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Research Article

Green Coconut Water Stimulates Follicular Growth and Development in the Ovary of Hyperprolactin Sprague- Dawley Rat

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ABSTRACT

Hyperprolactinaemia is a common endocrine cause of infertility in women. It is essentially associated with decrease in the secretion of follicle stimulating hormone which consequently leads to the inhibition of follicular development. Green coconut water (GCW) is the liquid in the inner cavity of an immature coconut fruit. Studies have demonstrated that GCW has estrogenic property that can regulate the endocrine system. This present study therefore investigated the effects of GCW on follicular growth and development in hyperprolactine rats to determine its endocrine regulating property on ovarian folliculogenesis. A total of fifty- five cyclic female Sprague-Dawley rats weighing 145-170 g were used for this study. The animals were randomly divided into four experimental study groups. In group 1, Metoclopramide hydrochloride (MCH) at 0.2 mg/100 g body weight was administered daily for 28 days to induce hyperprolactinaemia and this was withdrawn for 8, 16 and 28 days to check for recovery. The animals in group 2 were post-treated with GCW. In group 3, animals were treated with GCW only and group 4, the control group received distilled water only. The ovaries were carefully dissected out, trimmed of fat and processed for histology with Haematoxylin & Eosin stains. The histological presentation of follicular growth and development pattern in the ovaries of the post-treatment groups were comparable with the control. Therefore, this study presented structural evidence that GCW stimulates follicular growth in the ovary of hyperprolactin rats.

Keywords: *Ovary, Follicles, Histology, Green coconut water, hyperprolactinaemia*

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INTRODUCTION

Hyperprolactinaemia is defined as a consistent elevation of serum prolactin (PRL) level above 25ng/ml in non-pregnant and non-lactating individuals (Kelly et al., 2013). Hyperprolactinaemia is a common endocrine cause of infertility in women. It is essentially associated with chronic anovulation which is a major implication in female infertility. Increase in PRL level decreases the release of other anterior pituitary hormones and vice versa (Saranya and Leila, 2016). High PRL level interferes with the release of gonadotropin-releasing hormone (GnRH) by the hypothalamus and subsequently decrease the release of gonadotropins which are basically follicle stimulating hormone (FSH) and luteinizing hormone (LH). This suggests that prolactin-induced suppression of GnRH release is the proximal cause of infertility (David et al., 2007). Following the reduction in FSH release, a decrease in granulosa cell proliferation occurs which prevents the production of mature egg (Nawroth, 2005; Carlos et al., 2015). Clearly hormone disorder is a critical factor in hyperprolactin-induced infertility. Hyperprolactinemia is a common form of endocrine-induced infertility in female and affects about one-third of infertile women (Idris et al., 2018). It has also been incident in other causes of female infertility such as; galactorrhoea, polycystic ovarian syndrome, luteal phase deficiency induced- infertility and hypergonadotropic hypogonadism (Ranko et al., 2015).

For many centuries before the arrival of modern medicine, widely available natural remedies were used to prevent illness and restore natural healthy function including fertility (Sofowora, 1993). Medicinal plants have proven their value as a source of ingredients with therapeutic potential and still represent an important pool for the discovery of novel drugs (Atanas et al., 2015). There is a large volume of

information from folk reports of the existence of medicinal plants suspected to have fertility enhancing effects, but just a few of them have been scientifically tested for such effects. Green coconut water (GCW) is the clear liquid occupying the inner cavity of an immature coconut fruit. Coconut water is technically referred to as the liquid endosperm due to its cytoplasmic origin which serves as a suspension for nourishing the embryo during fruit development ((Patrick and Offler, 2001). Hence, the coconut water of an immature fruit is at its purest and contains more inorganic ions than the mature fruit which contribute to its therapeutic properties (Anurag and Rajamohan, 2003). It has long been believed that the water from an immature coconut is useful in treating many reproductive-related problems such as combating nausea and fatigue during pregnancy, treating irregular or painful menstruation and also taken during pregnancy to give the unborn baby strength and vitality. A number of these claims have been confirmed by scientific investigations. Studies have confirmed that GCW promotes diuresis with minimal loss of electrolytes to prevent threatened or recurrent abortion (Pragya, 2010; Kennedy et al., 2013). It was also confirmed through laboratory investigation to have properties that regulate the endocrine system when administered to a group of menopausal rats. The estrogen levels of menopausal rats when administered GCW were comparable to rats that were still ovulating (Nisaudah et al., 2009). In a preliminary study in our laboratory, we also confirmed that GCW reversed estrous acycling to normal cycling pattern when administered in hyperprolactin female rats (Bakare et al., 2013). Therefore, this present study was designed to further correlate this effect with follicular growth and development in the ovary through histological evaluation of ovarian tissue.

MATERIALS AND METHODS

Plant material: The immature coconut fruits were purchased from a coconut farm in Ajara, Topa, Badagry, Lagos. The average weight of the fruit was 1.55kg. The fruit was authenticated at the forest herbarium, Ibadan by a qualified Botanist. The plant's ascension number is No FHI 109665. The unripe coconut fruits were washed and dehusked. The extraction of the water was done through the germinal pore, poured directly into an airtight bottle, kept in the refrigerator and replaced every three weeks (Alexia, 2012).

Animal: A total of fifty- five female Sprague- Dawley rats of weight ranging from 145-170g were obtained from the Nigerian Institute of Medical Research, Yaba, Lagos and were authenticated by a taxonomist in the department of Zoology of the University of Lagos. The animals were allowed to acclimatize for two weeks under standard laboratory conditions of room temperature 27ºC with a photoperiodicity of twelve hours light alternating with twelve hours of darkness. The animals had free access to clean tap water and pellets. The animals were allowed to acclimatize for two weeks in the animal house of the Department of Anatomy, University of Lagos. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002) as approved by the University Ethical Committee (No: RGEEC/46/2015) of University of Lagos, Nigeria.

Estrous cycle study: The animals went through a recruitment phase of established estrous cycling determined by cytological examination of vaginal smears obtained daily between 8.00 a.m. and 10.00 a.m. Normal saline was drawn into the tip of the pipette, which was inserted 2mm deep into the vaginal canal and emptied into the vaginal cavity. The mixture of vaginal fluid and normal saline was then suctioned back into the tip of the pipette. The collected smear was placed onto the glass slide and examined under the light microscope. The metestrus phase showed leukocytes amidst large squamous cells. The diestrus phase showed predominance of leukocytes and a few large nucleated cells. The smear that showed large nucleated cells with the leukocytes was designated as the proestrus phase. The phase designated as the estrus phase showed large flakes of squamous cells (Marcondes *et al.*, 2002). Only animals with established estrous cycle were recruited for this study.

Experimental Design: A total of fifty- five animals with established approximately 4days cyclicity were randomly divided into four major experimental groups 1 to 4.

Group 1: Negative control group received distilled water
Group 2: Induction and Withdrawal group
a. 0.2mg/100g of MCH for 28 days
b. 0.2mg/100g of MCH for 28 days - withdrawal for 8 days
c. 0.2mg/100g of MCH for 28 days - withdrawal for 16 days
d. 0.2mg/100g of MCH for 28 days - withdrawal for 28 days
Group 3: Post- treatment group
a. 0.2mg/100g of MCH for 28 days -5ml/110g of GCW for 8 days
b. 0.2mg/100g of MCH for 28 days - 5ml/110g of GCW for 16 days
c. 0.2mg/100g of MCH for 28 days - 5ml/110g of GCW for 28 days
Group 4: Positive control group
a. 5ml/100g of GCW for 8 days
b. 5ml/100g of GCW for 16 days
c. 5ml/100g of GCW for 28 days

Each experimental rat was served with volumes of the extract according to their weight using simple percentile faction. The administration was done by the use of an oropharyngeal canula.

Animal sacrifice and organ harvest: Twenty-four hours after the final administration, the animals were anaesthetized using chloroform vapor in an enclosed jar. The animals were then dissected through a ventral abdominal transverse incision to remove the reproductive tube where the ovaries were separated from the spiral oviduct tube and carefully trimmed of fat. The tissues were immediately fixed in 10% formal saline for histological processing (Deepak *et al.*, 2012).

Histological processing: The fixed tissues were transferred into ascending grades of alcohol and finally cleared in xylene. The

tissues were then infiltrated in molten paraffin wax. Three changes of molten paraffin wax were made, after which the tissues were embedded in wax and blocked out. Serial sections of 5 µm thick were cut by the rotary microtome and floated in water bath. The sections were picked with clean slides onto which egg albumin had been coated and dried on the hot plate. The mounted sections were dewaxed in xylene and then hydrated in descending grades of alcohol. The sections were then stained with Haematoxylin for 10 minutes after which rinsed and differentiated in 1% acid alcohol for 10 seconds before rinsing in water. The sections were then counter stained in Eosin and dehydrated in ascending grades of alcohol. The sections were finally cleared in xylene and a drop of mountant was placed on the section and covered with cover slip (Hani *et al.,* 2015) Prepared histological slides were viewed under the microscope and photomicrographs were taken.

Histological Analysis: The score of follicular growth and development was evaluated by the pattern of growth and development in the ovarian section; the follicles were categorized into 4 groups based on their morphology: Primary follicles, Secondary follicles, Preantral follicles and Mature follicles. The primary follicles present a single layer of granulosa cells, the secondary follicle shows multiple layers of granulosa cells around the cell, the preantral follicle is seen with free fluid filled spaces within the granulose cells and the mature follicles are seen with mature oocyte with a single large fluid filled space called the antrum. More so, the mature follicles are seen with granulosa cell differentiations into corona radiata, cumulus oophorous and membrana granulosa (Mohammad and Takashi, 2010).

Statistical Analysis: Data were analysed using SPSS 16.0 computer software package (SPSS Inc; Chicago U.S.A). The results were expressed as mean ± standard error. Statistical comparisons were made using analysis of variance (ANOVA) with Scheffe's post hoc test for within group and between groups comparison. The level of significance was considered at $p < 0.05$.

RESULTS

The analysis of follicles at different stages of development in the ovarian section is shown in Table 1. There were significantly higher numbers of mature follicles in the post-treated and the positive control groups when compared with the induced group, the expressions of which were comparable with the negative control group.

The histological section of the control group showed follicles at different stages of maturation with more in the mature graafian stage as shown in Plate 1. There was visibility of the theca cells alignment around the follicle. There was also presence of corpus luteum which reflects recent ovulation. The induced group showed poor follicular differentiation with only visible primary follicles and absence of corpus luteum (Plate 2a). The histological sections of Plate 2b, c and d are withdrawal groups which were established to check for recovery. The maximal grade of follicular development was seen in Plate 2d which showed lower number of mature follicles.

Table 1:
Analysis of the different stages of follicular growth and development

GROUP	Sub-group	PF	SF	PAF	MF
1	Negative control	2.17 ±0.22	1.22 ±0.00	1.32 ±0.00	3.33 ±0.12*
2	a.	6.21 ±0.32	0	0	0
	b.	2.11 ±0.10	1.22 ±0.03	0	0
	c.	1.01 ±0.02	1.32 ±0.03	1.43 ±0.01	0
	d.	1.10 ±0.03	2.54 ±0.20	0	1.05 ±0.00
3	a.	2.11 ±0.20	2.01 ±0.00	0	0
	b.	1.23 ±0.00	1.93 ±0.03	1.21 ±0.00	1.83 ±0.01
	c.	1.43 ±0.03	2.45 ±0.10	0	3.23 ±0.20 *
4	a.	122 ±0.02	2.42 ±0.13	2.17 ±0.00	2.92 ±0.00 *
	b.	1.82 ±0.02	1.32 ±0.01	2.12 ±0.11	3.16 ±0.06*
	c.	1.21 ±0.01	1.17 ±0.00	1.46 ±0.00	3.22 ±0.09*

Key: *Primary follicles (PF), Secondary follicles (SF), Preantral follicles (PAF) and Mature follicles (MF).*

The histological sections of the groups post-treated for 16 days (Plate 3b) showed a number of mature follicles while in groups post-treated for 28days (Plate 3c), a comparable number of graafian follicles with the control group were seen. The section showed follicles at the mature stage with well defined theca cell alignment and a single large antrum. The histological sections of the ovary of all green coconut water treated groups were similar to the expression shown in the control group. (Plate 4a, b and c).

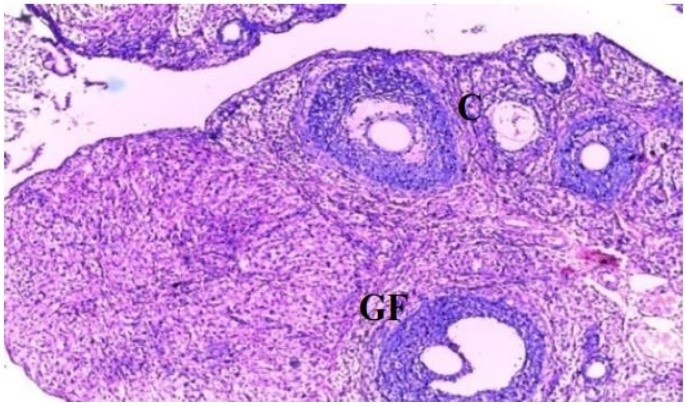

Plate 1:
Photomicrograph of the ovary of the control Sprague-Dawley rat at magnification of X100. Stains: Haematoxylin and Eosin. Abbreviations: PF (Primary Follicle); SF (Secondary Follicle); GF (Graafian Follicle); C(corpus luteum).

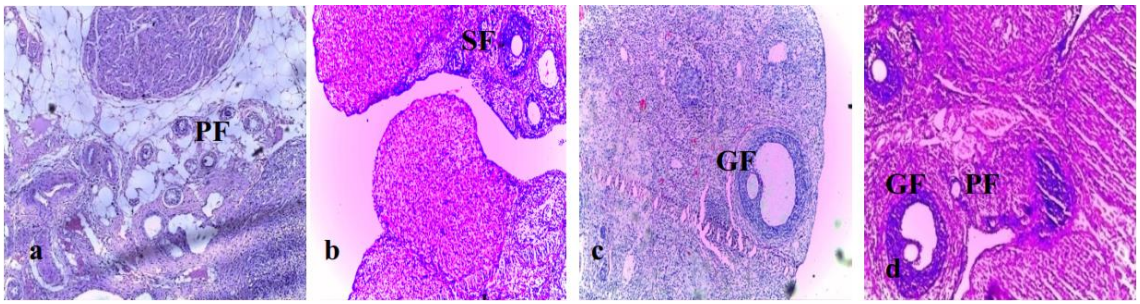

Plate 2:
Photomicrograph of the ovary of the induced, withdrawal groups at 8, 16 and 28 days at Magnification of X100. Stains: Haematoxylin and Eosin. Abbreviations: PF (Primary Follicle), SF (Secondary Follicle); MF (Mature Follicle).

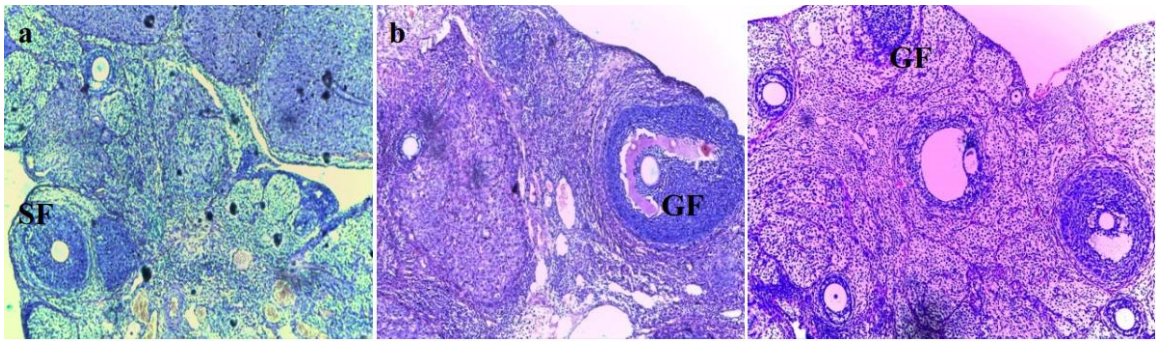

Plate 3
Photomicrograph of the ovary of GCW Post-treated groups 8, 16 and 28 days at magnification of X100. Stains: Haematoxylin and Eosin. Abbreviations: PF (Primary Follicle); SF (Secondary Follicle); MF (Mature Follicle).

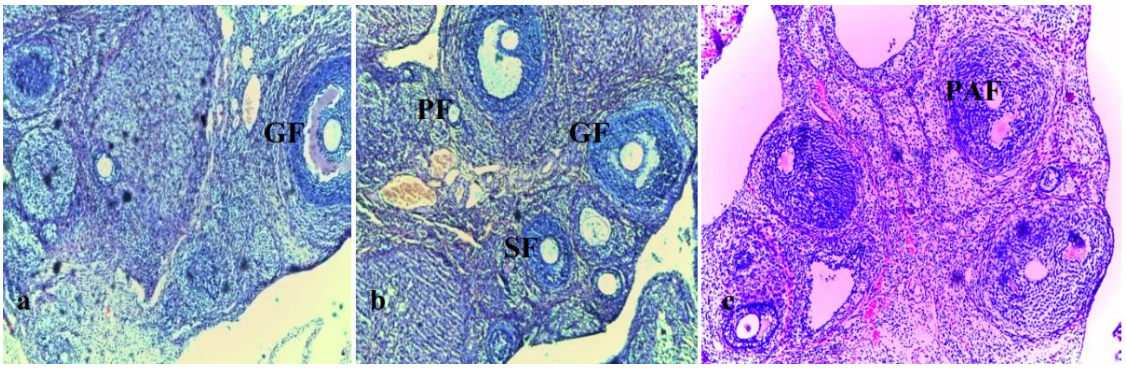

Plate 4
Photomicrograph of the ovary of the of the GCW- treated rats for groups 8, 16 and 28 days at magnification of X100. Stains: Haematoxylin and Eosin. Abbreviations: PF (Primary Follicle); SF (Secondary Follicle) ; MF (Mature Follicle).

DISCUSSION

Folliculogenesis is demonstrated by increase in granulosa cell proliferation and the formation of follicular fluid. Follicular growth and development is controlled by the hypothalamus through the release of GnRH which stimulates the release of pituitary FSH and LH (Matsuzaki *et al.*, 1994). It has been well established that increase in the secretion of prolactin is sufficient to affect the integrity of the hypothalamic-pituitary-gonadal axis (Anita , 2016; Patricia, 2016) Hyperprolactinaemia is associated with hypogonadotropic hypogonadism that results from low gonadotropin secretion. This leads to poor follicular growth and a consequent decrease in gonadal estrogen secretion with consequent inhibition of follicular growth and development. There is also the loss of LH-surge which is necessary to cause the releases of egg (Ursula and Kaiser 2012). In this present study, analysis of follicular growth pattern revealed that GCW stimulate follicular growth and development in the ovary of hyperprolactin rats. This expression may be attributed to the estrogenic characteristics of GCW which has been previously established. Phytosterols which facilitates the synthesis of endogenous estrogen was reported to be present in GCW (Punghmatharith, 1998). Another study confirmed estrogenic characteristic of GCW on serum estrogen level when it was administered to menopausal rats (Nisaudah *et al.*, 2009).
Estrogen is known to exert force during follicular development through regulated mechanism (Monique *et al.*, 2014). Estrogen is primarily produced by the ovarian follicle and as the follicles grow and develop, more estrogen is produced. As increasing amounts of estrogen is released into the blood stream, it travels to the anterior pituitary to stimulate

the release of FSH and LH which are essential for folliculogenesis. Therefore in agreement with Laura *et al.* (2012), any interruption in ovarian steroidogenesis will lead to hormonal imbalance; hence disruption of the reproductive cycle, morphology and function. Estrogens are therefore known to exert positive effects on granulosa cell growth and differentiation in association with the release of gonadotropins. More so, the result from this study is in conformity with an ovarian weight estimation study which showed increases in ovarian weight in hyperprolactin rats administered GCW (Bakare *et al.*, 2018). This further affirms that increase in the structural development of the ovarian follicles correlates with the consequent increase in ovarian weight and reversal of estrous demonstrated in previous studies. This investigation therefore suggest that estrogen was able to re-launch normal hormonal profile by stimulating the pituitary gland to act in a positive feedback fashion to release gonadotropins needed for follicular growth and development. The findings from study clearly depict that GCW stimulates follicular growth and development in hyperprolactin rats. Green coconut water is a promising substance in reversing infertility caused as a result of high prolactin in female S-D rat. The results from this study would serve as a preliminary template for further comparative studies and subsequent research work on hyperprolactin-induced infertility in higher female animals and in human.

REFERENCES

Alexia P., Manuel D., Nafissatou D., Jean-Pierre P. (2012): Coconut water preservation and processing. Fruits 67(3),157-171

Anita R. (2016): Oestrogens, prolactin, hypothalamic-pituitary-gonadal axis, and schizophrenic psychoses. The Lancet Psychiatry 4(1), 63-72.

Anurag P. and Rajamohan T. (2003). Cardioprotective effect of tendercoconut water in experimental myocardial infarction. Plant Foods for Human Nutrition, 58,1–12.

Atanas G. A., Birgit W., Eva-Maria P., Thomas L., Christoph W., Pavel U, Veronika T., Et al. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 33(8): 1582–1614.

Bakare A.A., Oremosu A.A., Akinsola O.J.(2013): Estrous cycle study on green coconut water in experimentally induced hyperprolactine in female Sprague-Dawleys rats. Sch. J. App. Med. Sci. 1(6), 1031-35.

Bakare A., Adam A., Rukayat A. (2018): Ovarian weight evaluation in hyperprolactin female Sprague-Dawley rats treated with Green coconut water. J exp. Cli ana., (in press).

Carlos S., Sarah C., Scott M., Musa A., Michelle F., Nicola J ., Bert S. (2015): FSH Regulates IGF-2 Expression in Human Granulosa Cells in an AKT-Dependent Manner. J Clin Endocrinol Metab.100(8),1046-55.

David R. Grattan Christine L. Jasoni Xinhuai Liu Greg M. Anderson Allan E. Herbison. (2007): Prolactin Regulation of Gonadotropin-Releasing Hormone Neurons to Suppress Luteinizing Hormone Secretion in Mice. Endocrin. 9 (148), 4344–51.

Deepak K., Rema R., Roy M. (2012): Description of a new method of ovariectomy in female rats. Rev. Bras. Reumatol. 52,3.

Hani A., Faris M. and Zuhair M. (2015): Histological Stains: A Literature Review and Case Study. Glob. J Health Sci. 8(3), 72–79.

Idris A., Ibrahim S., Rasheed Y., Isah A., Randawa A., Adesiyun G.(2018): Hyperprolactinemia and female infertility; Pattern of clinical presentation in a tertiary health facility in Northern Nigeria. Sahel Med J., 21 (1) 1-5

Kennedy I., Noel N., Wannang S. and Nanloh S. J. (2013): Preliminary studies on Cocos nucifera water for conceptive and antiabortive properties. Sch. J Med. 3(2), 19-22.

Laura D. R., Betina G., Petteri A., Noelia P. G., Matti P., Ricardo S. C. (2012): Short-Term Pharmacological Suppression of the Hyperprolactinemia of Infertile hCG-Overproducing Female mice persistently restores their fertility. Endocrinol. 153 (12), 5980–5992.

Marcondes F. K., Bianchi F. J. and Tanno T. A. (2002): Determination of the estrous cycle phases of rats: some helpful considerations. Braz. J. Biol. 62, 4a.

Matsuzaki T., Azuma K., Irahara M., Yasui T., Aono T. (1994): Mechanism of anovulation in hyperprolactinemic amenorrhea determined by pulsatile gonadotropin-releasing hormone injection combined with human chorionic gonadotropin. Fertil. Steril. 62, 1143–1149.

16. Mohammad M and Takashi M. (2010). Growth of primordial oocyte in Neonatal amd adult mammals. J Rep Dev., 6(56), 559-566.

Monique T., Bernardo G., Gustavo F., Rogério F., Rodrigo C., Raj D., Vilceu B. and Paulo B. (2014): Expression and molecular consequences of inhibition of estrogen receptors in granulosa cells of bovine follicles Journal of Ovarian Research 7, 96

Nawroth F. (2005): Hyperprolactinaemia and the regular menstrual cycle in asymptomatic women: should it be treated during therapy for infertility? Reprod. Biomed. 11(5), 581-8.

Nisaudah R., Farid S., Kitja S., Wilart R., Boornsirm W. and James R. C. (2009). Young coconut juice significantly reduces histopathological changes in the brain that is induced by hormonal imbalance: A possible implication to postmenopausal women. Journal of Hist. and Histopath., 24, 667-674.

Patricia F., Renato B., Loren W. (2016): . Physiology of the Hypothalamic Pituitary Gonadal Axis in the Male. Urologic Clinics of North America 43(2) ,):151-62

Patrick J. W. and Offler C.E. (2001): Compartmentation of transport and transfer events in developing seeds. J. Exp. Bot. 52, 551–564.

Pragya T. (2010): Coconut Water during Pregnancy. J. Sci. Food Agri. 2244, 875–88.

Punghmatharith B. (1998): Sex hormone-like substances in young coconut juice and their effects on uterine growth in rats. Songklanakarin J. Sci. Tech. 10, 221–226.

Ranko K., Jasmina P., Milan S., Dragana R., Radomir Z., Aleksandra P., Milan T. (2015): Ciation between hyperprolactinemia and other causes of female infertility. Med and Biol. 17(1) pp. 36-41

Saranya N. and Leila J. (2016): Prevalence of hyperprolactinemia in infertile women and its association with hypothyroidism. Int. J Adv. in Med. 1(2), 3925-33.

Sofowora A.(1993): Medicinal Plants and Traditional Medicines in Africa. New York: Chichester John Wiley & Sons. 97-145.

Ursula B. and Kaiser M.D. (2012): Hyperprolactinaemia and infertility: new insights. Journal of Clinical Investig. 122 (10), 3467–3468.