Coconut Water Improves Reproductive Functions in Offspring of High Fat Diet Fed Rats

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ABSTRACT

Objective: Maternal high fat diet adversely affects fertility of offspring. Coconut (*Cocos nucifera* L.) water has hypolipidemic actions in the presence of high fat diets. This study was carried out to determine if coconut water (CW) can protect the reproductive functions of offspring against the effects of maternal high fat diet (HFD).

Methods: Twenty-four pregnant rats were randomly assigned into four groups namely; Control, CW (10 ml/kg coconut water), HFD (30% butter: 70% chow) and HFD+CW and treated from gestation days 1 to 21. They were allowed to litter naturally and nurse their young until Post-Natal Day (PND) 28. Weaned offspring were housed separately according to sex. Estrus cycles were monitored for two weeks before sacrifice in female offspring. Offspring were sacrificed on PND 120, at which time epididymal sperms were analyzed in male offspring. Sex hormones, gonadotropins, gonadal malondialdehyde and interleukin 1β levels were measured in offspring.

Results: Puberty was delayed and reproductive organ weights were reduced in HFD offspring. Sperm counts were reduced in HFD male offspring, with a significant increase in testicular interleukin 1β levels. Female HFD offspring showed reduced luteinizing hormone levels. Architectural alterations were observed in reproductive organs of HFD offspring when compared with the control offspring. These changes were not apparent in HFD+CW offspring.

Conclusion: Coconut water ameliorates the effects of maternal high fat diet on reproductive functions of offspring.

Keywords: Coconut water, fertility, maternal high fat diet, offspring

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INTRODUCTION

The concept of foetal programming of adult disease has raised great awareness about the hazardous effects of maternal malnutrition during pregnancy on the health outcomes of offspring (1,2). If mothers consume a high fat diet during pregnancy, they risk compromising the reproductive health of their offspring (3–5). Unfortunately, economic and social pressures make the avoidance of high calorie diets during pregnancy an uphill task (6,7). Therefore, in addition to health education, other practical solutions should also be explored. Sandhya and Rajamohan (8,9) have reported the hypolipidemic effects of coconut (*Cocos nucifera* L.) water in the presence of high fat in diets. Kunle-Alabi et al. (10, 11) have also reported the enhancing effects of coconut water on both male and female reproductive function. This study therefore explored the hypolipidemic effect of coconut water on the reproductive functions of offspring from rat dams fed a high fat diet during gestation.

METHODS

Animals

Twenty-four virgin female Wistar rats weighing 140 - 150 g obtained from AFOKOL Animal Breeders Limited, Ibadan, Nigeria and twelve proven breeder male Wistar rats (males which had successfully sired pups previously), weighing 150 - 170 g obtained from the Laboratory for Reproductive Physiology and Developmental Programming (LRPDP), Department of Physiology, College of Medicine, University of Ibadan where used for the study. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the guidelines for laboratory animal care of the National Institutes of Health (NIH publication no. 85-23, revised 1996).

Coconut water

Coconut (*Cocos nucifera* L.) fruits were obtained from a coconut plantation in Oyo state, Nigeria and verified by a botanist from the Department of Botany, University of Ibadan, Ibadan, Nigeria. Fresh coconut water was administered daily via oral gavage.

High fat diet

Seventy percent (70%) of standard rat chow (67% carbohydrate, 18% protein, 5% fat, 5% fibre, 5% mineral and vitamin mixture) was supplemented with thirty percent (30%) butter containing 82% milk fat to obtain the high fat diet feed (16).

Experimental protocol

Mating was carried out at a 1:2 male to female ratio, and confirmed by the presence of spermatozoa in vaginal smears. The day of detection of spermatozoa was taken as Gestation Day (GD) 1 for each dam. Pregnant rats were then randomly assigned into four groups of six dams each as follows;

Control group; received standard rat chow *ad libitum* + 10 ml/kg body weight distilled from GD 1 to 21,

Coconut water group (CW); received standard rat chow *ad libitum* + 10 ml/kg body weight coconut water from GD 1 to 21,

High fat diet group (HFD); received high fat feed *ad libitum* + 10 ml/kg body weight distilled from GD 1 to 21,

High fat diet + coconut water group (HFD+CW); High fat feed *ad libitum* + 10 ml/kg body weight coconut water from GD 1 to 21.

The dams in each group were allowed to litter naturally and also allowed to nurse their own young from Post-Natal Day (PND) 1 to 28 when the pups were weaned (12). One male and one

female offspring were randomly selected from each dam in each group. Males were housed separately from females. Offspring were fed standard rat pellets (Ladokun feeds, Nigeria) *ad libitum* after weaning. Birth weight and anogenital distance index (AGDi) (13) were measured and calculated for each pup on PND 1 and PND 120. Testis descent day, day of preputial separation (in males) and vaginal opening (in females) were noted for each pup. Oestrus cycles were monitored in the female offspring for two weeks before sacrifice. Offspring were sacrificed under sodium thiopentone anaesthesia (14) on PND 120. Blood was obtained via cardiac puncture for serum assay of luteinizing hormone (LH), follicle stimulating hormone (FSH), oestrogen and testosterone levels using ELISA kits according to the manufacturer's instructions (Fortress Diagnostics, UK). Histological analysis of the reproductive organs was carried out. Malondialdehyde (MDA) and interleukin 1β (IL-1β) levels were analysed in the testis and ovaries using spectrophotometry kits according to the manufacturer's instructions (Oxford Diagnostics, USA and Ray Biotech, USA, respectively). Data were presented as mean ± Standard Error of the Mean (SEM) and analysed using ANOVA on the SPSS statistical software package version 20 (SPSS, USA). Statistical significance was placed at $p < 0.05$.

RESULTS

Testis descent and preputial separation were delayed in male offspring from all the test groups (Table 1). Maternal high fat diet delayed vaginal opening in female offspring (Table 1). Female offspring of coconut water (CW) dams had increased anogenital distance index (AGDi) at birth, while female high fat diet (HFD) offspring had reduced AGDi at birth (Table 2). This effect was nullified in the HFD+CW female offspring, who showed increased AGDi in adulthood (Table 2).

Weights of the epididymides were reduced in male HFD offspring (Table 3). Similarly, weights of the ovaries and uteri were reduced in female HFD offspring (Table 3). Sperm count was reduced with a relative increase in sperm viability in male HFD offspring (Table 4). Co-administration of high fat diet with coconut water (HFD+CW) reduced the frequency of the oestrus phase of the female oestrus cycle when compared with either the CW or HFD offspring (Table 4). Female HFD offspring showed reductions in luteinizing hormone levels (Table 5), while male offspring did not show any significant change in sex hormone or gonadotropin levels (Table 5 and 6).

The architectural distortions observed in the reproductive organs (Plates 1 and 2) were accompanied by marked increases in testicular interleukin 1-beta levels (Table 7), but no significant change in gonadal malondialdehyde levels (Table 7).

DISCUSSION

Maternal high fat diet during gestation adversely programmes the reproductive system of offspring *in utero*, predisposing them to infertility in adulthood (3, 5). This study was carried out to determine if the hypolipidemic activity of coconut water which has been reported by Sandhya and Rajamohan (8, 9) could prevent these effects. The delayed puberty onset observed in both male and female offspring of high fat diet fed dams supports reports of adverse programming of the reproductive system of offspring by maternal fat diets (15). We had previously reported that high fat diet during gestation increases estradiol levels in dams (16). Such high maternal levels of estradiol may have contributed to the delayed onset of puberty observed in their offspring, as excess maternal oestrogen levels have previously been reported to do so (17,18). This corroborates the report that high levels of oestrogen in the maternal circulation can cross the placental barrier and impair sexual development of offspring (19).

The reduction in anogenital distance index (AGDi) observed in the female offspring may also be as a result of this increased maternal estradiol levels. Anogenital distance is used as a measure of fertility as it is sensitive to hormone-induced changes in the reproductive system (20). The observed effects, however wore off in adulthood in the offspring of coconut water fed dams, while resulting in reduced reproductive organ weights in high fat diet offspring. These observations further support previous reports that maternal high fat diet adversely affects reproductive function of offspring (3, 21), and the premise that coconut water can alleviate these changes.

Male offspring of high fat diet fed dams showed defective testicular structure. The increase in testicular interleukin 1 beta levels with no change in testicular malondialdehyde concentrations observed in these offspring, suggest that maternal high fat diet alters reproductive function of male offspring by inducing gonadal inflammation. Coconut water was able to ameliorate the reduction in sperm counts caused by maternal high fat diet. The mechanism for this is still being explored.

The delayed puberty onset, reduced reproductive organ weight and luteinizing hormone level reduction observed in female offspring of high fat diet dams suggests that maternal high fat diet also programmes female offspring for infertility. Ettinger and Feldman (22) have reported that even when puberty is delayed, once female reproductive cycles are eventually initiated (at menarche), they occur normally throughout the reproductive life of a female. This suggests that the delayed puberty observed if female offspring of high fat diet fed dams may not have any adverse effects on their fertility in adulthood.

CONCLUSION

It was concluded that coconut water can attenuate the adverse effects of maternal high fat diet during gestation on reproductive functions of offspring.

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Table 1: Effects of maternal high fat diet and/or coconut water administration during GD 1-21 on testis descent and onset of puberty in offspring

Group	**Testis descent (postnatal days)**	**Preputial separation (postnatal days)**	**Vaginal opening (postnatal days)**

CONTROL	29.20 ± 0.24	52.88 ± 0.07	53.81 ± 0.19
CW	30.33 ± 0.27**a**	55.38 ± 0.13**a**	53.88 ± 0.22
HFD	33.76 ± 0.40**a,b**	56.00 ± 0.00**a,b**	56.00 ± 0.00**a,b**
HFD+CW	30.71 ± 0.32**a,c**	55.60 ± 0.34**a**	54.06 ± 0.34**c**

$p < 0.05$ compared with control group (**a**), coconut water group (**b**) and high fat diet group (**c**).

Table 2: Effects of maternal high fat diet and/or coconut water administration during GD 1-21 on anogenital distance index (AGDi) of offspring

AGDi	**CONTROL**	**CW**	**HFD**	**HFD+CW**
Male	5.37 ± 0.12	5.64 ± 0.16	5.82 ± 0.08**a**	5.79 ± 0.13**a**
Female	2.15 ± 0.10	2.28 ± 0.04	2.19 ± 0.04	2.52 ± 0.07**a,b,c**

$p < 0.05$ compared with control group (**a**), coconut water group (**b**) and high fat diet group (**c**).

Table 3. Effects of maternal high fat diet and/or coconut water administration during GD 1-21 on relative weights of the reproductive organs of offspring

	CONTROL	**CW**	**HFD**	**HFD+CW**
Testis	0.63 ± 0.02	0.54 ± 0.02a	0.59 ± 0.02	0.66 ± 0.03b,c
Epididymis (%)	0.26 ± 0.01	0.22 ± 0.01a	0.22 ± 0.01a	0.24 ± 0.01
Seminal vesicle (%)	0.41 ± 0.02	0.45 ± 0.03	0.41 ± 0.02	0.38 ± 0.03
Prostate gland (%)	0.12 ± 0.01	0.16 ± 0.01a	0.11 ± 0.01b	0.10 ± 0.01a,b
Ovary (%)	0.07 ± .0.01	0.06 ± 0.00	0.05 ± 0.01a	0.07 ± 0.01
Uterus (%)	0.32 ± 0.04	0.30 ± 0.05	0.19 ± 0.02a,b	0.15 ± 0.02a,b

$p < 0.05$ compared with control group (**a**), coconut water group (**b**) and high fat diet group (**c**).

Table 4: Effects of maternal high fat diet and/or coconut water administration during GD 1-21 on epididymal volume and sperm indices of adult male offspring

Group	Volume of epididymis (ml)	Sperm count (million/ml)	Sperm motility (%)	Sperm viability (%)	Oestrus length (days)	Oestrus frequency (%)
CONTROL	0.23 ± 0.01	112.57 ± 9.85	78.18 ± 5.81	86.83 ± 3.90	4.43 ± 0.30	10.21 ± 4.08
CW	0.22 ± 0.01	109.95 ± 6.84	89.44 ± 2.42	93.89 ± 1.33**a**	4.11 ± 0.20	12.70 ± 2.31
HFD	0.21 ± 0.01	88.40 ± 7.57 **a, b**	85.00 ± 9.46	96.10 ± 0.38**a**	3.70 ± 0.21	15.72 ± 2.08
HFD+CW	0.21 ± 0.01	96.64 ± 6.51	92.50 ± 1.31	95.42 ± 0.61**a**	4.14 ± 0.40	4.76 ± 2.06 **b, c**

$p < 0.05$ compared with control group (**a**), coconut water group (**b**) and high fat diet group (**c**).

Table 5: Effects of maternal high fat diet and/or coconut water administration during GD 1-21 on serum Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) levels in adult offspring

	LH (mIU/mL)		**FSH (mIU/mL)**	
Groups	Male	Female	Male	Female
CONTROL	2.32 ± 0.12	3.27 ± 0.38	10.33 ± 0.25	11.09 ± 0.92
CW	2.17 ± 0.09	2.86 ± 0.31	10.79 ± 0.19	10.11 ± 0.13
HFD	2.22 ± 0.05	1.99 ± 0.04a,b	10.44 ± 0.08	10.54 ± 0.31
HFD+CW	2.49 ± 0.15	2.07 ± 0.04a,b	10.45 ± 0.10	10.49 ± 0.13

$p < 0.05$ compared with control group (**a**), coconut water group (**b**) and high fat diet group (**c**).

Table 6: Effects of maternal high fat diet and coconut water administration during GD 1-21 on serum estradiol and testosterone levels in adult offspring

	Estradiol (pg/ml)		**Testosterone (ng/ml)**	
Groups	Male	Female	Male	Female
CONTROL	42.42 ± 4.25	117.77 ± 4.38	3.01 ± 0.13	2.75 ± 0.03
CW	50.90 ± 7.11	104.60 ± 5.38	2.93 ± 0.15	2.89 ± 0.15
HFD	60.85 ± 7.21	96.90 ± 13.86	2.82 ± 0.08	2.71 ± 0.08
HFD+CW	55.70 ± 10.61	68.97 ± 7.21a,b,c	3.31 ± 0.22c	2.65 ± 0.06

$p < 0.05$ compared with control group (**a**), coconut water group (**b**) and high fat diet group (**c**).

Table 7: Effects of maternal high fat diet and coconut water administration during GD 1-21 on gonadal Interleukin 1β and Malondialdehyde levels in adult offspring

	Interleukin 1β (pg/mL)		**Malondialdehyde (µM)**	
Groups	**Testis**	**Ovary**	**Testis**	**Ovary**
CONTROL	1853.33 ± 263.64	13466.67 ± 2563.85	42.16 ± 4.90	40.25 ± 9.01
CW	6880.00 ± 732.85**a**	15440.00 ± 1413.05	64.02 ± 8.20	44.49 ± 7.88
HFD	7146.67 ± 1972.64**a**	16013.33 ± 1633.46	58.14 ± 11.27	34.51 ± 6.61
HFD+CW	7346.67 ± 950.42**a**	18422.22 ± 1356.10	33.85 ± 2.91**b,c**	33.18 ± 7.26

$p < 0.05$ compared with control group (**a**), coconut water group (**b**) and high fat diet group (**c**).

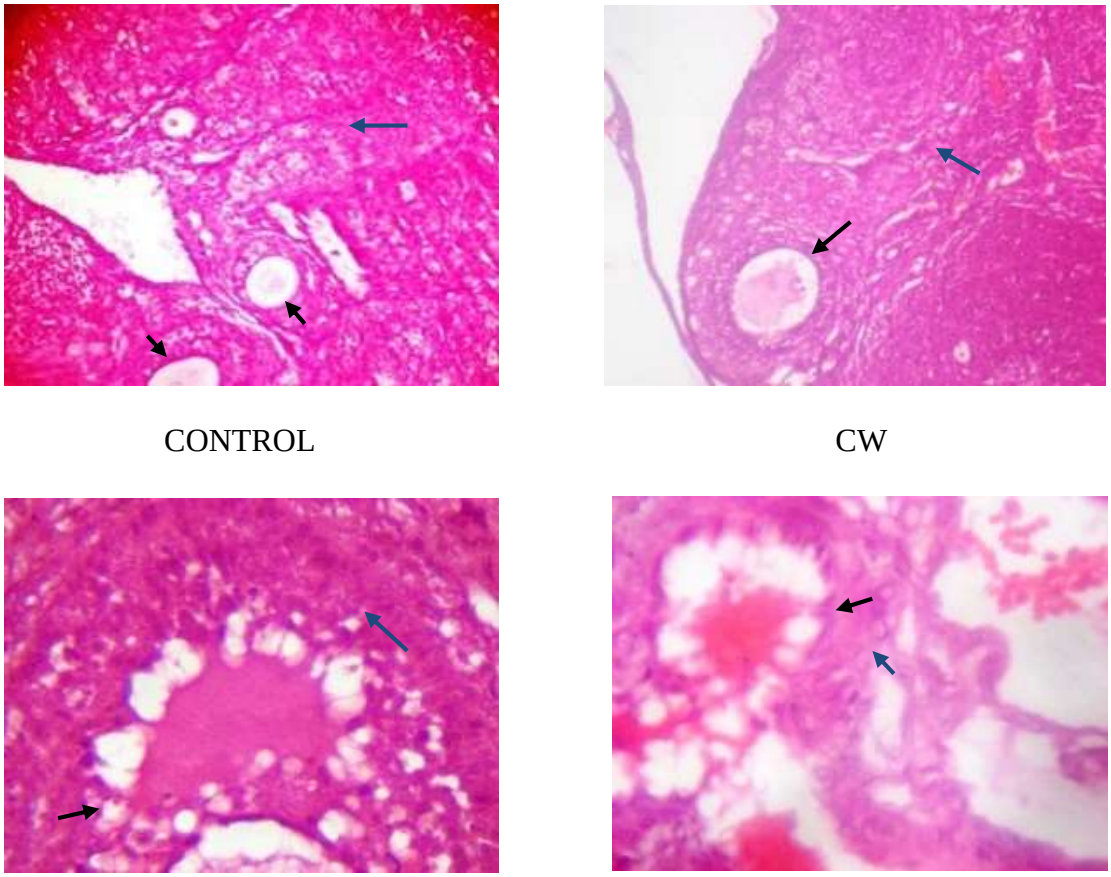

Fig. 1: Photomicrographs of ovary sections from female offspring of rats treated with high fat diet and/or coconut water from GD 1-21 stained by H&E magnification X400. Black arrows = follicles at different stages of development; blue arrows = ovarian stroma.

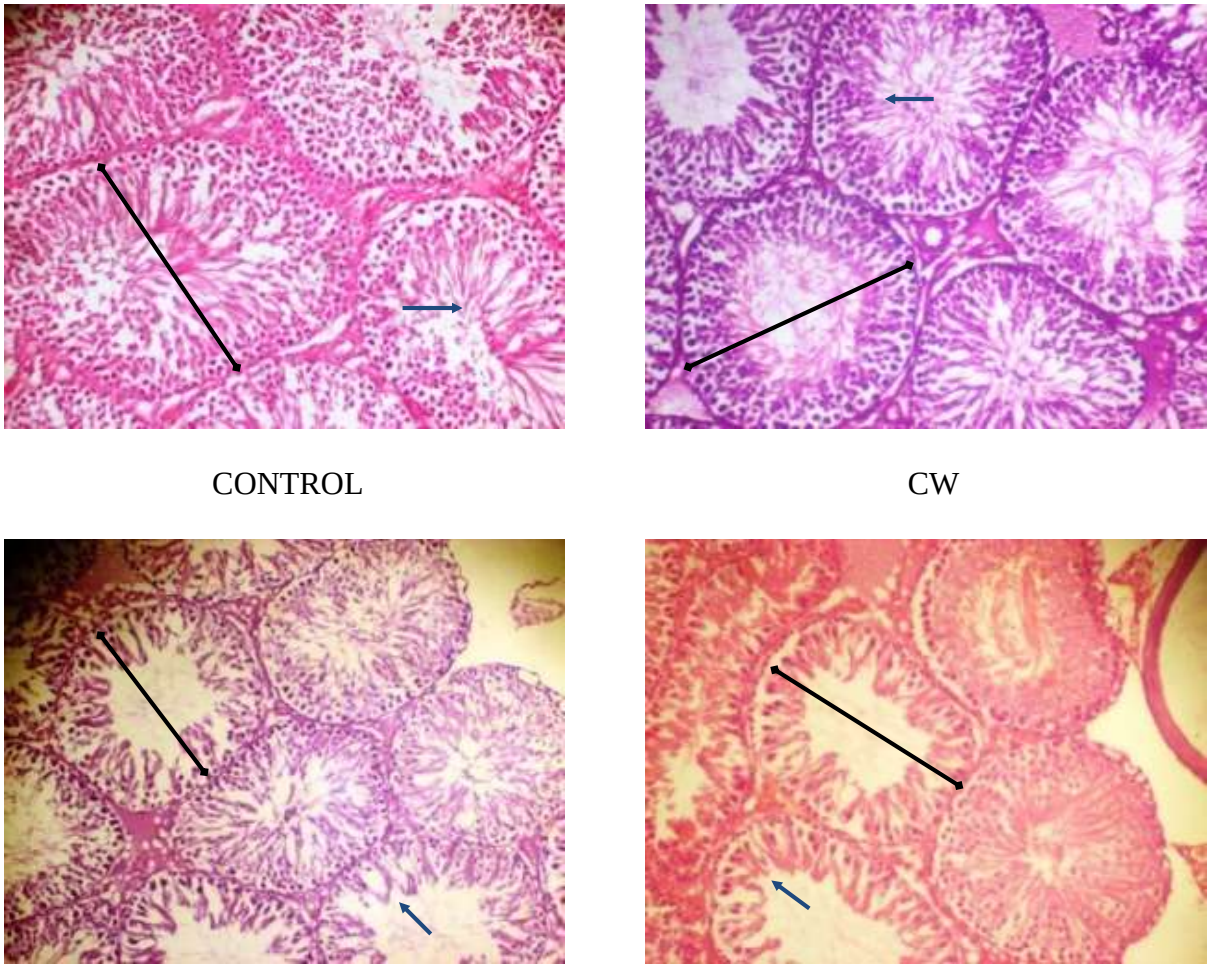

Fig. 2: Photomicrographs of testicular sections from male offspring of rats treated with high fat diet and/or coconut water from GD 1-21 stained by H&E magnification X100. Spanned = germinal cell layer within seminiferous tubules; blue arrows = spermatogonia and Sertoli cells.