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Molecular basis of male infertility phenotypes in DCAF17 knockout mice model

Thesis presented by

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For M.V.Sc. Degree
In Biochemistry and Chemistry of Nutrition

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2017

Dedication

This thesis is dedicated to:

The sake of Allah, my Creator and my Master, My great teacher and messenger, Mohammed (May Allah bless and grant him), who taught us the purpose of life, The my second magnificent home; My great parents, who never stop giving of themselves in countless ways, My dearest husband, who leads me through the valley of darkness with light of hope and support, My beloved brothers and sisters. My beloved kids: Jana, and Ahmed, whom I can't force myself to stop loving. To all my family, the symbol of love and giving, my friends who encourage and support me, all the people in my life who touch my heart, I dedicate this research

ACKNOWLEDGEMENT

In the Name of Allah, the Most Merciful, the Most Compassionate all praise be to Allah, the Lord of the worlds; and prayers and peace be upon Mohamed His servant and messenger. First and foremost, I must acknowledge my limitless thanks to Allah, the Ever-Magnificent; the Ever-Thankful, for His help and bless. I am totally sure that this work would have never become truth, without His guidance.

So, i would like to express my gratitude to all those who gave me the possibility to complete this thesis. First of all, I am deeply indebted to my supervisor **Prof.Dr. Hassan Amer** for his professional counsel and guidance on my project. I am deeply inspired by his enthusiasm and Insistence on high-quality research work.

Special gratitude is also deeply extended to my supervisor **Dr. Abdulla assiri** for offering me a chance to be a master candidate under his supervision and all the valuable instructions during the study. This project could not be accomplished without his valuable guidance. Also I would like to thank **Dr. Abdelbary prince** for his valuable guidance and support.

I would like to express my sincere appreciation for the assistance, courage and support from my laboratory mates **Dr. Bhavesh Mistry** for his valuable supports. The same appreciation also goes to **Ms. Hala Ahmed and Ms. Razan Abdulla** for their excellent technical support and the care rendered to me during my study.

Last but not least, I would like to appreciate the care, love and encouragement of my **husband**, **my parents**, **my sister** as well as my **Parents-in-law** Their love is the power of my life.

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Abstract: DDB1– and CUL4 –associated factor 17 (*Dcaf17*) is a member of DCAF family genes that encode substrate receptor proteins for Cullin-RING E3 ubiquitin ligases (CRLs). CRLs play an important role in diverse cellular processes such as cell proliferation, cell differentiation, DNA replication, DNA repair, gene expression and apoptosis. Proteins encoded by DCAF family genes are predicted to determine specificity of the DDB1-CUL4-E3 ubiquitin ligase complexes. Mutations in *Dcaf17* gene in humans causes Woodhouse-Sakati Syndrome (WSS) that is characterized by hypogonadism, alopecia, diabetes, intellectual disability and extrapyramidal symptoms. However, the function of DCAF17 and molecular pathogenesis of WSS are unknown. To unravel the function of DCAF17 and molecular underpinnings of WSS, we performed expression profiling of *Dcaf17* in different tissues of wild type mouse by qRT-PCR and generated *Dcaf17* knockout mice by targeted deletion that resulted into disruption of exon 4 of *Dcaf17* gene. Expression profiling of *Dcaf17* showed that it is highly expressed in testis with low level of expression in brain, liver, skin and pancreas. Analyses of *Dcaf17* mRNA transcripts in post-natal mice testis revealed that its level increased by 4, 7 and 9-fold at 14, 23 and 32 PND, respectively. Although *Dcaf17* disruption did not have any effect on female fertility, *Dcaf17* deletion led to male infertility with severe defects in sperm morphology with normal testicular mass. This male infertility resulted from decreased number of spermatozoa, reduced sperm motility and abnormal sperm morphology resulting from disrupted nuclei, displacement of acrosome and mid piece with disorganization of the mitochondrial sheath. Histological examination of the mutant testis showed impaired spermatogenesis with vacuoles in the seminiferous tubules and presence of degenerated sloughed germ cells and increased cell apoptosis at various stages.

Keywords: Male Infertility, gene knockout, globozoospermia, Spermatogenesis, Ubiquitination.

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Introduction

Male infertility is a major medical problem worldwide, where about 75% of these defects are idiopathic due to the fact that the molecular mechanisms controlling these defects are largely unknown. A large body of data indicates that male infertility is caused by genetic defects including chromosome aberrations, gene mutations and single nucleotide polymorphism **(Jamsai and O'Bryan, 2011; McLachlan and O'Bryan, 2010)**. Recently male infertility has been reported as phenotype in mice that are deficient in various single genes .There are at least 400 genes have been identified as essential for male infertility in human and mice **(O'Bryan and de Kretser, 2006; Wu *et al.*, 2004).**

Recent research has provided molecular data concerning the relation between impact of acrosome biogenesis and nuclear shaping as any defect in their formation leads to round–head sperm. The main characteristic feature of globozoospermia is the mal formation or loss of acrosome with abnormal nuclear shape as well as abnormal arrangement of sperm mitochondria **(Battaglia *et al.*, 1997).** Various genes were found to be associated with globozoospermia by gene targeting experiments including Hrb **(Kang-Decker *et al.*, 2001)** and the GOPC-deficient mice **(Yao *et***

***al*., 2002)** ,and CsnK2a2 **(Xu *et al*., 1999)**. Woodhouse-Sakati Syndrome (WSS) is rare autosomal recessive disorder characterized by hypogonadism , alopecia ,diabetes mellitus and mental retardation. WSS was reported in 1983 by group of clinicians and scientists at King Faisal Specialist Hospital and Research Center (KFSH&RC) **(Woodhouse and Sakati, 1983).** Recent findings revealed that mutations in C2orf37, now named DDB1 and CUL4A associated factor 17 (DCAF17), are underlying cause of WSS **(Alazami *et al*., 2008)**. To understand the molecular mechanism of WSS , DAF17 KO mice were generated by targeted mutation where deletion of exon4 caused frame shift deletion. Initial characterization of this model showed that males are infertile with normal body and gonadal weight while females have normal fertility.

Spermatogenesis is a cell differentiation process by which diploid cell become haploid cell that is able to fertilize oocytes. This process occurs continuously within the seminiferous tubules of the testes during male reproductive life in mammals. In mice, spermatogenic cycle consists of 12 stages and the cycle lasts for 35 days **(Clermont and Trott, 1969)** . It consists of three phases:

1) Mitotic proliferation and differentiation of spermatogonia into spermatocytes.
2) Meiotic division of diploid spermatocytes into haploid spermatid.

3) Morphogenesis of haploid spermatids into spermatozoa by different changes including condensation and elongation of the sperm head, cytoplasmic redistribution and reduction, acrosome formation and tail formation.

The mature spermazoa are then released from the supporting sertoli cells and the process called spermiation **(Tarulli *et al*., 2012).** Defects that occur in any of these steps can lead to male infertility especially at spermiogenesis, where the morphological changes are very critical for production of viable sperm able to fertilize the ova. Various microtubule based structure play an important role in shaping mouse spermatid head and assembly of the flagellar tail**.**The last phase of spermatogenesis involves spermatide elongation (spermiogenesis), where the chromatin condensation of the nucleus ,the excess cytoplasm are removed and the acrosome and sperm tail are formed . DCAF17 mutant mice has defect in head and tail morphology so, it seems that DCAF17 is required for microtubules formation and the disruption in the microtubule based protein (manchette) which is transient skirt - like structure surrounding the elongating spermatid head only during spermatid elongation around steps 8 - 12,is important.

Ubiquitination is very important process that is required in mammalian spermatogenesis . Cullin gene family is the largest family in ubiquitin in ligase family in mammals. Cullin-RING-