Predicting the Effect of Prolonged Dihydrotestosterone and Estradiol Valerate Administration on Male Albino Rats

Acha, Chigozie K[1*], Udochukwu Ahaiwe[2]

[1] Department of Statistics, Michael Okpara University of Agriculture Umudike, Abia State,Nigeria
[2] Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

Email Address

specialgozie@yahoo.com (Acha, Chigozie K), uahaiwe_eyahoo.co.uk (Udochukwu Ahaiwe)
*Correspondence: specialgozie@yahoo.com

Received: 30 November 2017; **Accepted:** 2 January 2018

Abstract:

Benign Prostatic Hyperplasia (BPH) can be chemically induced in male albino rat using a combination of dihydrotestosterone (DHT) and estradiol valerate (ESV). This paper predicts the effect of BHP induction on body weight and prostrate weight of male albino rats. The study used a primary data set of ten male albino rats divided into two groups of five rats each. Group 1 (Test) was administered subcutaneous injection of DHT and ESV dissolved in olive oil once in every two days for 28 days while Group 2 (control) was administered subcutaneous injection of olive oil without DHT and ESV once in every two days for 28 days. The rats were weighed daily and histochemical examination of the prostate was carried out to ascertain the presence of BPH. The result shows that the regression coefficients of the body weight and prostrate weight have positive relationship with BPH and also highly significant at 5% level. This study highlights the predictive potentials of body weight changes as a non-assay marker for BPH in male Wister rat and all the variables are highly statistically significant ($p < 0.05$). The R –squared and the coefficient of determination are 74.92% and 68% respectively, indicating how much of the total variation in the dependent variable, Benign Prostatic Hyperplasia (BPH) , can be explained by the independent variables Mean Prostrate Weight and Mean Body Weight Test. Also the body weight and the prostate weight prediction result shows that they are statistically significant to BPH, their correlation result established that they are also inversely proportional.

Keywords:

Dihydrotestosterone, Estradiol Valerate, Prostrate, Body Weight, Regression Model.

1. Introduction

BPH is the most common cause of lower urinary tract symptoms (LUTS), which are divided into storage, voiding, and symptoms which occur after urination, Figure 1, (https://en.wikipedia.org/wiki/Benign_prostatic_hyperplasia). **Benign prostatic hyperplasia** (**BPH**), also called **prostate enlargement**, is a noncancerous increase in size of the prostate. Prostate Enlargement (Benign Prostatic Hyperplasia) symptoms

may include frequent urination, trouble starting to urinate, weak stream, inability to urinate, or loss of bladder control. BPH complications can include urinary tract infections, bladder stones, and chronic kidney problems. The cause is unclear.

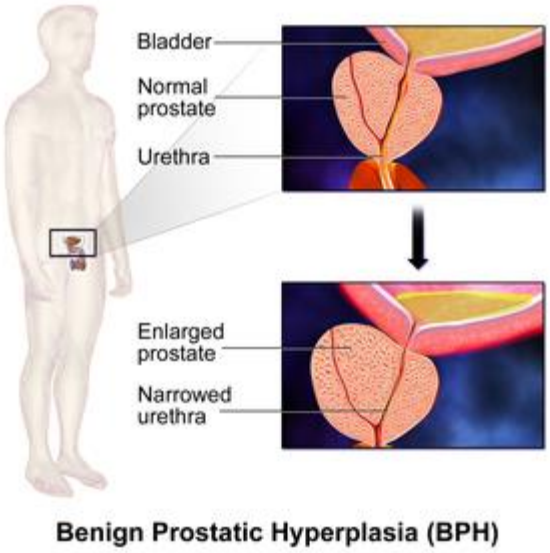

Figure 1. *Normal and Enlarged Prostates in Human Anatomy.*

Source: https://en.wikipedia.org/wiki/Benign_prostatic_hyperplasia

Clinical guideline [CG97]; (2015)confirms that Storage symptoms include the need to urinate frequently, walking at night to urinate, urgency (compelling need to void that cannot be deferred),involuntary urination, including involuntary urination at night, or urge incontinence (urine leak following a strong sudden need to urinate). Also, most urge incontinence and voiding symptoms include urinary hesitancy (a delay between trying to urinate and the flow actually beginning), intermittency (not continuous), White, et al., (1990); involuntary interruption of voiding, weak urinary stream, straining to void, a sensation of incomplete emptying, and terminal dribbling (uncontrollable leaking after the end of urination, also called post-micturition dribbling Robinson, J (2008) and Sarma & Wei (2012). These symptoms may be accompanied by bladder pain or pain while urinating, called Dysuria. It is pertinent to note that when drawing up a list of reasons that rodents are ideal for research and biomedical study, we often find ourselves first pointing to the logistics of experimentation and noting that it's helpful to have an animal that's cheap, available in large quantities and small. With those criteria, it's no wonder that creatures like fruit flies, roundworms and mice are helpful to have around the lab. But in some cases, having a small subject isn't entirely ideal; consider that researchers testing, for example, the efficacy of a physical intervention like surgery would consider a larger animal such as a rat a friend indeed; (see, https://emice.nci.nih.gov/research-uses/rats/experimental-therapeutics-research).

This paper predicts the effect of BHP induction on body weight and prostrate weight of male albino rats. The study used a primary data set of ten male albino rats divided into two groups of five rats each. Group 1 (Test) was administered subcutaneous injection of DHT and ESV dissolved in olive oil once in every two days for 28 days while Group 2 (control) was administered subcutaneous injection of olive oil without DHT and ESV once in every two days for 28 days. The rats were weighed daily and histochemical examination of the prostate was carried out to ascertain the

presence of BPH. In this study, the Benign Prostatic Hyperplasia (BPH) will be chemically induced in male albino rat using a combination of dihydrotestosterone (DHT) and estradiol valerate (ESV).

2. Literature Review

Benign Prostatic Hyperplasia (BPH) is a non-malignant proliferation of the prostate gland characterized by prostate enlargement that is accompanied with lower urinary tract symptoms (LUTs), (Rosenberg, 1980). Epidemiological studies of BPH reveal that BPH is an age-related disorder that occurs in nearly all men starting from the fifth decade of life (Barry, *et al.*, 1984). Histological BHP around the world shows the prevalence of BPH to be 10% of men in their 30s, 20% for men in their 40s,50% to 60% for men in their 60s and 80% to 90% for men in their 70s and 80s (Roehrorn, 2005: Roehrborn, and McConnell (2002).

This has prompted vigorous research on BPH using animal models to study disease progression and possible points of therapeutic interventions. Recent studies have shown that BPH can be induced in albino Wistar rat model using a combination of Dihydrotestosterone and estradiol Valerate (Ahaiwe, Ijeh, and Ejike, 2013). Dihydrotestosterone is the major prostatic androgen. It plays crucial role in the formation of the prostate gland during fetal life and in the development of BPH in late adult life. Dihydrotestosterone is a potent growth factor and its levels are increased in BPH, particularly in the periurethral area.

Animal models are important for defining the molecular basis of some diseases so as to unveil possible point at which therapeutic interventions will yield positive result with minimal side effects. Unlike most other animals and non-human primates, rats are among the few animal species known to develop BPH spontaneously (Lamb and Zhang, 2005). In addition to mimicking the progression of prostate tumor in humans, rat model is widely employed in the study of pathogenesis and chemoprevention strategy of BPH in the most third worlds. However, in more advanced countries, models like cell line and genetically engineered mouse model are more frequently employed.

Many researchers have worked extensively on dihydrotestosterone and estradiol valerate administration, for example, Sotomayor-Zárate, Tiszavari, Cruz, HE (2011), studied the Neonatal exposure to single doses of estradiol or testosterone programs ovarian follicular development-modified hypothalamic neurotransmitters and causes polycystic ovary during adulthood in the rat. Matsumoto, Sakari, Okada, Yokoyama, Takahashi, Kouzmenko, Kato (2013), worked on the androgen receptor (AR) in health and disease in males. They also offered a perspective on the use of animal genetic model systems aimed at eventually developing novel therapeutic AR ligands. Anesetti, Chávez-Genaro, (2015) established that Neonatal testosterone exposure induces early development of follicular cysts followed by sympathetic ovarian hyperinnervation. Also in 2016, Anesetti, Chávez-Genaro, worked on Ovarian follicular dynamics after aromatizable or non aromatizable neonatal androgenization. Uchida, (2015) studied the Sympathetic regulation of estradiolsecretion from the ovary. In 2016, Rosenfield, Ehrmann , revisited the Pathogenesis of Polycystic Ovary Syndrome (PCOS) taking the Hypothesis of PCOS as Functional Ovarian Hyperandrogenism. More research are still going on especially on PCOS, Morales-ledesma, Ramos, and Hernandez,(2017), in their study evaluated the role of the sympathetic innervation running through the superior ovarian nerve (SON) in

polycystic ovary syndrome (PCOS) persistence at pubertal and adult rats. They concluded in their result that exposure to testosterone propionate at birth results in the development of PCOS, and, in this model, the syndrome is not due to noradrenergic innervation hyperactivity. Caldwell, Edwards, Desai, Jimenez, Gilchrist, Handelsman, Walters, (2017) said Neuroendocrine androgen action is a key extraovarian mediator in the development of polycystic ovary syndrome.

A number of studies on BPH induction in albino Wistar rat model has been published (Ejike and Ezeanyika, 2010; Ahaiwe, et al 2013) but there is no agreement among researchers on the statistical specific duration of BPH induction. This study therefore aims to highlight the possible predictive potential of body weight changes with respect to BPH induction as a non-assay maker for successful BPH induction in albino Wistar rat. It is also pertinent to estimate for the actual statistical inference for immediate and future prediction and forecasting, as emphasized in Acha (2011; 2012). To achieve this, the next section reviews the BPH literature; the third section describes the materials and methods, including the statistical analysis. In the fourth section data is analyzed using the Classical linear regression model (CLRM). This paper is summarized and concluded in the fifth section with few recommendations.

3. Research Methodology

3.1. Materials and Methods

Ten male albino Wistar rats aged between 9-10 weeks old were used for this study. The rats were breed in the animal breeding unit of University of Nigeria Nsukka and transported in a stainless steel cage with a plastic base to the Animal house of the Biochemistry Department, Michael Okpara University of Agriculture, Umudike. The rats were acclimatized for seven days in the animal house and exposed to 12 hour light/dark cycle. Vita feed grower's mesh and clean tap water was daily provided for the animals' *adlibitum.*

The rats were sorted into two groups of five rats each with an inter-group weight difference of± 5 after the acclimatization. Group 1 was induced with BPH using the method described by Ejike and Ezeanyika (2010). BPH induction was done by subcutaneous injection of 9mg/kg body weight dihydrotestosterone and 0.9mg/kg body weight estradiol valerate dissolved in olive oil once in every two days for 28 days. Group 2 served as the control group. Body weight changes of both groups were monitored and recorded. After 28 days both groups were fasted overnight and scarified by cardiac puncture. The prostate gland was excised and weighed for each rat. Relative prostate weight was calculated and histochemical analysis carried out on the prostate to confirm the presence of BPH.

3.2. Historical Analysis

Three prostate glands were selected at random from each group for histological analysis. The prostates were transformed to a sterile sample container with 10% formalin solution. The organs were then dehydrated in ascending grade of alcohol (50%, 70%, 90% and 100%) twice. Xylene was then used to render the organs transparent by removing alcohol from dehydrated sections. The tissues were impregnated with paraffin wax and embedded in an oven at$62 - 68^{O}C$. Block cast for each tissue was provided and the set up was allowed to solidify at room temperature. The blocks were appropriately labeled for sectioning. The paraffin wax was trimmed

before sectioning with a microtone. Staining was done with hematoxylin and eosin (Hєiε). A drop of Dipex mountant was then placed on the specimen slide and covered carefully with a cover slip before the slides were photomicrograph.

3.3. Statistical Analysis

In this section the statistical analysis of the relationship between BPH and various biological weights (body and prostate) variables is carried out. The essence of this is to review practically if the theorized relationship between interest rate and these variables hold true in the experiment. This goal will be achieved by using the specified albino Wistar rat models.

Albino Wistar rat linear regression model takes the form

$$yi = \beta_1 x_{i1} + \cdots + \beta_p x_{ip} + \varepsilon_i = X_i^T \beta + \varepsilon_i, \quad i = 1, \ldots, n.$$

where ^{T} denotes the transpose, so that $\boldsymbol{x}_i^T\boldsymbol{\beta}$ is the inner product between vectors $\boldsymbol{x}_i$ and $\boldsymbol{\beta}$.

Often these *n* equations are stacked together and written in vector form as

$$y = X\beta + \varepsilon$$

Where,

$$y = \begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{pmatrix}, X = \begin{pmatrix} X_1^T \\ X_2^T \\ \vdots \\ X_n^T \end{pmatrix} = \begin{pmatrix} x_{11} & \cdots & x_{1p} \\ x_{21} & \cdots & x_{2p} \\ & \vdots & \\ x_{n1} & \cdots & x_{np} \end{pmatrix}, \beta = \begin{pmatrix} \beta_1 \\ \beta_2 \\ \vdots \\ \beta_n \end{pmatrix}, \epsilon = \begin{pmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{pmatrix} \quad (1)$$

Where Xi is a row vector of observations on k regressors, n =2, is the number of observations or statistical units, β is a k-vector and ε_i is the error term. A linear regression model assumes that the relationship between the dependent variable y_i and the p-vector of regressors x_i is linear. This relationship is modeled through a disturbance term or error variable — an unobserved random variable that adds noise to the linear relationship between the dependent variable and regressors. (Acha 2011;2012).

4. Statistical Result and Interpretation

Correlations			
		Test group	Control group
Test group	Pearson Correlation	1	.331**
	Sig. (2-tailed)		.000
	N	140	140
Control group	Pearson Correlation	.331**	1
	Sig. (2-tailed)	.000	
	N	140	140
**. Correlation is significant at the 0.01 level (2-tailed).			

Apart from the correlation result showing that they are statistically significant at o.o1 level (2-tailed, it also shows that they are also inversely proportional, figure 2.

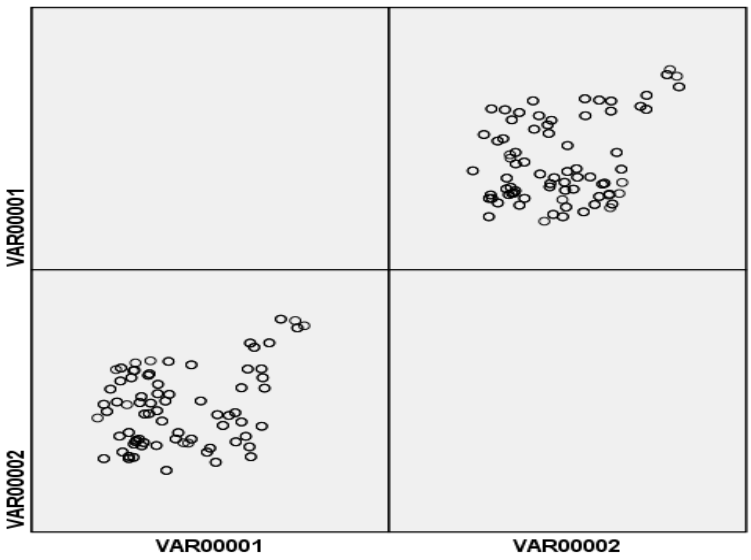

Figure 2. *Correlation graph for variables (test group (var00001); Control group (var00002)).*

In this section, the three main tables will be required to explain the regression results, assuming that no assumptions have been violated. The first table of interest is the **Model Summary** table, as shown below:

Table 1. *Model Summary.*

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.865[a]	.749	.498	16.23328
a. Predictors: (Constant), Mean Prostrate Weight and Mean Body Weight Test				

This table provides the R and R^2 values. The R value represents the simple correlation and is 0.865 (the "**R**" Column), which indicates a high degree of correlation. The R^2 value (the "**R Square**" column) indicates how much of the total variation in the dependent variable, Benign Prostatic Hyperplasia (BPH) , can be explained by the independent variables Mean Prostrate Weight and Mean Body Weight Test. In this case, 74.92% can be explained, which is very large.

The next table is the **ANOVA** table, which reports how well the regression equation fits the data (i.e., predicts the dependent variable) and is shown below:

Table 2. *Analysis of Variance (ANOVA) a table.*

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1572.253	2	786.127	2.983	.002[b]
	Residual	527.039	2	263.519		
	Total	2099.292	4			
a. Predictors: (Constant), Mean Prostrate Weight, Mean Body Weight						
b. Dependent Variable: Benign Prostatic Hyperplasia (BPH)						

This table indicates that the regression model predicts the dependent variable significantly well. Here, $p < 0.002$, which is less than 0.05, and indicates that, overall, the regression model statistically significantly predicts the outcome variables. This indicates the statistical significance of the regression model that was run (i.e., it is a good fit for the data).

The **Coefficients** table provides us with the necessary information to predict Benign Prostatic Hyperplasia (BPH) from Mean Prostrate Weight and Mean Body Weight as well as determine whether Mean Prostrate Weight and Mean Body Weight contribute statistically significantly to the model.

Table 3. *Coefficient* [a].

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	516.374	223.951		2.306	.000
	Mean Body Weight Test	-711.570	347.031	-.748	-2.050	.002
	Mean Prostrate Weight	.388	.484	.292	.800	0.00
a. Dependent Variable: Benign Prostatic Hyperplasia (BPH)						

The estimated regression equation of BPH:
BPH = 516.374 + 0.388MPW -711.570MBW
Where;
MPW - Mean Prostrate Weight
MBW - Mean Body Weight
BPH - Benign Prostatic Hyperplasia

From Table 3; the "**Unstandardized Coefficients**" values in the "**B**" column, x, shows that the model predicts that one unit decrease in the Mean Body Weight (MBW); the Benign Prostatic Hyperplasia (BPH) will reduce by -711.570 units holding Mean Prostrate Weight constant. In addition, the model predicts that one unit increase in the Mean Prostrate Weight (MPW); the Benign Prostatic Hyperplasia (BPH) will increase by 0.388 units holding Mean Body Weight constant.

Furthermore, the **Standardized Coefficients "Beta"** in table 3 indicates that one standard deviation increase in MPW, the model predicts that the BPH will increase by 0.292 standard deviations while for one standard deviation decrease in MBW, the model predicts that the BPH will decrease by -0.748 standard deviations.

The coefficient of determination is 0.68, which means that 68% of the variation in mean body can be predicted from the relationship between Mean Prostrate Weight and Mean Body Weight. (Conversely, 32% of the variation in mean body weight cannot be explained.

Thus, prostrate weight is inversely proportional to body weight, mathematically represented by $Prostrate\ weight = \frac{c}{Body\ weight}$; where c is the constant.

5. Conclusions

The result of this study shows that body weight changes following chemical induction of BPH can serve as a non-assay maker for successful induction of BPH in albino Wistar rats. The onset of BPH is characterized by lower urinary tract syndrome (LUTs) which results in decreased quality of life. This reduced quality of life in turn leads to loss of body weight which is demonstrated in this study. However, further

analysis will elucidate the threshold at which body weight loss corresponds to BPH induction.

Conflicts of Interest

The authors declare that there is no conflict of interest between them in publishing this paper.

References

[1] Acha, C.K. Comparative Analysis of Quadratic and Cubic Models on Egg Production. *Animal Production Research Advances,* 2011, 7(2), 110-115.

[2] Acha, C.K. Regression and Principal Component Analyses: A Comparison Using Few Regressors. *American Journal of Mathematics and Statistics,* 2012, 2(1), 1-5.

[3] Ahaiwe, U.E.; Ijeh, I.I.; Ejike C. Methanol extract of Vernonia amygdalina may protect against experimental benign prostatic hyperplasia in an albino Wistar rat model. *J. Med. and Arom. plant sci.* 2013, (3-4), 115-120.

[4] Anesetti G.; Chávez-Genaro R. Neonatal testosterone exposure induces early development of follicular cysts followed by sympathetic ovarian hyperinnervation. *Reprod Fertil Dev.* 2015, 28(11), 1753-1761.

[5] Anesetti G.; Chávez-Genaro R. Ovarian follicular dynamics after aromatizable or non aromatizable neonatal androgenization. *J Mol Histol.* 2016, 47(5), 491-501. Available online: https://emice.nci.nih.gov/research-uses/rats/experimental-therapeutics-research (accessed on 30 November 2017).

[6] Barry, S.J.; Coffey, D.S.; Walsh, D.C.; Ewing, L.L. The development of human benign prostatic hyperplasia with age. *J Urol.* 1984, 132(3), 474-497.

[7] Caldwell A.S.L.; Edwards M.C.; Desai R.; Jimenez M., Gilchrist R.B.; Handelsman D.J.; Walters K.A. Neuroendocrine androgen action is a key extraovarian mediator in the development of polycystic ovary syndrome. *Proc Natl Acad Sci U S A.* 2017, 114(16), 3334-3343.

[8] Carlos, J.; Almeida, Flavio. Residual Urinary Volume and Urinary Tract Infection- when are they linked? *The Journal of Urology,* 2008, 180 (1), 182-185.

[9] Chang R.J., Cook-Andersen H. Disordered follicle development. *Mol Cell Endocrinol.* 2013, 373(1-2), 51-60.

[10] Clinical guideline [CG97]. Lower urinary tract symptoms in men: management. NICE (National Institute for Health and Care Excellence), 2015. Available online: https://www.nice.org.uk/guidance/cg97 (accessed on 30 November 2017).

[11] Ejike, C.C.E.; Ezeanyika, L.U.S. Management of experimental benign prostatict hyperplasia in rats using a food-based therapy containing Telfairia occidentalis seed. *Afri. J. Tradit. Compl. Altern. Med.* 2010, 8(4), 398-404.

[12] Lamb, D.J.; Zhang, L. Challenges in prostate cancer research: Animal models for nutritional studies of chemoprevention and disease progression. International conference on diet, nutrition and cancer. *J. Nutr.* 2005, 135(12), 3009S-3015S.

[13] Matsumoto T.; Sakari M.; Okada M.; Yokoyama A.; Takahashi S.; Kouzmenko A.; Kato S. The androgen receptor in health and disease. *Annu Rev Physiol.* 2013, 75(13), 201-224.

[14] Morales-ledesma, L.; Ramos, J.A.D.; Hernandez, A.T. Polycystic Ovary Syndrome induced by Exposure to Testosterone Propionate and effects of Sympathectomy on the Persistence if the Syndrome. *Reprod Biol Endocrinol.* 2017; 15(50), 1-10.

[15] Prizant H.; Gleicher N.; Sen A. Androgen Actions in the Ovary: Balance is Key. *J Endocrinol.* 2014, 222(3), 141-151.

[16] Prostate Enlargement (Benign Prostatic Hyperplasia). NIDDK. September 2014. Archived from the original on 4 October 2017. Retrieved 19 October 2017.

[17] Robinson, J. Post-micturition dribble in men: causes and treatment. *Nurs Stand.* 2008, 22 (30), 43-46.

[18] Roehrborn, C.G. Benign prostatic hyperplasia: An overview. *Rev. Urol.* 2005, 7 (Suppl 9), S3-S14.

[19] Roehrborn, C.G.; McConnell, J.D. Etiology, pathophysiology, epidemiology and natural history of benign prostatic hyperplasi In: Walsh, P. C., Retik, A. W. and Vaugh, E. (eds). Campbell's Urology. Philadelphia Linpincot Williams and Wilkins, 2002, 1297-1336.

[20] Rosenberg, B. Metabolic control and disease. W. B. Saunders Company, 1980, 1563.

[21] Rosenfield R.L.; Ehrmann D.A. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. *Endocr Rev.* 2017, 37(5), 467-520.

[22] Sarma, A.V.; Wei, J.T. Clinical practice. Benign prostatic hyperplasia and lower urinary tract symptoms. *N Engl J Med.* 2012, 367(3), 248-257.

[23] Sotomayor-Zárate R.; Tiszavari M.; Cruz G.; Lara H.E. Neonatal exposure to single doses of estradiol or testosterone programs ovarian follicular development-modified hypothalamic neurotransmitters and causes polycystic ovary during adulthood in the rat. *Fertil Steril.* 2011, 96(6), 1490-1496.

[24] Uchida S. Sympathetic regulation of estradiol secretion from the ovary. *Auton Neurosci.* 2015, 187, 27-35.

[25] Urge incontinence. MedlinePlus. US National Library of Medicine. Archived from the original on 6 October 2015. Retrieved 26 October 2015.
Available online: https://medlineplus.gov/ency/article/001270.htm (accessed on 6 November 2017).

[26] Urination - painful. MedlinePlus. US National Library of Medicine. Archived from the original on 6 October 2015. Retrieved 26 October 2015.
Available online: https://www.nlm.nih.gov/ (accessed on 30 November 2017)

[27] White, J.M.; O'Brien, D.P.; Walker, H.K.; Hall, W.D.; Hurst, J.W. Incontinence and Stream Abnormalities, Butterworths, Boston, 1990, PMID 21250138.

[28] Wu, C.P.; Gu, F.L. The prostate in eunuchs. *Prog Clin Biol Res.* 1991, 370, 249-55.

© 2017 by the author(s); licensee International Technology and Science Publications (ITS), this work for open access publication is under the Creative Commons Attribution International License (CC BY 4.0). (http://creativecommons.org/licenses/by/4.0/)