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Research Paper

Comparative Effect of Virgin Coconut Oil (VCO) and Coconut Milk on Fertility Prarameters (Fertility Testing) In Adult Male Rats

* [1]EBISINTEI P, [2]ELENDU M U,[3]Ugwu I P

1. *Department of Biological Sciences, Faculty of Basic and Applied Sciences, University of Africa Toru Orua,Bayelsa State, Nigeria*
2. *Department of Physiology, College of Health Sciences, Imo State University, Owerri, Nigeria*
3. *Department of Physiology, Faculty of Basic Medical Sciences, University of Nigeria*

SUMMARY: *The comparative effects of Virgin Coconut oil (Cocos Nucifera) and Coconut milk on fertility parameters using male rats as model was studied. A total of 72 rats (40 adults male and 32 female) weighing between 200-250g were used in this study. Group 1 served as the control and received distilled water only. Group 2-4 were the test groups and were fed orally with the coconut extracts. Group 2 was fed with virgin coconut oil (VCO), group 3 fed coconut milk (CM) while group 4 was fed the mixture of coconut oil and coconut milk. The male animals at the end of a given duration respectively were used to cohabit with the female rats and after which fertility testing was conducted. The result of the study showed that there was no significant difference between the experimental groups and control (P>0.05) based on the number of litters. Furthermore, that there is a significant decrease (P > .05) in the fertility rate of the female rats with the Coconut Milk and Virgin Coconut Oil but in the mixture of CM + VCO has no significant changes (P<0.05)*
The administration of Virgin Coconut oil could have deleterious effect on fertility by significantly reducing the fertility rate, litter size as well as number of pregnant rats.
KEYWORDS*: Virgin coconut oil, coconut milk, number of litters, size of litters, fertility rate.*

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I. INTRODUCTION

Fertility is the natural capability to produce children. When a man and woman cannot perform this function or capability to produce children, they are termed 'infertile'. That means fertility problems can happen both in women and men, which account for about 40-50% (Hirsh A, 2003).

Male infertility: This is a health issue in a man that lowers the chances of his female partner getting pregnant after 12 months of unprotected and regular sex. It affects 7% of all men (Loffi F, Maggi M, 2004).

Male infertility is usually caused by testicular damage leading to inability to produce sperm; the damaged testicle will not usually regain its sperm-making abilities (Purvisk, Christiansen E, 1992). Furthermore, dramatic changes in the semen quality have been seen during the past three decades(Nagiahet al, 2015) High intake of antioxidants, fruits, vegetables, poultry, sea foods, skim milk and shellfish as well as low intake of full-fat diary, sweet and processed meat, especially with high-saturated fat foods, has favorable association with sperm quality(Daniel N et al 2019). Study also has shown that sperm motility can be change in a short period and seems to be closely coupled to diet (Daniel Natt et al., 2019).

Plants like coconuts, have been used for many studies ranging from treatment of heart diseases to stress reduction (Laurence E et al 2016). Coconuts have also proven to be strong antioxidant, and source of hydration in the prevention of kidney stones and reduction of blood pressure (Saat M *et al.*, 2002,Bhagya D, *et al.*, 2012)

Medicinal herbs gotten from plants and plants extract have been shown to improve infertility problems in men (Gonzales GF 2002).

Infertility is a growing problem worldwide (Okanufa FE, Odunsi OA, 2003).Epidemiologic reports indicated that prevalence of infertility ranges from 3.5% to 16.7% in developed countries and from 6.9-9.3% in developing countries(Boivin J et al., 2007).

About 8-12% of couples globally are experiencing infertility during their reproductive lives estimated by world health organization in 1991, thus affecting 50-80 million couples with 20-35 million in Africa (WHO, 1991).

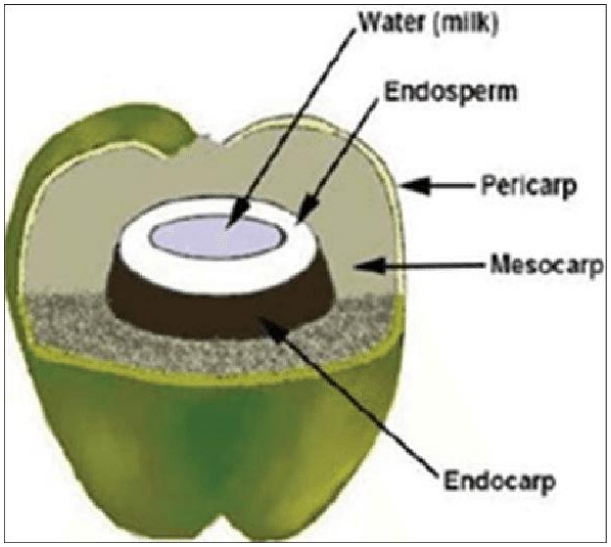
It affects about one in every six couples and researchers estimate about one in every three cases is due to fertility problems in the male partner alone (Brugo-Olmedos, Chilik C, etal., 2001). Also despite the numerous advance methods of treatment, there have been increased number of infertile couples at the past decade (Heitman E, 1995).

Assisted reproduction methods have been developed to overcome infertility. However, due to it cost most couples cannot afford it (Heitman E, 1995).

Fertility incidence has remained very high, despite the increased use of assisted reproductive technologies (ART) in recent years. (Salas-Huktos A, et al., 2017).

In Nigeria, it is estimated that 3-4 million couples were affected by infertility issues (Thomas K D et al., 1995).

In Nigeria, infertility comes with a high financial and physical price for many couples, this has made a lot of couples discontinue from their treatments (Akinoye O, Truter EJ, 2011) which has led to significant increase in psychological trauma among couples (Umezulike A C et al., 2004).Despite its high prevalence, not much efforts has been made in tackling this problem. The impact of male infertility is likely to increase if adequate measures are not taken.



Source:

Although researchers have suggested that the kind of food we eat can play an important role in altering fertility related outcomes in both men and women, the current research examining the effect of dairy on fertility is limited in scope. (Cousineau TM et al., 2018).

Plants like coconuts, have been used for many studies arising from heart diseases, stress reduction, antioxidant, and source of hydration, kidney stone prevention and reduction of blood pressure. Most recently, the water of immature coconut has been associated with fertility by increasing sperm motility and count (Augustine A. et al., 2019), but no substantial work has been done in consumption of coconut meat which contain the coconut oil and milk. Considering studies done in 2019, the consumption of coconut has increased in Nigeria by over 500% in the past five years (m-guardian-ng.cdn.ampproject.org/2019)

SAMPLE IDENTIFICATION AND COLLECTION.

Solid dry mature coconut was purchase from Swali market, Yenagoa, Bayelsa state, Nigeria. The coconuts were identified by the Department of crop and pest management, Faculty of Agriculture, University of Africa. Healthy Wister rats, two months old and weighing 160-200g were procured from Human Physiology Department, University of Port Harcourt.

Experimental design .The animals were randomized and grouped for two(2) weeks in accordance to the method of Morton and Han (Morton D.B, Han J, 2010) and placed in a wooden netted cages and maintained under environmentally controlled room provided with 12:12 hours light and dark cycle approximately at 25C. They were grouped into 4 (n=10 per group)

- Group 1 (control)
- Group 2 (VCO)

- Group 3 (CM)
- Group 4 (VCO+CM)

The test group (2-4) were feed orally with the extracts while the control were feed distilled water and normal feed. Furthermore, the procedures involving the animal models conformed to the guiding principles in the care and the use of animals by the American Physiological society. (American Physiological society 2002)

Preparation of plant extracts. Coconut milk: the coconut were broken, its meat scrapped from the shell and cut into small piece using a sharp knife. The cut pieces were grinded in a grinding machine into viscous slurry and therefore squeezed through cheese cloth (filter) to obtain coconut milk which was put into a glass jar.(Nevin kG, Rajmohan T. 2006). The glass jar containing coconut milk were kept in a refrigerator for preservation.

Virgin coconut oil: This is done by the method as described by Nevin KG and Rajamohan T.(Nevin kG, Rajmohan T. 2006).

The solid matured coconut were crushed manually and the meat was removed and cut into pieces using a sharp knife, the pieces was grinded in a grinding machine into viscous and slurry and therefore squeezed through the cheese cloth to obtain coconut milk. The coconut milk produced was left for 24 hours to aid the gravitational separation of the milk, which was in accordance with (Nevin kG, Rajmohan T. 2006).

Three phases resulted: The lower aqueous phase, a middle emulsion phase and an upper oily phase. The upper oily phase was then decanaled and heated for 10 minutes to remove moisture.

The resultant virgin coconut oil (V.C.O) was then filtered with a fine sieve, stored in bottles at room temperature and used for experiment.

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The Acute Toxicity test of the study. The minimum dose that is required to cause 50% death of the rat was determined using Arithmetic method of Lorke (Dietrich L., 1983)

Following a 24-hour period of Acute toxicity study, no deaths were recorded in the animal groups 1, 2, 3, 4, 5, 6 and 7 treated with 500, 1000, 2000, 3000, 4000, 5000, 6000, mg/kg body weight of Cocos *Nucifera* extract. The animals spontaneously regained their activity within one hour of treatment and all survived the acute test.

In contrast, one death has recorded in group 8 which was treated with 7000mg/kg. Hence the acute toxicity value obtained by the application of looke's after shown in formula was 4.582.56 mg/kg

Mating and fertility test

During 30 days of administration of the extract to the male rats, the female untreated rats were cohabitated with the adult male (2:1) on the 20^{th} day till the 30^{th} day making a total of ten days of cohabitation with the male.

After 10 days of cohabitation, the female rats are separated from the male and kept in a cage according to the group they were paired to, and they were left for 7 days making it a total of 17 days from the day at which they were paired with the males.

The Females were then sacrificed on the 18^{th} day before gestation period (Before 21 days) with the use of chloroform to make the unconscious, after which the number of litters (Fetuses) were removed and counted and weighed. The male rat from each group were chosen and suitably marked.

Statistical analysis. Data were express as mean+ SEM and their group will be evaluated by one-way analysis of variance (ANOVA)

II. RESULTS

Table1: COMPARISON ON FERTILITY RATE IN RAT TREATED WITH COCONUT MILK (CM), VIRGIN COCONUT OIL (VCO) AND MIXTURE OF CM + VCO

GROUP	TREATMENT	FERTILITY RATE (%) MEAN±SEM	TEST FOR SIGNIFICANCE
1	Control (a)	98.7%	
2	Coconut Milk (CM) (b)	41.67%	

3	Virgin Coconut Oil (VCO) (c)	25%	
4	Mixture of VCM + VCO (d)	83.33%	

Results expressed as Mean±SEM

EFFECT OF THE EXTRACT ON THE FERTILITY RATE OF THE RAT.

From the table 1 as illustrated above the female rats used for mating in the control group had 98.7% fertility rate, while coconut milk (CM), virgin coconut oil (VCO) and mixture of coconut milk and virgin coconut oil had 41.67%, 25% and 83.33% respectively. This showed that there is a significant decrease (P > .05) in the fertility rate of the female rats with the Coconut Milk and Virgin Coconut Oil but in the mixture of CM + VCO has no significant changes (P<0.05)

i) FERTILITY RATE

A fertility test has been calculated using the following formula:

% fertility success = Pregnant females X 100

Mated females (Raji Y*et al*, 2006)

The female rats used for mating in the control group had 98.7% fertility rate whereas the female rats used for the extracts CM, VCO and CM+VCO had 41.67%, 25% and 83.33% respectively.

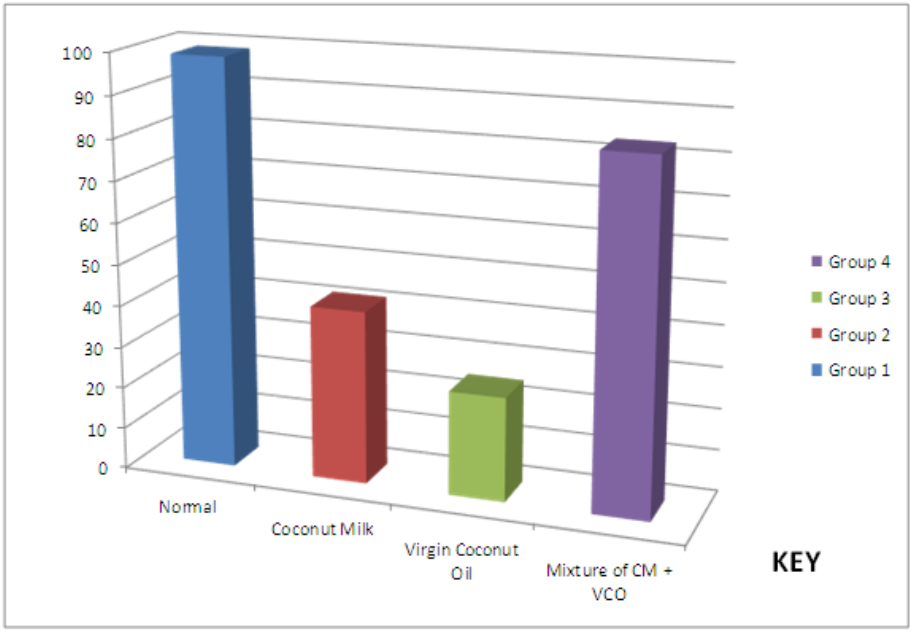

Figure 1: comparison on fertility rate in rat treated with virgin coconut milk (CM), Virgin Coconut oil (VCO) and mixture of CM and VCO

Table2: COMPARISON OF THE WEIGHT OF LITTERS IN FEMALE RATS TREATED WITH MILK (VCM), VIRGIN COCONUT OIL (VCO) AND MIXTURE OF VCM, VCO AND CW

GROUP	TREATMENT	MEAN±SEM (g) (weight of litters)	TEST OF SIGNIFICANNCE
1	Normal (a)	6.24 ± 0.1	
2	Virgin Coconut Milk (450mg/kg) (b)	4.3 ± 0.1**	aVs b* P<.05
3	Virgin Coconut Oil (450mg/kg) (c)	2.0 ± 0.3**	aVs c* P<.05
4	Mixture of VCM + VCO (450mg/kg) (d)	6.01 ± 0.05**	aVs d* P>.05

***Results** = Mean±SEM. Means marked * is significantly different from control while means marked ** is significantly different from other test.*

Results obtained from the effect of extracts on the weight of litters.

As represented in table 2 above, the average weight of litters are as follows6.24 ± 0.1, 4.3 ± 0.1, 2.0 ± 0.3 and 6.01 ± 0.05 for group 1, 2, 3 and 4 respectively, female rats used for mating in the control group had an average litter weight of 6.24 ± 0.1.

An average litter weight of 4.3 ± 0.1, 2.0 ± 0.3 and 5.47 ± 0.05 was produced by female rats used in mating male rats treated with CM, VCO and CM+VCO respectively.

This shows that the weight of the litters decreased significantly (P<0.05) when treated with the extracts as compared to the control. This is illustrated in figure below.

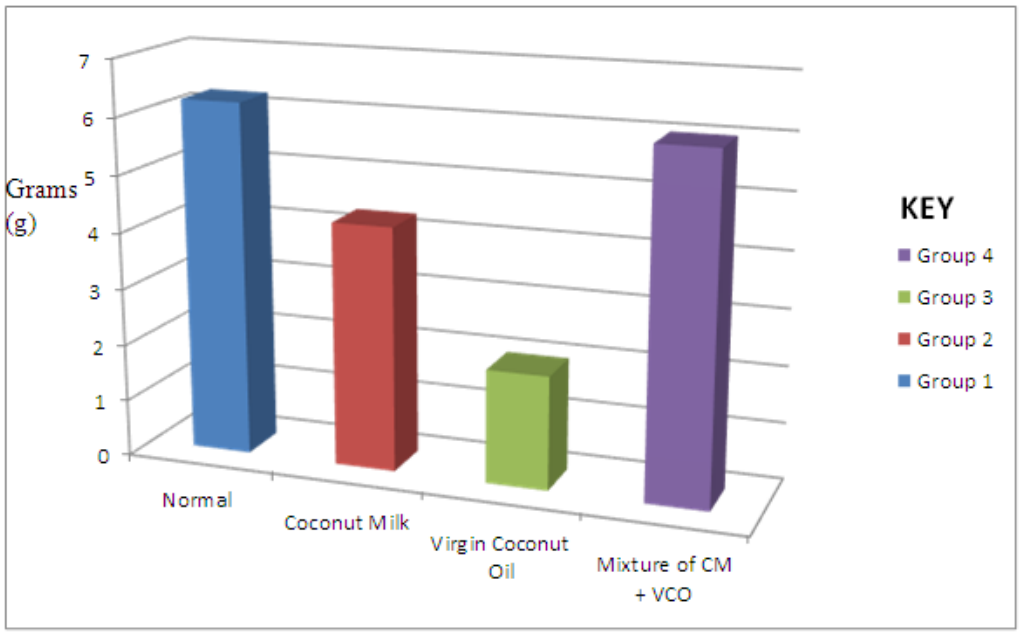

Figure 2: Comparing the weight of litters against the different extracts of Cocos *nucifera* and the control

Table 3: COMPARISON OF THE NUMBER OF LITTERS IN RAT TREATED WITH COCONUTMILK (CM), VIRGIN COCONUT OIL (VCO) AND MIXTURE OF CM AND VCO

GROUP	EXTRACTS	MEAN±SEM (Number of Litters)	TEST OF SIGNIFICANCE
1	Normal (a)	8.20± 1.32	
2	Coconut Milk (VCM) (b)	4.5±1. 27**	aVs b* P<.05
3	Virgin Coconut Oil (VCO) (c)	2.90±1.26**	aVs c* P<.05
4	Mixture of VCM + VCO (d)	7.10± 1.44**	aVs d* P>0.5

***Results** = Mean±SEM. Means marked * is significantly different from control while means marked ** is significantly different from other test.*

ii) NUMBER OF LITTERS:

As indicated in table 3, the female rat used for mating in the control group had an average number of litters of about 8.20± 1.32. An average of 4.50 ± 1.27, 2.90 ± 1.26 and 7.10 ± 1.44 was produced by female rats used to mat male rats that received CM, VCO and CM + VCO respectively.

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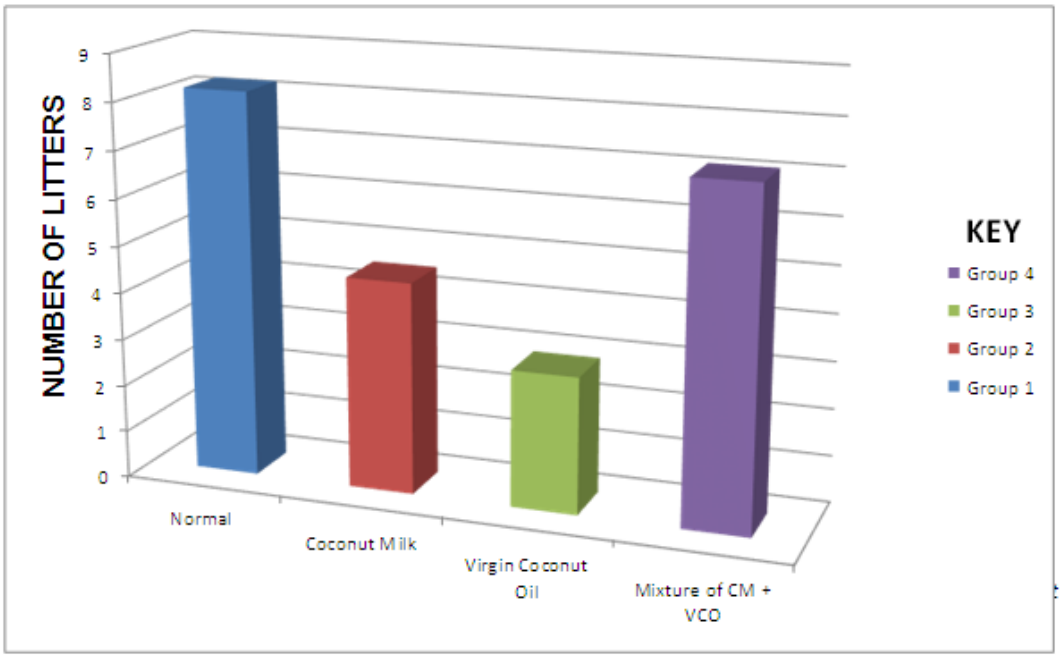

Figure 3: showing the *comparison of the number of litters in rat treated with Coconut milk (CM), virgin coconut oil (VCO) and mixture of CM and VCO.*

Table 4: COMPARISON OF THE SIZE OF LITTERS IN RAT TREATED WITH MILK (VCM), VIRGIN COCONUT OIL (VCO) AND MIXTURE OF VCM, VCO

GROUP	EXTRACTS	MEAN±SEM (size of Litters)	TEST FOR SIGNIFICANCE
1	Normal (a)	8.4 ± 1.7	
2	Coconut Milk (VCM) (b)	8.1 ± 2.23**	
3	Virgin Coconut Oil (VCO) (c)	8.1 ± 4.4.5**	
4	Mixture of VCM + VCO (d)	82 ± 4.0**	

Results *= Mean±SEM. Means marked * is significantly different from control while means marked ** is significantly different from other test.*

iii) LITTER SIZES:

Also from table 4, the female rats used for mating in the control group produced an average litter size of 8.4 ± 1.7. An average of 8.1 ± 2.3, 8.1 ± 4.5 and 9.5 ± 2.0 was produced by female rats used to mate male rats that received CM, VCO and CM + VCO respectively. There was no significant difference between the experimental groups and control (P>0.05)

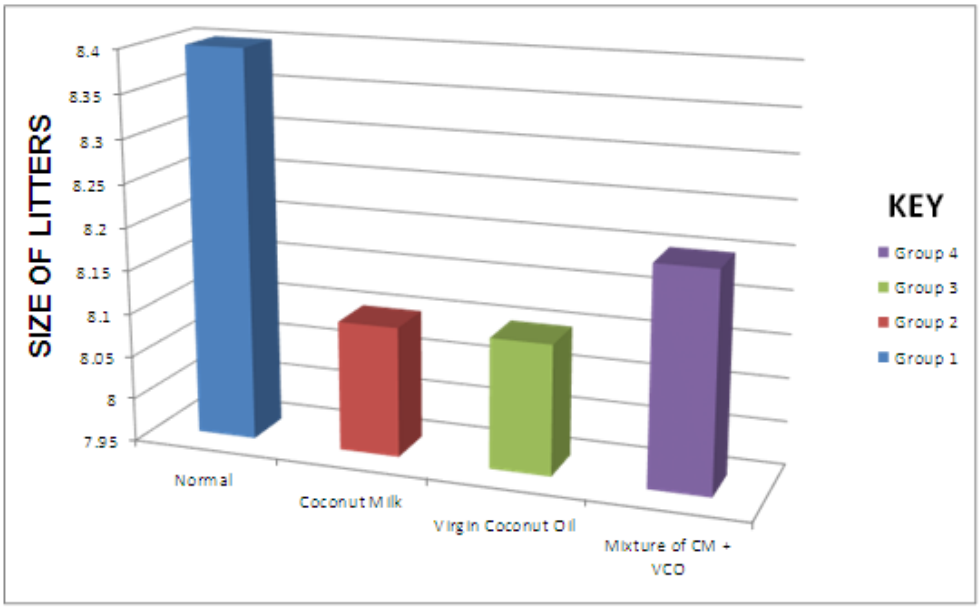

Figure 4: showing the *comparison of the size of litters in rat treated with Coconut milk (CM), virgin coconut oil (VCO) and mixture of CM and VCO.*

Table 5: COMPARISON OF THE NUMBER OF PREGNANT FEMALE RATS TREATED WITH COCONUT MILK (CM), VIRGIN COCONUT OIL (VCO) AND MIXTURE OF CM AND VCO

GROUP	TREATMENT	MEAN±SEM (Number of Pregnant Females)	TEST OF SIGNIFICANNCE
1	Normal (a)	11	
2	Virgin Coconut Milk (450mg/kg) (b)	5	aVs b* P<.05
3	Virgin Coconut Oil (450mg/kg) (c)	3	aVs c* P<.05
4	Mixture of VCM + VCO (450mg/kg) (d)	10	aVs d* P>.05

***Results** = Mean±SEM. Means marked * is significantly different from control while means marked ** is significantly different from other test.*

EFFECT OF THE EXTRACT ON THE NUMBER OF PREGNANT FEMALE OF THE RAT.

From the table 5 as illustrated above, 11 out of the 12 female rats used for mating in the control group were pregnant, while coconut milk (CM), virgin coconut oil (VCO) and mixture of coconut milk and virgin coconut oil had 5, 3 and 11 respectively. This showed that there is a significant decrease in the number of pregnant female rats with extracts when compared with that of the control (P<0.05)

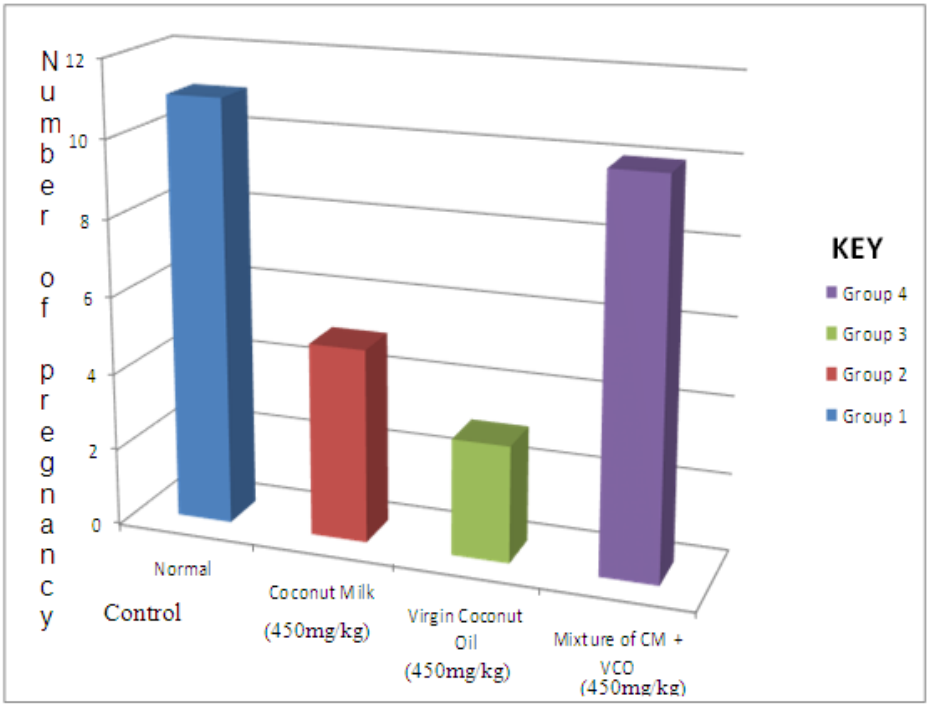

Figure 5. Showing the comparison of the size of litters in rat treated with Coconut milk (CM), virgin coconut oil (VCO) and mixture of CM and VCO.

Table 6: SUMMARY OF THE COMPARISON ON THE FERTILITY PARAMETERS IN RAT TREATED WITH VIRGIN COCONUT MILK (VCM), VIRGIN COCONUT OIL (VCO) AND MIXTURE OF VCM, VCO

FERTILITY PARAMETER	TREATMENT			
	CONTROL	COCONUT MILK	VIRGIN COCONUT OIL	MIXTURE OF CM + VCO
Number of Males mated	6	6	6	6
Number of females mated	12	12	12	12
Number of pregnant females	11	5	3	10
Number of fetuses (Mean ± SEM)	8.20 ± 1.32	4.50 ± 1.27	2.90 ± 1.26	7.10 ± 1.44
Size of litters	8.4 ± 1.7	8.1± 2.3	8.1± 4.5	8.2 ± 4.0
Weight of litters	6.24 ± 01	4.3± 0.1	2.0± 0.3	6.1 ± 0.05
Fertility Rate (%)	98.7%	41.67%	25%	83.33%

Values are expressed as Means ± SEM of 6 male rats per group mated with 12 female rats per group mated.

III. DISCUSSION

Fertility percentage (Rate)

The fertility success rate was done by calculating the number of pregnant female divided by mated untreated females multiply by 100 (Raji Y *et al.*,2006).

In this research work done, fertility rate or percentage show a significant decrease in untreated female rat that cohabited with male rat treated with VCO when compared with other extracts and that of the control. This decrease in fertility rate may be probably associated with the decrease in serum testosterone, LH and FSH as seen in previous study (Castro ACS, 2002, Benghuzzi *et al.*, 2008, Puranik NV *et al.*, 2019) and also sex hormone (Testosterone, LH and FSH) has been known to play on important role in male fertility, and sperm's health and production (Mooradian AD *et al.*, 1987).

Number of pregnancy

Number of pregnant untreated female rats that cohabited with male rats treated with VCO extracts had a very reduced number of pregnancy (a total of three) when compared with the control that had 98.9% fertility rate and gives a total of 11 pregnant untreated female ,out of the 12, CM 5 and mixture of CM + VCO has 10.

This reduction in the number of untreated female rats may be due to the number of factors as seen in previous work and this present study in this research reduction of sex hormones level, reduction of sperm count (A Haman *et al.*, 2012, Hishi Kava D *et al.*, 2017) reduction of height of the testis as a result of saturated fats (Oyeyemi *et al.*, 2002, Salhab SA *et al*., 2001).

Litter size

A report shows that, in rats early over nutrition can cause reduction in the size of the litter (Kennedy G C 1957).

Studies have shown a relationship between testes size and litter size (Flowers., 2001; Kaweeka *et al*., 2013) and the size and weight of the testes can directly reflected the quality and quantity of sperm (Knight TW 1997, Raadsma and Edey 1984).

Similarly, studies have suggested that low reduction of weight could decrease litter size during the suckling period (Pietruzka A *et al*., 2017,) while Alm K *et al*.,(2006) reported that there is a strong relationship between the litter size and sperm morphology.

In this study, administration of 450mg/kg of Cocos nucifera extracts (VCO) caused a reduction in the litter size of the untreated female rats cohabited with the rats than that of the control and other extract (CM). This is the agreement with (Knight TW, 1997) and Raadsma Edey, 1984). So it is not out of place to conclude that a reduction of the size and weight of the testis has a negative impact on the quality of the semen (Salhab SA *et al*.,2001) may have resulted in the reduction of the litter size.

It is our belief that the decrease in fertility rate of the female rats in our study might be as a result of the presence of high amount saturated fat found in the Virgin Coconut oil extracts.Virgin Coconut milk extracts is preferred to Virgin Coconut oil extracts to improve or burst fertility in men.

***Correspondence Author.**
Ebisintei P. Department of Biological Sciences, Faculty of Basic and Applied Sciences, University of Africa Toru Orua,Bayelsa State

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