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PII: S0303-7207(19)30057-7

DOI: https://doi.org/10.1016/j.mce.2019.02.011

Reference: MCE 10399

To appear in: *Molecular and Cellular Endocrinology*

Received Date: 15 August 2018

Revised Date: 30 November 2018

Accepted Date: 18 February 2019

Please cite this article as: Meneses, Maria.Joã., Borges, D.O., Dias, Tâ.R., Martins, Fá.O., Oliveira, P.F., Paula Macedo, M., Alves, M.G., Knockout of insulin degrading enzyme leads to mice testicular morphological changes and impaired sperm quality, *Molecular and Cellular Endocrinology* (2019), doi: https://doi.org/10.1016/j.mce.2019.02.011.

Knockout of insulin degrading enzyme leads to mice testicular morphological changes and impaired sperm quality

Maria João Meneses[a,b,c], Diego O Borges[a], Tânia R Dias[b], Fátima O Martins[a], Pedro F Oliveira[b,d,e], M Paula Macedo [a,f,g*], Marco G Alves[b*]✉

[a] CEDOC – Centro de Estudos de Doenças Crónicas, NOVA Medical School/Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal;

[b] Department of Microscopy, Laboratory of Cell Biology and Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal;

[c] ProRegeM PhD Programme, NOVA Medical School/Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal;

[d] I3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal;

[e] Department of Genetics, Faculty of Medicine, University of Porto, Portugal;

[f] Portuguese Diabetes Association - Education and Research Center (APDP-ERC), Lisbon, Portugal;

[g] Department of Medical Sciences, University of Aveiro, Portugal

* both senior authors contributed equally to this work

✉ Address correspondence to: Marco G. Alves - Department of Microscopy, Laboratory of Cell Biology, Abel Salazar Institute of Biomedical Sciences (ICBAS), University of Porto, 4050-313 Porto, Portugal. Ph: +351 220428000 E-mail: alvesmarc@gmail.com

Abstract

Insulin degrading enzyme (IDE) is a zinc metalloprotease responsible for degrading and inactivating several bioactive peptides, including insulin. Individuals without this enzyme or with a loss-of-function mutation in the gene that codifies it, present hyperinsulinemia. In addition, impairment of IDE-mediated insulin clearance is associated with the development of metabolic diseases, namely prediabetes. Although insulin regulates male fertility, the role of IDE on male reproductive function remains unknown. We proposed to study the influence of IDE in the reproductive potential of males. As insulin mediates key events for the normal occurrence of spermatogenesis, we hypothesized that IDE functioning might be linked with sperm quality. We used C57BL/6N mice that were divided in three groups according to its genotype: wild type (WT), heterozygous and knockout (KO) male mice for *Ide*. Spermatozoa were collected from the cauda of epididymis and sperm parameters were evaluated. Testicular tissue morphology was assessed through hematoxylin and eosin stain. Mitochondrial complex protein levels and lipid peroxidation were also evaluated in the testicular tissue. Our results show that KO mice present a 50 % decrease in testes weight compared to WT mice as well as a decrease in seminiferous tubules diameter. Moreover, KO mice present impaired sperm quality, namely a decrease in both sperm viability and morphology. These results provide evidence that IDE plays an important role in determining the reproductive potential of males.

Keywords: insulin degrading enzyme; male fertility; spermatozoa; spermatogenesis; testes

Abbreviations

IDE – insulin degrading enzyme

4-HNE – 4-hydroxy-2-nonenal

PVDF – polyvinylidene difluoride

OXPHOS – oxidative phosphorylation

WT – wild type

KO – knockout

1. Introduction

Insulin degrading enzyme (IDE), also known as insulysin, is a thiol zinc-metalloendopeptidase that may be found in the cytosol, peroxisomes, endosomes, mitochondria and cell membrane (Leissring, Farris, Wu et al., 2004, Vekrellis, Ye, Qiu et al., 2000). IDE is found in most organisms and is ubiquitously expressed independently of the sensitivity of the cells to insulin (Pivovarova, Höhn, Grune et al., 2016). Furthermore, its levels and conformation may be controlled by many signals, including cellular stress, glucagon, and free fatty acids (Tundo, Sbardella, Ciaccio et al., 2013, Wei, Ke, Zhao et al., 2014) supporting a multifunctional role of the protein and showing that IDE is finely tuned depending on the state of the cell. In fact, IDE was found to have a heat shock protein-like behavior and to interact with the ubiquitin-proteasome system (Tundo, Sbardella, Ciaccio et al., 2013). Due to its configuration that changes between active and inactive states IDE cleaves small peptides of assorted sequence, namely amyloid β-protein (Farris, Mansourian, Chang et al., 2003, Leissring and Selkoe, 2006) thus being associated to Alzheimer's disease. Moreover, this metallopeptidase cleaves insulin, being considered the major intervenient in insulin regulation. Actually, IDE has a crucial role in insulin metabolism and in inhibiting insulin translocation and accumulation in the cell nucleus (Authier, Posner and Bergeron, 1996, Harada, Smith, Smith et al., 1993). Thus, loss-of-function mutations of IDE cause hyperinsulinemia (Farris, Mansourian, Leissring et al., 2004), one of the main features of prediabetes and type 2 diabetes *mellitus*. Inhibitors of IDE were already hypothesized as possible treatment for those disorders (Costes and Butler, 2014) but as IDE degrades other substrates besides insulin, this is a very complex topic.

Insulin regulation is crucial for several essential cellular processes, namely glucose uptake. Impairment of cell metabolism by insulin has a major impact in fertility, particularly in the reproductive potential of males. Insulin dysregulation, as occurs in prediabetes and other metabolic diseases, affects the nutritional support of

spermatogenesis by changing glucose transport, acetate and lactate production and the expression of some metabolism-related genes in testicular cells (Alves, Socorro, Silva et al., 2012). These effects have a negative impact in sperm production and quality and are reported to be mainly mediated by Sertoli cells, the somatic testicular cells responsible for give physical and nutritional support to developing germ cells (Griswold, 1995). Data from several studies show that both prediabetes and diabetes *mellitus* alter sperm quality. In fact, decreased sperm concentration, motility and normal morphology (Amaral, Moreno, Santos et al., 2006, Schoeller, Albanna, Frolova et al., 2012, Shrilatha and Muralidhara, 2007) and increased nuclear DNA damage (Mangoli, Talebi, Anvari et al., 2013, Rama Raju, Jaya Prakash, Murali Krishna et al., 2012) have been reported in diabetic individuals. Moreover, mitochondria was also reported to be affected namely by an increase in mitochondrial DNA damage (Agbaje, Rogers, McVicar et al., 2007) as well as altered sperm mitochondrial function (La Vignera, Condorelli, Di Mauro et al., 2015). However, the exact mechanisms by which insulin dysfunction mediates these effects remain largely unknown. Thus, it is imperative to study the impact of primary hyperinsulinemia derived from insulin clearance on male reproductive function. Herein, we hypothesized that IDE may be a key player in insulin dysfunction-associated male infertility.

2. Material and Methods

2.1. Chemicals

Mammalian Protein Extraction Reagent and BCA Protein Assay Kit were purchased from Thermo Scientific (Whalthan, MA, USA). Dried milk was purchased from Regilait (Saint-Martin-Belle-Roche, France). ECF™ substrate was purchased from GE Healthcare (Weßling, Germany). NZYColour Protein Marker II was purchased from NZYTech (Lisbon, Portugal). Coverquick 2000 was purchased from VWR (Radnor, PA,

USA). Diff quick was purchased from Baxter Dale Diagnostics AG (Dubinger, Switzerland). All other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA), unless stated otherwise.

2.2. Animals

C57BL6/N, full body *Ide* heterozygous mice were purchased from the European Mouse Mutant Archive (EMMA) and provided by Wellcome Trust Sanger Institute (Cambridge, United Kingdom). Heterozygous breeding generated the wild type (WT), heterozygous (Het) and knockout (KO) animals used in the experiments. Animals were maintained on a 12 h light/dark cycle with standard chow diet (Special Diets Services, United Kingdom) and water *ad libitum* at Instituto Gulbenkian de Ciência (Oeiras, Portugal). Mice were monitored weekly for body weight, blood glucose levels, as well as for distress signals. All procedures followed ARRIVE guidelines and the Europeans laws (Directive 2010/63/EU) that rule the use of animals in research.

2.3. Tissue preparation

Testes were weighed to determine gonadosomatic index (GSI = [testes weight/body weight]×100). The right testis was placed in Davidson's modified fixative and then dehydrated in a graded ethanol series, embedded in paraffin, cut into 4 µm sections, mounted onto glass slides and stained with hematoxylin & eosin. The diameter of 25 round seminiferous tubules per animal was measured in Cell^B (Olympus, Japan).

2.4. Evaluation of Sperm Parameters

The cauda of the left epididymis of each mouse was minced to allow sperm dispersion in Hank's balanced salt solution and sperm parameters were evaluated according to World Health Organization indications (World Health Organization, 1999). To assess sperm motility, a drop of 100 µL of the sperm suspension was placed in a prewarmed (37ºC) slide and covered with a cover slip. The percentage of motile sperm was assessed under a light microscope (x100 magnification) in 10 random fields, and the

average was used as the final motility. Sperm concentration was evaluated with a Neubauer chamber. Sperm viability was evaluated by the assessment of sperm membrane integrity using an eosin/nigrosin staining. A total of 5 µL of the sperm suspension obtained as described above was mixed with 5 µL of 0.5 % eosin/nigrosin stain and placed on a prewarmed glass microscope slide. The number of viable and nonviable spermatozoa was determined by counting a total of 100 spermatozoa per slide in continuous random fields under a light microscope under ×1000 magnification, to determine the percentage of viable sperm (Rato, Alves, Dias et al., 2013). Viable sperm remained white, whereas unviable sperm stained pink, due to increased membrane permeability, which allowed the dye uptake. Sperm morphology was assessed through Papanicolaou staining as described (World Health Organization, 1999).

2.5. Protein extraction

Total protein was extracted from testicular tissue using Mammalian Protein Extraction Reagent (supplemented with 1 % protease inhibitor cocktail and 100 mM sodium orthovanadate) following the manufacturer's instructions. Protein concentration was determined by Pierce™ BCA Protein Assay Kit according to the manufacturer's instructions.

2.6. Evaluation of Lipid Peroxidation

Lipid peroxidation was evaluated through the analysis of 4-hydroxy-2-nonenal (4-HNE) protein adducts by Slot Blot technique using a Hybri-slot manifold system (Biometra, Göttingen, Germany). The proteins (5 µg) were transferred to previously activated polyvinylidene difluoride (PVDF) membranes. The latter were then blocked for 90 min with 5 % (w/v) non-fat milk and incubated overnight (4℃) with goat anti-4-HNE (1:5000, AB5605, Merck Millipore, Temecula, USA). Afterwards, membranes were incubated with rabbit anti-goat (1:5000, A4187, Sigma–Aldrich, St. Louis, MO, USA) for 90 min and then reacted with ECF™ and read using a Bio-Rad GelDoc XR (Bio-Rad,

Hemel Hempstead, UK). Densities from each band were quantified using the Quantity One Software (Bio-Rad, Hemel Hempstead, UK).

2.7. Evaluation of Protein Carbonylation and Nitration

Carbonyl groups are produced on protein side chains when oxidized and thus, carbonyl content is generally used as an indicator for protein oxidation. Protein nitration is a marker of inflammation, cell damage, and nitric oxide production and can be evaluated by measuring the resulting products, namely nitro-tyrosine. These parameters were evaluated using slot-blot technique as explained above. The resulting membranes were incubated overnight (4ºC) with rabbit anti-DNP (1:5000, D9656, Sigma–Aldrich, St. Louis, MO, USA) or rabbit anti-nitro-tyrosine (1:5000, 9691, Cell Signaling Technology, Leiden, Netherlands).

2.8. Western Blot

Protein samples (75 µg) were mixed with sample buffer (1.5% Tris, 20% glycerol, 4.1% SDS, 2% β-mercaptoethanol, 0.02% bromophenol blue, pH 6.8), sonicated for 10 minutes at 4℃ and denatured for 15 minutes at 55℃ . Proteins were fractionated in 15% polyacrylamide gels and electrophoresis was carried out for 85 min. The proteins were transferred from gels to activated PVDF membranes in a Mini Trans-Blot® cell (Bio-Rad, Hemel Hempstead, UK) and then blocked for 3 hours in a 5% non-fat milk solution at room temperature. The membranes were then incubated overnight at 4℃ with MitoProfile® Total OXPHOS Antibody Cocktail (1:1000, MitoSciences, Oregon, USA, MS604). Mouse anti-β-actin (1:5000, Thermo Scientific, Rockford, IL, USA, MA5-15739) was used as protein loading control. The immune-reactive proteins were detected with goat anti-mouse IgG-AP (1:5000, A3562). Membranes were reacted with ECF™ and read with the Bio-Rad GelDoc XR (Bio-Rad, Hemel Hempstead, UK). Quantity One Software (Bio-Rad, Hemel Hempstead, UK) was used to obtain band densities, which were divided by the respective β-actin band density and then normalized with the wild type group value.

188 *2.9. Reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR)*

189 The total RNA (RNAt) of the samples was extracted using the EZNA Total RNA Kit
190 (Omega Biotek, Norcross, USA) and its concentrations were determined by Nanodrop
191 2000 (ThermoFisher, Waltham, USA). RNAt was then reversely transcribed to
192 complementary DNA (cDNA) as previously described (Rato et al., 2013).

193 qRT-PCR was used to analyze the mRNA expression levels of *Bax*, *Bcl2* and *Casp3*
194 was performed in a BioRad CFX96 system (Biorad, Califórnia, USA) using specific
195 primers (Table 1). Amplification conditions comprised an initial denaturation step of 5
196 min at 95ºC, followed by 40 runs of a 3 steps cycle: a denaturation step of 30 s at 95ºC;
197 an annealing step of 30 s with a specific temperature for each set of primers (Table 1);
198 and an extension step of 1 min at 72ºC. The quantification cycle (C_q) was determined
199 through cycle threshold and fold variation of the expression levels was calculated
200 following Pfaffl's mathematical model (Pfaffl, 2001), using β-2-microglobulin and
201 glyceraldehyde 3-phosphate dehydrogenase as reference genes.

202 *2.10. Statistical Analysis*

203 The statistical significance among the experimental groups was assessed by one-way
204 ANOVA. All experimental data are shown as mean ± SEM. Statistical analysis was
205 performed using GraphPad Prism 6 (GraphPad software, San Diego, CA, USA).
206 Possible outliers were removed using Grubbs' method, $\alpha=0.2$. $p<0.05$ was considered
207 significant.

208 **3. Results**

209 *3.1. Effect of the absence of insulin degrading enzyme on testicular morphology*

210 Testes weight was decreased in heterozygous mice (91.75 ± 9.38 mg) and further
211 decreased in the Knockout (KO) mice (59.75 ± 2.59 mg) when compared with the Wild
212 Type (WT) group (121.03 ± 1.47 mg; Figure 1A). Concomitantly, the GSI which is a
213 measure that links the body weight to the gonads weight was decreased in Het (0.54 ±

0.04%) and *Ide* KO mice (0.44 ± 0.06%) when compared to WT (0.73 ± 0.03%; Fig. 1B). However, when observing the seminiferous tubules diameter, only *Ide* KO mice presented a significant decrease (185.56 ± 4.71 μm) when compared to the WT group (214.92 ± 7.11 μm; Figure 1C-D).

3.2. Insulin degrading enzyme and mice sperm morphology

As insulin regulation is crucial for spermatogenesis and IDE is the enzyme responsible for insulin inactivation, we hypothesized that IDE could affect sperm quality. Our results showed no differences in sperm motility (Figure 2A) and concentration (Figure 2B) of neither heterozygous nor KO mice for *Ide*. However, *Ide* KO mice showed decreased sperm viability (43.17 ± 2.18%) when compared to the WT (55.00 ± 2.74%; Fig. 2C). Moreover, abnormal sperm morphology was increased in KO (60.00 ± 2.73%) when compared to WT (43.50 ± 2.63%) and Het (37.83 ± 5.44%; Fig. 2D).

3.3. Oxidative stress markers in the testicular tissue of Ide KO mice

Oxidative stress is one of the major causes for decreased sperm quality. It damages lipids and proteins compromising sperm quality. Thus, we evaluated lipid peroxidation, protein nitration and carboxylation in testicular tissue of the different mice. When observing both carbonyl groups and protein nitration levels in testicular tissue of *Ide* KO and heterozygous mice, our results showed no significant differences when comparing with the WT mice (Fig. 3A and B). However, there was an increase in testicular tissue lipid peroxidation of *Ide* KO mice (1.29 ± 0.04 – fold variation to WT), when compared to both, heterozygous (1.03 ± 0.01 – fold variation to WT) and WT mice (1.00 ± 0.02; Fig. 3C). As one of the causes for lipid peroxidation is the impairment of oxidative phosphorylation, we measured the levels of mitochondrial complexes (Fig. 3D) and a decrease in complex IV in the testes of KO (0.54 ± 0.09 – fold variation to WT) when compared to WT (1.00 ± 0.05) was found.

3.4. Apoptotic markers in the testicular tissue of Ide KO mice

Oxidative stress plays a key role in apoptosis thus we analyzed the expression of some key players of this programmed cell death mechanism in the testicular tissue of mice. There was an increase of *Bax* mRNA expression levels in the testicular tissue of *Ide* KO mice (2.43 ± 0.44), when compared to both WT (0.57 ± 0.25) and Het (0.83 ± 0.47) mice (Fig. 4A). However, when analyzing both *Bcl2* (Fig. 4B) and *Casp3* mRNA levels (Fig. 4C), no significant differences were found between the different groups.

4. Discussion

The prevalence of prediabetes/diabetes in men in reproductive age has been increasing (Chen, Magliano and Zimmet, 2012). Several reports discussed the impact of prediabetes and diabetes *mellitus* in male fertility and concluded that dysregulated insulin is a key factor for this connection (Alves, Martins, Cavaco et al., 2013, Alves et al., 2012, Rato, Meneses, Silva et al., 2016). IDE is a metallopeptidase that degrades and inactivates insulin and several other peptides, thus having a crucial role in insulin clearance (Pivovarova, Bernigau, Bobbert et al., 2013). Although it is already known that insulin dysregulation (hypo- and hyperinsulinemia), affects male reproductive potential (Alves et al., 2012), the role of IDE on male reproductive tract remains completely unknown. Our data shows that IDE affects testicular morphology. In fact, heterozygous mice for *Ide* showed decreased testes weight, that was further noticed in KO mice. Concomitantly, the gonadosomatic index was decreased in KO animals, illustrating that *Ide* KO mice have testicular atrophy and morphological alterations when compared to the testes of the WT group. However, only KO mice showed decreased seminiferous tubules diameter. Testes' weight, along with seminiferous tubules diameter, are largely dependent on the mass and quantity of the different sperm cells, and are associated with compromised spermatogenesis (Rossi and Aeschlimann, 1982). Several studies revealed decreased testes weight and seminiferous tubules diameter in animal models of diabetes *mellitus* (Cai, Chen, Evans et al., 2000, Frenkel,

Homonnai, Drasnin et al., 1978, Rossi and Aeschlimann, 1982). Our results suggest that IDE is pivotal for a normal testicular weight and seminiferous tubule structure and may be involved in the mechanisms by which prediabetic and diabetic individuals present subfertility/infertility.

Reproductive dysfunction and abnormal sperm production is well documented in men suffering with metabolic diseases, namely diabetes *mellitus*, and infertility/subfertility is a common characteristic of men with diabetes (Rama Raju et al., 2012). It is known that insulin dysregulation has a huge impact on spermatozoa, mainly due to studies focused on insulin deprivation rather than hyperinsulinemia (Alves et al., 2012, Dias, Rato, Martins et al., 2013). Moreover, studies on animal models of diabetes do not distinguish between hyperglycemia and/or hyperinsulinemia. In fact, most studies are focused on the effects of hyperglycemia rather than the effects of hyperinsulinemia (Agbaje et al., 2007, Du Plessis, Cabler, McAlister et al., 2010). The KO mice for *Ide* present hyperinsulinemia, which is an age-dependent feature of this model (Abdul-Hay, Kang, McBride et al., 2011), and we observed defective sperm parameters that may be a consequence of the detected defective testicular morphology. Although sperm concentration and motility remained similar between the different groups, there were differences in what concerns to sperm viability and morphology. Sperm viability was decreased in KO mice for *Ide*. In addition, sperm morphology defects were also increased in KO mice. Those defects were mostly detected in the head of sperm cells, which normal morphology is very important for processes like its transport through female reproductive tract, capacitation, or even sperm-oocyte fusion (Kot and Handel, 1987). Normal head morphology is associated with higher pregnancy rates and lower miscarriage rates in cases of assisted reproductive techniques, namely intracytoplasmic sperm injection (Bartoov, Berkovitz, Eltes et al., 2003, Berkovitz, Eltes, Lederman et al., 2006). Our results point towards a role for IDE in sperm differentiation/maturation. One main cause for abnormal sperm morphology is the

unbalance in oxidative status (Gomez, Irvine and Aitken, 1998, Hosseinzadeh Colagar, Karimi and Jorsaraei, 2013). Oxidative stress is associated with augmented production of reactive oxygen species that modify phospholipids and proteins leading to peroxidation and oxidation of thiol groups (Aitken, Clarkson and Fishel, 1989, Hosseinzadeh Colagar et al., 2013). Spermatozoa are particularly susceptible to reactive oxygen species as they present a significant concentration of polyunsaturated fatty acids (Koppers, De Iuliis, Finnie et al., 2008) and many possess sensitive double bonds between carbon atoms that easily undergo non-enzymatic oxidation and fragmentation. In fact, spermatozoa were already found to be affected by endocrine disruptors that cause increased oxidative environment on testes, such as bisphenol A (D'Cruz, Jubendradass, Jayakanthan et al., 2012). Our data shows an increase on testicular tissue lipid peroxidation in *Ide* KO mice when compared to both WT and heterozygous mice for *Ide* illustrating that the testes of these mice have a prooxidant environment. These results illustrate that lack of *Ide* induces an unbalance in oxidative status towards a pro-oxidant status. The major intracellular biological source of reactive oxygen species is mitochondrial respiration (Aly, Domenech and Abdel-Naim, 2009). We tested this hypothesis and our results show that the increase in lipid peroxidation was not accompanied by dysfunction in total OXPHOS protein illustrating that the pro-oxidant status may be caused by a decrease in antioxidant defenses mediated by lack of IDE rather than an increase in mitochondrial dysfunction (Valko, Leibfritz, Moncol et al., 2007). Apoptosis is associated with increased oxidative stress and may be triggered by both internal or external signals. As one of the apoptotic mechanisms converge on mitochondria, we analyzed some proteins associated with this process (Hengartner, 2000), namely the proapoptotic factor Bax and the antiapoptotic Bcl-2. *Bax* expression was increased in KO mice for *Ide* when compared to wildtype animals, pointing towards a proapoptotic state in the testicular tissue of those mice. Having into account that KO mice have increased insulin levels, these results point out that insulin

levels affect *Bax* levels which is supported by previous data that show a decrease in *Bax* levels in cases of insulin deprivation in rat testicular cells (Dias et al., 2013).

The emerging number of men in reproductive age with metabolic diseases, along with an increasing incidence of metabolic disorders in children, adolescents and young adults highlight the need for molecular studies linking these two conditions that must unveil the mechanisms responsible for such connection. Spermatogenesis, the process by which spermatozoa are formed, is a highly-regulated process with enormous energetic needs being insulin a crucial player. In fact, while peripheral insulin levels oscillate due to diet and even the circadian rhythm, testes provide a constitutive source of insulin for spermatogenesis (Gomez, Ballester, Romero et al., 2009), in order to minimize these fluctuations. Moreover, Sertoli cells secrete insulin to provide adequate levels of this hormone to germ cells that do not have direct access to plasma insulin due to the blood-testis barrier (Schoeller et al., 2012). Furthermore, the absence of insulin shifts the glycolytic metabolism of Sertoli cells to Krebs cycle, which compromises germ cell development (Alves et al., 2012, Rato et al., 2016). This work illustrates that IDE acts on glucose metabolism beyond liver, in peripheral organs such as testes. Notably, lack of this enzyme induces lower sperm quality with a lower percentage of viable spermatozoa as well as more defective sperm cells. Besides the relevance for the understanding of subfertility/infertility in men suffering with metabolic diseases, we must highlight that most of infertility cases, about 60-75% in men, are idiopathic, which means that the causes remain obscure. Our results show that when IDE, which should be present and functional in all individuals, is lacking or with heterozygous phenotype, fertility problems may arise in men.

Acknowledgements

This work was funded by FEDER funds POCI - COMPETE 2020 – Programa Operacional para a Competitividade e Internacionalização no Eixo 1 (Project POCI-01-0145-FEDER-007491) and by “Fundação para a Ciência e a Tecnologia” – FCT to

Maria J. Meneses (PD/BD/114256/2016 and); Tânia R. Dias (SFRH/BD/109284/2015) Pedro F. Oliveira (PTDC/BBB-BQB/1368/2014 and IFCT2015); M. Paula Macedo (PTDC/DTP-EPI/0207/2012, PTDC/BIM-MET/4265/2014, PTDC/BIM-MET/2115/2014); Marco G. Alves (PTDC/BIM-MET/4712/2014; PTDC/MEC-AND/28691/2017 and IFCT2015); iNOVA4Health (UID/Multi/04462/2013); and UMIB (PEst-OE/SAU/UI0215/2014).

Declarations of interest

None

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496

497 **Table 1. Primers used in this study.**

Gene	Primer sequence (5' - 3')	Annealing temperature (ºC)	Amplicon size (bp)
Bax	Fwd: CTCAAGGCCCTGTGCACTAA Rvs: CAGCCACCCTGGTCTTGGAT	60	110
Bcl2	Fwd: GTGAACCTGGGGGAGGATTGT Rvs: CATGCTGGGGCCATATAGTTC	60	195
Casp3	Fwd: GAGCTTGGAACGGTACGCTAA Rvs: GAGTCCACTGACTTGCTCCC	59	118
Gapdh	Fwd: AAGAGGGATGCTGCCCTTAC Rvs: TACGGCCAAATCCGTTCACA	61	123
β2M	Fwd: GCTTCAGTCGTCAGCATGGC Rvs: GGATTTCAATGTGAGGCGGGT	57	187

498

Figure Legends

Figure 1 – Effect of insulin degrading enzyme on mice testicular morphology. The figure shows data of testis weight (panel A), gonadosomatic index (panel B), seminiferous tubules diameter (panel C) and representative images from seminiferous tubules of wild type (WT) and knockout (KO) mice (panel D) for *Ide*. Results are expressed as mean ± SEM (n = 4 for each condition). Significantly different results are indicated as: * − P < 0.05; ** − P < 0.01; **** − P < 0.0001.

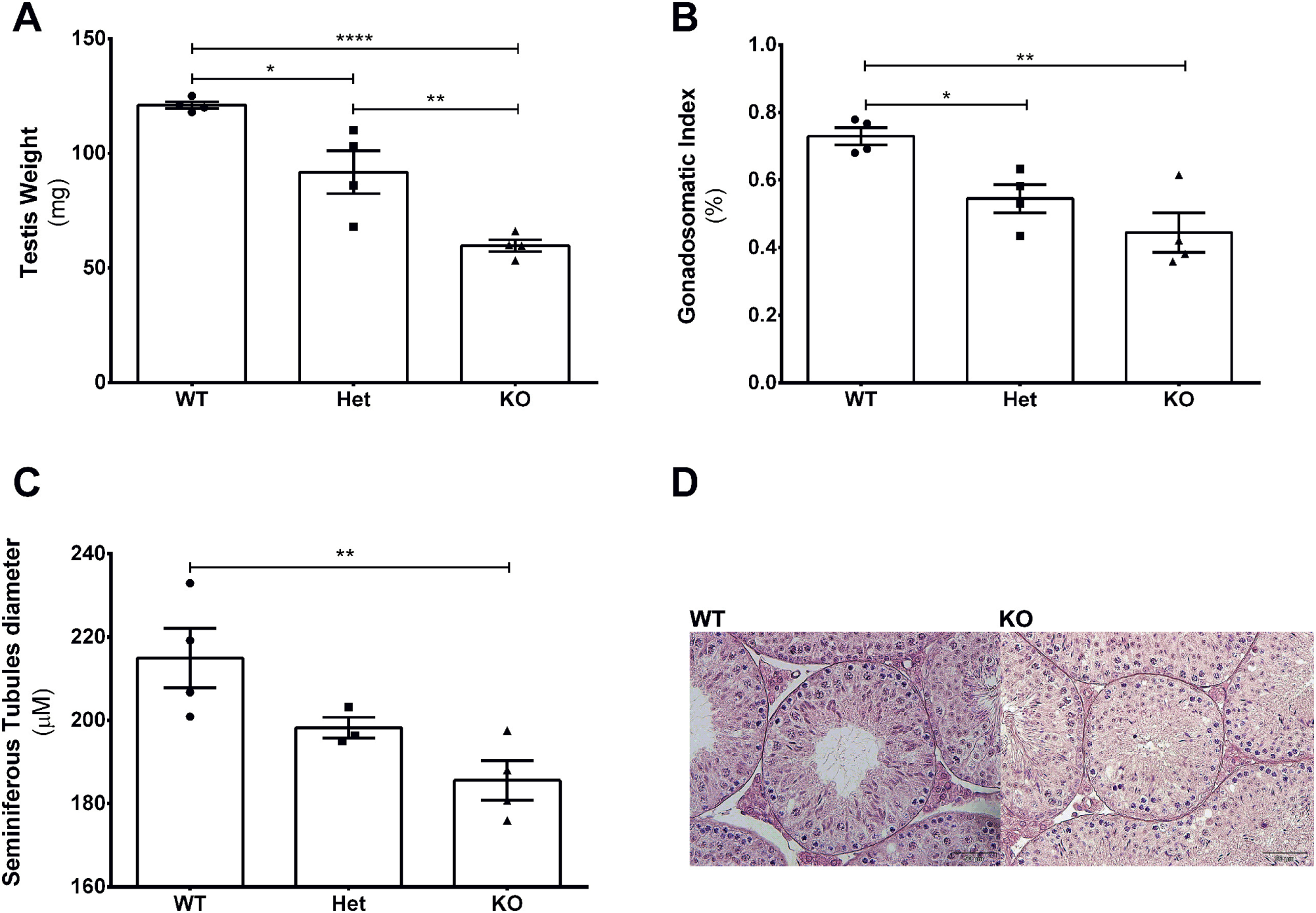
Figure 2 - Effect of insulin degrading enzyme on mice sperm parameters. The figure shows data of sperm motility (panel A), concentration (panel B), viability (panel C) and morphology (panel D) from heterozygous (Het) or knockout (KO) mice for *Ide* and wild type (WT) mice. Results are expressed as mean ± SEM (n = 4 for each condition). Significantly different results are indicated as: * − P < 0.05; ** − P < 0.01.

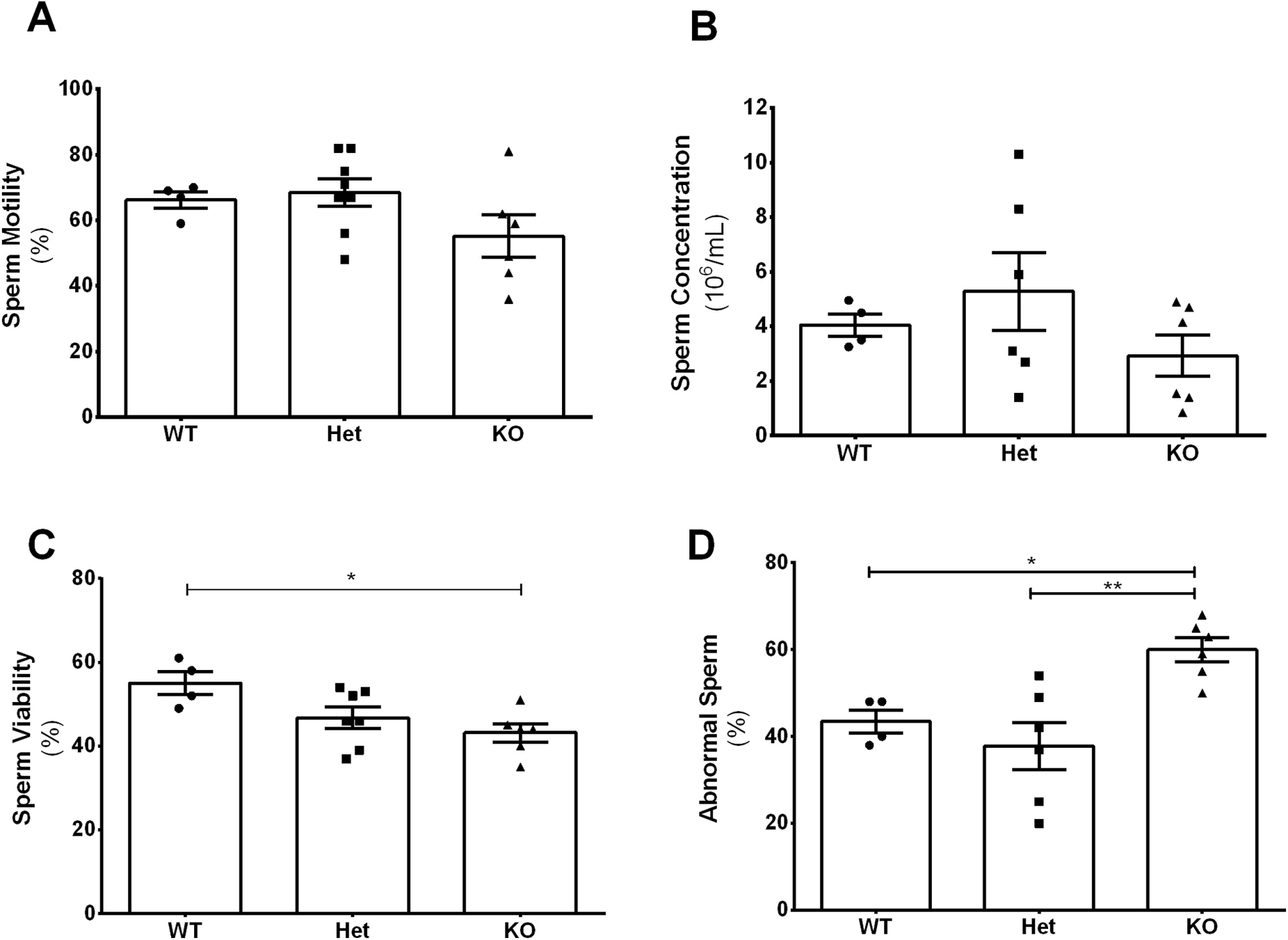
Figure 3 - Effect of insulin degrading enzyme on oxidative stress markers in testicular tissue of mice. The figure shows data of protein nitration (panel A), carbonyl groups levels (panel B), lipid peroxidation (panel C) and oxidative phosphorylation (OXPHOS) protein levels (panel D) in testicular tissue from heterozygous (Het) or knockout (KO) mice for *Ide* and wild type (WT) mice. Results are expressed as mean ± SEM (n = 4 for each condition). Significantly different results are indicated as: * − P < 0.05; ** − P < 0.01.

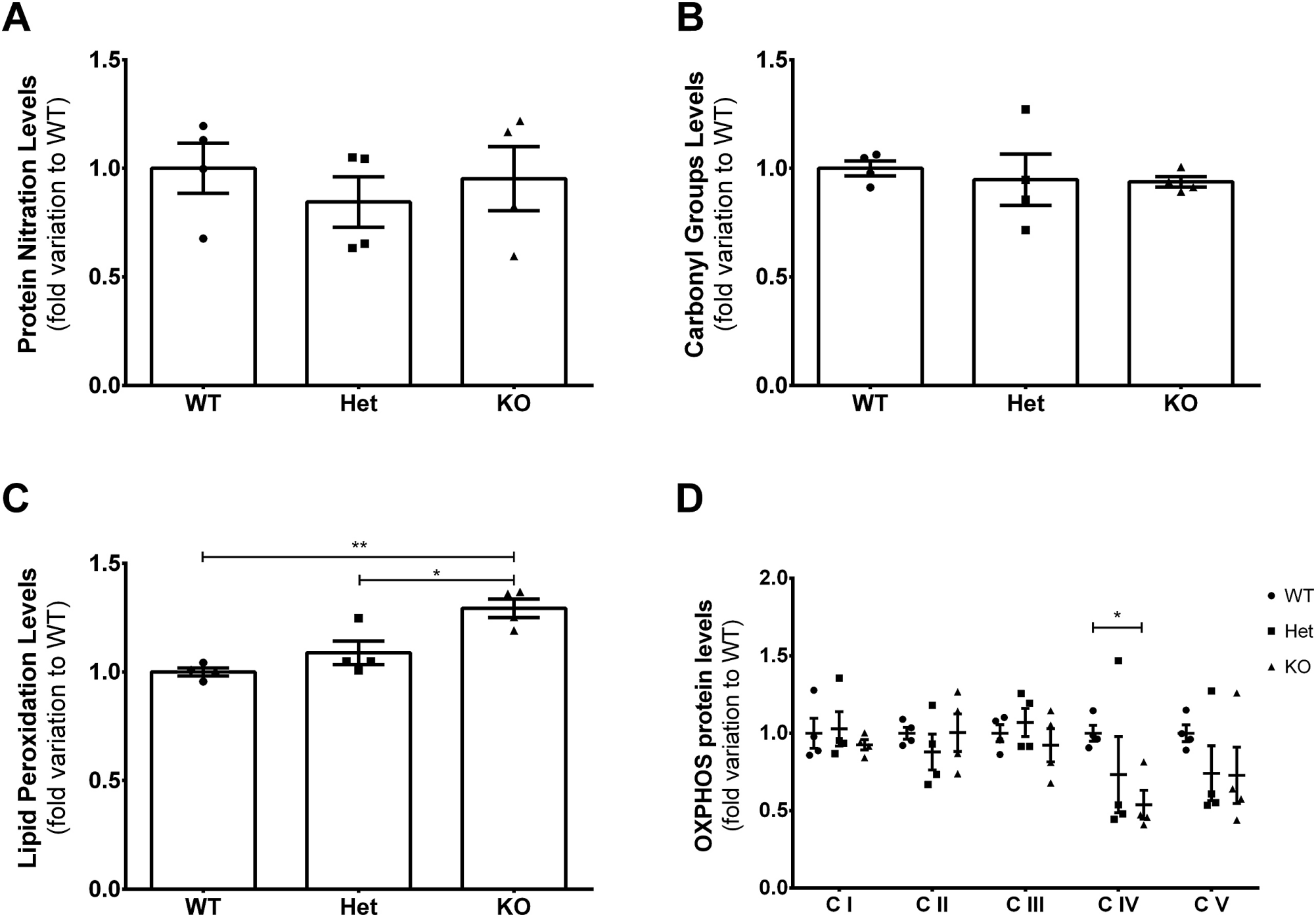
Figure 4 - Effect of insulin degrading enzyme on apoptotic markers in the testicular tissue of mice. The figure shows data of Bax (panel A), Bcl2 (panel B) and Casp3 (panel C) mRNA expression levels in the testicular tissue from heterozygous (Het) or knockout (KO) mice for *Ide* and wild type (WT) mice. Results are expressed as mean ±

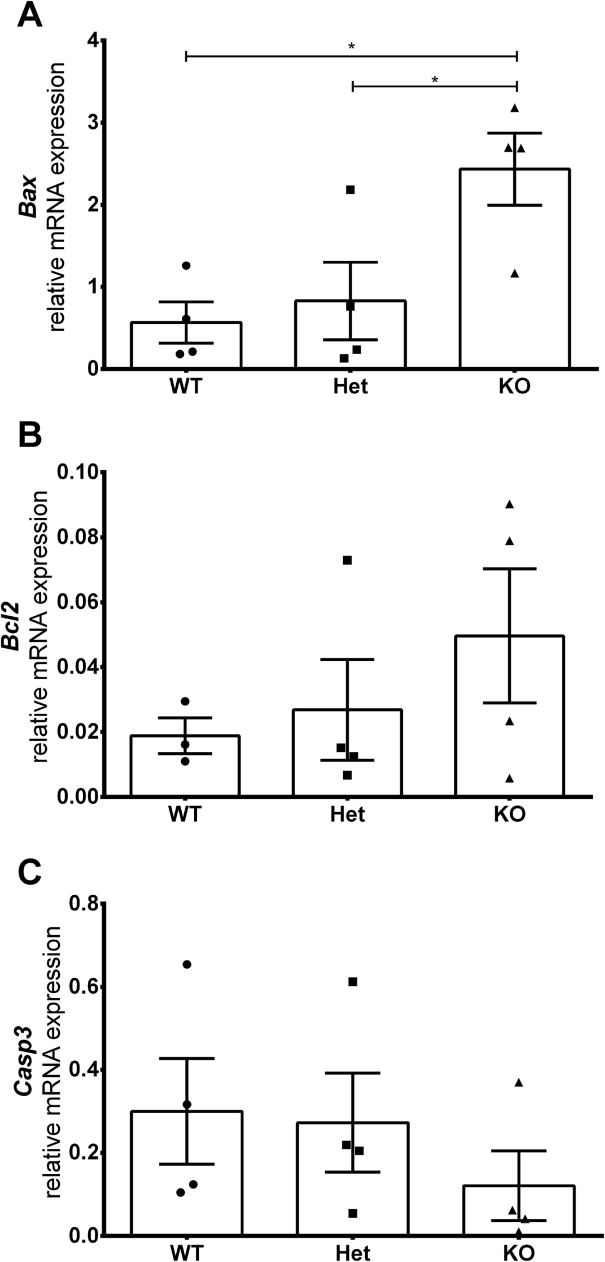
525 SEM (n = 4 for each condition). Significantly different results are indicated as: * − P <

526 0.05.


A
Testis Weight
(mg)
150
100
50
0
****
*
**
WT
Het
KO
B
Gonadosomatic Index
(%)
1.0
0.8
0.6
0.4
0.2
0.0
**
*
WT
Het
KO
C
Seminiferous Tubules diameter
(μM)
240
220
200
180
160
**
WT
Het
KO
D
WT
KO


A
Sperm Motility (%)
100
80
60
40
20
0
WT
Het
KO
B
Sperm Concentration (10^6/mL)
12
10
8
6
4
2
0
WT
Het
KO
C
Sperm Viability (%)
80
60
40
20
0
*
WT
Het
KO
D
Abnormal Sperm (%)
80
60
40
20
0
*
**
WT
Het
KO


A
Protein Nitration Levels
(fold variation to WT)
1.5
1.0
0.5
0.0
WT
Het
KO
B
Carbonyl Groups Levels
(fold variation to WT)
1.5
1.0
0.5
0.0
WT
Het
KO
C
Lipid Peroxidation Levels
(fold variation to WT)
**
*
1.5
1.0
0.5
0.0
WT
Het
KO
D
OXPHOS protein levels
(fold variation to WT)
*
2.0
1.5
1.0
0.5
0.0
C I
C II
C III
C IV
C V
WT
Het
KO


A
Bax
relative mRNA expression
4
3
2
1
0
*
*
WT
Het
KO
B
Bcl2
relative mRNA expression
0.10
0.08
0.06
0.04
0.02
0.00
WT
Het
KO
C
Casp3
relative mRNA expression
0.8
0.6
0.4
0.2
0.0
WT
Het
KO

Highlights

- Insulin-degrading enzyme (IDE) is the major responsible for insulin metabolism
- IDE malfunction leads to hyperinsulinemia, a feature of prediabetes
- Absence of IDE affects testicular morphology and seminiferous tubules diameter
- IDE absence decreases sperm viability and morphology
- Absence of IDE leads to increased lipid peroxidation in testicular tissue