaluated over a six-week period. The Wistar rats were divided into three experimental groups consisting of 10 male and 27 female rats. Each male rat was paired with three female rats and assigned to one control group and two experimental groups. Pregnancy tests, live delivery outcomes, estrogen and progesterone levels in females, and sperm parameters in males were methodically documented. Pregnancies were recorded in 6 (66%), 6 (66%), and 7 (77%) of the female rats in groups 1, 2, and 3, respectively, resulting in the delivery of 14, 15, and 16 offspring in the three groups. The mean number of pregnancies and deliveries was similar across the three groups, with no statistically significant difference (p>0.05). All male rats showed normal semen parameters. The comparison of mean semen analysis findings among the three groups was not statistically significant, except for sperm motility, which was higher in groups 2 and 3. OPV did not have any detrimental effect on the reproductive performance of both female and male rats. This study demonstrated that OPVs have no effect on the reproductive outcomes in both male and female rats exposed to OPVs.

Keywords: OPVs (Oral Polio Vaccines), Reproductive profile, Mortality indices, Wistar Rats.

INTRODUCTION

Poliomyelitis, commonly known as polio or infantile paralysis, is an infectious disease caused by the poliovirus (Hamborsky *et al.*, 2015). This highly contagious viral illness primarily affects young children. The virus spreads from person to person, mainly via the fecal-oral route or, less frequently, through a common vehicle (e.g., contaminated food or water). It multiplies in the intestine, from which it can invade the nervous system and may lead to paralysis (WHO 2017). Evidence suggests that poliomyelitis has been present for nearly 6000 years, as indicated by some Egyptian mummies' withered and deformed limbs. However, it wasn't until the 1950s that a vaccine became available (Vidyadara, 2017). Initial symptoms of polio include fever, fatigue, headache, vomiting, neck stiffness, and limb pain. In a small proportion of cases, the disease results in paralysis, which is often permanent. There is no cure for polio; it can only be prevented through immunization (WHO 2017).

As of 2012, Nigeria accounted for more than half of all polio cases worldwide, but the country made significant strides, marking two years without a case of polio as of July 2016. This progress resulted from a concerted effort by all levels of government, civil society, religious leaders, and many dedicated health workers. However, on August 12, 2016, after more than two years without wild poliovirus in Nigeria, the government reported that the disease had paralyzed two children in the northern Borno state. (WHO 2016). Among the possible explanations for the resurgence of the disease was the inaccessibility of certain communities in northeastern Nigeria for vaccinations due to the Boko Haram conflict, as well as a persistent rejection of the vaccines due to rumors about possible side effects. The rumors regarding

potential side effects from the vaccine were most prominent in Northern Nigeria, leading to widespread rejection of the vaccines. Paramount among these rumors were fears that polio vaccines would lead to infertility in immunized children. (Michael 2014, Chen 2004). With the rejections of the vaccines, disease transmission has never been stopped in Nigeria.

However, little attention has been paid to confirming or refuting the fears of various communities in Northern Nigeria regarding the potential for the OPV to cause infertility in immunized children. As a result, vaccine rejection persists among many concerned parents who fear for the safety of these vaccines in their children due to a lack of credible information. There are no comprehensive studies that have been conducted to determine whether these vaccines can actually cause infertility. In the absence of reliable information, parents and some Islamic clerics continue to spread these rumors, leading to widespread rejection of the vaccines. As we approach the final milestone in eradicating poliomyelitis, the need for a study of this nature becomes evident. This study aims to test whether these vaccines potentially affect fertility. The goal of conducting this study is to provide concrete information that will either allay or confirm parents' concerns regarding the vaccines' safety. Therefore, the specific objectives of this research are to determine pregnancy and live delivery rates in female Wistar rats exposed to polio vaccines (study groups) and compare them with the pregnancy and live delivery rates in female Wistar rats that were exposed to placebo (control group consequently, sperm analysis in the male Wistar rats in the study groups and the sperm analysis in the male Wistar rats in the control group were determined therefore comparing the sperm analysis in the study and control groups

METHODOLOGY

The study was conducted in the animal laboratory of the Department of Physiology, Umaru Musa Yaradua University, Katsina. Yaradua University is a tertiary educational center located in Katsina, one of the worst hit of the polio vaccine refusals. The animal lab is located at the university's Faculty of Basic Medical Sciences and has been accredited by the Nigerian University Commission for this purpose. The animals were taken care of by an experienced animal handler specifically employed for that purpose. The animal handling was done per the international guidelines for animal research developed by the National Advisory Committee for Laboratory Animal Research. (National Advisory Committee for Laboratory Animal Research, 2004). The housing was in the form of well-ventilated cages of 3 female rats with one male rat with a light: darkness cycle of 12:12 hours.

TABLE A: Comparison of human and rat days for administration of opv

OPV TYPE	OPV 1	OPV2	OPV3	OPV4
Time administered in humans (weeks of life)	0 (birth)	6	10	14
Time administered in humans (days of life)	0	42	70	98
Time administered in rats (days of life)	0	1.5 (2)	2.59 (3)	3.6 (4)

Source: John Hopkins University 2017

Dosages of OPV/ Placebo: As shown in Table A, human: rat days comparison and human: rat days doses were calculated as outlined. To calculate the dosage of the OPV and placebo, it was considered that the dose of OPV administered at each session to humans is 0.5 mL. To calculate the commensurate dosage of OPV for the rats, the average weight of a rat at birth (5g) (Johns Hopkins University, 2017) was compared to the average weight of a human baby at birth (3065g) (Onankpa, 2006). Using the weight comparison, the appropriate volume of OPV for the rat was estimated to be 0.05mL and this was administered to the rats in study group 1 while 0.1mL was administered to those in study group 2. The same volume of placebo (0.05mL) was administered to the rats in the control group. As shown in Table B, Insulin syringes were used to administer the OPV and placebo.

Table B: Dosage and Timing of Administration of OPV and Placebo Among the Groups

	Control Group	Study Group 1	Study Group 2
Placebo	0.05mL	-	-
OPV	-	0.05mL	**0.1mL**
Time of Administration (day of life)	**Birth, 2,3,4**	**Birth, 2,3,4**	**Birth, 2,3,4**

Semen analysis.

The semen analysis of the 9 male rats involved sacrificing all the male rats at the end of the study and surgical extraction of the epididymis, which was then sent to the laboratory, and extraction of spermatozoa from it for further

analysis. The sperm analysis was conducted by a trained laboratory scientist as follows:

Evaluation of sperm motility

Semen samples from the different treatment groups were dropped on a glass slide and

viewed under the microscope. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 spermatozoa for each rat. The percentage of sperm motility was then analyzed.

Estimation of mean sperm count

Sperm count was carried out by diluting the semen (1 in 20) using sodium bicarbonate-formalin diluting fluid. The well-mixed diluted semen was then applied to an Improved Neubauer-ruled chamber and appropriately filled. It was then waited for about 3 minutes for the spermatozoa to settle. The number of spermatozoa in an area of 2 sq mm (i.e., 2 large squares) was counted. The number of spermatozoa in 1ml of fluid was calculated by multiplying the number counted by 100, 000.

Estimation of sperm viability

This was estimated using the improved one-step eosin staining technique. A fraction of each suspension of the sperm samples was mixed with an equal volume of eosin stain were prepared on glass slides for each sample; after 2 minutes, the slides were examined under the microscope for percentage viability. Normal live sperm cells (viable) exuded the eosin, while dead sperm cells took up the stain. Percentage viable spermatozoa were counted using 40 x objectives.

Estimation of semen pH

The pH of semen was measured using a specially treated calibrated paper blot that changes color according to the pH of the semen that it is exposed to.

Data Management

The mean number of live births and the mean numbers of pregnancies within three months in the three groups were determined and compared. Also, the mean sperm counts, mean sperm motility and mean normal sperm morphology in the male rats in the three groups were compared using Analysis of Variance (ANOVA) determined through the Minitab software. The level of statistical significance will be set a p-value < 0.05.

Limitations

This study is best performed with the addition of a positive control group that will have their reproduction enhanced to further promote comparison on the effect of the OPV on reproduction. However, this was not done in this study as there are no known drugs that will enhance reproduction in rats

Also, this study is best done with transgenic animals genetically modulated for this study. This was not done due to the lack of these animals and their cost implication

Ethical Consideration

Ethical clearance was sought and obtained from the ethical committee of the Katsina State Ministry of Health with approval reference number 1044. The animals were cared for by an experienced animal handler specifically hired for that purpose. The handling of the animals followed the international guidelines for animal research established by the National Advisory Committee for Laboratory Animal Research.

This study assessed the reproductive outcomes and seminal fluid parameters of Wistar rats following exposure to different doses of oral polio vaccines (OPVs), comparing them with a control group that received a placebo. The analysis included female and male rats, evaluating pregnancy rates, live births, and comprehensive semen analyses.

RESULTS

Pregnancy and Delivery Outcomes

As shown in Table 1, pregnancies were successfully achieved in all three experimental groups. Specifically:

Group 1 (Control): 6 out of 9 female rats conceived, representing **66.6%** conception rate, and delivered a total of **14 live pups**.

Group 2 (OPV 0.05 ml): 6 out of 9 also conceived (**66.6%**), with **15 live births** recorded.

Group 3 (OPV 0.1 ml): 7 out of 9 rats conceived (**77.7%**), resulting in **16 live births**.

Notably, **all pups born were alive** across all groups, giving each group a 100% live birth rate.

There were pregnancies recorded among rats in all the three groups as shown in:

Table 1. Pregnancies were recorded in 6 (66%), 6 (66%) and 7 (77%) of the rats in groups 1, 2 and 3. This culminated in the delivery of 14, 15 and 16 rats in the three groups respectively.

Table 1: Outcome of pregnancy and delivery among the female rats in the three groups

GROUP	No of rats that conceived (% of total no of female rats)	No of offspring delivered	No of offspring born alive (% of those delivered)
1	6 (66.6)	14	14 (100)
2	6 (66.6)	15	15 (100)
3	7 (77.7)	16	16 (100)

A comparative analysis of pregnancy and delivery outcomes across the three groups is presented in Table 2. The mean number of pregnancies and mean number of live offspring were statistically not significantly different across the groups. For instance, the mean number of rats that conceived was approximately 0.44–0.50 across groups, and the mean live births ranged from 1.5 to 1.6 pups per rat. ANOVA results yielded p-values of 0.96 and 0.99, respectively, confirming no statistically significant difference in reproductive outcomes ($p > 0.05$)

Table 2. shows the comparison of the pregnancy and delivery among the three groups. The mean number of pregnancies and deliveries were similar among the three groups and the difference as determined by ANOVA was not statistically significant ($p > 0.05$).

Table 3 presents the detailed seminal fluid analysis of three male rats from Group 1 (Control), focusing on six key semen quality parameters: semen volume, pH, motility, viability, sperm count, and morphology. These parameters are essential indicators of male reproductive health.

Table 4 and Figure 1 show the comparison of the mean semen analysis as determined by ANOVA. The only statistically significant finding was in the mean motility, which was statistically significantly higher in groups 2 and 3 ($p < 0.05$). Comparison of morphology was not done as the finding was the same in all the groups (morphological defects was not up to 10%).

Table 2: Comparison of pregnancy and delivery among the female rats

Variable	Group 1 (N=9)	Group 2 (N=9)	Group 3 (N=9)	P-value
Mean no of rats that conceived	0.44±0.52	0.44±0.52	0.5±0.52	0.96
Mean no of live offsprings	1.5±1.87	1.6±2	1.6±1.77	0.99

Table 3: Seminal Fluid Analysis Findings Among the Male Rats

GROUP1(CONTROL)	SV (ml)	PH	MOTILITY (%)	VIABILITY (%)	SC (*10 ^{6}/ml)	NM
1	0.1	6.8	75	90	50	>90%
2	0.07	6.9	60	80	48	>90%
3	0.08	6.4	74	80	47	>90%
GROUP2						
1	0.07	6.8	90	80	49	>90%
2	0.07	6.5	85	70	52	>90%
3	0.08	6.7	86	70	48	>90%
GROUP3						
1	0.1	6.9	95	80	55	>90%
2	0.09	6.8	88	75	49	>90%
3	0.08	6.7	87	75	50	>90%

SV = SEMEN VOLUME; SC = SPERM COUNT; NM = NORMAL MORPHOLOGY

Table 4: Comparison of Mean Semen Analysis Findings Among the Groups

VARIABLE	Group 1 N=3	Group 2 N=3	Group 3 N=3	P Value
Mean Semen Volume (ml) ± SD	0.08±0.01	0.07±0.05	0.09±0.01	0.25
Mean PH ± SD	6.7±0.26	6.6±0.15	6.8±0.1	0.67
Mean Motility (%) ± SD	83.3±5.7	87±2.6	90±4.3	0.00*
Mean Sperm Viability (%) ± SD	69.6±8.3	73.3±5.7	76.6±2.8	0.11

*** Statistically significant value (p <0.05)**

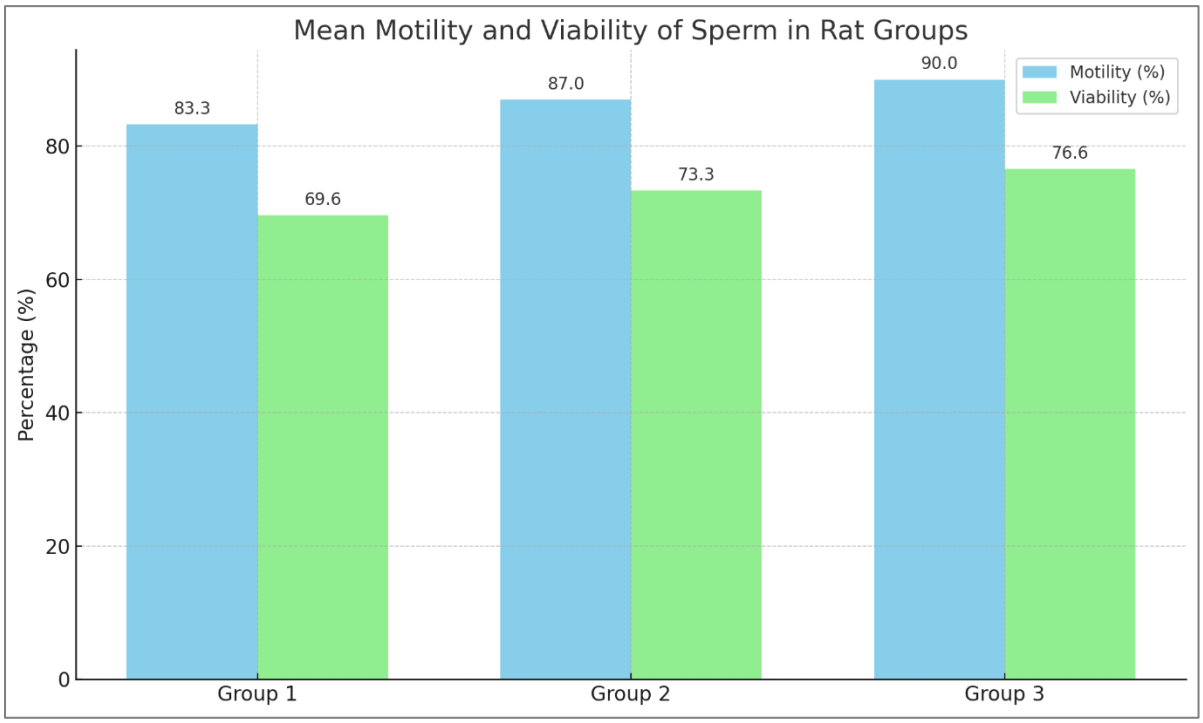

Figure 1: Bar chart illustrating the mean sperm motility and viability percentages among the three rat groups

Motility increases progressively from Group 1 (83.3%) to Group 3 (90%).

Viability also shows an increasing trend from Group 1 (69.6%) to Group 3 (76.6%).

This visual supports the data in Table 4 and highlights the higher motility (statistically significant) and viability (not statistically significant) in Groups 2 and 3. Let me know if you'd like a version including error bars or additional variables like sperm count.

The mortality index is typically calculated as:

Mortality Index=100%−Viability (%)

Using the mean viability values from Table 4 for each group, the mortality index is as shown in Table 5.

Table 5: Mortality Index

Group	Mean Viability (%)	Mortality Index (%)
Group 1	69.6	30.4
Group 2	73.3	26.7
Group 3	76.6	23.4

So, the mortality indices are:

Group 1: 30.4%

Group 2: 26.7%

Group 3: 23.4%

These indicate a declining trend in mortality from Group 1 to Group 3, suggesting improved sperm survival in the latter groups.

DISCUSSION

The key finding in this study was that there were no statistically significant differences in the pregnancy and live delivery rates among the female rats exposed to placebo as compared to those exposed to the OPV vaccine. A similar finding was recorded for the sperm parameters in the male rats except for the sperm motility, which was statistically significantly higher in those exposed to the OPV vaccine. These findings imply that the OPV vaccine has no deleterious effect on the reproductive outcome in the rats that were studied. Group three had twice the normal dose of OPV vaccine administered to the rats and yet, the reproductive outcome in both the male and female rats was similar to that of those that had placebo. In the sperm analysis, the motility appeared higher in the groups exposed to OPV vaccine than in those administered placebo. The explanation for this finding is not entirely clear. It could be that the OPV enhances motility of spermatozoa which is a beneficial effect for the attainment of reproduction. It could also reflect individual differences in the male rats studied, bearing in mind that the semen parameters in the male rats exposed to placebo was also within normal ranges. These findings warrant further analysis in future studies on this subject.

However, it is reassuring to note that it was an increase rather than a reduction in semen motility that was found and that it was in those male rats exposed to the OPV vaccine.

The rumors in Northern Nigeria alleged that oral polio vaccine has a sterilizing effect or spreads AIDS because it is contaminated with human immunodeficiency virus (Jegede 2007). Although they are baseless and may easily be refuted on scientific grounds, these allegations have the potential to discredit and damage immunization programs. They are major reason for refusal of vaccines by parents. (Samba, Nkrumah and Leke 2004). The rumour about the effect of OPV on causing HIV/AIDS is easy to discredit because there has been no report of any child who suffered from HIV/AIDS after immunisation with OPV. The rumour that appears to give parents greater concern, including educated parents, is that of the possibility of an agent in the OPV capable of impairing the reproductive potential of their children in the future. It gives a greater concern since the effect is not immediately seen, unlike that of HIV/AIDS, and makes the parents develop a feeling of hopelessness. However, this study will reassure parents about the safety of the vaccine.

The scientific community has not previously addressed the concerns of the parents as there were no studies identified in the literature which addressed the rumours and reassured the parents on the safety of the vaccines. This study directly addressed the rumours and generated evidence aimed at reassuring the concerned parents. The evidence generated has refuted the allegations of the rumours and showed that the vaccines have no effect on the reproductive performance of the rats. The evidence generated is expected to satisfy parents that their concerns have been addressed and willingly present their children for vaccinations. This partnership between concerned parents and the scientific community is what is needed as a way forward towards the global elimination of polio. This is even more important now that the world is on the verge of the elimination of polio. Currently, parents are being forced to bring their children for vaccinations whether they are willing or not. A partnership between parents and health workers following the presentation of this evidence rather than the compulsion of parents to bring their children for immunisation campaigns is likely to yield a better result.

There is an urgency attached to the global elimination of polio. The WHO already targeted 2018 as the year for the global eradication of Polio (Mundel and Orenstein 2013). There is an urgent need for wide dissemination of the findings on OPV safety regarding reproduction. Hopefully, this will make parents bring their children for immunisation.

CONCLUSION

This study showed that OPV has no effect on the reproductive outcomes in male and female rats exposed to OPV. There is a need for widespread dissemination of these findings through mass media to reassure parents about the safety of OPV concerning reproductive outcomes. This will help improve attendance at immunization clinics, as better-informed parents will be more likely to bring their children. Ultimately, this will assist in the goal of achieving a polio-free world by 2025.

RECOMMENDATIONS

The government and stakeholders should inform the public about the importance of OPV immunization and use the evidence from this research to further convince parents and caregivers that the rumors attributed to OPV are baseless and that they truly need to allow their children to be immunized in order to create a productive, polio-free population.

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