

Elevated Corticosterone Level Due To Chronic Stress on Hb- Egf Expression as a Marker of Endometrial Receptivity Disorder in *Rattus norvegicus*

by Risya Secha Primindari

Submission date: 20-Jun-2022 12:05PM (UTC+0800)

Submission ID: 1859866846

File name: 10._Elevated_Corticosterone_Level_Due_To_Chronic_Stress_on.pdf (914.01K)

Word count: 2928

Character count: 16615

Elevated Corticosterone Level Due To Chronic Stress on Hb-Egf Expression as a Marker of Endometrial Receptivity Disorder in *Rattus norvegicus*

Risya Secha Primindari¹, Ashon Sa'adi², Reny I'tishom³

¹ Master degree of Reproductive Health Science, ² Department of Obstetrics and Gynecology,

³ Departemen of Biomedical Science, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia

Abstract

Endometrial receptivity is a physiological state in which the endometrium obtains an adhesive phenotype that allows embryo implantation and have a role in the problem of implantation failure. Endometrial receptivity disorders can be caused by interference with the Hypothalamus-Pituitary-Gonad (HPG) axis due to the activation of the Hypothalamus-Pituitary-Adrenal (HPA) axis by stress. HB-EGF is a biomarker of endometrial receptivity that plays a role in the decidualization of endometrial stromal cells to reach the receptive state and initiation of implantation. Corticosterone is the dominant glucocorticoid hormone in rodents, as is cortisol in humans. High corticosterone due to chronic stress triggers disruption of homeostasis in the endometrium by resulting in decreased levels of HB-EGF. This study aims to find out the effect of increased corticosterone levels due to chronic stress on HB-EGF expression in endometrium *Rattus norvegicus*. This research has obtained ethical eligibility from the Research Ethics Commission of the Faculty of Medicine Airlangga University. The samples on this study were 34 rat (*Rattus norvegicus*) which were divided into 2 groups, the control group and the stress treatment group using the Chronic Unpredictable Mild Stress (CUMS) method. Corticosterone level were obtain from blood serum detected via ELISA and HB-EGF expression was obtained from endometrial in diestrus phase was evaluated by immunohistochemical methods. Corticosterone levels in the stress treatment group were higher (72.84 ± 64.03) than in the control group (23.29 ± 8.42). HB-EGF expression in the stress treatment group was lower (82.06 ± 5.91) than in the control group (118.76 ± 13.20). Statistical tests showed significant differences in HB-EGF expression in endometrium *Rattus norvegicus* $p = 0,000$ ($p < 0.05$). Elevated level of corticosterone can decrease HB-EGF expression in endometrium *Rattus norvegicus*.

Keywords : corticosterone, chronic stress, HB-EGF expression, endometrial receptivity

Introduction

Endometrial receptivity is a physiological state in which the endometrium obtains an adhesive phenotype that allows embryo implantation and occurs over a period of time known as the implantation window in the middle phase of endometrial secretion. In this phase endometrium is very dependent on the presence of endogenous or exogenous progesterone and stimulation

of 17 β -estradiol. Implantation can only occur when the endometrium is in its receptive phase^(1,2).

A study shows that endometrial receptivity has a 60% role in the incidence of implantation failure⁽³⁾. Another study conducted in India found that 25% of implantation failures are known to be caused by endometrial receptivity⁽⁴⁾. As many as 30% of secondary infertility in women is caused by implantation failure and endometrial receptivity disorders being one of them^(5,6,7). One factor that can interfere with endometrial receptivity is stress⁽⁸⁾.

Endometrial receptivity disorders can be caused by several factors, such as interference with the

Corresponding Author:

Dr. Reny I'tishom, M.Sc; Tambaksari, Surabaya,
East Java, Indonesia; ritishom@fk.unair.ac.id;
+628121644432

Hypothalamus-Pituitary-Gonad (HPG) axis due to the activation of the Hypothalamus-Pituitary-Adrenal (HPA) axis by stress. Excessive activity of Corticotrophin Releasing Hormone (CRH) in the hypothalamus, in response to stress, is a mechanism for suppression of GnRH. Furthermore, as the body's effort to adapt to stress causes the production of a glucocorticoid hormone, cortisol, by the adrenal glands. Increased cortisol levels can indirectly cause interference with GnRH pulsation, causing a decrease in the amount of gonadotropin hormone produced by the anterior pituitary⁽⁹⁾. Increased cortisol results in impaired levels of the hormones estrogen and progesterone in the ovary. Decreased levels of the hormones estrogen and progesterone in the ovary also have an impact on estrogen and progesterone levels in the endometrium⁽¹⁰⁾.

One of the biomarkers that plays a role in determining endometrial receptivity is Heparin Binding Epidermal Growth Factor (HB-EGF) which affects cell-to-cell interactions and is a dependent estrogen progesterone⁽¹⁾. HB-EGF is needed for normal decidualization of endometrial stromal cells to reach the receptive state in the endometrium and for the initiation of implantation. HB-EGF has been identified as an initial mediator of embryo-uterine interactions during implantation and is expressed both in the blastocyst and in the endometrium during implantation and plays a role in stimulating embryonic development during hatching. The high cortisol hormone due to chronic stress triggers homeostasis in the endometrium due to the inhibition of the formation of the hormone progesterone which results in decreased levels of HB-EGF⁽⁶⁾. When HB-EGF levels decrease, the number of mature ErbB4 and HB-EGF receptors released in the endometrium decreases. ErbB4 has an important role in stimulating blastocyte implantation. ErbB4 in endometrium will communicate in juxtacrine with ErbB1 contained in blastocytes. Communication between these two receptors is an important key in mediating implantation^(10,11).

Chronic stress in this study uses the Chronic Unpredictable Mild Stress (CUMS) method. Chronic Unpredictable Mild Stress is giving various treatments as a stressor and resembles a stressor of everyday life that is not too heavy but continuously⁽¹²⁾. This method has been shown to significantly increase corticosterone levels (cortisol in mice) within 20 days⁽¹³⁾. In Rattus, cortisol

secretion is replaced by corticosterone⁽¹⁴⁾. Chronic stress increases glucocorticoid synthesis and secretion (cortisol in humans and corticosterone in rodents). Increased glucocorticoids are biomarkers for stress and depression disorders⁽¹⁵⁾. Based on this background, this study was aim of to find the effect of increased corticosterone on HB-EGF expression as a marker of interference with endometrial receptivity.

Material and Method

This study was true laboratory experimental designs with randomized post test only control group design. The study was conducted from April to June 2019 at the Experimental Animal Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlangga. The samples of this study were female rats (*Rattus norvegicus*) Wistar strains aged 5-6 months, have ever given birth to the consideration of mice in fertile conditions, never been used as an object of research before, and in healthy condition. All animals are injected with PGF2 α at a dose of 25 μ g / g body weight intraperitoneally to synchronize the lust cycle. The purpose of synchronizing the lust cycle is to equalize and obtain the diestrus phase during surgery. Female rat was taken randomly into 2 groups, each group consisting 17 female rat. The control group (K1) consisting rat with negative treatment were mice that were not given a stressor and the treatment group (K2) consisting rat with stressors treatment. Stressors treatment in rat using Chronic Unpredictable Mild Stress (CUMS) method for 20 days.

Corticosterone level were obtain from blood serum detected via ELISA and HB-EGF expression was obtained from endometrial in diestrus phase was evaluated by immunohistochemical methods. HB-EGF was assessed semi-quantitatively on the scale of Rammele-Stegner using the Immuno Reactive Score (IRS). Statistical analysis uses IBM SPSS Statistic 25. Data with normal distribution was tested by Independent T test. If the data was not normally distributed, the data was tested by the Mann Whitney test. This study uses a significance level of 0.05 with a confidence level of 95%.

Findings

1. Corticosterone Levels

26 **Table 1. The mean and standard deviation of corticosterone levels in serum *Rattus norvegicus***

Group	n	Corticosterone Levels
		Mean \pm Standard Deviation
K1	15	23.29 \pm 8.42
K2	16	72.84 \pm 64.03

The results showed a mean of corticosterone levels in the treatment group was higher (72.84 \pm 64.03) than control group (23.29 \pm 8.42).

Table 2. Mann Whitney analysis results of corticosterone levels in serum *Rattus norvegicus*

Group		P Value	Different test analysis
K1	K2	0,000*	Mann Whitney Test

14 *Significantly different $P < 0,05$

10 Analysis of the Mann Whitney test showed that there were significant differences in the corticosterone levels between the control and treatment groups with a value of $P = 0,000$ ($P < 0,05$).

2. HB-EGF Expressions

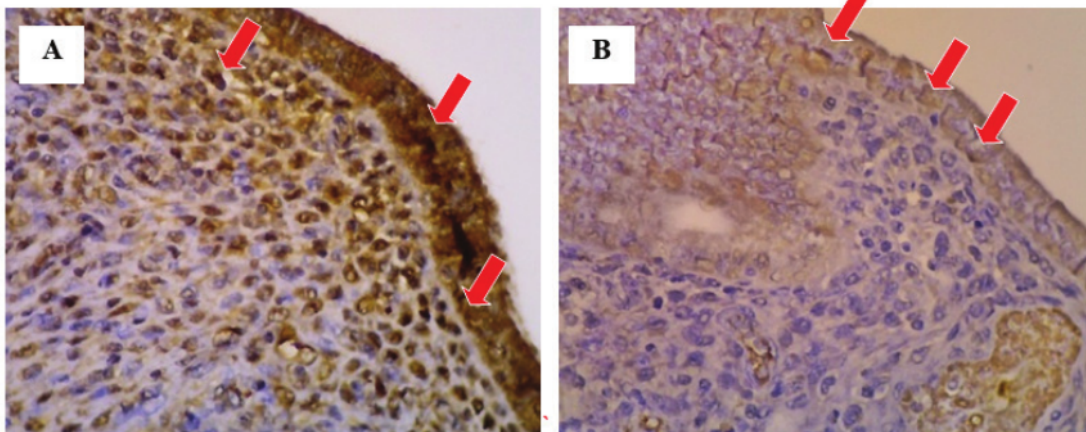


Figure 1. Comparison of HB-EGF expressions represented by chromogen brown color on the endometrial tissue, red arrows showing the maximum expression area using immunohistochemical staining, 400x magnification; Miconos microscope MCX50LED; Optilab Plus camera. (A) HB-EGF expression in the control group (B) HB-EGF expression in the treatment group.

Table 3. Mean and standard deviation of endometrium HBEGF expression

Group	n	HBEGF expression (IRS)
		Mean ± Standard Deviation
K1	15	118.76 ± 13.20
K2	16	82.068 ± 5.91

The examination results of the endometrium HBEGF expression showed a mean and deviation standard in the stress treatment group (82.068 ± 5.916) lower than the control group (118.76 ± 13.208).

Table 4. Results of Independent T analysis of endometrium HBEGF expression

Group		P Value	Different test analysis
K1	K2	0,000*	Independent T

*Significantly different $P < 0,05$

The results of the Independent T test showed that there was a significant difference in the HBEGF expression between the control and treatment groups with a value of $P = 0,000$ ($p < 0,05$).

Discussion

Chronic stress increases glucocorticoid synthesis and secretion (cortisol in humans and corticosterone in rodents). Increased glucocorticoids are biomarkers for stress and depression disorders⁽¹⁵⁾. Corticosterone is the dominant glucocorticoid hormone in rodents, as is cortisol in humans⁽¹⁴⁾.

In normal conditions, glucocorticoids will provide negative feedback by suppressing CRH secretion to prevent excessive stress responses, but this does not occur in chronic stress where the activity of the HPA axis continues⁽¹³⁾. Increased of glucocorticoids also affects the HPO axis directly by releasing GnRH release from the hypothalamus and suppressing the release of the gonadotropin hormone from the pituitary which can interfere with various mechanisms that occur in the ovaries so that it can lead to reproductive disorders⁽⁹⁾.

There are many theories that present the mechanism of chronic stress on changes in reproductive function. Apart from the activity of CRH and glucocorticoids, which will be discussed next with the effect on the gonadotropin hormone, the disturbed circadian cycle also becomes the mechanism used in this study. The sample unit in this study is *Rattus norvegicus* which is a nocturnal animal⁽¹⁶⁾, while all treatment activities are carried out in the morning - afternoon so that it changes the circular cycle of *Rattus norvegicus*. Disorders of sleep patterns that are applied to experimental animals cause disruption of the circadian rhythm in the central arrangement of the circadian rhythm in the hypothalamus, the Supra Chiasmatic Nucleus (SCN). Circadian rhythm is the body's biological clock 24 hours a day. This system regulates hormone secretion, and one of the physiological processes of the sleep cycle is awake⁽¹⁷⁾. Disorders of sleep patterns that are not sought to overcome it will fall in a state of psychosocial stress. In addition to the physical stress that is given, the change in circular rhythm also has an impact on the psychological stress of the experimental animal, thereby increasing the existing stress. The circadian cycle is an integral part of the reproductive system, when the 24-hour program is irregular the endocrine system can be disrupted. The high corticosterone levels in *Rattus norvegicus* in this study are in line with the Lopez-Lopes *et al.* study which states that CUMS can increase corticosterone levels with a minimum of treatment for 20 days⁽¹³⁾.

HB-EGF has been identified as an initial mediator of embryo-uterine interactions during implantation and is expressed both in the blastocyst and endometrium during implantation and is a dependent estrogen and progesterone^(1,6). Chronic stress causes elevated serum corticosterone levels, resulting in impaired estrogen and progesterone levels in the ovaries. Decreased levels of the hormones estrogen and progesterone in the ovary also have an impact on estrogen and progesterone levels in the endometrium⁽¹⁰⁾. Increased cortisol has an impact on homeostasis disruption in the endometrium resulting in decreased levels of HB-EGF which is a dependent estrogen and progesterone⁽⁶⁾. HB-EGF is expressed in luminal endometrial epithelial cells and on the surface of pinopods. Disregulation in HB-EGF expression in the endometrium has been associated with infertility whose causes cannot be explained (unexplained infertility). HB-EGF is needed for normal decidualization of endometrial stromal cells to reach the

receptive state in the endometrium and for the initiation of implantation^(1,18).

HB-EGF performs two simultaneous functions during the human implantation as an attachment factor and a growth factor. It was reported that HB-EGF plays an important role in the preparation of the uterine luminal epithelium for blastocyst attachment at the beginning of pregnancy. HB-EGF, as a growth factor, accelerates the development of human embryos to the blastocyst stage and their subsequent hatching from the zona pellucida. HB-EGF, as an attachment factor, upregulates many important proteins expressed from a uterine luminal epithelial surface such as integrin β 3, leukemia inhibitory factor (LIF), and HOXA10. Integrin α β 3 serves for osteopontin to mediate the embryo attachment. LIF stimulates human embryo development to the blastocyst stage and is required for embryo implantation. HOXA10 was found in the human endometrium during the mid-secretory phase abundantly and may be involved in implantation and decidualization of endometrium during early pregnancy. HOXA10 induction by progesterone during the window of implantation leads to a blockage in the stromal cell cycle, facilitating decidualization⁽¹⁹⁾.

HB-EGF induces endometrial cell proliferation through activating the cascade of ERK1 / 2 signals in epithelial cells and increasing DNA synthesis and cyclin D3 in stroma cells^(10,20). Decreased levels of HB-EGF have an impact on disruption of endometrial proliferation and angiogenesis, this can have an impact on the inability of the endometrium to reach its receptive phase. The sequence of endometrial proliferation and angiogenesis disorders can be interpreted as a disturbance in endometrial receptivity.

Conclusion

Elevated level of corticosterone can decrease HB-EGF expression in endometrium *Rattus norvegicus*.

Ethical Clearance: Ethical clearance of this study was taken from Ethical Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. Number: 129/EC/KEPK/FKUA/2019

Source of Funding: This study was self funding by authors.

Conflict of Interest: The authors have no conflicts of interest.

References

- Horcajadas JA, Jaime G. Reproductomics - The Omics Revolution and its Impact on Human Reproductive Medicine. London : Elsevier Inc; 2018. 279 – 301.
- Skinner MK. Encyclopedia of Reproduction. 2nd ed. London : Elsevier Inc; 2018. 231-8.
- Song M, Xian X, Yongli L, Rong H, Junli Z, Lingxia H, et al. Expression of heparin-binding epidermal growth factor in the endometrium is positively correlated with IVF-ET pregnancy outcomes. Int J Clin Pathol. 2016 August 15; 9(8) : 8280-85.
- Mahajan N. Endometrial receptivity array : Clinical application. Journal of Reproductive Science. 2015 July 10; 8(3) : 121–9.
- Mascarenhas MN, Seth RF, Ties B, Sheryl V, Gretchen A, Steven. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Medicine. 2012 December 18; 9(12) : 1-13.
- Wang H, Guangwen S, Ming L, Hong F, Hong M, Li S. Correlation of IL-1 and HB-EGF with endometrial receptivity. Experimental and Therapeutic Medicine. 2018 September 4; 16 : 5130-6.
- Zhao LH, Xiang ZC, Hong JY, Bong L, Liang-liang Z, Yu XL, et al. Restrain stress inhibits mouse implantation : temporal window and the involvement of HB-EGF, estrogen, and progesterone. PLoS Medicine. 2013 November 14; 8(11) : 1-8.
- Fritz MA, Leon S. Clinical Gynecologic Endocrinology and Infertility. 8th ed. Philadelphia : Lippincott Williams & Wilkins. 2011.
- Whirledge S, John AC. Glucocorticoids, stress, and fertility. Minerva endocrinol. 2010 June; 35(2) : 109-25.
- Lim HJ, Dey SK. HB-EGF : a unique mediator of embryo-uterine interactions during implantation. Experimental Cell Research. 2009 February 15; 315(4) : 619-26.
- Fang CX, Ying-Qi N, Feng-Hua L, Lin F, Yen C. Heparin binding epidermal growth factor like growth factor enhances aquaporin 3 expression and function during mouse embryo implantation. Reproductive Science. 2016 July 19; 24(3) : 1-8.
- Maramis MM. Mekanisme Gangguan Fungsi

- Memori Kerja Spasial dan Fleksibilitas Kognitif Melalui HSP70, IL-6,5-HT, BDNF dan pCREB Pada Model Tikus Putih (*Rattus norvegicus*) Depresi [Disertasi]. Surabaya : Universitas Airlangga ; 2015.
13. López-López AL, Herlinda BJ, María DCEV, Mailinalli BP, Gonzalo VP, Fransisco JAA. Chronic unpredictable mild stress generates oxidative stress and systemic inflammation in rats. *Physiology & Behavior*. 2016 April 7; 161 : 15-23.
 14. Hall J. Guyton dan Hall : Buku Ajar Fisiologi Kedokteran. 12nd ed. Singapore : Elsevier Pte Ltd; 2016.
 15. Joseph DN, Shannon W. Stress and the HPA axis: balancing homeostasis and fertility. *Molecular Science*. 2017 October 24; 18(10) : 2224-39.
 16. Kusumawati D. *Bersahabat dengan Hewan Coba*. 4th ed. Yogyakarta : UGM Press; 2016.
 17. Zee PL, Hayr A, Aleksandar V. Circadian rhythm abnormalities. *Learning in Neurology*. 2013 February; 19(1): 132-47.
 18. Lessey BA, Yaoting G, Apparao KBJ, Steven LY, Joy M. Regulated expression of heparin-binding EGF-like growth factor (HB-EGF) in the human endometrium : a potential paracrine role during implantation. *Molecular Reproduction and Development*. 2016; 62 : 446-55.
 19. Ozbilgin K, Fatma K, Afsin T, Can K, Seda V, Tayfun O. The higher heparin-binding epidermal growth factor (HB-EGF) in missed abortion. *Taiwanese Journal of Obstetrics & Gynecology*. 2015 February; 54 : 13-8.
 20. Strauss JF, Robert LB. *Yen & Jaffe's Reproductive Endocrinology, Physiology, Pathophysiology, and Clinical Management*. 7th Edition. Philadelphia : Elsevier Inc. 2014.

Elevated Corticosterone Level Due To Chronic Stress on Hb-Egf Expression as a Marker of Endometrial Receptivity Disorder in Rattus norvegicus

ORIGINALITY REPORT

16%

SIMILARITY INDEX

12%

INTERNET SOURCES

10%

PUBLICATIONS

0%

STUDENT PAPERS

PRIMARY SOURCES

1	eprints.ums.ac.id Internet Source	1%
2	snis.ch Internet Source	1%
3	repository.unair.ac.id Internet Source	1%
4	tessera.spandidos-publications.com Internet Source	1%
5	link.springer.com Internet Source	1%
6	repositori.usu.ac.id Internet Source	1%
7	www.nature.com Internet Source	1%
8	C. M. M. Pyper. "Fertility awareness and natural family planning", The European	1%

Journal of Contraception & Reproductive Health Care, 2009

Publication

9	www.jpmer.com Internet Source	1 %
10	www.researchgate.net Internet Source	1 %
11	garuda.ristekbrin.go.id Internet Source	1 %
12	repository.upi.edu Internet Source	1 %
13	Takatsu, K, and TJ Acosta. "Expression of Heparin-Binding EGF-Like Growth Factor (HB-EGF) in Bovine Endometrium: Effects of HB-EGF and Interferon- τ on Prostaglandin Production", <i>Reproduction in Domestic Animals</i> , 2015. Publication	1 %
14	pt.scribd.com Internet Source	1 %
15	eprints.skums.ac.ir Internet Source	<1 %
16	www.ncbi.nlm.nih.gov Internet Source	<1 %
17	E. Dimitriadis. "Cytokines, chemokines and growth factors in endometrium related to	<1 %

implantation", Human Reproduction Update, 08/25/2005

Publication

18

I. A. Greer. "The potential role of heparin in assisted conception", Human Reproduction Update, 09/29/2008

Publication

19

journals.pan.pl

Internet Source

20

www.orthopaper.com

Internet Source

21

Ioannis Zalachoras, Fiona Hollis, Eva Ramos-Fernández, Laura Trovo et al. "Therapeutic potential of glutathione-enhancers in stress-related psychopathologies", Neuroscience & Biobehavioral Reviews, 2020

Publication

22

Khalili, Mostafa, Mohammad Reza Soleyman, Maryam Baazm, and Cordian Beyer. "High-level expression and purification of soluble bioactive recombinant human heparin-binding epidermal growth factor in Escherichia coli : High-level expression of soluble HB-EGF", Cell Biology International, 2015.

Publication

<1 %

<1 %

<1 %

<1 %

<1 %

23

M. Cavagna, J.C. Mantese. "Biomarkers of Endometrial Receptivity—A Review", Placenta, 2003

Publication

<1 %

24

Merve Karpuz, Mine Silindir-Gunay, Asuman Yekta Ozer. "Current and Future Approaches for Effective Cancer Imaging and Treatment", Cancer Biotherapy and Radiopharmaceuticals, 2018

Publication

<1 %

25

Pinar H. Kodaman, Hugh S. Taylor. "Hormonal regulation of implantation", Obstetrics and Gynecology Clinics of North America, 2004

Publication

<1 %

26

[Www.Sid.Ir](http://www.sid.ir)

Internet Source

<1 %

27

Xiao Xi Sun, Kristina Gemzell-Danielsson, Hong Zhen Li, Berit Stâbi, Anneli Stavreus-Evers. "Expression of heparin-binding epidermal growth factor-like growth factor and its receptors in the human fallopian tube and endometrium after treatment with mifepristone", Fertility and Sterility, 2006

Publication

<1 %

28

www.medicopublication.com

Internet Source

<1 %

29

Bufang Xu, Xiaoxi Sun, Lu Li, Ling Wu, Aijun Zhang, Yun Feng. "Pinopodes, leukemia inhibitory factor, integrin- β 3, and mucin-1 expression in the peri-implantation endometrium of women with unexplained recurrent pregnancy loss", *Fertility and Sterility*, 2012

Publication

<1 %

30

L. Aghajanova. "HB-EGF but Not Amphiregulin or Their Receptors HER1 and HER4 Is Altered in Endometrium of Women With Unexplained Infertility", *Reproductive Sciences*, 03/10/2008

Publication

<1 %

31

worldwidescience.org

Internet Source

<1 %

32

Lessey, Bruce A., and Steven L. Young. "The Structure, Function, and Evaluation of the Female Reproductive Tract", *Yen & Jaffe s Reproductive Endocrinology*, 2014.

Publication

<1 %

Exclude quotes On

Exclude matches Off

Exclude bibliography On

Elevated Corticosterone Level Due To Chronic Stress on Hb-Egf Expression as a Marker of Endometrial Receptivity Disorder in Rattus norvegicus

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6
