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# **Molecular basis of male infertility phenotypes in DCAF17 knockout mice model**

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## **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*

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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.

| <b>CONTENTS</b>  | <b>Page</b> |
|--|-------------|
| <b>1. Introduction.....</b>                                | <b>1</b>    |
| <b>2. Review of Literature.....</b>                        | <b>6</b>    |
| <b>2.1. Male infertility.....</b>                          | <b>6</b>    |
| 2.1.1. Causes of male infertility.....                     | 6           |
| 2.1.2. Goboospermia.....                                   | 6           |
| 2.1.3. Mouse models of male infertility.....               | 9           |
| 2.1.4. Knockout mouse model for gene functional study..... | 10          |
| <b>2.2. Testes Histology.....</b>                          | <b>15</b>   |
| <b>2.3. Spermatogenesis.....</b>                           | <b>19</b>   |
| 2.3.1. Spermatogonial proliferation.....                   | 20          |
| 2.3.2. Meiotic Phase.....                                  | 20          |
| 2.3.3. Spermiogenesis.....                                 | 23          |
| 2.3.3.1. Nuclear DNA condensation.....                     | 26          |
| 2.3.3.2. Development of acrosome.....                      | 26          |
| 2.3.3.2.1. Acrosome biogenesis.....                        | 27          |
| 2.3.3.2.2. Acrosome-Acroplaxome-manchette complex.....     | 28          |
| 2.3.3.3. Flagellum formation.....                          | 31          |
| 2.3.3.4. Elimination of spermatid cytoplasm.....           | 32          |
| <b>2.4. Apoptosis in the testis.....</b>                   | <b>34</b>   |
| 2.4.1. Apoptosis pathways.....                             | 34          |
| <b>2.5. Ubiquitination during spermatogenesis.....</b>     | <b>37</b>   |
| <b>2.6. Protein ubiquitination.....</b>                    | <b>39</b>   |
| <b>2.7. E3 Ligases: Two major families.....</b>            | <b>41</b>   |
| 2.7.1. HECT E3 ligase.....                                 | 42          |
| 2.7.2. RING type E3 ligases.....                           | 42          |
| <b>2.8. 26S Proteasome.....</b>                            | <b>43</b>   |

|   |           |
|---|-----------|
| <b>2.9. Cullin-RING Ligases.....</b>  | <b>44</b> |
| <b>2.10. The CUL4 subfamily of CRLs.....</b>  | <b>45</b> |
| <b>2.11. DCAF proteins family.....</b>  | <b>48</b> |
| <b>2.12. Bioinformatics analyses DCAF17 gene.....</b>   | <b>48</b> |
| <b>3. Materials and Methods.....</b>  | <b>50</b> |
| <b>3.1. Construction of DCAF17 null mice.....</b>   | <b>50</b> |
| <b>3.2. Mice care.....</b>  | <b>52</b> |
| <b>3.3. Genotyping.....</b>   | <b>52</b> |
| 3.3.1. DNA Extraction from mice tail.....   | 53        |
| 3.3.2. Quantity and purity assessment of DNA.....   | 54        |
| 3.3.3. Polymerase chain reaction using specific primers.....  | 55        |
| 3.3.4. Agarose gel electrophoresis.....   | 56        |
| <b>3.4. Male sexual behavior and fertility test.....</b>  | <b>57</b> |
| <b>3.5. Evaluation of DCAF17 KO mice sperm (quantity and quality).....</b>                                      | <b>58</b> |
| 3.5.1. Evaluation of sperm count and motility.....  | 58        |
| 3.5.1.1. Mouse dissecting and samples collection.....   | 58        |
| 3.5.1.2. Determination the concentration of sperm and Motility % by Hemocytometer.....                          | 59        |
| 3.5.1.3. Calculation of sperm concentration and Motility %.....   | 61        |
| <b>3.5.2. Evaluation of sperm morphology.....</b>   | <b>62</b> |
| 3.5.2.1. Diff Quick Stain.....  | 62        |
| 3.5.2.2. Mitotracker, PNA and DAPI staining (to study sperm mitochondrial function and acrosome integrity)..... | 63        |
| <b>3.6. DCAF17 gene expression analysis by real –time PCR (RT-qPCR).....</b>                                    | <b>66</b> |
| 3.6.1. RNA isolation.....   | 66        |

|  |           |
|--|-----------|
| 3.6.2. Quantity and purity assessment of RNA.....  | 70        |
| 3.6.3. Reverse Transcription PCR.....  | 71        |
| 3.6.3.1. cDNA synthesis for mRNA detection.....  | 71        |
| 3.6.4. Quantitative real – time PCR (RT-qPCR).....   | 73        |
| 3.6.4.1. Primer design.....  | 74        |
| 3.6.4.2. Real-time PCR set up.....   | 75        |
| 3.6.4.2.1. Procedure of RT-qPCR .....  | 76        |
| 3.6.4.2.2. Analysis of RT-q PCR data.....  | 77        |
| <b>3.7. Histological analysis of testis and epididymis.....</b>  | <b>80</b> |
| <b>3.8. Detection of apoptosis by TUNEL assay.....</b>   | <b>80</b> |
| <b>3.9. Testes squashed preparation and immunofluorescence.....</b>  | <b>83</b> |
| 3.9.1. Testis Squash Preparation method for immunostaining of<br>spermatogenic cells.....                          | 83        |
| 3.9.2. Immunofluorescence staining method.....   | 84        |
| <b>3.10. Transmission electron microscopy (TEM).....</b>   | <b>86</b> |
| <b>3.11. Statistical analysis.....</b>   | <b>87</b> |
| <b>4. Results.....</b>   | <b>88</b> |
| <b>4.1. Generation and detection of DCAF17<sup>-/-</sup> mice by genotype.....</b>                                 | <b>88</b> |
| <b>4.2. Male sexual behavior and fertility test showed infertility of<br/>DCAF17<sup>-/-</sup> males.....</b>      | <b>90</b> |
| <b>4.3. Sperm Production &amp; Motility .....</b>  | <b>93</b> |
| <b>4.4. DCAF17<sup>-/-</sup> male mice exhibited different morphological<br/>abnormalities of spermatozoa.....</b> | <b>95</b> |
| <b>4.5. DCAF17 gene expression analysis.....</b>   | <b>99</b> |
| 4.5.1. Quantification.....   | 99        |
| 4.5.2. PCR efficiency.....   | 100       |

|  |            |
|--|------------|
| <b>4.6. Histological analysis of DCAF17 -/- testes.....</b>  | <b>104</b> |
| <b>4.7. Germ cells apoptosis in DCAF17 mutant mice by TUNEL assay.....</b>                         | <b>107</b> |
| <b>4.8. Pattern of chromosome pairing and formation of XY-body during meiotic progression.....</b> | <b>109</b> |
| <b>4.9. Manchette formation during spermiogenesis.....</b>   | <b>111</b> |
| <b>5. Discussion.....</b>  | <b>114</b> |
| <b>6. Conclusion.....</b>  | <b>127</b> |
| <b>7. Summary.....</b>   | <b>128</b> |
| <b>8. References.....</b>  | <b>131</b> |
| <b>9. List of Abbreviations.....</b>   | <b>155</b> |
| <b>10. Arabic summary.....</b>   | <b>1</b>   |

## LIST OF TABLES

|                    |   |
|--------------------|---|
| <b>Table (1):</b>  | WSS primers.  |
| <b>Table (2):</b>  | PCR reaction master mix.  |
| <b>Table (3):</b>  | Master Mix for reverse transcription PCR for mRNA detection   |
| <b>Table (4):</b>  | Thermal Cycler program.   |
| <b>Table (5):</b>  | Primers of DCAF17 & 18s RNA.  |
| <b>Table (6):</b>  | Reaction mix for real - time PCR, mRNA.   |
| <b>Table (7):</b>  | Programing the Real-time cycler condition for DCAF17 gene.  |
| <b>Table (8):</b>  | Primary and secondry antibodies.  |
| <b>Table (9):</b>  | Excitation and Emission wavelengths for secondry antibodies.  |
| <b>Table (10):</b> | Male sexual behavior & fertility test.  |
| <b>Table (11):</b> | Body weight and Testes weight of DCAf17 <sup>+/+</sup> and DCAF17 <sup>-/-</sup> at 8 and 32 weeks. |

## LIST OF FIGURES

- Figure (1):** Illustration for the generation of knockout mice by gene targeting.
- Figure (2):** Generation of tissue/cell specific knockout mouse.
- Figure (3):** Cross section of testis and epididymis.
- Figure (4):** Testicular and seminiferous epithelium organization and spermatogenesis.
- Figure (5):** Phases of spermatogenesis.
- Figure (6):** Spermatogenic stage and spermiogenic step in mouse.
- Figure (7):** Phases of acrosome biogenesis.
- Figure (8):** Schematic diagrams of the microtubule- and actin-based transport system in the elongating mouse spermatid.
- Figure (9):** Structure of spermatozoa flagellum.
- Figure (10):** Extrinsic and intrinsic apoptosis signaling in the testis.
- Figure (11):** The overview of ubiquitination during Spermatogenesis.
- Figure (12):** Depiction of target protein ubiquitination via the ubiquitin proteasome system (UPS).
- Figure(13):** Crystal structure of the HECT E3 ligase E6AP complexed to UbcH7.
- Figure (14):** Illustration of CRL assembly.
- Figure (15):** Finer illustration of interaction of CUL4.
- Figure (16):** Steps for the generation of DCAF17 knockout mice by gene targeting.
- Figure (17):** Genotyping Steps.

- Figure (18):** Hemocytometer.
- Figure (19):** Syber Green DNA binding in Real –Time PCR
- Figure (20):** Amplification curve with the threshold line and Ct- values.
- Figure (21):** PCR genotyping of representative Dcaf17 mutant mice.
- Figure (22):** Breeding test.
- Figure (23):** Morphology of testes collected from wild type and Dcaf17 mutant mice.
- Figure (24):** Epididimal sperm count and motility in DCAF17 mutant mice compared to wild type mice
- Figure (25):** Malformation of spermatozoa in DCAF17-deficient mice.
- Figure (26):** Ultra-structure analysis by electron microscopy of epididimal spermatozoa of WT versus DCAF17 mutant mice.
- Figure (27):** Amplification curve with the threshold line, Ct- values, Standard curve and melting curve
- Figure (28):** Standard curve.
- Figure (29):** Expression profiling of DCAF17 by RT-qPCR.
- Figure (30):** Histological comparison of WT and DCAF17<sup>-/-</sup> testes in different postnatal days.
- Figure (31):** TUNEL assay to assess germ cell apoptosis in wild type and dcaf17<sup>-/-</sup> adult mice testes sections.
- Figure (32):** Normal meiotic prophase progression in DCAF17<sup>-/-</sup> mouse testes.
- Figure (33):** Manchette formation during spermiogenesis.

## 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 (DAF17), 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 spermatozoa 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 structures 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 is removed and the acrosome and sperm tail are formed. DCAF17 mutant mice have a 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 a transient skirt-like structure surrounding the elongating spermatid head only during spermatid elongation around steps 8 - 12, is important.

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