PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

Ministry of Higher Education and Scientific Research

University Abdelhamid Ibn Badis of Mostaganem

Natural and Life Sciences Faculty

Biology Department

Domain: Natural and Life Sciences

Field: Biological Sciences

Specialty: Animal biology and physiology

Laboratory: Sciences and Techniques of Animal Production (LSTPA)

PhD dissertation LMD 3rd cycle

THEME

Physiological and physiopathological modulation of GPER1 in the reproductive system of the Wistar rat and the sand rat

Disserted by:
Miss **FERNINI Meriem**

On: July 10th , 2025

In front of jury:

Jury chair: BENAKRICHE Ben Mehel	Prof University of Mostaganem
Research director: BELHOCINE Mansouria	Prof University of Mostaganem
Research co-director: MENAD Rafik	Prof University of Algiers
Reviewer: GRAR Hadria	Lect University of Mostaganem
Reviewer: SAHRAOUI Tewfik	Prof University of Oran
Guest: DAHOU Abdelkader El Amine	Lect University of Mostaganem

Academic year: 2024-2025

Acknowledgements

I thank Allah the Creator, the Almighty, the All-Merciful, the Most Merciful.

اللهم لك الحمد حمدا كثيرا مباركا فيه

Thank you for giving me life, health, courage, strength, and the opportunity to achieve this work.

I am grateful for **Mrs. BELHOCINE Mansouria** for offering me the chance to pursue my dream as a PhD student and be a researcher, thank you for incorporating me in academic pathway, I would not have made it this far without the opportunities that you have made for us, thank you very much

I would like to express my sincere thankfulness to **Mr. MENAD Rafik** for encouraging and helping me along my research pathway, thank you very much for your valuable advice and your support.

I would like to thank **BENTAIBA Karima,** my friend and my colleague for what she has done for me, the help, the assistance, and the encouragement, thank you very much.

MAMA and **BABA**, I am grateful and lucky to have such supportive parents, thank you for encouraging me, thank you for being patient for me, I hope you are proud of me.

I also would like to thank everyone who participated directly or indirectly in carrying out this modest work.

Dedication

To MAMA and BABA,

To Mrs BELHOCINE Mansouria,

To Mr MENAD Rafik,

To my sister AMINA, my brothers HAROUN, IDRIS and OUSSAMA,

To all my family and beloved, To KARIMA and my friends,

To MYSELF, I dedicate this work.

Meriem

Abstract

The journey of research in reproductive physiology, infertility, and cancer, requires deep understanding of GPER1 pathway to promotes estrogenic effects in the male reproductive physiology. GPER1 mediates several intracellular pathways leading to various physiological effects. While free-ranging animals, having a seasonal reproductive cycle, seem to be the perfect animal models to perform such studies. Our work aimed in the first part to investigate GPER1 localisation in the reproductive system of the sand rat (*Psammomys obesus*) and the gerbil (*Gerbillus gerbillus*), seasonally breeding rodents from North Africa, employing immunohistochemical approach. In the second part, *in silico* approach was used to clarify the interaction between different endocrine disrupting chemicals, phytochemicals and known ligands with the GPER1. The histology of the testis, the efferent ducts and the epididymis of both rodents underwent remarkable alterations during the resting season. The expression of the GPER1 during the breeding season, was found all over the testis, the efferent ducts, and the epididymis. While during the resting season, we noticed the absence of the GPER1 in Sertoli cells and spermatogonia, but it was found in the Leydig cells and spermatocytes, as spermatogenesis was blocked at the stage of spermatocytes. In the efferent ducts, not all ciliated cells, non-ciliated cells and basal cells were immunostained, while in the epididymis, only principal cells and basal cells expressed GPER1. Interestingly, the endocrine disruptors showed the highest affinity among the tested substances, for instance, Bisphenol A, Polychlorinated Biphenyls and Acrylamide had ΔG of -16.47 kcal/mol, -10.35 kcal/mol and -8.78 kcal/mol respectively. While phytochemicals that had lower binding energies were Coumestrol with ΔG of -9.03 kcal/mol, Genistein with ΔG of -8.47 kcal/mol and Biochanin A with ΔG of -8.33 kcal/mol. In addition, Estramustin, a chemotherapeutic agent derived from oestrogen displayed low binding energy ΔG of -7.45 kcal/mol. In summary, the presence of GPER1 in the breeding season and its absence during the resting season signal the influence of the seasonal fluctuations

on estrogenic actions via GPER1 and highlight the importance of the GPER1 in normal reproductive physiology of the male. Nevertheless, the low binding energies and the high affinity displayed by endocrine disruptors witness their emerging effects on endocrine system and the need to supervise the use of these harmful chemicals. Furthermore, the strong interactions between phytochemicals and Estramustin chemotherapeutic agent with the GPER1 advocate the possibility of using these chemicals as therapeutic agents targeting the GPER1 in several types of pathologies prompted by the GPER1.

keywords: GPER1, testis, efferent ducts, epididymis, oestrogen, phytochemicals, endocrine disruptors.

الملخص

تتطلب رحلة البحث في علم وظائف الأعضاء التناسلية، العقم والسرطان فهمًا عميقًا لتأثيرات الاستروجين عن طريق GPER1 في وظائف الأعضاء التناسلية الذكرية. في حين أن الحيوانات ذات الدورة الإنجابية الموسمية، تبدو النماذج الحيوانية المثالية لإجراء مثل هذه الدراسات. يهدف عملنا في الجزء الأول إلى دراسة تواجد GPER1 في الجهاز التناسلي لجرذ الصحراء *Psammomys obesus* و للجربوع *Gerbillus gerbillus* ، اللذان هما قوارض ذات تكاثر موسمي تعيش في شمال إفريقيا، باستخدام نهج كيميائي مناعي. في الجزء الثاني، تم استخدام طريقة السيليكو *in silico* لتوضيح التفاعل بين المواد الكيميائية المختلفة المسببة لاضطرابات الغدد الصماء، المواد الكيميائية النباتية والروابط المعروفة مع GPER1.خضعت أنسجة الخصية و القنوات الصادرة و البربخ لتغيرات ملحوظة خلال موسم الراحة. تم العثور على المستقبل GPER1 خلال موسم التكاثر في جميع أنحاء الخصية والقنوات الصادرة والبربخ. بينما خلال موسم الراحة لاحظنا غياب الـ GPER1 في خلايا سيرتولي والخلايا المنوية الأصلية، لكنه وجد في خلايا لايديغ والخلايا المنوية الثانوية، حيث تكوين الحيوانات المنوية توقف في مرحلة الخلايا المنوية الثانوية. في القنوات الصادرة، لا تبدي جميع الخلايا الهدبية والخلايا غير الهدبية والخلايا القاعدية هذا المستقبل، بينما في البربخ، فقط الخلايا الرئيسية والخلايا القاعدية من أظهرت GPER1. من المثير للاهتمام أن مواد التي تسبب اضطراب الغدد الصماء أظهرت أقل طاقة ربط بين المواد التي تم اختبارها، على سبيل المثال، ثنائي الفينول أ، وثنائي الفينيل متعدد الكلور والأكريلاميد كان لديهم ΔG بقيمة -16.47 كيلو كالوري/مول، -10.35 كيلو كالوري/مول، و-8.78 كيلو كالوري/مول على التوالي. في حين أن جميع المواد الكيميائية النباتية لها طاقات ربط أقل مثل كوماسترول مع ΔG من -9.03 كيلو كالوري/مول، جينيستين مع ΔG من -8.47 كيلو كالوري/مول وبيوشانين أ مع ΔG من -8.33 كيلو كالوري/مول. بالإضافة إلى ذلك، أظهر الإستراموستين، وهو عامل علاج كيميائي مشتق من الإستروجين، طاقة ربط منخفضة ΔG تبلغ -7.45 كيلو كالوري/مول. باختصار، يشير وجود GPER1في موسم التكاثر وغيابه خلال موسم الراحة إلى تأثير التقلبات الموسمية على فعالية الاستروجين عبر GPER1 ويسلط الضوء على أهمية GPER1 في فسيولوجيا الإنجاب الطبيعية للذكر. كما أن طاقات الارتباط المنخفضة والتقارب المرتفع اللذن تظهرهما المواد المسبب لاضطرابات الغدد الصماء تشهد على آثارها الفتاكة على نظام الغدد الصماء والحاجة إلى تقييد استخدام هذه المواد الكيميائية الضارة. علاوة على ذلك، فإن التفاعلات القوية بين المواد الكيميائية النباتية وعوامل العلاج الكيميائي الإستراموستين مع GPER1 تدعم إمكانية استخدام هذه المواد الكيميائية كعوامل علاجية تستهدف GPER1 في عدة أنواع من الأمراض التي يحفزهاGPER1 .

الكلمات المفتاحية :GPER1 ، الخصية، البربخ، القنوات الصادرة، الاستروجين، المواد الكيميائية النباتية، مثبطات الغدد الصماء.

Résumé

Le parcours de recherche sur la physiologie de la reproduction, l'infertilité et le cancer nécessite une compréhension approfondie de la voie oestrogénique via le GPER1 dans la physiologie de la reproduction masculine. Le GPER1 active plusieurs voix de signalisation intracellulaire pour susciter différents processus physiologique et physiopathologique. Les animaux sauvages, ayant un cycle de reproduction saisonnier, semblent être les modèles animaux parfaits pour réaliser de telles études. Notre travail visait dans une première partie à étudier la localisation du GPER1 dans le système reproducteur du rat des sable (*Psammomys obesus)* et de la gerbille (*Gerbillus gerbillus)*, des rongeurs sahariens d'Afrique du Nord, en utilisant une approche immunohistochimique. Dans la deuxième partie, une approche *in Silico* a été utilisée pour clarifier l'interaction entre différents perturbateurs endocriniens, composés phyto-chimiques et ligands connus avec le GPER1. Pendant la saison d'activité, les testicules, les canaux efférents et l'épididyme ont conservé leur architecture fonctionnelle. En outre, le GPER1 a été trouvé partout a travers le system reproducteur. Néanmoins, la saison de repos, les testicules, les canaux efférents et l'épididyme sont fortement atrophiés. L'expression du GPER1 pendant la saison de reproduction a été trouvée partout dans le testicule, les canaux efférents et l'épididyme. Cependant, au cours la saison de repos, nous avons noté l'absence de GPER1 dans les cellules de Sertoli et les spermatogonies, mais il a été trouvé dans les cellules de Leydig et les spermatocytes, car la spermatogenèse était bloquée au stade des spermatocytes. Dans les canaux efférents, toutes les cellules ciliées, les cellules non ciliées et les cellules basales n'étaient pas marquées, tandis que dans l'épididyme, seules les cellules principales et les cellules basales exprimaient GPER1. Il est intéressant de noter que les perturbateurs endocriniens ont montré l'affinité la plus forte parmi les substances testées. Le Bisphénol A, les Biphényles Polychlorés et l'Acrylamide avaient une ΔG de -16,47 kcal/mol, -10,35 kcal/mol et -8,78 kcal/mol respectivement. Alors que tous les composés phytochimiques ont eu des énergies

de liaison très faibles : Coumestrol avec une ΔG de -9.03 kcal/mol, Génistéine avec une ΔG de -8,47 kcal/mol et le Biochanin A avec une ΔG de -8.33 kcal/mol. De plus, l'Estramustin, un agent chimio-thérapeutique dérivé de l'œstrogène, présentait une faible énergie de liaison ΔG de -7,45 kcal/mol. En conclusion, la forte présence du GPER1 pendant la saison de reproduction et sa présence réduite pendant la saison de repos signalent l'influence des fluctuations saisonnières sur les actions œstrogéniques via le GPER1 et mettent en évidence l'importance de GPER1 dans la physiologie reproductive normale du mâle, l’activité cellulaire étant ralentie en saison d’inactivité sexuelle traduisant l’estompement du signal. Néanmoins, les faibles énergies de liaison et les fortes affinités affichées par les perturbateurs endocriniens témoignent de leurs effets émergents sur le système endocrinien et de la nécessité de superviser l’utilisation de ces produits chimiques nocifs. De plus, les fortes interactions entre les composés phytochimiques et les agents chimio-thérapeutiques tel que l’Estramustin plaident en faveur de la possibilité d'utiliser ces produits chimiques comme agents thérapeutiques ciblant le GPER1 dans plusieurs types de pathologies provoquées par le GPER1.

Mots-clés : Œstrogène, GPER1, testicule, canaux efférents, épididyme, perturbateurs endocriniens, composés phyto-chimiques, agents chimio-thérapeutiques.

Table of Contents

- Abstract

- List of tables

- List of figures

- List of abbreviations

- Introduction **1**

Literature Review

Chapter I: Male reproductive system 4

A. Structural organization of the male reproductive system 4

A.1. The testis 4

A.2. The genital tract 7

A.3. The accessory glands 11

B. Male reproductive physiology 13

B.1. Spermatogenesis 13

B.2. Regulation of the male reproductive functions 14

Chapter II: G protein-coupled oestrogen receptor 1 16

A. Oestrogens 16

A.1. Biosynthesis and localization 16

A.2. Oestrogen receptors 17

A.3. Ligands 18

A.4. Signalization 19

A.5. Effects 20

B. GPER1 21

B.1. History and discovery 21

B.2. Structure and variation. 21

B.3. Localization 22

B.4. Ligands..... 23

B.5. Signalization 24

B.6. Physiological and physio-pathological effects 25

B.7. Cross talk between the GPER1 and the classical ESRs..... 27

Materials and methods

I. *in vivo* study 29

I.A. Animal model..... 29

I.A.1. Scientific classification 30

I.A.2. Habitat and distribution..... 31

I.A.3. Food, foraging and reproduction. 31

I.A.4. Animal capture 32

I.B. Experimentation 32

I.C. Methods 32

I.C.1. Histology 32

I.C.2. Immunohistochemistry..... 33

I.C.3. Microscopic observation 34

I.C.4. Statistical analysis 34

II. *In silico* study..... 34

II.A. Databases 35

II.A.1. Uniprot..... 35

II.A.2. PubChem..... 35

II.A.3. Phytochemical databases 36

II.B. Software 37

II.B.1. OpenBabel..... 37

II.B.2. BioVia Discovery Studio ... 37

II.B.3. AutoDock Vina ... 38

II.C. Methods ... 38

II.C.1. Definition and principal of molecular docking ... 38

II.C.2. Protocol and steps. ... 39

II.C.2.1. Receptor and ligand structure downloading ... 39

II.C.2.2. Receptor-ligand docking ... 41

II.C.2.3. Results analysis ... 42

Results

I. *In vivo* study ... 43

I.A. Organ weight ... 43

I.B. Histology ... 43

I.B.1. The testis ... 43

I.B.2. The efferent ducts ... 45

I.B.3. The proximal epididymis ... 47

I.B.4. The distal epididymis ... 49

I.C. Immunohistochemistry ... 51

I.C.1. The testis ... 54

I.C.2. The efferent ducts ... 56

I.C.3. The proximal epididymis ... 58

I.C.4. The distal epididymis ... 60

II. *In silico* study ... 62

II.A. Known ligands. ... 62

II.B. Phytochemicals ... 68

II.C. Heavy metals ... 82

II.D. Pesticides 85

II.E. Food additives 89

II.F. Personal care chemicals 94

II.G. Plasticizers and flame retardants 100

II.H. Chemotherapy 105

- Discussion 112

- Conclusion 138

- References

- Appendices

- Scientific publications

List of tables

Table 01: Types of ligands tested (part1). 40

Table 01: Types of ligands tested (part2). 41

Table 02: Immunolocalization of GPER1 in the gerbil *Gerbillus gerbillus* 52-53

Table 03: Immunolocalization of GPER1 in the sand rat *Psammomys obesus*................53-54

Table 04: Molecular docking results of GPER1 with the known ligands. 64-66

Table 05: Molecular docking results of GPER1 with chemicals. 69-75

Table 06: Molecular docking results of GPER1 with heavy metals. 83

Table 07: Molecular docking results of GPER1 with pesticides.....................................85-86

Table 08: Molecular docking results of GPER1 with food additives..............................90-91

Table 09: Molecular docking results of GPER1 with personal care chemicals. 95-97

Table 10: Molecular docking results of GPER1 with plasticizers and flame retardants101-102

Table 11: Molecular docking results of GPER1 with chemotherapy. 106-108

List of Figures

Figure 01: Anatomy of the male reproductive system 4

Figure 02: Anatomy and histology of the testis. Adapted from Principles of anatomy and physiology. 5

Figure 03: Anatomy of the epididymis 8

Figure 04: Schematic representation of the epididymal histology 8

Figure 05: Schematic illustration of spermatogenesis 14

Figure 06: Regulation of testis functions 14

Figure 07: The oestrogen biosynthetic pathway 17

Figure 08: Structure of classical oestrogen receptors ESRs 18

Figure 09: Mechanisms of nuclear oestrogen receptor pathways 20

Figure 10: The G protein-coupled oestrogen receptor. 22

Figure 11: GPER1 modulators: agonists and antagonists. 24

Figure 12: GPER1 and ESRs signalling pathway. 25

Figure 13: Physiological and physio-pathological effects of the GPER1 27

Figure 14: Crosstalk between GPER1 (GPR30) and the classical ERs 28

Figure 15 : The sand rat *Psammomys obesus* 29

Figure 16 : The Lesser Egyptian gerbil *Gerbillus gerbillus* 30

Figure 17: The basics of immunohistochemistry. 33

Figure 18: UniProt database 35

Figure 19: PubChem database...36

Figure 20: Dr. Duke's Phytochemical and Ethnobotanical Databases 36

Figure 21: Coconut database...37

Figure 22: OpenBabel software version 2.4.1...37

Figure 23: BIOVIA Discovery Studio version 21.1.0.20298...38

Figure 24: Autodock Vina 1.5.7...38

Figure 25: Average testicular weight in *Gerbillus gerbillus* during the breeding season and the resting season. 43

Figure 26: Histology of the testis of *Gerbillus gerbillus*. 44

Figure 27: Histology of the testis of *Psammomys obesus*..45

Figure 28: Histology of the efferent ducts of *Gerbillus gerbillus*.......................................46

Figure 29: Histology of the efferent ducts of *Psammomys obesus*. 47

Figure 30: Histology of the proximal epididymis of *Gerbillus gerbillus*. 48

Figure 31: Histology of the proximal epididymis of *Psammomys obesus*...........................49

Figure 32: Histology the distal epididymis of *Gerbillus gerbillus*. 50

Figure 33: Histology the distal epididymis of *Psammomys obesus*....................................51

Figure 34: Immunolocalization of GPER1 in the testis of *Gerbillus gerbillus*....................55

Figure 35: Immunolocalization of GPER1 in the testis of *Psammomys obesus*..................56

Figure 36: Immunolocalization of GPER1 in the efferent ducts of *Gerbillus gerbillus*.......57

Figure 37: Immunolocalization of GPER1 in the efferent ducts of *Psammomys obesus* 58

Figure 38: Immunolocalization of GPER1 in the proximal epididymis of *Gerbillus gerbillus*. 59

Figure 39: Immunolocalization of GPER1 in the proximal epididymis of *Psammomys obesus*. 60

Figure 40: Immunolocalization of GPER1 in the distal epididymis of *Gerbillus gerbillus*. 61

Figure 41: Immunolocalization of GPER1 in the distal epididymis of *Psammomys obesus*.62

Figure 42: Predicted binding sites of known ligands in the GPER1, as well as the 2D representation of the amino acids involved in the interactions between the known ligands and the GPER1........67-68

Figure 43: Predicted binding sites of phytoestrogens in the GPER1, as well as the 2D representation of the amino acids involved in the interactions between the phytochemicals and the GPER1........78-81

Figure 44: Predicted binding sites of heavy metals in the GPER1, as well as the 2D representation of the amino acids involved in the interactions between heavy metals and the GPER1........84

Figure 45: Predicted binding sites of pesticides in the GPER1, as well as the 2D representation of the amino acids involved in the interactions between the pesticides and the GPER1..88-89

Figure 46: Predicted binding sites of food additives in the GPER1, as well as the 2D representation of the amino acids involved in the interactions between the food additives and the GPER1........93-94

Figure 47: Predicted binding sites of Personal care chemicals in the GPER1, as well as the 2D representation of the amino acids involved in the interactions between the personal care chemicals and the GPER1........99-100

Figure 48: Predicted binding sites of plasticizers and flame retardants in the GPER1, as well as the 2D representation of the amino acids involved in the interactions between the plasticizers and flame retardants and the GPER1. 104-105

Figure 49: Predicted binding sites of chemotherapy in the GPER1, as well as the 2D representation of the amino acids involved in the interactions between the chemotherapy and the GPER1. 110-111

Figure 50: Schematic representation of the histology of the testis in *Gerbillus gerbillus* and *Psammomys obesus* during the breeding season and the resting season. 106

Figure 51: Schematic representation of the histology of the efferent ducts in *Gerbillus gerbillus* and *Psammomys obesus* during the breeding season and the resting season. 107

Figure 52: Schematic representation of the histology of the proximal epididymis in *Gerbillus gerbillus* and *Psammomys obesus* during the breeding season and the resting season. 108

Figure 53: Schematic representation of the histology of the distal epididymis in *Gerbillus gerbillus* and *Psammomys obesus* during the breeding season and the resting season. 109

Figure 54: Schematic representation of the GPER1 distribution in the testis of *Gerbillus gerbillus* during the breeding season and the resting season. 112

Figure 55: Schematic representation of the GPER1 distribution in the testis of *Gerbillus gerbillus* during the breeding season and the resting season. 114

Figure 56: Schematic representation of the GPER1 distribution in the efferent ducts of *Psammomys obesus* during the breeding season and the resting season. 115

Figure 57: Schematic representation of the GPER1 distribution in the efferent ducts of *Psammomys obesus* during the breeding season and the resting season. 116

Figure 58: Schematic representation of the GPER1 distribution in the proximal epididymis of *Gerbillus gerbillus* during the breeding season and the resting season. 117

Figure 59: Schematic representation of the GPER1 distribution in the proximal epididymis of *Psammomys obesus* during the breeding season and the resting season. 118

Figure 60: Schematic representation of the GPER1 distribution in the distal epididymis of *Gerbillus gerbillus* during the breeding season and the resting season. 119

Figure 61: Schematic representation of the GPER1 distribution in the distal epididymis of *Psammomys obesus* during the breeding season and the resting season. 120

Figure 62: Chemicals that are presumed to be selective ligands for the GPER1 123

Figure 63: Chemicals that are presumed to be nonselective ligands for the GPER1 123

Figure 64: The different tested chemicals classified according to their binding affinity 124

List of abbreviations

ΔG: Free binding energy.

.pdb: Protein data bank.

.pdbqt: Protein Data Bank, Partial Charge (Q), & Atom Type (T).

.sdf: Syntax definition formalism.

3β HSD: 3β Hydroxysteroid dehydrogenase.

2D: Two dimensions.

ABP: Androgen binding protein.

AEC: Amio ethyl carbazole.

AF: Activation factor.

AKT: Protein kinase B.

Ala: Alanine.

ANOVA: Analysis of variance.

ANP: Atrial natriuretic peptide.

AP: Activation protein.

Arg: Arginine.

Asn: Asparagine.

ArKO: Androgen receptor knock out.

Asp: Aspartic acid.

ATP: Adenosine triphosphate.

BPA: Bisphenol A.

cAMP: Cyclic adenosine monophosphate.

cGMP: Cyclic guanosine monophosphate.

CRE: cAMP response element.

CREB: cAMP Response element-binding protein.

Cyc : Cysteine.

CYP : Cytochrome P450.

DAB: Di amino benzidine.

DBD: DNA binding domain.

DDT: Dichloro-diphenyl-trichloroethane.

DHT: Dihydrotestosterone.

DNA: Deoxyribonucleic acid.

EDs: Endocrine disruption chemicals.

EGF: Epidermal growth factor.

ElK1: ETS Like-1 protein.

Epp: Proximal epididymis.

Epd: Distal epididymis.

ERα: Oestrogen receptor α.

ERβ: Oestrogen receptor β.

ER: Oestrogen receptor.

ERC: Cytoplasmic oestrogen receptor.

ERE: Oestrogen responsive element.

ERK1/2: Extracellular signal-regulated kinase 1/2.

ESR1: Oestrogen receptor 1 (ERα).

ESR2: Oestrogen receptor 2 (ERβ).

ESRs: Oestrogen receptors.

ErKO: Oestrogen receptor Knock out.

ET : Endothelin.

ETS : Erythroblast Transformation Specific.

FOXO : The forkhead box O.

FSH: Follicle stimulating hormone.

GA runs: Genetic algorithms run.

GDNF: Glial cell-derived neurotrophic factor.

Gln : Glutamine.

Glu : Glutamic acid.

Gly: Glycine.

GnRH : Gonadotropin-releasing hormone.

GPCRs: G protein coupled receptors.

GPER1: G protein-coupled oestrogen receptor 1.

GPR30: G protein-coupled receptor 30.

GPR54 : The KiSS1-derived peptide receptor (the Kisspeptin receptor).

HB-EGF: Heparin-binding EGF-like growth factor.

His: Histidine.

HSD : Hydroxysteroid dehydrogenase.

ICI 182.780: Fulvestrant.

IGF: Insulin-like growth factor 1.

ICL2: Second intracellular loop.

Ile : Isoleucine.

INSL: Insulin like hormone 3.

IUHAR: International union of pharmacology.

Ki: Inhibition factor.

LBD: Ligand binding domain.

Leu : Leucine.

LH: Luteinizing hormone.

Lys : Lysine.

MAPK: Mitogen-activated protein kinases.

Met: Methionine.

MMPs: Metalloproteinase.

mRNA : Messenger ribonucleic acid.

NADPH: Nicotinamide Adenine Dinucleotide Phosphate Hydrogen.

NIH: National institutes of health.

NO: Nitric oxide.

NOS: Nitric oxide synthase.

NOX : NADPH Oxidases.

NTD: Amino terminal domain.

OT: Oxytocin.

PBS: Phosphate buffered saline.

PCBs: Polychlorinated biphenyls.

PDGF: Platelet-derived growth factor.

PFOSA: Perfluorooctanesulfonamide.

Phe: Phenylalanine.

PI3K: Phosphatidylinositol 3-kinase.

PIP3: Phosphatidylinositol 3,4,5 Trisphosphate.

PKA: Protein kinase A.

PKC: Protein kinase C.

PKG: Protein kinase G.

PLC: Phospholipase C.

PPT: Agonists selective for ERα.

PRL: Prolactin.

Pro: Proline.

PSA: Prostate-Specific Antigen.

Rh-ROCK: Rho/Rho-associated protein kinases.

Ser: Serine.

SERDs: Selective oestrogen receptor degrader.

SERMs: Selective oestrogen receptor modulators.

SF: Specific factor.

SNP: Single nucleotide polymorphism.

SP: Specifity protein.

SRC: Non-receptor cytoplasmic tyrosine kinase.

SRE: Serum response element.

SRF: Serum response factor.

T3 : Triiodothyronine.

T4 : Thyroxine.

Thr: Threonine.

TM: Transmembrane.

Trp: Tryptophan.

Tyr : Tyrosine.

US: United state.

Val : Valine.

VAP: Average path velocity.

VCL: Curvy linear velocity.

VEGF: Vascular endothelial growth factor.

YAP-TAZ : Yes-associated protein 1 (YAP) and Transcriptional coactivator.

WHO: World health organisation.

Introduction

Introduction

Male reproductive physiology is widely studied to elucidate its functional basis and physio-pathological processes, especially infertility, since it became a major concern in modern world. According to the WHO, the global prevalence of male infertility is about 9% to 15%, consequently 30% of infertility cases are induced by male factors **(Barratt *et al.,* 2017; WHO, 2023)**. Moreover, infertility can be acquired or congenital **(Ferlin *et al.,* 2007)**, and it is classified to obstructive infertility, non-obstructive infertility and coital infertility **(Naz *et al.,* 2017)**. Actually, infertility is caused by several factors such as: hormonal imbalance, genetic disorders, testicular dysfunctions, malnutrition, cancer as well as exposure to heat and toxic substances **(Cocuzza *et al.*, 2013; Harlev *et al.*, 2015; Rodprasert *et al.*, 2023)**, yet 45% of infertility is due to unknown reasons (idiopathic infertility) **(Bracke *et al.,* 2018)**.

Meanwhile, the male reproductive physiology is under control of hypothalamic-pituitary-testis axis and mainly by androgen **(Patrão *et al.*, 2009b)**, which is systematically or locally synthetised by Leydig cells. In the same manner, oestrogens play key roles in male reproductive physiology, as they were ubiquitously found in the testis and the genital tract **(Hess & Cooke, 2018; Hess *et al.*, 2021)**, also oestrogen deficiency can cause fertility problems **(Schweikert, 2019)**. In addition, oestrogens exert their effects through the classical nuclear receptors ESRs (ESR1 and ESR2) responsible for genomic response **(Heldring *et al.,* 2007b)**, as well as through the new membrane receptor GPER1, that prompts rapid non genomic response **(Prossnitz *et al.*, 2007b; Prossnitz & Barton, 2023)**.

Nevertheless, GPER1 real function in the male reproductive system still poorly studied, in contrast to its role in other systems. In fact, GPER1 is involved in regulating cardiovascular, pulmonary, nervous, kidney and digestive functions as well as metabolism and immune response **(Meyer *et al.*, 2011b; Sharma *et al.*, 2013; Rodenas & Cabas, 2017a; Tsai *et al.*, 2018; Ahmadian *et al.*, 2020; Zheng *et al.*, 2020; de Souza *et al.*, 2021; Gohar *et al.*, 2021;**

Prossnitz & Barton, 2023). Moreover, GPER1 was reported to be responsible for different types of carcinogenesis, including: breast **(Scaling *et al.,* 2014)**, ovaries **(Smith *et al.,* 2009)**, pancreas **(Natale *et al.,* 2020)**, prostate **(Chan *et al.,* 2010)**, endometrium **(Vivacqua *et al.*, 2006b; Bubb *et al.*, 2022)**, colon **(Gilligan *et al.,* 2017)**, lung **(Liu *et al.,* 2019)**, and thyroid cancers **(Vivacqua *et al.,* 2006a)**.

Studying reproductive physiology in free-ranging animals can be an effective approach to elucidate human reproductive physiology, especially in animals having seasonal reproductive cycle. Interestingly, the gerbil *Gerbillus gerbillus*, a nocturnal rodent from north Africa **(Thomas, 1902)**, exerts seasonal reproductive pattern characterised by breeding season from winter to spring and resting season from spring to autumn **(Zaina Amirat *et al.,* 1977)**. While the sand rat *Psammomys obesus*, a diurnal rodent, breeds from autumn through early spring and rests from late spring through summer **(Khammar, 1987)**. In the same species, previous studies reported seasonal variations of the reproductive system, marked by cytological, histological, and biochemical remodelling of the reproductive system simultaneously with hormonal fluctuations **(Klein *et al.*, 1975; Zaina Amirat et al., 1977; Khammar & Brudieux, 1987; Gernigon-Spychalowicz, 1992a; Belhocine & Gernigon-Spychalowicz, 1994; Boufermes *et al.*, 2014)**.

However, given the lack of information regarding free-ranging animals and their adaptations to environmental shifts as well as the obvious implication of the GPER1 in male reproductive physiology, there is still work need to be done to fully clarify these aspects. For these purposes, our study was conducted to investigate the localisation of the GPER1 in the reproductive system of the sand rat *Psammomys obesus* and the gerbil *Gerbillus gerbillus* during the reproductive cycle, subsequently elucidating the impact of the seasonal fluctuations on the estrogenic regulation of reproductive physiology via the GPER1.

Furthermore, our work was completed with *in silico* study to define the chemicals that have possible interactions with the GPER1. Hence, we tested endocrine disrupting chemicals and chemotherapeutic substances to prove their ability to modulate GPER1, which may explain the carcinogenesis induced by these chemicals via GPER1. Then, we examined the interactions with phytochemicals that could be used to treat some kinds of pathologies such as cancer and infertility, by targeting the GPER1. Finally, we compared the results with those of the known ligands which were set as reference compounds.

Literature Review

Chapter I: Male reproductive system

The male reproductive system is the set of organs that are responsible for the reproduction process under the strict regulation of the endocrine and nervous systems to produce a very well functional gamete (spermatozoa). Some of these organs are located outside the body and other in the pelvis.

A. Structural organisation of the male reproductive system

The male reproductive system is divided into three main parts: gonads (testis), genital tract and accessory glands **(Figure 01)**.

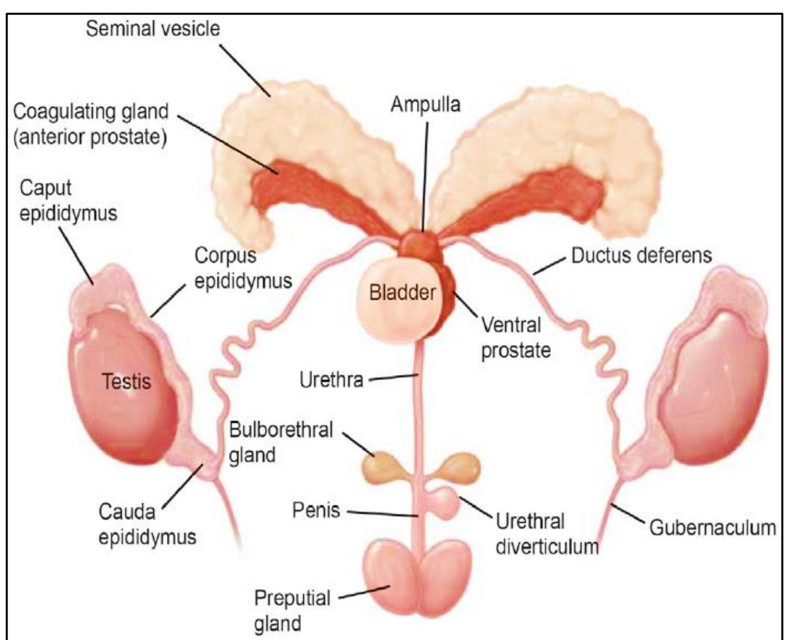


Figure 01: Anatomy of the male reproductive system. Source: Adapted from Anatomy of a Laboratory Mouse **(Cook, 1965)**.

A.1. The testis: The two testicles are oval shaped organs, housed within the scrotum, primarily responsible for spermatogenesis, but also have an endocrine and exocrine functions **(Jiménez-Reina *et al.,* 2016)**. They are covered by thick capsule composed of three layers: tunica vaginalis with two layers (external parietal layer and internal visceral) and tunica albuginea **(Figure 02, Panel A)**. These layers are mainly made up of connective tissue **(Steger**

& Weidner, 2011). The extending of tunica albuginea (septa of the testis) inside the testicular parenchyma forms many lobulus testis, each one contains 1–4 convoluted seminiferous tubules of 150–250 µm diameter and 30–80 cm long, bounded by peritubular tissue (lamina propria). These tubules gather to make straight tubules named *tubuli recti* connected to the *rete testis* **(Steger & Weidner, 2011)**.

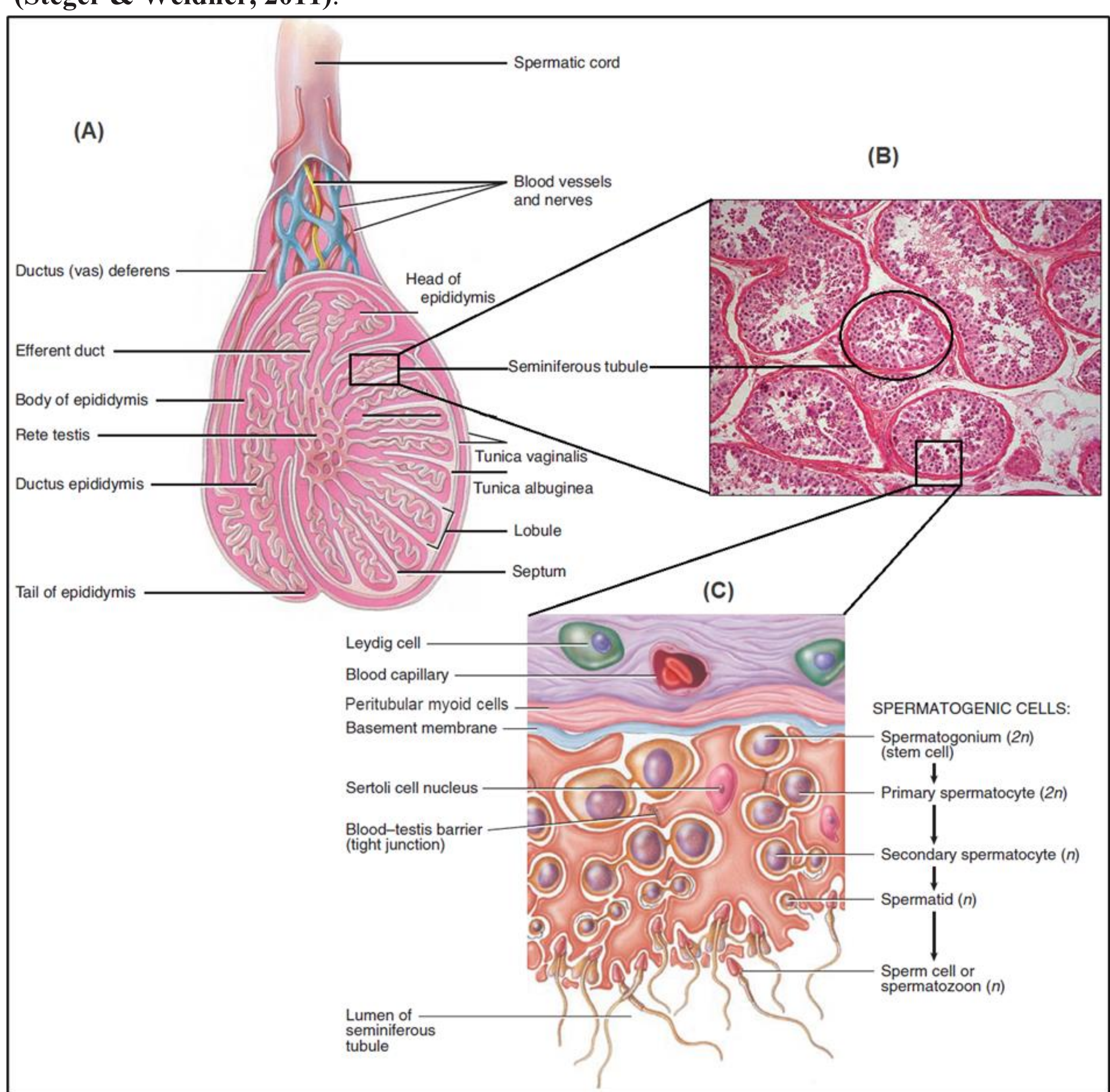


Figure 02: Anatomy and histology of the testis. Adapted from Principles of anatomy and physiology **(Tortora & Derrickson, 2014)**

The stratified epithelium lining the seminiferous tubules is built of two types of cells **(Figure 02, Panel B):**

Sertoli cells: Columnar shaped cells with basal nuclei, extend from the basal lamina of the seminiferous tubules towards the lumen **(Hess & Vogl, 2015)**. Neighbouring Sertoli cells and germinal cells are attached with different types of junctions (Zonula Occludens, zonula adherens and zonula communicans) to form blood-testis barrier, which has a role in regulating the exchange of large proteins and certain xenobiotics between blood and seminal fluid in the seminiferous tubules **(Plöen & Setchell, 1992; Mruk & Cheng, 2015)**.

Sertoli cells play pivotal role in the testis **(Johnson *et al.,* 2015)**, they:

- Support germinal cells, provide nutrient, growth factors, control spermatogenesis and release sperm in the lumen (spermiation).
- Eliminate residual cytoplasm and deteriorated germ cells.
- Produce and secret testicular fluid, inhibin and ABP (Androgen binding protein).

Furthermore, Sertoli cells express several types of hormone receptors like those of: FSH, androgens, oestrogens and thyroid hormones, thus its functions are regulated by these hormones **(Lara *et al.,* 2018)**.

Germ cells: Between the Sertoli cells, align germ cells at different differentiation stages and organized accordingly from the basement membrane to the lumen: spermatogonia, spermatocytes, spermatids, and spermatozoa **(Lew *et al.,* 2022)**.

The peritubular tissue or lamina propria is formed of connective tissue filled with blood and lymphatic vessels, nerves, as well as fibroblasts **(Figure 02, Panel C)**. We can also find:

Peritubular myoid cells: Type of smooth muscle cells that border the seminiferous epithelium and contract to transport the immobile sperm. They express several types of proteins such as: growth factor receptors that bind to Endothelins (ETs), vasopressin, and platelet-derived growth factor beta (PDGFβ) **(Thompson *et al.,* 2018)**.

Leydig cells: Could be found singly or in clusters, they have a round nucleus, an abundant network of smooth endoplasmic reticulum, mitochondria with tubulovesicular cristae, Golgi

apparatus and several large lipid droplets **(Haider, 2004)**. Under LH control, these cells are mainly involved in testosterone secretion to regulate spermatogenesis and are implicated in various endocrine and anabolic effects systemically **(Teerds & Huhtaniemi, 2015)**. In addition, during foetal life Leydig cells secrete the INSL3 hormone (Insulin like hormone 3) involved in testicular descent during foetal life **(Ivell *et al.,* 2017)**.

A.2. The genital tract: Also named sperm ducts **(Figure 02 Panel A)**, transport sperm form the testis to the outside of the body:

Efferent ducts: Vasa efferentia are small, convoluted tubules between the rete testis and the epididymis **(Ilio & Hess, 1994)**. Their number varies between 1 and 33 ductules **(Hess, 2018a)**, and their columnar epithelium is formed of several cell types such as:

- **Ciliated cells:** Represent up to 80% of the epithelium, and characterised by the presence of motile cilia responsible for stirring and homogenising the luminal fluids so it can be reabsorbed by the non-ciliated cells **(Hess, 2014)**. Ciliated cells have apical mitochondria to energetically support the motile cilia, a nucleus and basal bodies maintaining the cilia **(Hess, 2018b)**, Ciliated cells beatings serve to maintain sperm in suspension, prevent sperm aggregation and agglutination **(Yuan *et al.*, 2019; Aprea *et al.*, 2021; Hoque *et al.*, 2022)** and facilitate reabsorption of luminal fluid **(Rosenfeld, 2019)**.
- **Non ciliated cells**: They are mainly involved in fluid reabsorption **(Clulow *et al.,* 1998)**, they have basal nuclei, large lipid droplets in the cytoplasm and a brush of microvilli in the apical cytoplasm **(Hermo *et al.,* 1994)**. This cell type is believed to have an apocrine secretion **(Hermo & Jacks, 2002)**.

Other cell types could be found in some species such as: basal cells and intraepithelial lymphocytes or macrophages **(Hess, 2002)**. The ducts epithelium lies on a basement membrane made of three layers of smooth muscle cells that contract to pulse sperm to the epididymis **(Ilio & Hess, 1994)**. Efferent ducts, using the non-ciliated cell, reabsorb tubular fluid which makes

sperm 28 times concentrated **(Clulow *et al.,* 1998)**. This implicates water and ion transport, endocytosis, and secretion **(Hansen *et al.,* 1999)**.

Epididymis: The epididymis is a single convoluted duct links the efferent ducts and the vas deferens, it is divided into three main regions: Caput, corpus, and cauda epididymis **(Figure 03) (Srivastav *et al.,* 2004)**. The pseudostratified epithelium of the epididymis is built of several cell types, **(Figure 04)**, including:

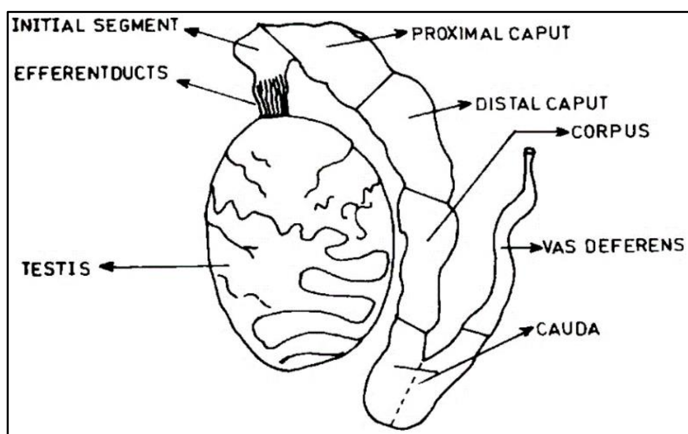


Figure 03: Anatomy of the epididymis **(Srivastav *et al.,* 2004)**.

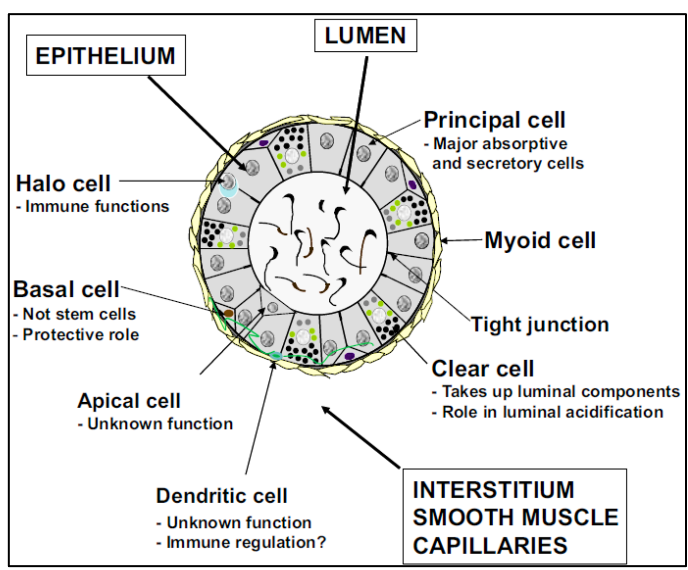


Figure 04: Schematic representation of the epididymal histology **(Robaire & Hinton, 2015b)**.

- **Principal cells:** Large prismatic cells which are the most abundant cell and constitute 65-80% of epididymal epithelium **(Hamilton, 1975)**. They synthetise many proteins that are

either secreted into the epididymal lumen or remain in the cell, they also play a role in endocytosis of luminal proteins and epididymal fluid reabsorption **(Cooper, 2007)**. These cells are attached to each other with tight junctions and desmosomes that form the blood-epididymal barrier **(Cornwall, 2009; James *et al.*, 2020)**.

- **Basal cells:** They represent about 10 to 20% of the total epididymal cell population **(Hamilton, 1975)**. These elongated cells lie on the basement membrane and adhere to the neighbouring principal cells by desmosomes, they also regulate principal cells and other cells using paracrine factors **(Hermo & Jacks, 2002)**. Moreover, Basal cells express apolipoprotein E and alcohol dehydrogenase, which have a role in the regulation of electrolytes and water transport by principal cells **(Leung *et al.,* 2004)**.

- **Apical cells:** Named so because of the apical localisation of the nucleus **(Robaire & Hermo, 1988)**. These cells have dense cytoplasm, very rich in mitochondria, contains lysosomes and carbonic anhydrase involved in H^+ ions secretion and bicarbonates (HCO_3^-) reabsorption **(Pietrement *et al.,* 2006)**. Thus, these cells contribute to the acidification of the epididymal fluid **(Cornwall, 2009)**, they also contain several proteolytic enzymes **(Serre & Robaire, 1998)**.

- **Narrow cells:** these cells are narrower than Principal cells. Their aim role is to protect spermatozoa from harmful luminal environments, by degenerating proteins and carbohydrate using carbonic anhydrase and their lysosomes **(Adamali & Hermo, 1996)**.

- **Halo cells:** These small cells with a narrow clear cytoplasm are present throughout the epididymal epithelium, usually in the basement **(Robaire & Hermo, 1988)**. Halo cells have been designated as lymphocytes **(Robaire & Hermo, 1988)** or monocytes **(Hamilton, 1975)**.

- **Clear cells:** These large prismatic cells could be found in corpus and cauda epididymis **(Abou-Haïla & Fain-Maurel, 1984)**. They are distinguished by the presence of several clear vesicles in the apical region, lysosomes in the middle region and numerous lipid inclusions in

their basal region **(Soranzo *et al.,* 1982)**. Clear cells have a more important endocytosis role than principal cells, since they eliminate the cytoplasmic droplets left by spermatozoa **(Robaire & Hermo, 1988)**. Together with the narrow cells, they contribute to the acidification of luminal fluid using carbonic anhydrase **(Robaire & Hinton, 2015a)**.

The epididymis plays several key roles such as:

- **Acquisition of mobility:** As sperm passes the epididymal tubule, spermatozoa gain the ability to move progressively and swim in the female genital tract **(Cornwall, 2018)**. This process requires different mechanism such us: increasing intracellular energy and cAMP **(Vadnais *et al.,* 2014)**.
- **Membrane modification:** Studies reported important modifications in protein, sugar, and lipid found in sperm membrane and acrosome **(Guyonnet *et al.*, 2012; Labas *et al.*, 2015)**. These changes can be a result of protein redistribution or suppression after proteolytic processes **(Métayer *et al.,* 2002)**, or insertion of newly synthesized proteins **(Cooper, 2007)**.
- **Sperm maturation and acquisition of fertilizing ability:** The cytoplasmic droplets associated with spermatozoa migrate from the head toward the tail to be released **(Cooper, 2007)**. Besides, spermatozoa receive several types of molecules (proteins, lipids, and mRNAs), required for sperm maturation, carried by the extracellular vesicles, micro vesicles (epididymosome) and exosomes **(Sullivan *et al.*, 2005; Rejraji *et al.*, 2006)**. Fertilizing ability can be achieved by gaining the capacity to phosphorylate protein tyrosine, bind to the oocyte (zone pellucida), lunch the acrosomal reaction, enter the oocyte and fecundate it **(Gervasi & Visconti, 2017)**.
- **Sperm storage:** The epididymis is the major site of sperm storage in mammals, where sperm can be kept in quiescent state up to 30 days **(Orgebin-Crist, 1967)**. This process is facilitated by increasing epididymal fluid viscosity using a protein named immobilin, which

make sperm immobile **(Usselman & Cone, 1983)** and reducing pH in the environment **(Acott & Carr, 1984)**.

Epididymal functions are under control of distinct factors. Androgen and oestrogen seem to be the main regulators as their receptors are widely expressed in the epididymal epithelium **(Zhou *et al.*, 2002; Pearl *et al.*, 2007)**. Several other hormones are also involved, such as: FSH **(Dahia *et al.*, 2006)**, corticoids and T3/T4 **(Cornwall, 2009)**. In addition, the secretion of epididymal fluid is modulated by different neuroendocrine factors including Atrial Natriuretic Peptide (ANP), Activin, Follistatin and the β subunit of Inhibin, Oxytocin (OT) and Prolactin (PRL), Serotonin, Bradykinin, Angiotensin and Endothelin **(Cornwall, 2018)**.

Ductus (Vas) Deferens: It comes after the epididymis and has columnar pseudostratified epithelium surrounded by three muscular layers and lies on lamina propria composed of collagen and elastic fibres **(Flannigan & Goldstein, 2018)**. This canal could be divided to five segments: scrotal, funicular, inguinal, iliac, and pelvic. Vas deferens main function is secreting a fluid to ease sperm transit through the canal with the assistance of the contraction of the smooth muscle cells. Sperm can be stored in this canal for months, where dead or unejaculated sperm is eliminated **(Flannigan & Goldstein, 2018)**.

A.3. The accessory glands

The seminal vesicle: The two seminal vesicles are coiled and curved glands, found behind the bladder, above the distal ureter and behind the rectum. Their dimensions differ depending on the species, in human they are up to 50 mm long, 10 mm wide, and 10 mm thick **(Jiménez-Reina *et al.*, 2016)**. Their pseudostratified columnar epithelium contains two main cells: Principal cells with abundant mitochondria, secretion vesicles, lipofuscin granules, lipid droplets and a well-developed rough endoplasmic reticulum important for protein secretion, while basal cells are stem cells, all lied on lamina propria of connective tissue **(Lew *et al.*, 2022)**. This epithelium lies on a basement membrane and supported by two layers of smooth

muscle cells. Seminal vesicle main role is to make seminal fluid as an adequate environment for the viability and functionality of sperm **(Bromfield *et al.,* 2018)**. Thus, this fluid is thick, viscous, and alkaline, and it is rich in energetic substrates, minerals and ions, various proteins and amino acids, enzymes, hormones, vitamins, buffering agents and antioxidants. The functions of seminal vesicle are essentially controlled by testosterone **(Schjenken *et al.,* 2018)**.

Cowper glands: Also named bulbourethral gland, they are pea-sized glands situated upper side of the urethral bulb. They are composed of lobules with tubuloalveolar glands and a central excretory duct ending up in the spongy urethra **(Lew *et al.,* 2022)**. The acini are formed of cuboidal cells specialized in mucus secretion surrounded by smooth muscle cells, while the excretory duct is lined with pseudostratified epithelium and wrapped with smooth muscle cells. The intratubular tissue contains connective tissue, skeletal muscle fibres **(Prins & Lindgren, 2015)**. The Cowper's gland contributes to urethral lubrication and sperm coagulation **(Whitney, 2018)**. In addition, they are involved in the immune defence of the genitourinary system and secrete numerous glycoproteins, including PSA **(Chen, 2023)**.

The Prostate: It is located at the base of the bladder and surrounds the urethra. Its epithelium is composed of three types of cells: Luminal secretory cells, basal cells, and neuroendocrine cells **(Gauntner & Prins, 2018)**. All underlined by a basement membrane, bordered with smooth muscle cells and fibroblasts **(Sasaki *et al.,* 2018)**. The prostate secrete a slightly acidic fluid (pH around 6.5), which contains several substances such as: (1) citric acid: used by spermatozoa to produce ATP, (2) several proteolytic enzymes, such as prostate specific antigen (PSA), pepsinogen, lysozyme, amylase and hyaluronidase, (3) acid phosphatase, (4) seminal plasmin which is an antibiotic **(Gauntner & Prins, 2018)**.

B. Male reproductive physiology

B.1. Spermatogenesis

Spermatogenesis is a very organized phenomenon that takes place in the seminiferous epithelium and is arranged by Sertoli cells **(Griswold, 2015)**. This physiological phenomenon involves several cellular processes such: multiplication, differentiation, and apoptosis to produce spermatozoa, haploid cells, from spermatogonia, diploid germinal stem cells **(Creasy & Chapin, 2018)**. Spermatogenesis can be divided into three phases, according to the morphological and functional features of the germ cells **(Figure 05)**:

• **Proliferative phase:** At this stage, spermatogonia give, by mitotic division, two cells: spermatogonia type A and spermatogonia type B. Spermatogonia type A remains near the basal lamina to preserve the germ line, while spermatogonia B moves towards the tubular lumen to give, by mitosis, spermatocyte I (preleptotene- leptotene- zygotene- diplotene) **(Waqas, 2021)**.

• **Meiotic phase:** Each spermatocyte I undergoes meiosis I to form two haploid spermatocyte II cells which quickly enter meiosis II to give four round spermatids **(Waqas, 2021)**.

• **Differentiation phase (spermiogenesis):** The longest process in spermatogenesis, aimed to morphologically and functionally transform haploid spermatids, into spermatozoa **(Lara *et al.,* 2018)**. During this phase, we notice: (a) organelle biogenesis; (b) nuclear chromatin compaction; (c) acrosomogenesis; (d) flagellogenesis; (e) establishment of ectoplasmic specializations between Sertoli cells and spermatids; (f) removal of residual body by spermatid; and (g) liberation of spermatozoa (spermiation). Considering the delicate and complex process of spermiogenesis, numerous key genes/proteins specifically intervene in this phase **(O'Donnell & Stanton, 2018)**.

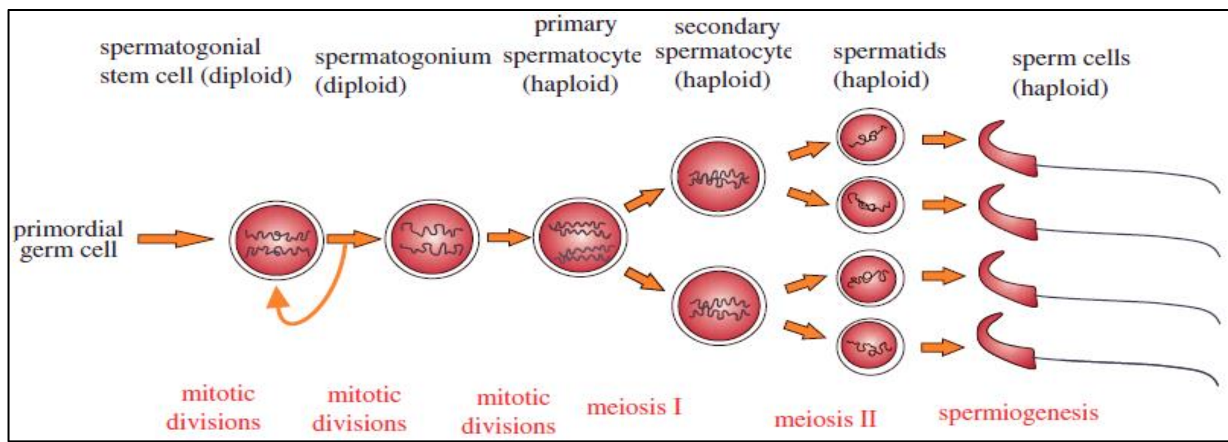


Figure 05: Schematic illustration of spermatogenesis **(Cheng & Mruk, 2010)**.

B.2. Regulation of male reproductive function

The physiology of reproduction in males is under the endocrine control of the hypothalamic-pituitary testis axis, and the interactions between different actors such as Sertoli cells and Leydig cells **(Kretser, 2018)**. Furthermore, the follicle-stimulating hormone (FSH) stimulates Sertoli cells to secrete an androgen-binding transport protein: ABP **(Kretser, 2018)**. In addition, Leydig cells secrete testosterone, which will act on Sertoli cells to maintain spermatogenesis, and inhibits germ cell apoptosis **(Huhtaniemi, 2018)**.

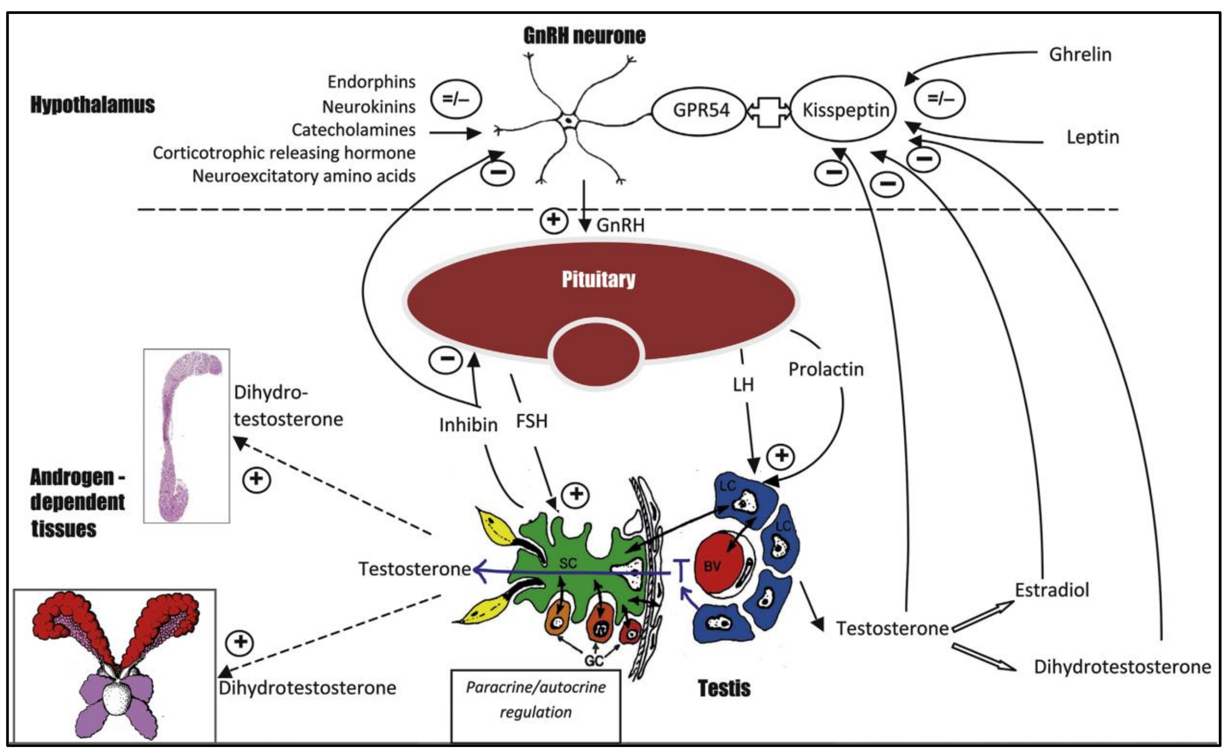


Figure 06: Regulation of testis functions **(Creasy & Chapin, 2013)**.

Moreover, epididymal functions are regulated by androgens **(Robaire & Hamzeh, 2011)**, oestrogens **(Joseph *et al.,* 2011)**, as well as by several other hormones such as: Atrial Natriuretic Peptide (ANP), Activin, Follistatin and the β subunit of Inhibin, Oxytocin (OT) and Prolactin (PRL) **(Lara et al., 2018; Carlson, 2019)**.

The paraventricular nucleus of the hypothalamus secretes GnRH, to prompt the pituitary gland to release FSH and LH **(Figure 06) (YOUNG *et al.,* 1983)**. Firstly, FSH regulates Sertoli cells function such as releasing inhibin **(Bilezikjian *et al.,* 2004)**. Secondly, LH acts on Leydig cells to produce testosterone, where Leydig cell sensitivity to LH, increases with prolactin from the pituitary gland **(Wetsel *et al.,* 1992)**. Testosterone is not only vital for spermatogenesis, but also has systematic role, found in seminal fluid. Oestradiol, formed by converting testosterone using aromatase is important in male reproductive physiology, while dihydrotestosterone (DHT) made by 5α-reductase mainly regulates the accessory sex organ functions **(Longcope *et al.,* 1969; Russell & Wilson, 1994)**. Inhibin, testosterone, oestradiol and dihydrotestosterone have negative feedback on GnRH, FSH and LH secretion by the hypothalamus and the pituitary gland respectively **(Corradi *et al.,* 2016)**. GnRH neurons, express GPR54 and they are modulated by different afference such as: energy balance and stress. The kisspeptin also controls leptin, ghrelin, and steroids action on GnRH neurons **(Rønnekleiv & Kelly, 2013)**. Furthermore, different cells in the testis have autocrine and paracrine communication to modulate adjacent cells and regulate testis function **(Creasy & Chapin, 2018)**.

Chapter II: G protein coupled oestrogen receptor 1

Male reproductive physiology is under control of different factors, among them oestrogen, that could be synthetise in reproductive system and acts via its nuclear receptors (ESRs) or via GPER1 **(Hess et al., 2021)**. Oestrogen importance in male reproduction has been widely reported, where lack of oestrogen secretion or oestrogen receptor inactivation cause fertility problems, disease, and cancer **(Matsuyama & DeFalco, 2024)**.

A. Oestrogen

Oestrogen is a steroid hormone, initially considered as a female hormone. Even though was found in the stallion's urine **(Zondek, 1934)**, it was until late 1960s and early 1970s that male production of oestrogen became evident, and numerous research groups became interested in studying oestrogen in the male of several species .

A.1. Biosynthesis and localisation: As a steroid hormone, oestrogen is biosynthesised by the conversion of cholesterol which is translocated into the mitochondria to make four types of oestrogen: estrone (E1), 17b-estradiol (E2), estriol (E3), and estetrol (E4) **(Figure 07)**. First, the cholesterol's side chain is cleaved by cytochrome P450 enzyme (CYP11A1 gene) to form pregnenolone **(Belfiore *et al.,* 1994)**. Then, CYP17A1 and 3β-HSD enzymes convert pregnenolone to androstenedione **(Simpson *et al.,* 2005)**. The aromatisation of androstenedione by the enzyme CYP19A1 makes estrone which is turned into oestradiol using 17β-HSD **(Cui *et al.,* 2013)**. According to the site of production, oestrogen biosynthesis can follow different mechanisms. As oestrogen can be produced in small quantities in different tissue such as: adipose, breast, bone, smooth muscle and brain tissues, liver, ovaries, pancreas, adrenal gland, and vascular endothelium **(Ryan, 1959; Simpson et al., 2005; Barakat *et al.*, 2016)**, during pregnancy, placenta also makes oestrogen **(Barakat *et al.,* 2016)**. In the male, testosterone can be metabolized to oestradiol under the action of P450 aromatase **(Basu, 2011)**, which is found

in several types of cells such as: Sertoli cells, Leydig cells, germ cells, epididymal cells and efferent ducts cells **(Cooke *et al.*, 2017)**.

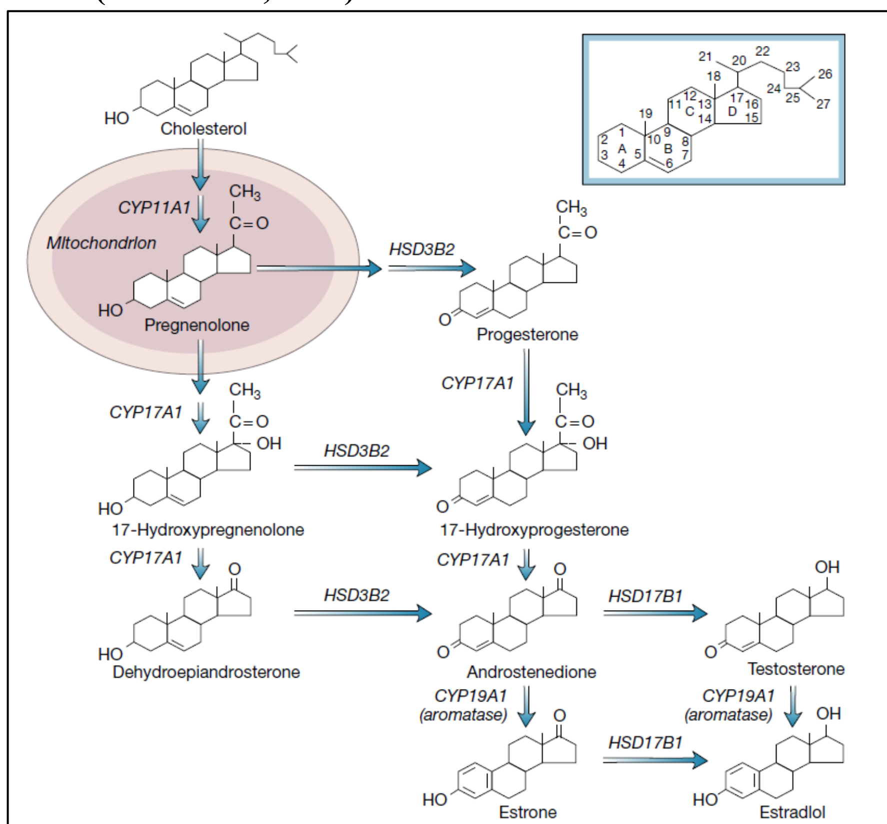


Figure 07: The oestrogen biosynthetic pathway **(Samavat & Kurzer, 2015)**.

A.2. Oestrogen receptors: Oestrogen receptors are composed of 4 domains (A/B, C, D, and E/F) **(Figure 08)**. ERα is encoded by ESR1 on chromosome 6 (6q25.1) and made of 595 amino acids with molecular mass of 66 kDa, while ERβ is encoded by ESR2 on chromosome 14 (14q23.2) and made of 530 amino acids with molecular mass of 59 kDa **(Gosden *et al.*, 1986; Enmark *et al.*, 1997)**. The A/B domain (N terminal domain) is responsible for gene transcription transactivation using the transcriptional activation function (AF)-1, and present 16% similarity between ERα and ERβ. The C domain (DNA-binding domain DBD) engages in ERs dimerization and DNA binding ability. ERα and ERβ have 97% analogy in the DBD domain **(Truss & Beato, 1993)**. The D domain (the hinge region) links the C domain and the E domain, it has the nuclear localization signal, and binds to the heat shock proteins **(Tora *et al.*, 1989)**. The E/F (ligand binding domain LBD) in the C terminal region

contains the activation function 2. A resemblance of 59% is documented between ERα and ERβ in this domain **(Kumar *et al.*, 2011)**.

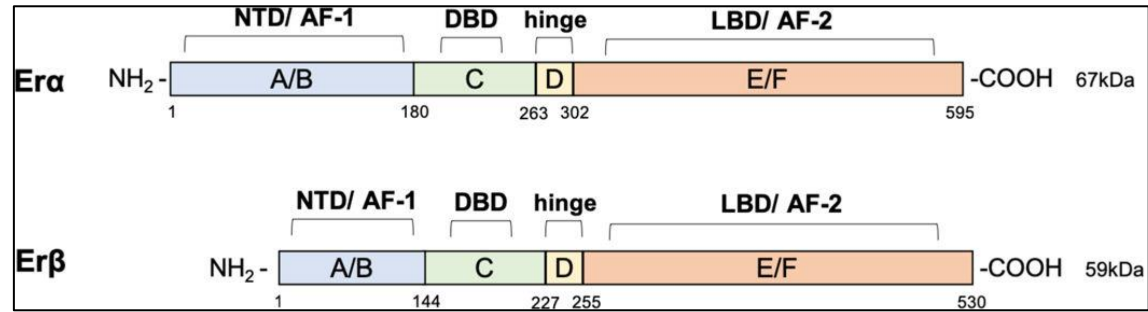


Figure 08: Structure of classical oestrogen receptors ESRs **(Fuentes & Silveyra, 2019)**.

A.3. Ligands: Besides oestrogens, several other types of molecules can bind and exhibit higher affinity to ESRs, thus prompt different signalisation cascades. These molecules can be agonists (activate ESRs) or antagonists (block ESRs). Ligands biding to ESRs can be divided into six types:

- Endoestrogens: oestradiol, estriol, estretrol, and estrone that are naturally produced **(Farooq, 2015)**.
- Phytoestrogens: These non-steroidal molecules made by plants can be categorized into three classes isoflavones (genistein, daidzein, formononetin, glycitein), coumestans (coumestrol, repensol, trifoliol), and lignans (matairesinol, pinoresinol, secoisolariciresinol, podophyllotoxin, steganacin) **(Basu & Maier, 2018)**.
- Xenoestrogens: These synthetic chemicals can be divided into five groups: medicinal drugs (diethylstilbestrol, ethinyl oestradiol), food additives (butylated hydroxyanisole, erythrosine), body cosmetics (4-methylbenzylidene camphor, methylparaben, ethylparaben, propylparaben), environmental pesticides (atrazine, dichlorodiphenyldichloroethylene, dichlorodiphenyltrichloroethane, methoxychlor, dieldrin, endosulfan, heptachlor, lindane), and industrial chemicals (bisphenol A, nonylphenol, monochlorobiphenyl and dichlorobiphenyl, di-2-ethylhexyl phthalate, diisodecyl phthalate, diisononyl phthalate) **(Farooq, 2015)**.

- The selective oestrogen receptor modulators (SERMs): these non-natural chemicals act as agonists of the ESRs in a specific tissue and as antagonists in other tissues **(Shang & Brown, 2002; Martinkovich *et al.*, 2014)**. The most known SERMs are tamoxifen, clomifene, toremifene, raloxifene, ormeloxifene.

- The selective oestrogen receptors degraders (SERDs): these chemicals antagonise ESRs and inhibit the signalling pathway. The most common SERDs are: Fulvestrant, imlunestrant, elacestrant **(Lee *et al.,* 2017)**.

- Metalloestrogens: these inorganic chemicals are heavy metals that includes aluminium, barium, cadmium, chromium, cobalt, copper, lead, mercury, nickel, arsenite, selenite, and vanadate **(Farooq, 2015)**.

A.4. Signalisation: Oestrogens penetrate intracellularly via the plasma membrane to exert their effects, by activating the nuclear receptors ERα and ERβ. Oestrogen signalling pathway via the classical nuclear receptors is called genomic response and can be divided into direct genomic response, indirect genomic response, and Ligand-independent signalling pathway **(Figure 09) (Fuentes & Silveyra, 2019)**. During the direct genomic response, oestrogen binding to ERs provokes receptor dimerization **(Le Dily & Beato, 2018)** and translocation into the nucleus. Then, the DBD domain of the receptor binds to ERE (oestrogen responsive element) region of the chromatin and regulates the transcription of the target genes **(Klinge, 2001)**. While in the indirect genomic response ER-oestrogen complex interacts with ERE via protein-protein interactions, where ERs bind to transcription factors, such as activator protein 1 (AP-1) or specificity protein 1 (SP-1) and recruit coregulators to controls gene expression **(Cheung *et al.*, 2005)**. Recent studies demonstrated that IGF1 activation leads to ER-mediated genomic response independent of oestrogen and described as ligand-independent signalling pathway **(Hewitt & Korach, 2018)**.

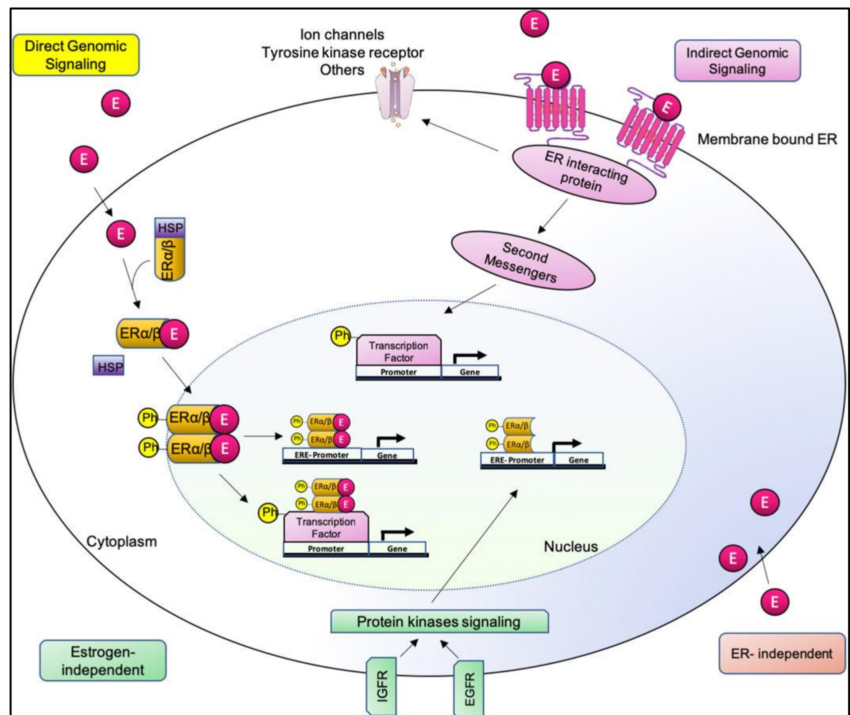


Figure 09: Mechanisms of nuclear oestrogen receptor pathways **(Fuentes & Silveyra, 2019)**.

A.5. Effects: ESRs expression has been widely investigated in male as in female. In male, ERs were found in the brain, liver, muscle, and kidney **(Mester & Baulieu, 1972; LI *et al.*, 1974; Dubé *et al.*, 1976)**. Oestrogen is responsible for bone maintenance, cholesterol metabolism, brain function, inflammation, breast, and reproductive organs development **(Liang & Shang, 2013)**. Furthermore, the broad expression of ERs all over the male reproductive tissue, is a clear evidence of its importance in maintaining male fertility, hence, oestrogen deficiency in male engenders sperm alteration, decreased fertility and sexual performance **(Schulster *et al.,* 2016)**.

Oestrogen is also involved in various physio pathological processes such as: Breast, lung, pancreatic, ovarian, and prostate cancers, cutaneous melanoma, bone, urogenital tract, liver, intestinal, gastric, oesophageal, cardiovascular, and neurodegenerative diseases, as well as endometriosis **(Chen *et al.,* 2022)**.

B. GPER1

Oestrogen, as a steroid hormone, acts via its classical receptors (ESR1 and ESR2) that belong to the nuclear receptor superfamily and trigger non rapid genomic response to regulate gene expression **(Heldring *et al.,* 2007a)**. Nevertheless, it has been recently documented that oestrogen can act on membrane receptors and trigger rapid non genomic effects via the GPER1 **(Rago *et al.,* 2011)**.

B.1. History and discovery: Studies on the GPCRs were largely conducted in the late of 1990s. Hence, in 1997 a novel GPCR was cloned and identified as an orphan receptor because no ligand has been identified for it, thus given the designation of GPR30 **(Owman *et al.*, 1996; Kvingedal & Smeland, 1997)**. It was until 2000, when Filardo and colleagues demonstrated that the activation of GPR30 by oestrogen mediates ERK1/2 pathway **(Filardo *et al.,* 2000)** and generates cAMP **(Filardo, 2002)**. Afterward, studies reported that oestrogen signals and binds with high affinity to GPR30 **(Revankar *et al.,* 2005; Thomas *et al.,* 2005)**, and therefore the International Union of Pharmacology (IUPHAR) gave it the name GPER1 (G protein-coupled oestrogen receptor 1). Since then, several studies have been carried out to identify potential GPER1 selective agonists and antagonists **(Bologa *et al.,* 2006; Dennis *et al.,* 2009; Dennis *et al.,* 2011b)**.

B.2. Structure and variation: GPER1 belongs to the family of the 7-transmembrane G protein-coupled receptors and acts through heterotrimeric guanine nucleotide-binding proteins to change effector's activity **(Figure 10) (Luttrell, 2006)**. GPER1 is classified within the Rhodopsin like receptors subfamily A2 and shares an identity of 28% with the angiotensin II 1A receptor and the interleukin 8A receptor **(Carmeci *et al.,* 1997)**. As no structure is available for this receptor, bovine Rhodopsin was used as a model to build the GPER1 structure using "*in silico*" approaches **(Kim *et al.,* 2004)**. The GPER1 final model had 375 amino acids, seven transmembrane domains, three extracellular loops and three cytoplasmic intracellular loops.

The α-helices had well conserved kinks, formed by proline residue, and responsible for the structural conformation to activate the G protein effectors **(Yohannan *et al.,* 2004)**. The class of Rhodopsin like receptors subfamily A2 are characterized by the presence of sequential motif Asp-Tyr-Arg between the third transmembrane domain (TMII) and the second intracellular loop (ICL2), while the G protein binds to the 7TM receptor using the third cytoplasmic loop **(Feng & Gregor, 1997)** . Furthermore, the N terminal region (Met1- Phe60) has N-acetylated methionine residue and three potential N-glycosylation sites (Asn25, Asn32, Asn44) **(Bonini *et al.,* 1997)**.

The human GPER1 gene is located on the 7th chromosome and composed of three exons. When compared with other species, GPER gene showed 95% similarity between human, mouse, rat, and hamster **(Wang *et al.,* 2007)**. The GPER1 is well conserved in many species **(Kadokawa *et al.,* 2018)** but presents over 300 single nucleotide polymorphisms (SNPs) **(Giess *et al.,* 2010)**.

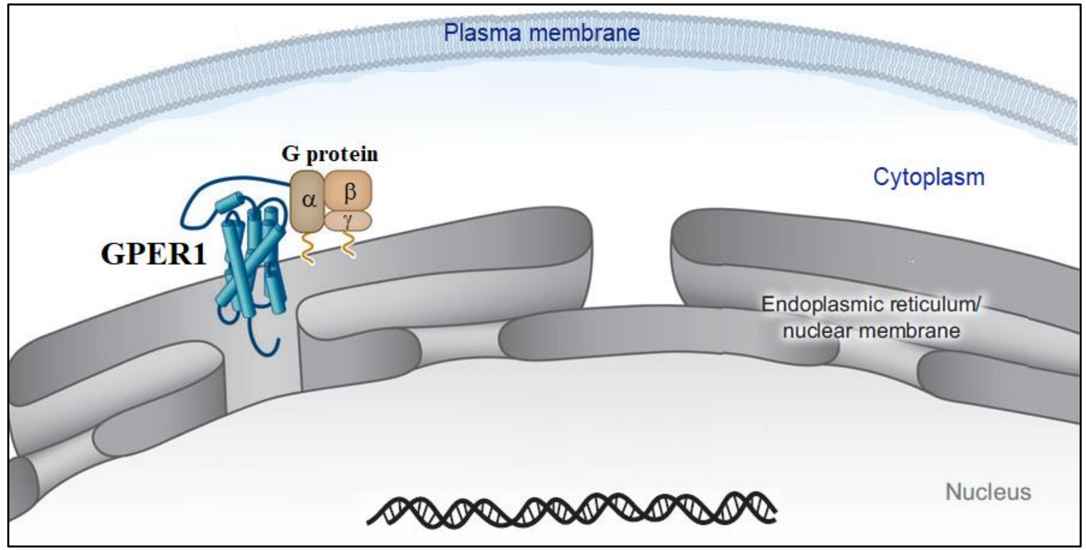


Figure 10: The G protein-coupled oestrogen receptor **Adapted from (Prossnitz *et al.,* 2008a)**.

B.3. Localisation: The subcellular localization of the GPER1 defines the signalling pathway of the GPER1, thus, the distribution patterns of this receptor have been widely investigated. As a GPCR, GPER1 is normally found in the plasma membrane **(S. B. Cheng *et***

***al.,* 2011)**, but larger amounts of it are found in the intracellular compartment such as: endoplasmic reticulum, Golgi apparatus, nuclear membrane **(Revankar *et al.,* 2005)**, mitochondria and, even more in the cytoplasm **(Ronda & Boland, 2016)**. The subcellular localization of the GPER1 depends on species, tissues, and cell types, which makes the GPER1 an atypical GPCR **(Luo & Liu, 2020)**.

B.4. Ligand: Since the description of the GPER1 as oestrogen receptor, several studies were performed to determine the possible ligands that bind to GPER using different methods **(Figure 11)**. Large amounts of ligands (agonists and antagonists) have been identified and subdivided into the following types:

- Natural compounds such as: Endoestrogen (oestradiol, estriol, estretrol, and estrone) acts as agonists **(Revankar et al., 2005; Thomas et al., 2005; Lappano *et al.*, 2010)**. Some oestrogen metabolites act as agonists (2-methoxy-oestradiol, 17β-oestradiol-17-d-glucuronide, dehydroepiandrosterone) and others act as antagonists (2-hydroxy-oestradiol, 7β-hydroxyepiandrosterone) **(Zucchetti *et al.*, 2014; Chourasia *et al.*, 2015)**. Phytoestrogens (genistein, daidzein, equol, resveratrol, oleuropein, icariin, green tea catechins, niacin, quercetin, flavonoids, chalcones, coumestans, stilbenes, lignans, ginsenosides and tetrahydrofurandiols) bind to GPER1 some of them are agonists, while others are antagonists **(Kang *et al.*, 2009; Rowlands *et al.*, 2011; Palmieri *et al.*, 2012)**. Furthermore, the mycoestrogen zearalenone binds to GPER1 and agonise it **(Chimento *et al.,* 2014)**.
- Synthetic compounds such as: Xenoestrogens (bisphenol A, PCBS, DBDPO, , methoxychlor, dieldrin, endosulfan, dimethyl phthalates, methyl parabens, acrylamide…) mainly acts as agonists **(P. Thomas & J. Dong, 2006; Albanito *et al.*, 2008; Pupo *et al.*, 2012)**. In addition, the selective oestrogen receptor modulators (SERMs) and the selective oestrogen receptor down regulators (SERDs) are mainly GPER1 agonists. Recently, highly GPER-selective ligands have been discovered and used in several studies as potential therapeutic

agents against various types of cancer, including the agonist G1 and the antagonists G15 and G36 **(Bologa *et al.,* 2006; Dennis *et al.,* 2009; Dennis *et al.,* 2011a)**.

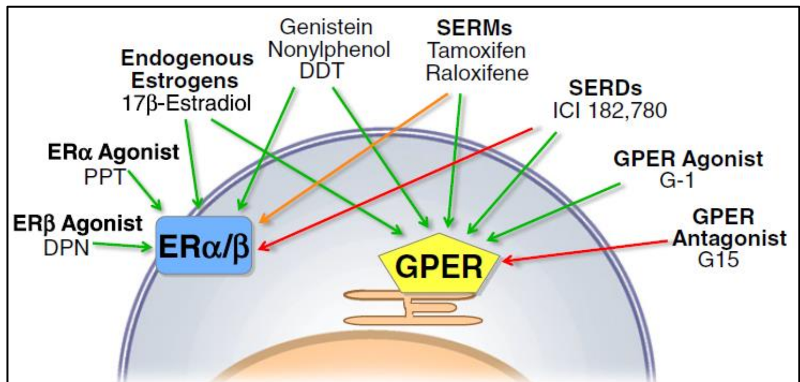


Figure 11: GPER1 modulators: agonists and antagonists. Green arrows: activation, red arrows: inhibition, and orange arrows: tissue dependent activation or inhibition **(Meyer *et al.,* 2011a)**.

B.5. Signalisation: Following ligand binding to GPER1 **(Figure 12)**, the G protein undergoes structural conformation to activate several types of enzymes such as: adenylate cyclase and phospholipase C **(Filardo *et al.,* 2002)**. The employment of adenylate cyclase increases cAMP production and activates PKA then CREB **(Sun *et al.,* 2017)**. While Phospholipase C increases calcium mobilization leading to PKC and plasma membrane calcium channel stimulation **(Sharma & Prossnitz, 2011)**. The PKC enzyme triggers Rho–ROCK pathway to activates gene expression using the YAP–TAZ transcription factors **(Zhou *et al.,* 2015)**. G protein also uses SRC to activate matrix metalloproteinases (MMPs) and cleaves proheparin-binding epidermal growth factor (HB-EGF) to release HB-EGF. The later stimulates the EGF receptor and MAPK (ERK1/2) cascade **(Filardo *et al.,* 2000)**. The MAPK acts on FOXO3 to control gene transcription **(Zekas & Prossnitz, 2015)**. Whereas Akt stimulates the l-arginine–endothelial nitric oxide synthase (NOS3)–NO–cGMP pathway, liberates nitric oxide (NO) from endothelial cells and trigger the adjacent vascular smooth muscle cells to recruit PKG **(Fredette *et al.,* 2018)**. Furthermore, GPER1 induces its effects via

the NOTCH and VEGF signalling pathway and promotes NOX1 gene expression **(De Francesco *et al.,* 2018)**.

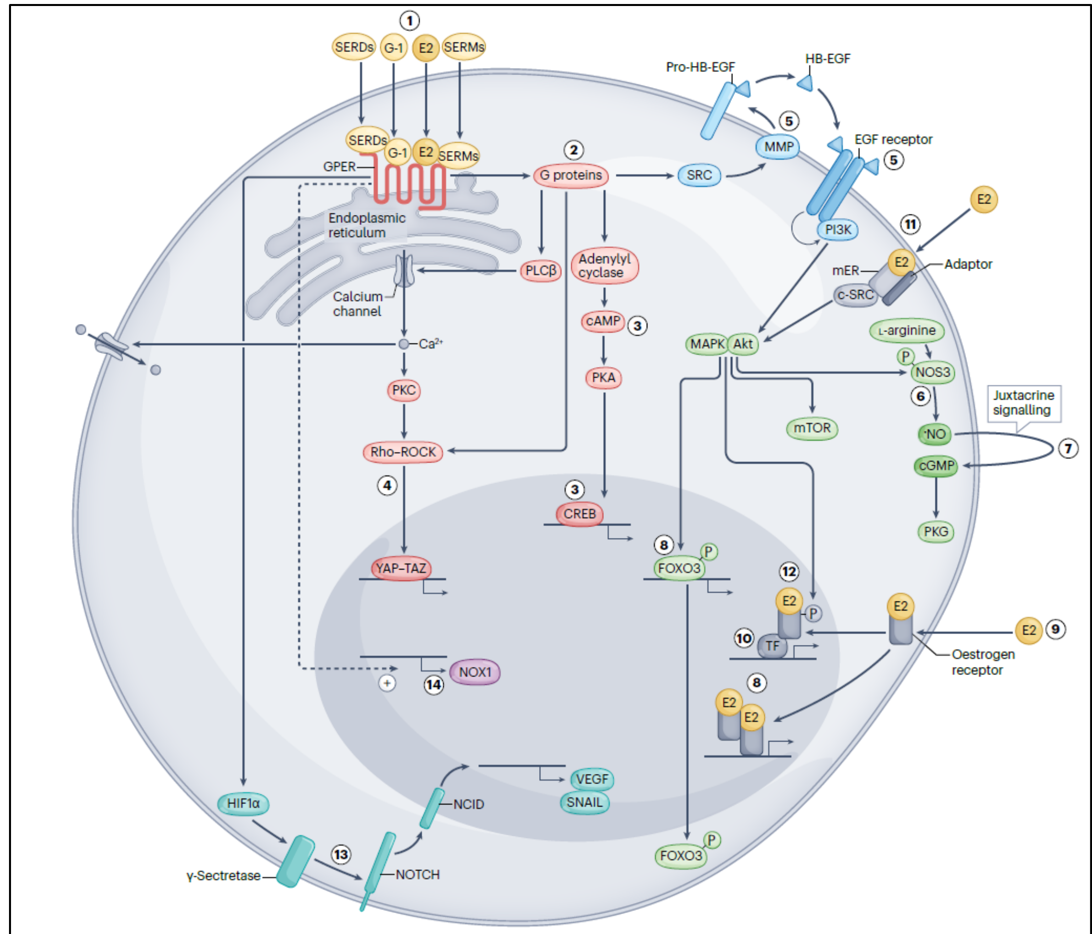


Figure 12: GPER1 and ESRs signalling pathway **(Prossnitz & Barton, 2023)**.

B.6. Physiological and physio pathological effects: GPER1 functions are mainly studied using GPER1-deficient mice, which showed different phenotype changes. In addition, using GPER1 selective ligands is also very useful to study GPER1 effects. The main findings are summarised in **(Figure 13)** and as follow:

- In the cardiovascular system, GPER1 is ubiquitously expressed, and is involved in: blood pressure regulation **(Feldman *et al.,* 2014)**, angiogenesis **(Prossnitz & Barton, 2014)**, vasodilatation **(Meyer *et al.,* 2010)**, myocardial contractility **(Whitcomb *et al.,* 2020)**, protecting the vascular system **(Yu *et al.,* 2011)**, reducing inflammation **(Meyer & Barton, 2016)**, preventing heart failure and atherosclerosis **(Prossnitz & Barton, 2011; Meyer *et al.*, 2014)**.

• GPER1 reduces cardiopulmonary injury **(Alencar *et al.*, 2017)** and ameliorates skeletal muscle performance in Pulmonary arterial hypertension disease **(Ahmadian *et al.*, 2020)**.

• GPER1 protects kidneys by controlling natriuresis **(Gohar *et al.*, 2020)**, reducing proteinuria **(Gohar *et al.*, 2021)** and tubular injury **(Meyer *et al.*, 2015)** caused by inflammation and oxidative stress **(Qiao *et al.*, 2018)**.

• The digestive system is also under control of the GPER1, which decreases oesophageal sphincter tone **(Tsai *et al.*, 2018)**, improves visceral pain and motility of large intestine **(Zielińska *et al.*, 2017)**. GPER1 also ameliorates digestive inflammatory conditions by reducing inflammation **(Jacenik *et al.*, 2019)** and protecting from gallstone formation **(DeLeon *et al.*, 2020)**.

• GPER1 exerts a significant effect on lipid **(Sharma *et al.*, 2013)** and glucose metabolism **(Mårtensson *et al.*, 2009)**, energy balance and insulin secretion **(Davis *et al.*, 2014)**. Moreover, GPER1 is important in managing obesity and associated pathologies **(Le May *et al.*, 2006; E. Haas *et al.*, 2009)**.

• GPER1 is largely found in several types of tumours such as: breast **(Scaling *et al.*, 2014)**, ovaries **(Smith *et al.*, 2009)**, pancreas **(Natale *et al.*, 2020)**, prostate **(Chan *et al.*, 2010)**, endometrium **(Vivacqua *et al.*, 2006b)**, colon **(Gilligan *et al.*, 2017)**, lung **(Liu *et al.*, 2019)**, and thyroid cancers **(Vivacqua *et al.*, 2006a)**. Nevertheless, its exact role is still not fully understood, as it promotes carcinogenesis in some cancer types, but induces cell apoptosis in other cancer types.

• In the immune system, GPER1 regulates immune cells functions **(Brunsing & Prossnitz, 2011; Rodenas & Cabas, 2017b)** and protects from infection **(Triplett *et al.*, 2019)**.

• In the nervous system, GPER1 has crucial role in psychiatric disorders (addiction, depression, and anxiety) **(Zheng et al., 2020; Wang *et al.*, 2021)** and protects nervous system from age related diseases such as: cerebral ischaemia **(Tang *et al.*, 2014)**, haemorrhagic stroke

(Peng *et al.,* 2019), Parkinson disease **(Bourque *et al.,* 2013)**, and multiple sclerosis **(Confavreux *et al.,* 1998)**, in addition to preserving long term memory **(de Souza *et al.,* 2021)**.

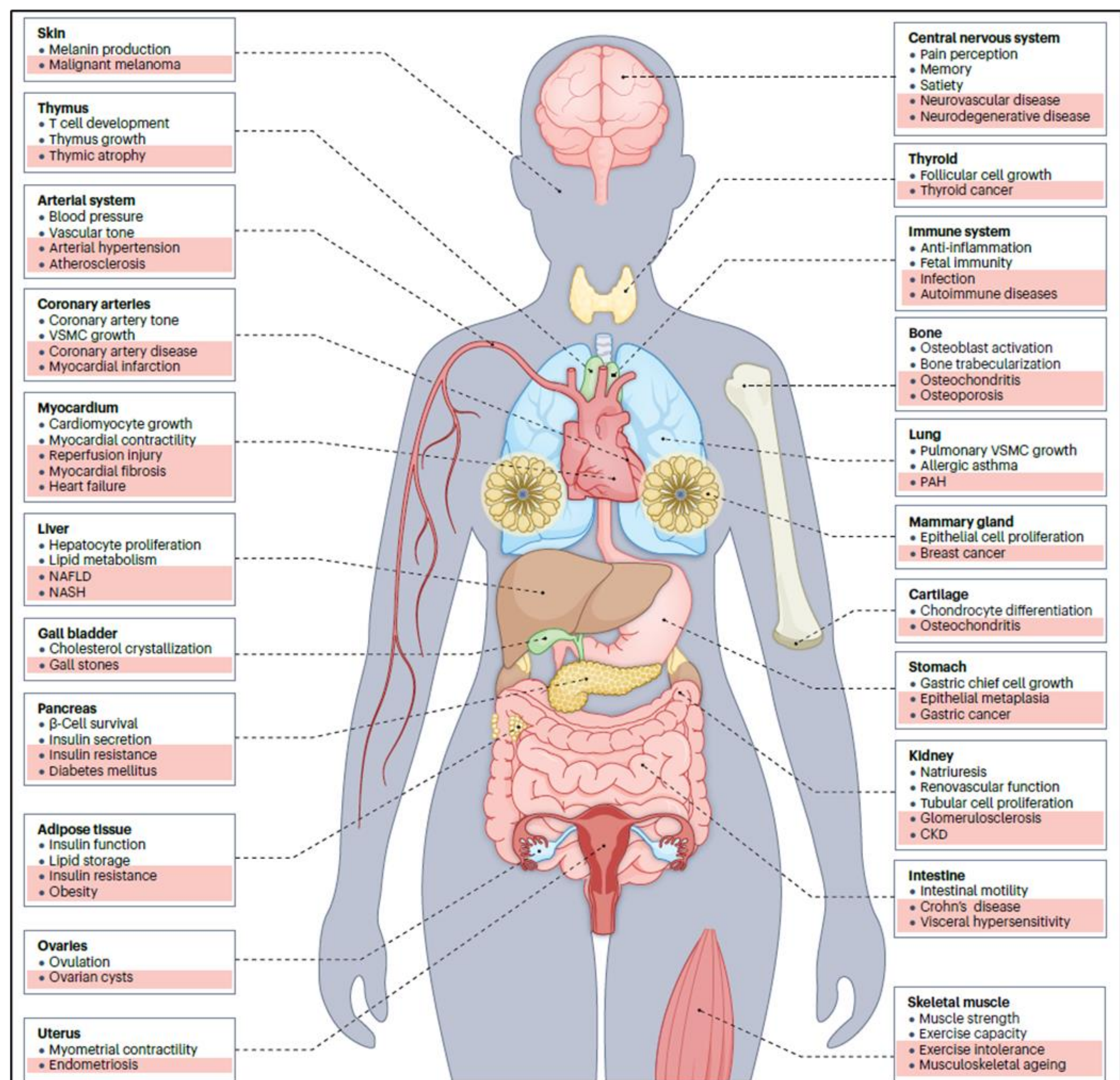


Figure 13: Physiological (white background) and physio pathological (pink background) effects of the GPER1 **(Prossnitz & Barton, 2023)**.

B.7. Crosstalk between GPER1 and ESRs: GPER1 and classical oestrogen receptors (ESR1 and ESR2) may have a crosstalk and interact together in a specific manner to induce the same effects **(Prossnitz *et al.,* 2008a)**. For instance, ERα and GPER1 increase intracellular

calcium using parallel pathway. While ERα activates PLC without EGF, GPER1 depends on EGF to activate PI3K and PIP3 in order to increase intracellular calcium **(Prossnitz & Barton, 2009)**. In addition, the genomic effects of ERs may be up regulated using GPER1. The later may stimulates gene transcription in oestrogen-dependent genes that do not have EREs **(Albanito *et al.,* 2007)**. This cascade engages MAPKs and PI3Ks that activate Elk-1/SRF transcription factors **(Duan *et al.,* 2001)**.

In addition, GPER1 and ERs signalling pathway are consecutively ordered, as one downstream the other **(Hadjimarkou & Vasudevan, 2017)**. Blocking GPER1 in lordosis female mice did not fully block oestrogen effect in these mice, while GPER1 stimulation increases ERα output in the ventral hippocampus of male mice **(Anchan *et al.,* 2014; Hart *et al.,* 2014)**. Furthermore, ERs and GPER1 may antagonise each other. Indeed, ERK activation via GPER1 reduced ERα-mediated signals, while GPER1 decreases Akt signal and suppresses ERβ pathway **(Gao *et al.,* 2011; Boscia *et al.,* 2015)**. Nevertheless, ESRs and GPER1 are highly regulated by protein degradation and desensitization mechanisms **(Prossnitz *et al.,* 2008b)**. In summary, ERs and GPER1 signalling cascade can interact positively or negatively and lead to different outputs depending on recruited effectors and second messengers.

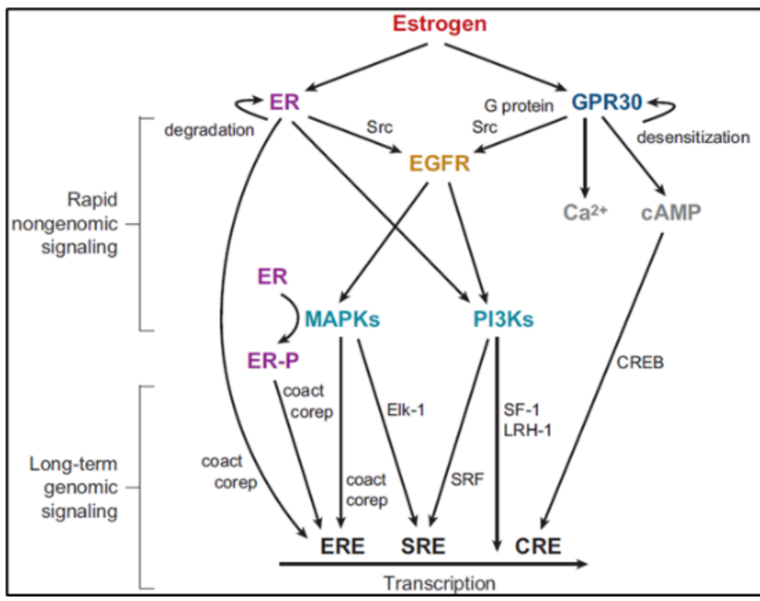


Figure 14: Crosstalk between GPER1 (GPR30) and the classical ERs **(Prossnitz *et al.,* 2008b)**.

Materials & methods

The aim of this study is to elucidate the importance of the GPER1 in male reproductive system especially in free-ranging animals having seasonal reproduction and are subjected to environmental fluctuations. In the present work, two investigations have been used: *in vivo* study and *in silico* study.

I. *In vivo* study

I.A. Animal model

The animal models used in this study were *Psammomys obesus* a diurnal rodent **(Figure 15)** and *Gerbillus gerbillus* a nocturnal rodent **(Figure 16)** from north Africa **(Thomas, 1902)**. The sand rat *Psammomys obesus*, is a fully haired animal with light brown to red dorsal fur and greyish to white belly hair **(Figure 15)**. Its length varies between 130 mm to 150mm with long tufted tail of 110mm to 150mm. The sand rat has large black eyes, robust limbs and black claws, and weights between 146 g and 207g **(Petter, 1962)**. Number of chromosomes in this species is 2n = 48 **(Benazzou *et al.*, 1984)**.

Figure 15 : The sand rat *Psammomys obesus* **(GBIF, 2023)**.

While the gerbil *Gerbillus gerbillus* is characterized by soft, sand coloured dorsal pelage and white ventral fur, white feet, sandy above and white below, a tail with light grey tuff ending **(Klein *et al.*, 1975)**. In addition, this small gerbil of approximatively 88 mm head-and-body

length has 117 mm tail length and long hind feet and digits **(Figure 16)**. Adults weight varies between 27-37g, have large ears covered with short hair, and round big eyes **(Klein *et al.*, 1975)**. Number of chromosomes in this species is 2n = 42 **(Qumsiyeh & Schlitter, 1991)**.

Figure 16 : The lesser Egyptian gerbil *Gerbillus gerbillus* **(GBIF, 2023)**.

I.A.1. Scientific classification:

Psammomys obesus **(Cretzschmar, 1828)** is classified according to **(Schoch *et al.*, 2020)**:

Domain:	Eukaryota
Kingdom:	Animalia
Phylum:	Chordata
Class:	Mammalia
Order:	Rodentia
Family:	Muridae
Subfamily:	*Gerbillinae*
Tribe:	*Gerbillini*
Subtribe:	*Merionina*
Genus:	*Psammomys*
Species:	*Psammomys obesus*

Gerbillus gerbillus **(Olivier, 1801)** is classified according to **(Schoch et al., 2020)**:

Domain: Eukaryota
Kingdom: Animalia
Phylum: Chordata
Class: Mammalia
Order: Rodentia
Family: Muridae
Subfamily: *Gerbillinae*
Tribe: *Gerbillini*
Subtribe: *Gerbillina*
Genus: *Gerbillus*
Species: *Gerbillus gerbillus*

I.A.2. Habitat and distribution: *Psammomys obesus* and *Gerbillus gerbillus* are basically distributed in the desert and semi-desert regions of North Africa, thus can be found on shifting sand, dunes, oases, and wadis **(Harrison & Bates, 1991)**. In Algeria, these species are mainly found in dunes and sandy soils **(Kowalski & Rzebik-Kowalska, 1991)**. Indeed, these locations dispose enough underground water and thin layers of sand, which make them resistant sand suitable to make burrows of 30–80 cm deep **(Kingdon, 2014)**. The openings of the burrow are not visible during daylight, and sometimes buried with sand motility **(Poulet, 2004)**.

I.A.3. Food, foraging and reproduction: *Psammomys obesus* is considered as the most demanding rodent, as its diet essentially contains fresh Chenopodiaceae, which it must obtain all year round and under which it digs its burrows. Gestation lasts 23-25 days and gives 1 to 7 pups. Both males and females are sexually mature by the age of 3 months, and breeds from autumn to spring and rest from late spring through summer **(Petter, 1962)**. In contrast, *Gerbillus gerbillus* is essentially granivorous, but herbivorous in winter and insectivorous in spring **(Bar *et al.*, 1984)**. Male *Gerbillus gerbillus* become sexually active as soon as their weight reaches 17g-18g ,while females became so when they reach 15g-16g weight **(Klein *et al.*, 1975)**. This rodent is characterised by seasonal reproduction, starting in winter (December to January) when foraging opportunities are available with favourable climate conditions, while

resting season is observed in spring to autumn **(Zaina Amirat *et al.*, 1977)**. Gestation lasts between 20 to 22 days and give 1 to 8 gerbil pups per litter **(Klein *et al.*, 1975)**.

I.A.4. Animal capture: adult males were captured in the wild of Béni Abbès region (30°07'N; 2°10'W), during the breeding season and the resting season using traps designed by Mr Bouzidi, a hunter from the Béni Abbès research station, and a dough made of wheat and peanuts to attract the rodents.

I.B. Experimentation

After capture, animals were housed in outdoor enclosures (120cm x 60cm x 60cm); given natural food and fresh water; exposed to natural environment, ambient temperature, and photoperiod. For each species, animals were divided into two groups as follow:

- **Group 01:** During the breeding season (n=08).
- **Group 02:** During the resting season (n=08).

Animals were euthanized by decapitation 48 h after capture. Their testis, epididymis and efferent ducts were quickly removed, weighted, and fixed in Bouin's solution for 2-7 days.

I.C. Methods

I.C.1. Histology: All organs, underwent classical histology procedure described by **(Martoja & Martoja-Pierson, 1967)** as follow:

- Dehydration in increasing concentrations of ethanol (70%, 95%, and 100%) for 10 mn.

- Cleaning for 10 mn in two baths of toluene.

- Embedded for 2 hours in 2 baths of melted paraffin (58°C).

- Organs were placed in paraffin moulds, and blocks were removed after cooling.

- Samples were then sectioned, using a Leitz Wetzler microtome, into 5µm slices put in warm water bath to float and dilates, then mounted on histological Superfrost®glass slides (Thermo Scientific, Menzel-Gläser, Brausschweig, Germany). Slides were left to dry to make sure they are well attached.

- Sections were dewaxed using heat and toluene then rehydrated in decreasing concentrations of ethanol (100%, 95%, and 70%) for 10 mn.

-Finally slides were stained with Masson's trichrome **(Gabe, 1976)**. This dye colours the cytoplasm with pink; nuclei appeared black; peritubular myoid cells were red; and collagen was green.

I.C.2. Immunohistochemistry: To investigate GPER1 distribution in the male reproductive system, we performed immunohistochemistry. This approach is based on antibody-antigen affinity. We used specific antibody that links GPER1 with high precision, the later was combined with biotinylated secondary antibody recognized by the avidin peroxidase to amplify the signal **(Figure 17)**.

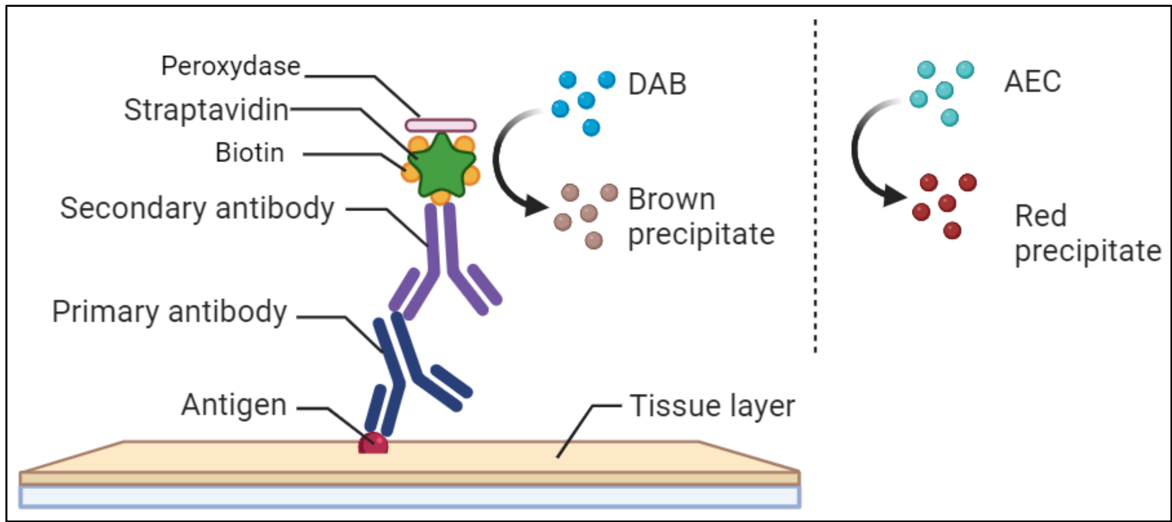


Figure 17: The basic of immunohistochemistry.

The steps followed in this study mirror the same procedure described by **(Menad *et al.*, 2017c)** as follow:

- Sections were deparaffinised, rehydrated, then washed in tap water and PBS (prepared) for 10 mn

- Incubation with 10 mM sodium citrate solution for 45 min at 95°C, then left to chill for 20 mn and plunged in distilled water.

- To block the endogenous peroxidase activity a 3% peroxidase solution was used as a bath for 20mn then dipped two times in distilled water.

- Using DakoPen, sections were bordered.

- To block the non-specific binding sites, sections were incubated with 10% normal goat serum for 1 h.

- Slides were incubated with GPER1 primary antibody in a wet chamber for 2 h, whereas some slides were incubated with normal goat serum instead to serve as negative controls.

- Prior cleaning with PBS was used before reincubation with the secondary biotinylated antibodies for 1 h in a wet chamber.

- Slides were washed with PBS for 5 min and incubated with a streptavidin-biotin-peroxidase complex for 1 h.

- Slides were dyed using a DAB chromogen, washed with PBS then counterstained with haematoxylin for 1 mn.

- Finally, slides were dehydrated and mounted using Permount mounting medium.

I.C.3. Microscopic observation: All histological as well as immunohistochemical slides were observed with the Nikon Eclipse E 400 light microscope and pictured with the Nikon DXM 1200 digital camera.

I.C.4. Statistical analysis: Normality was verified using Shapiro-Wilk test, which allows to test a null hypothesis coming from a normally distributed population. Next, organ weights were subjected to the Student's t test and ANOVA test using OriginPro 8.0 software (OriginLab Corp., Northampton, MA, USA). A probability below 0.001 was considered significant.

II. *In silico* study

In silico approaches are being widely used in toxicological studies, that were originally conducted *in vivo* on animal models and were time/money consuming, in addition of having

ethical constraints **(Dearden, 2003)**. Therefore, computational methods aim to decrease animal usage, experiment time and fees, moreover, forecast chemical toxicity before being synthesized. Furthermore, *in silico* approaches' main goal is to orientate research and minimize studies failure, that's why *in silico* studies compliment those of *in vivo* **(Raies & Bajic, 2016)**.

II.A. Databases

II.A.1. UniProt: UniProt is a universal database collecting over 120 million proteins of different living organisms **(Apweiler *et al.*, 2004) (Figure 18)**. UniProt provides several information on specific proteins such as: structure, family, domain, sequence, expression, variants, subcellular localisation, similar proteins, physiological function and physio pathological implications **(Consortium, 2018)**.

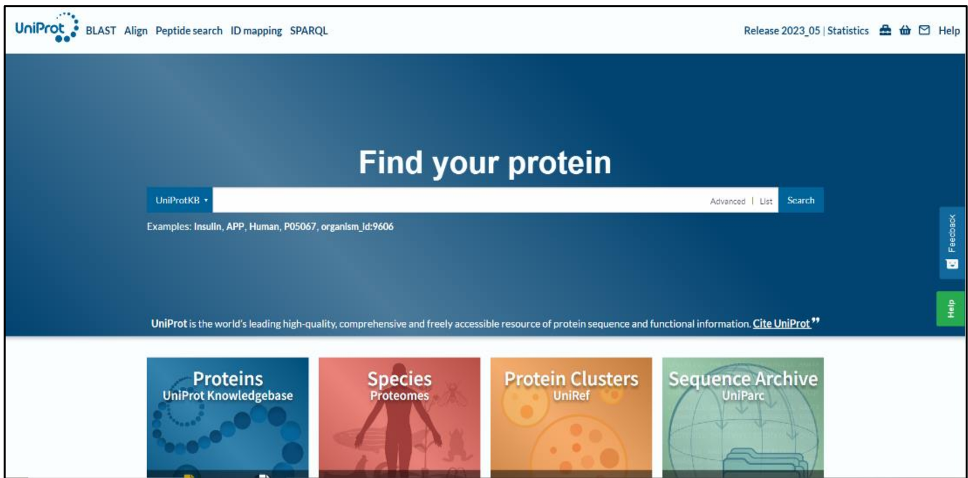


Figure 18: UniProt database (uniprot.org).

II.A.2. PubChem: PubChem is a public database maintained by the US National Institutes of Health (NIH) **(Kim *et al.*, 2015) (Figure 19)**. It contains different types of chemicals and provide large information about their structure, chemical and physical properties, pharmacology, safety, toxicity, associated pathologies , and other biochemical information **(Ihlenfeldt, 2018)**.

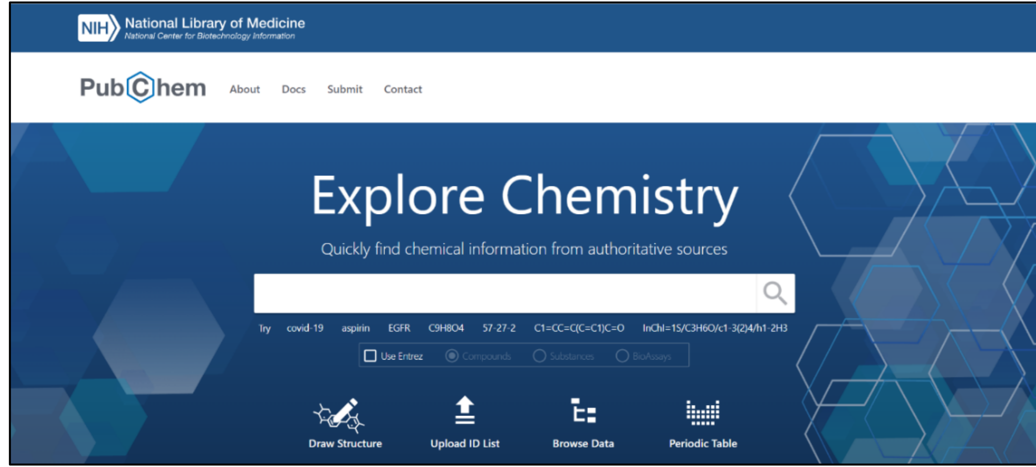


Figure 19: PubChem database (pubchem.ncbi.nlm.nih.gov)

II.A.3. Phytochemicals database: As for searching on phytochemicals several databases were used such as: Plant Secondary Compounds database **(Valdés-Jiménez *et al.*, 2021)**, PhytoHub database **(da Silva *et al.*, 2016)**, Dr. Duke's Phytochemical and Ethnobotanical databases **(Duke & Bogenschutz, 1994) (Figure 20)** and Coconut database **(Sorokina *et al.*, 2021) (Figure 21)**. These open sources repositories provide structural, functional, pharmacological, and toxicological information about plant's chemicals.

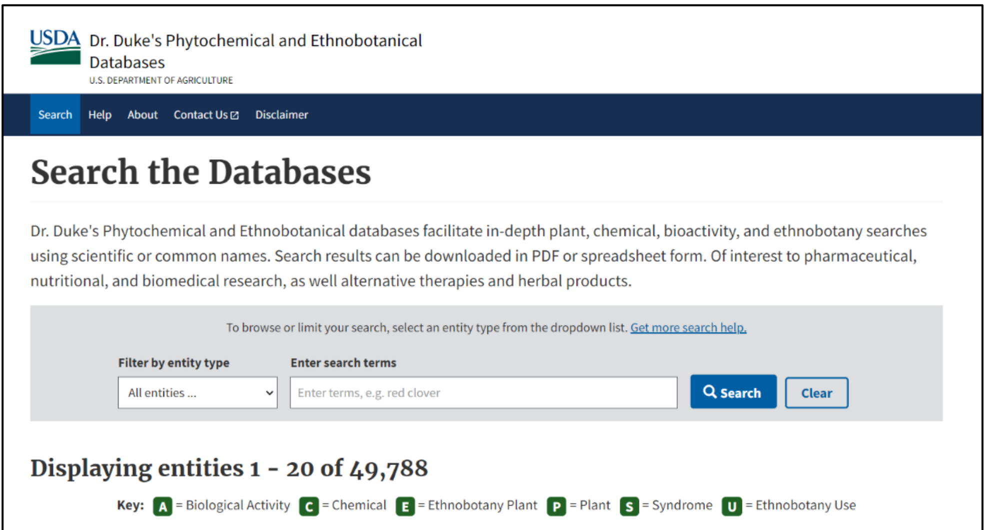


Figure 20: Dr. Duke's Phytochemical and Ethnobotanical databases (phytochem.nal.usda.gov).

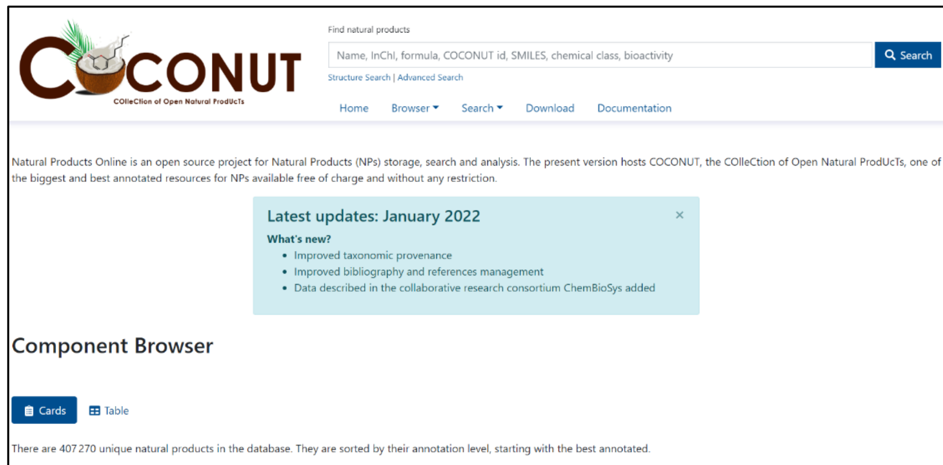


Figure 21: Coconut database (coconut.naturalproducts.net).

II.B. Software

II.B.1. OpenBabel: OpenBabel is a free software used to convert chemical structures between different formats (version 2.3 has 110 formats) so they can easily be used for *in silico* approaches such as molecular docking **(Figure 22)**. It can also be used to search for structural conformations, 2D descriptions, similarity, and substructures **(O'Boyle *et al.*, 2011)**.

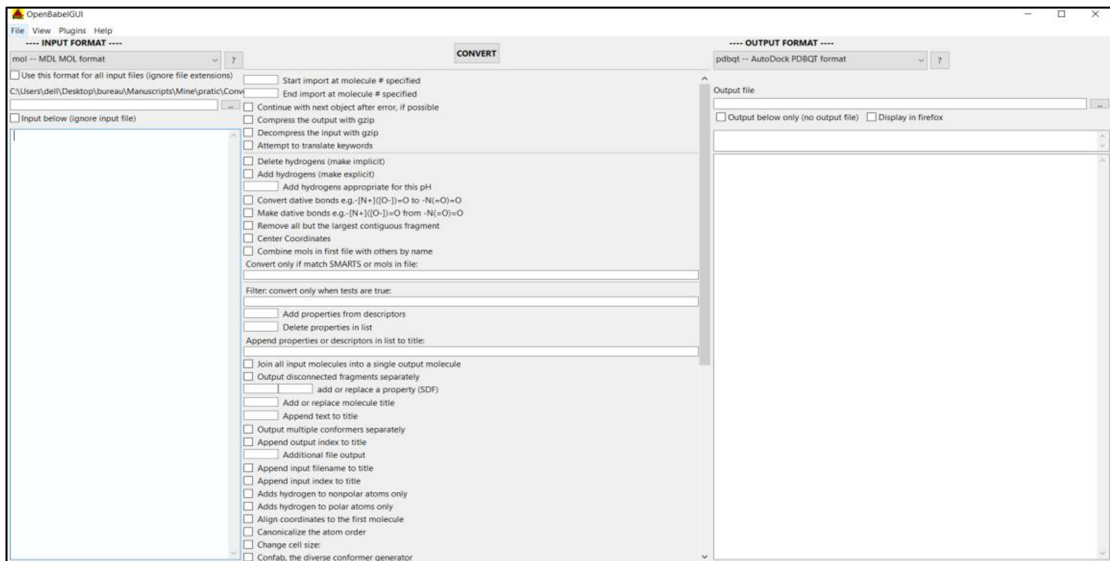


Figure 22: OpenBabel software version 2.4.1.

II.B.2. BIOVIA Discovery Studio: BIOVIA Discovery Studio is a visualization tool for analysing proteins, complexes, modelling data and protein-ligand interactions **(Jejurikar & Rohane, 2021) (Figure 23)**.

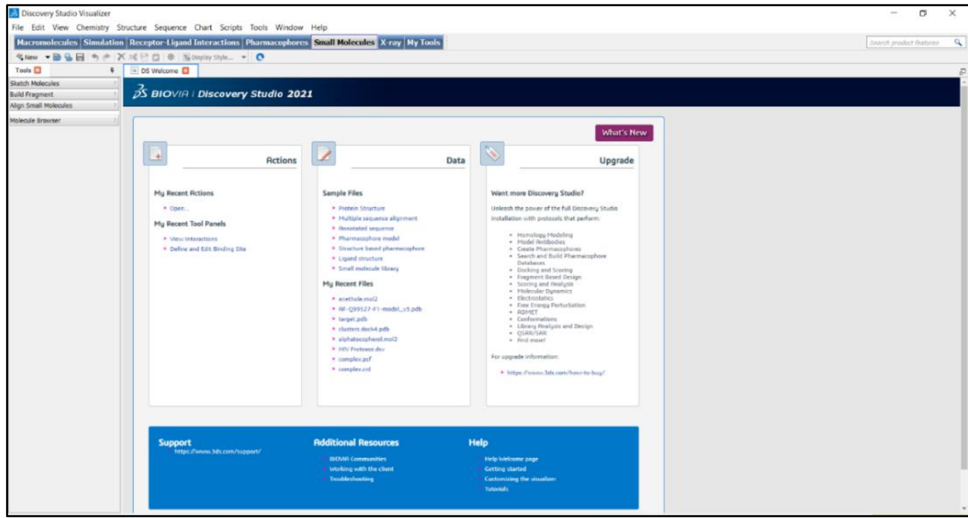


Figure 23: BIOVIA Discovery Studio version 21.1.0.20298.

II.B.3. AutoDock tools: AutoDock tools is widely used open-source software to predict the interactions between a protein and a ligand **(Ravi & Kannabiran, 2016) (Figure 24)**.

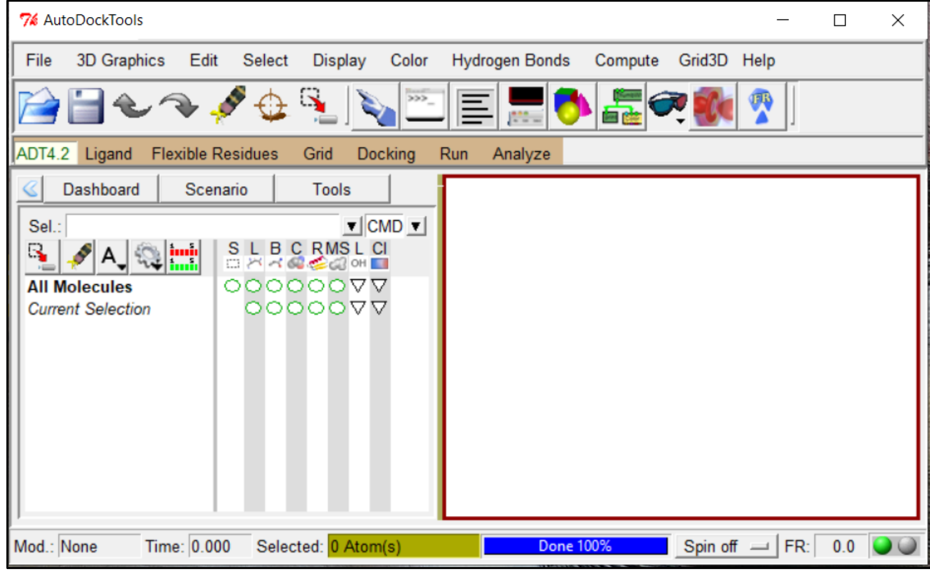


Figure 24: AutoDock tools 1.5.7.

II.C. Methods

II.C.1. Definition and principles of molecular docking: Molecular docking is computer-assisted method used to anticipate the suitable binding mode(s) of a ligand with a protein, these two molecules interact with each other to form a complex **(Meng *et al.,* 2011)**. Results are scored and can be visualized to determine binding sites and atoms, to predict the affinity and activity of the ligand toward the protein **(Morris & Lim-Wilby, 2008)**. This, *in*

silico method, is widely used in drug design and discovery as well as in toxicological studies **(Chaudhary & Mishra, 2016)**.

Binding poses are classified according to a giving score. The scoring function is based on the free binding energy that can be calculated using van der Waals interactions energy, hydrogen bonds energy and entropic energy, so it can be written as follow:

$\Delta G_{binding} = c_1 \Delta G_{vdW} + c_2 \Delta G_{Hbond} + c_3 \Delta G_{entropy}$ (c_i is heightening coefficient)

The aim of this score is to evaluate protein-ligand best conformation that is thought to be the closest to the real conformation.

II.C.2. Protocols and steps

II.C.2.1. Receptor and ligand's structure downloading: Ligands used in this study were divided into four categories, each downloaded from a specific database as follow:

* Known natural ligands and synthetic ligands: these ligands will serve as reference to be compared with other molecules; they were all downloaded from the PubChem database.

* Endocrine disruptors: several types of endocrine disrupting molecules including pesticides, plastics/plasticizers, electronic wastes, flame retardants, metals, food additives, and personal care chemicals. They were downloaded from Pubchem database and listed according to the international endocrine disruptors list (https://edlists.org/).

* Phytochemicals: classified as phytoestrogens or documented to have effects on endocrine system, were downloaded from Plant Secondary Compounds database, PhytoHub database, Dr. Duke's Phytochemical and Ethnobotanical databases, and Coconut database.

* Chemotherapeutic agents: used in cancer treatment such as: doxorubicin, cyclophosphamide, carboplatin, methotrexate, colchicine, and the chemotherapeutic agent derived from oestrogen, Estramustin, all downloaded from PubChem database.

All downloaded ligands **(Table 01)** are in format .sdf so they were converted into .pdbqt format using OpenBebel software.

As for the GPER1, no crystal structure of it is available. In our study we used the AlphaFold GPER1 predicted structure (AF-O08878-F1) recently clustered employing MMseq2 and Foldseek, while cluster should satisfy two criteria: highest sequence identity and attaining 90% sequence overlap with the cluster indicative **(Jumper *et al.*, 2021; Varadi *et al.*, 2021)**. This structure was developed based on Wistar rat *Rattus norvegicus* GPER1 protein structure **(Varadi *et al.*, 2023)** and is currently widely used given its credibility compared to the homology modelled structures **(Lu *et al.*, 2023; Pratiwi *et al.*, 2023)**.

Type	Name	Source
Known natural and synthetic ligands	E2	PubChem CID: 5757
	Fulvestrant	PubChem CID: 104741
	Tamoxifen	PubChem CID: 2733526
	G1	PubChem SID: 481131179
	G36	PubChem CID: 73755224
Phytochemicals	Coumestrol	PubChem CID: 5281707
	Sesamol	PubChem CID: 68289
	Anethole	PubChem CID: 637563
	Lupalbigenin	PubChem CID: 10001388
	Biochanin A	PubChem CID: 5280373
	Piceatannol	PubChem CID: 667639
	Enterodiol	PubChem CID: 115089
	Wedelolactone	PubChem CID: 5281813
	Eugenol	PubChem CID: 3314
	Genistein	Coconut: CNP0174866
	Resveratrol	Coconut: CNP0247661
	Glabrene	PubChem CID: 480774
Heavy metals	Lead Acetate	PubChem CID: 9317
	Cadmium Chloride	PubChem CID: 24947
	Dimethylmercury	PubChem CID: 11645

Table 01: Types of tested ligands (part1).

Type	Name	Source
Pesticides	Acetamiprid	PubChem CID: 213021
	Propoxur	PubChem CID: 4944
	DDT	PubChem CID: 3036
	Fenvalerate	PubChem CID: 3347
	Captan	PubChem CID: 8606
Food additives	Furan	PubChem CID: 8029
	Monosodium glutamate	PubChem CID: 23672308
	Indigo carmine	PubChem CID: 2723854
	Carboxymethyl cellulose	PubChem CID: 24748
	Quinoline Yellow	PubChem CID: 6731
Personal care	Dimethyl Phthalate	PubChem CID: 8554
	Homosalate	PubChem CID: 8362
	Methyl paraben	PubChem CID: 7456
	Tricresyl phosphate	PubChem CID: 6529
	Resorcinol	41PubChem CID: 5054
Plasticizers and flame retardant	Bisphenol A (BPA)	PubChem CID: 6623
	Acrylamide	PubChem CID: 6579
	Decabromodiphenyl oxide	PubChem CID: 14410
	Polychlorinated biphenyls (PCBs)	PubChem CID: 40470
	PFOSA	PubChem CID: 69785
Chemotherapy	Estramustine phosphate	PubChem CID: 259329
	Cyclophosphamide	PubChem CID: 2907
	Doxorubicin	PubChem CID: 31703
	Colchicine	PubChem CID: 6167
	Methotrexate	PubChem CID: 126941
	Carboplatin	PubChem CID: 426756

Table 01: Types of tested ligands (part2).

II.C.2.2. Receptor-ligand docking: Before launching docking, protein and ligand must be prepared according to the following steps using AutoDock tools:

(1) Set the input file or the working space.

(2) Add the GPER1 molecule. (3) Remove water molecules.

(4) Check for missing atoms and repair them. (5) Add polar hydrogen bonds.

(6) Add Kollman charges and check if they have been equally distributed.

(7) Add ligand. Charges and hydrogen bonds will be added automatically.

(8) Detect the root of the ligand. (9) Set number of torsions.

(10) Choose the macromolecules and the ligand to set the grid box.

(11) Set the grid box to its highest to perform blind docking. Save current parameters, then run the autogrid.

(12) Select the receptor and ligand for rigid docking

(13) Select the Genetic Algorithm and set the number of GA runs to 50 and population size to 300.

(14) Select the docking output Lamarkian GA.

(15) Run docking.

II.C.2.3. Result analysis: At the end of the docking session, we will have different types of results, but in our study, we are only interested in the following:

* Free binding energy ΔG. A key metric that reflects how favourably a ligand binds to a protein, guiding predictions of efficacy, stability, and potential biological function.

* The inhibitor constant Ki.

* The interactions between the GPER1 and the ligands.

* The Amino acids involved in the interactions.

Results are visualised using BIOVIA discovery studio, Pymol or Protein plus (online server), to generate high resolution images showing the best pose of the ligand in the pocket of the receptor as well as the interaction between them.

Results

I. *in vivo* study

A. Organ weight: The mean testicular weight of the gerbil *Gerbillus gerbillus* decreased significantly during the resting season compared to the breeding season (mean±SD Standard deviation, respectively: 0.183±0.014g versus0.108±0.011g ; p <0.001) **(Figure 25)**.

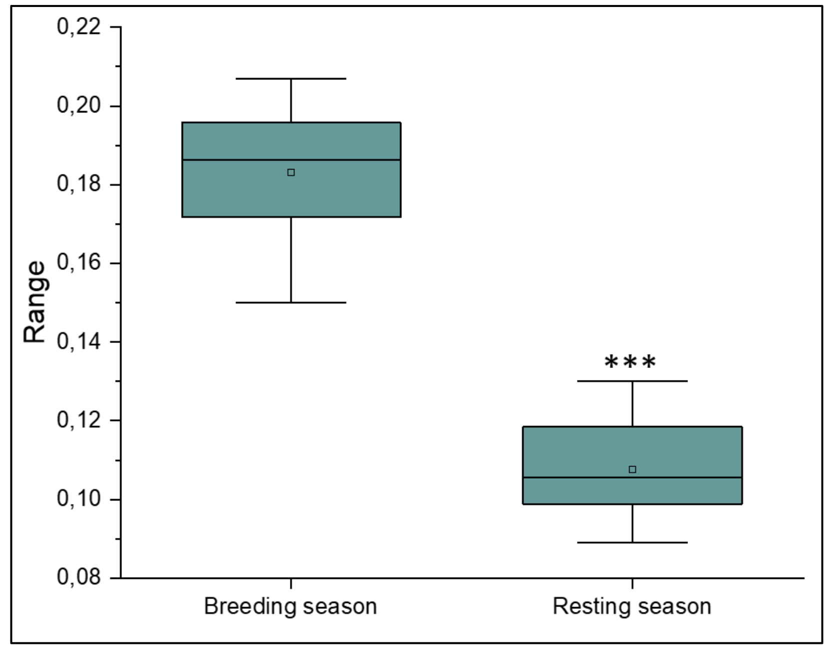


Figure 25: Average testicular weight in *Gerbillus gerbillus* during the breeding season and the resting season, p <0.001.

I.B. Histology

I.B.1. The testis: During the breeding season, the testicles of the sand rat *Psammomys obesus* and the gerbil *Gerbillus gerbillus* appeared as a set of well organised seminiferous tubules within the interstitial tissue, all enclosed in a thick fibrous tunic named the albuginea **(Figure 26, Panel A; Figure 27, Panel A)**. The interstitial tissue is primarily made up of connective tissue, in which we observe dispersed fibroblasts and clusters of Leydig cells around blood vessels. In addition, the seminiferous tubules were highly organised with a germinal epithelium formed of Sertoli cells maintaining germ cells (spermatogonia, spermatocytes, spermatids, and spermatozoa), their lumens were filled with sperm, while the basement membrane was laying on peritubular myoid cells **(Figure 26, Panel B; Figure 27, Panel B)**.

However, during the resting period, in the lumen of the seminiferous tubules, no spermatozoa were observed, and spermatogenesis was stopped at the spermatocyte stage, as the epithelium contained only spermatogonia and spermatocytes enclosed between Sertoli cells **(Figure 26, Panel C; Figure 27, Panel C)**. Albuginea appeared very thick and in the inter-tubular compartment, the connective tissue contained Leydig cells around blood vessels, fibroblast and peritubular cells surrounding the tubules **(Figure 26, Panel D; Figure 27, Panel D)**.

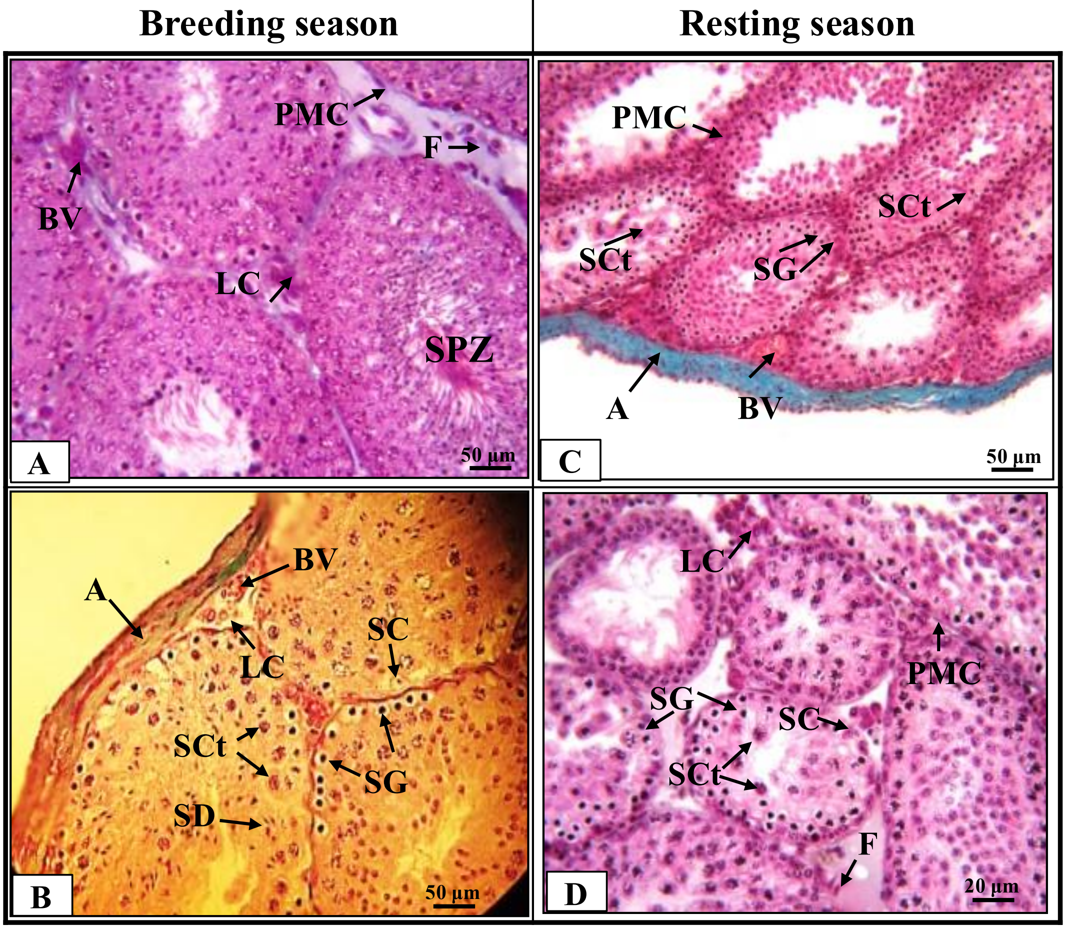


Figure 26: Histology of the testis of *Gerbillus gerbillus*. Panels A and B during the breeding season, Panels C and D during the resting season. Spermatogonia (SG), spermatocytes (SCt), spermatids (SD), spermatozoa (SPZ), Leydig cells (LC), Sertoli cells (SC), blood vessel (BV), albuginea (A), fibroblasts (F) and peritubular myoid cells (PMC). Masson's trichrome stain.

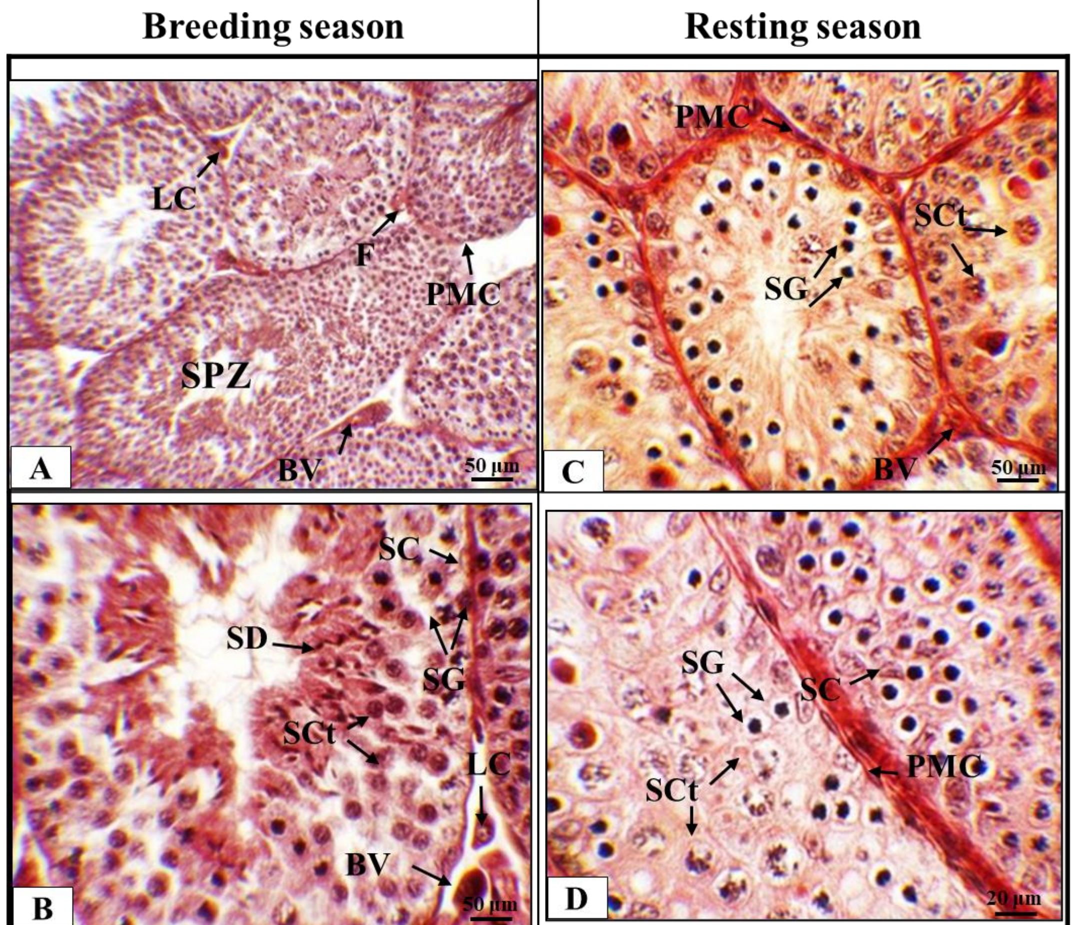


Figure 27: Histology of the testis of *Psammomys obesus*. Panels A and B during the breeding season, Panels C and D during the resting season. Spermatogonia (SG), spermatocytes (SCt), spermatids (SD), spermatozoa (SPZ), Leydig cells (LC), Sertoli cells (SC), blood vessel (BV), albuginea (A), fibroblasts (F) and peritubular myoid cells (PMC). Masson's trichrome stain.

I.B.2. The efferent ducts: During the breeding season, the efferent ducts of *Gerbillus gerbillus* and *Psammomys obesus* were composed of epithelial sections dispersed in connective tissue **(Figure 28, Panel A ; Figure 29, Panel A)**. The pseudostratified epithelium was made up of three types of cells: columnar ciliated cells with basal nuclei and cilia in the apical cytoplasm, non-ciliated cells which are secretory cells, and basal cells resting on a basal lamina but not reaching lumen between the ciliated and non-ciliated cells **(Figure 28, Panel B; Figure 29, Panel B)**. Tubules are surrounded by three layers of smooth muscle cells and connective tissue containing fibroblasts. In the lumen we found spermatozoa.

During the resting season, we noticed a proliferation of the intertubular connective tissue and decrease of the height of the epithelium as the ciliated cells and non-ciliated cells looked cuboid **(Figure 28, Panel C; Figure 29, Panel C)**. In addition, spermatozoa were absent **(Figure 28, Panel D; Figure 29, Panel D)**.

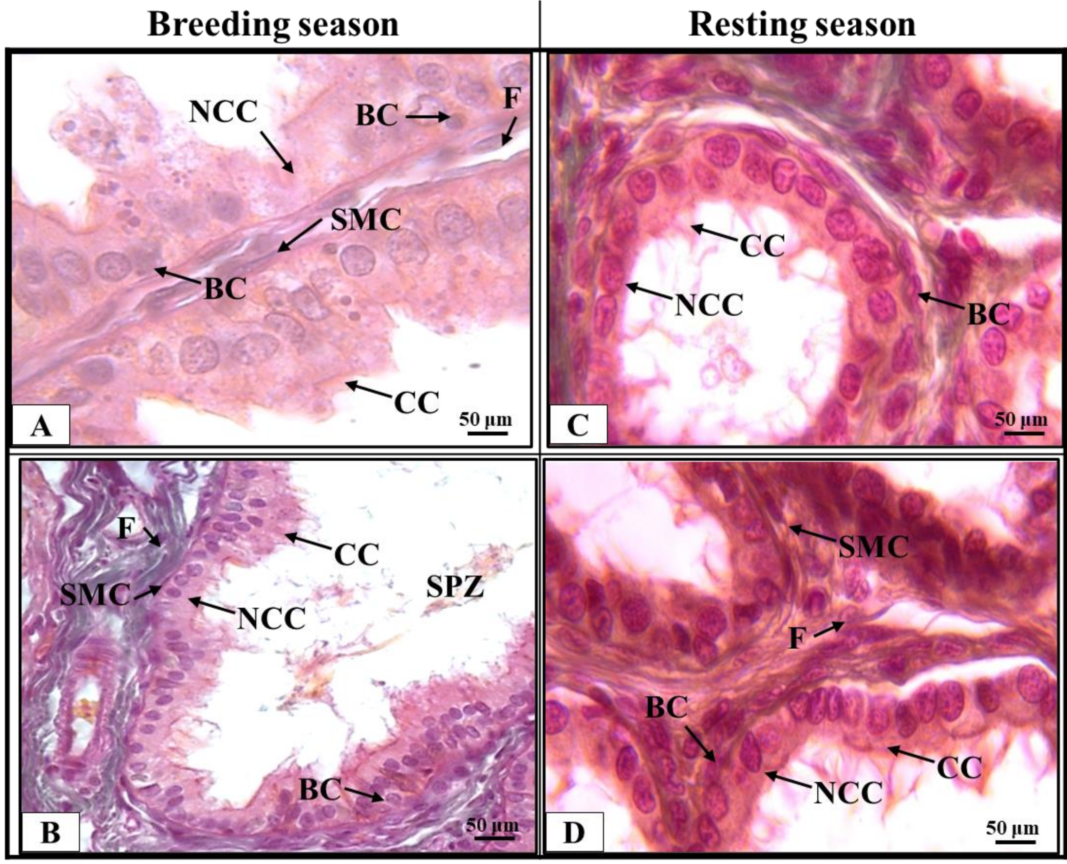


Figure 28: Histology of the efferent ducts of *Gerbillus gerbillus*. Panels A and B during the breeding season, Panels C and D during the resting season, ciliated cells (CC), non-ciliated cells (NCC), basal cells (BC), spermatozoa (SPZ), fibroblasts (F) and smooth muscle cells (SMC). Masson's trichrome stain.

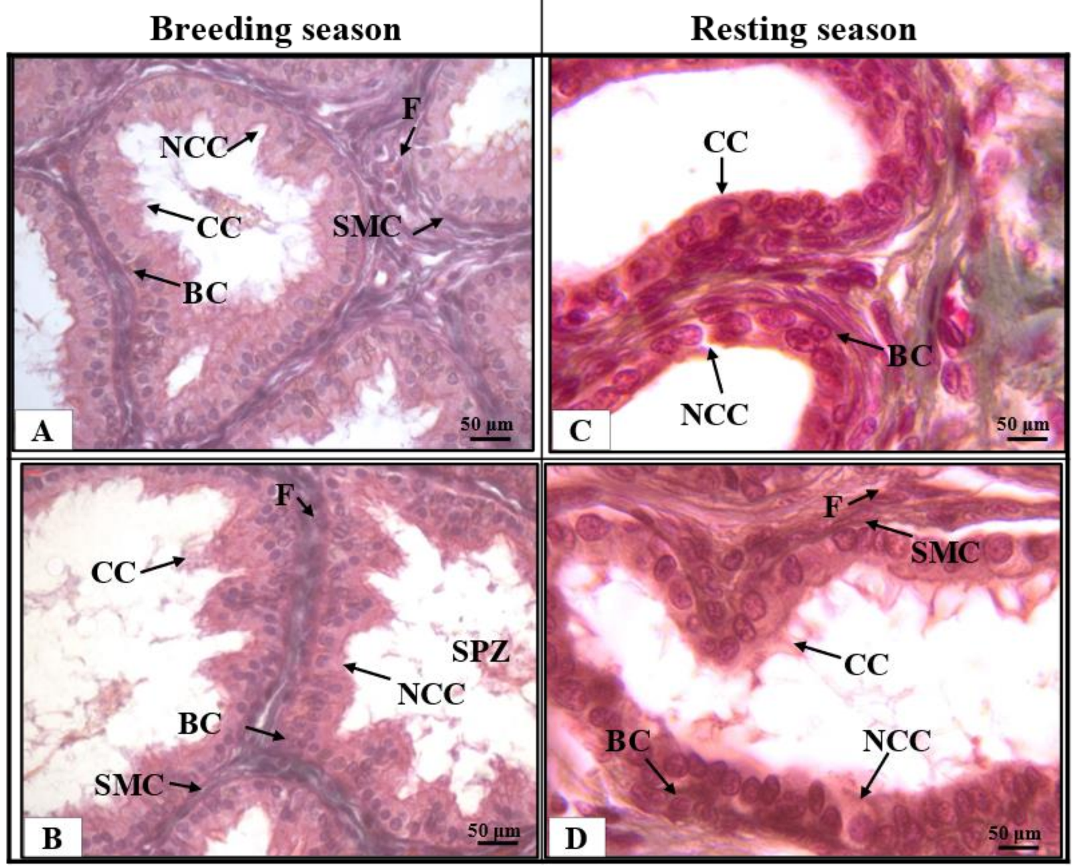


Figure 29: Histology of the efferent ducts of *Psammomys obesus*. Panels A and B during the breeding season, Panels C and D during the resting season, ciliated cells (CC), non-ciliated cells (NCC), basal cells (BC), spermatozoa (SPZ), fibroblasts (F) and smooth muscle cells (SMC). Masson's trichrome stain.

I.B.3. The proximal epididymis: During the breeding season, the proximal epididymis of the gerbil *Gerbillus gerbillus* and the sand rat *Psammomys obesus* appeared as sections of epididymal tubule surrounded with smooth muscle cells and loose connective tissue containing fibroblasts and blood vessels, the lumen was full of sperm **(Figure 30, Panel A; Figure 31, Panel A)**. The epithelium of the tubules consisted mainly of principal cells, which are columnar cells with apical secretions, between which there are basal cells having oval nuclei, localized in contact of the basement membrane. In addition, a particular type of cells was encountered in the upper part of the epithelium: apical cells **(Figure 30, Panel B; Figure 31, Panel B)**.

During the resting season, the epithelium appeared narrower, and the interstitial compartment was larger than usual **(Figure 30, Panel C; Figure 31, Panel C)**. We observed

several extruded cells in the lumen of the tubules. Smooth muscle cells seemed thicker, principal cells and basal cells were present unlike apical cells that were not **(Figure 30, Panel D; Figure 31, Panel D)**.

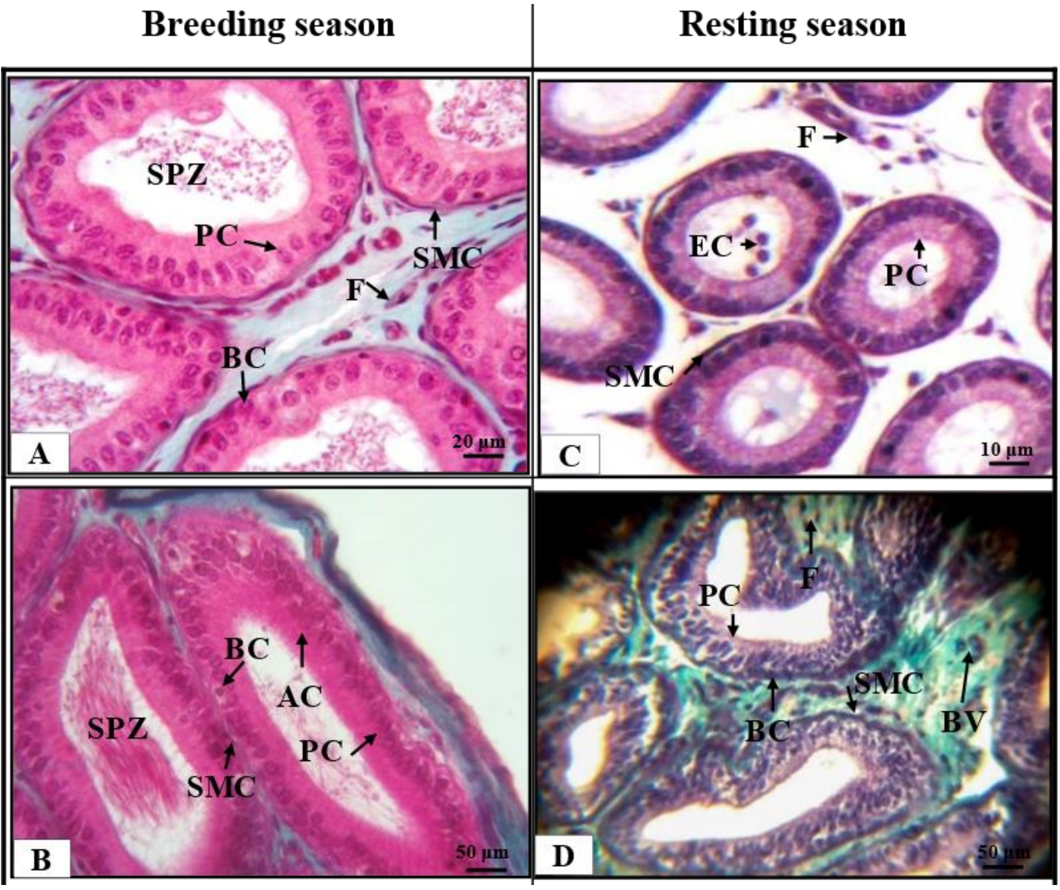


Figure 30: Histology of the proximal epididymis of *Gerbillus gerbillus*. Panels A and B during the breeding season, Panels C and D during the resting season, principal cells (PC), apical cells (AC), basal cells (BC), spermatozoa (SPZ), extruded cells (EC), blood vessels (BV), fibroblasts (F) and smooth muscle cells (SMC). Masson's trichrome stain.

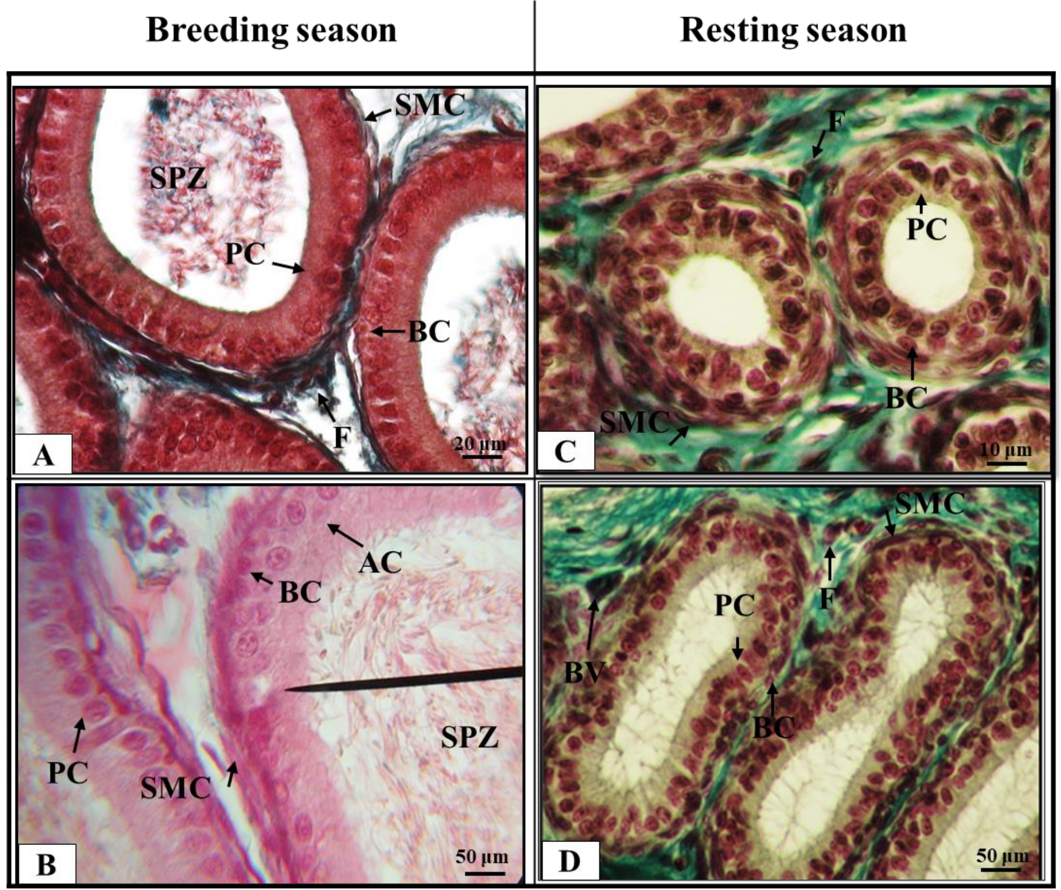


Figure 31: Histology of the proximal epididymis of *Psammomys obesus*. Panels A and B during the breeding season, Panels C and D during the resting season, principal cells (PC), apical cells (AC), basal cells (BC), spermatozoa (SPZ), blood vessels (BV), fibroblasts (F) and smooth muscle cells (SMC). Masson's trichrome stain.

I.B.4. The distal epididymis: During the breeding season, the distal epididymis of the gerbil *Gerbillus gerbillus* and the sand rat *Psammomys obesus* were made up of large tubules full of sperm and lined with pseudostratified epithelium surrounded with smooth muscle cells and loose connective tissue **(Figure 32, Panel A; Figure 33, Panel A)**. In the epithelium we found principal cells, basal cells and clear cells characterising the distal segment of the epididymis, which are morphologically closed to the principal cells but appear clearer **(Figure 32, Panel B; Figure 33, Panel B)**.

During the resting season, we noticed a disorganisation of the epithelium, lumen was narrow and empty **(Figure 32, Panel C; Figure 33, Panel C)**. The connective tissue surrounding the tubules was thicker and well developed **(Figure 32, Panel D; Figure 33, Panel D)**.

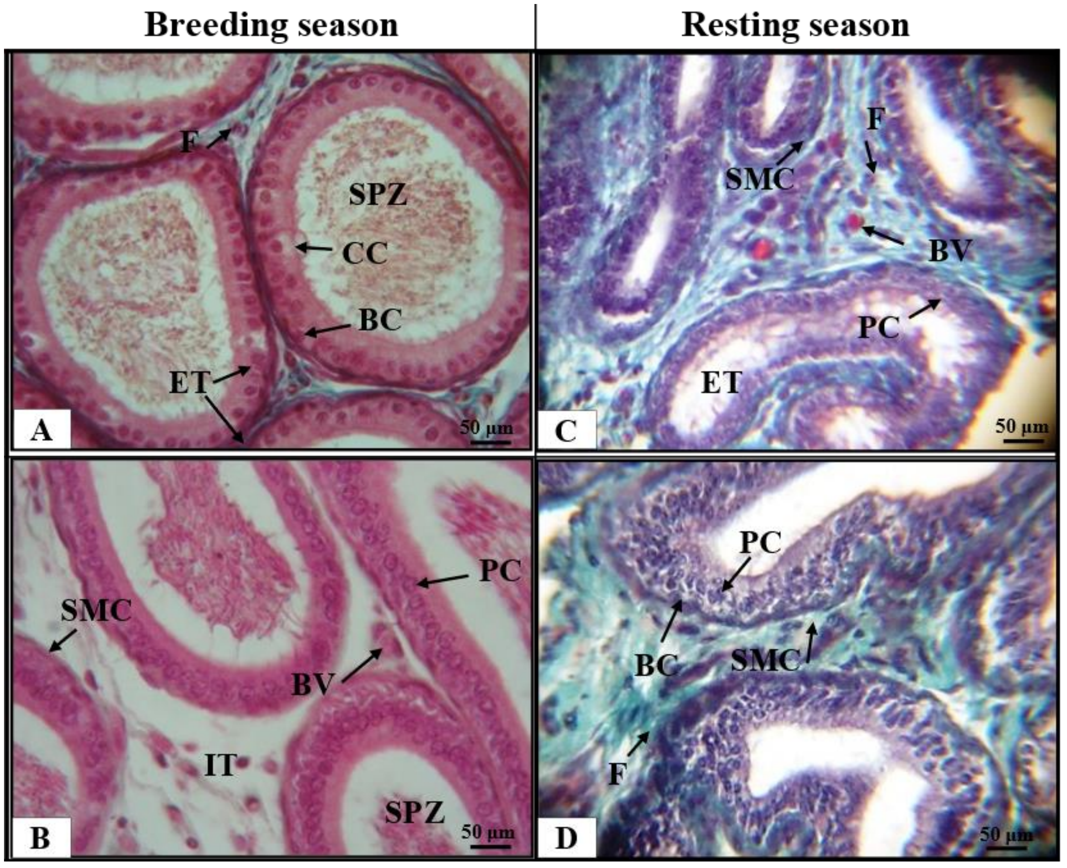


Figure 32: Histology of the distal epididymis of *Gerbillus gerbillus*. Panels A and B during the breeding season, Panels C and D during the resting season, epididymal tubules (ET), principal cells (PC), clear cells (CC), basal cells (BC), spermatozoa (SPZ), interstitial tissue (IT), blood vessels (BV) fibroblasts (F) and smooth muscle cells (SMC). Masson's trichrome stain.

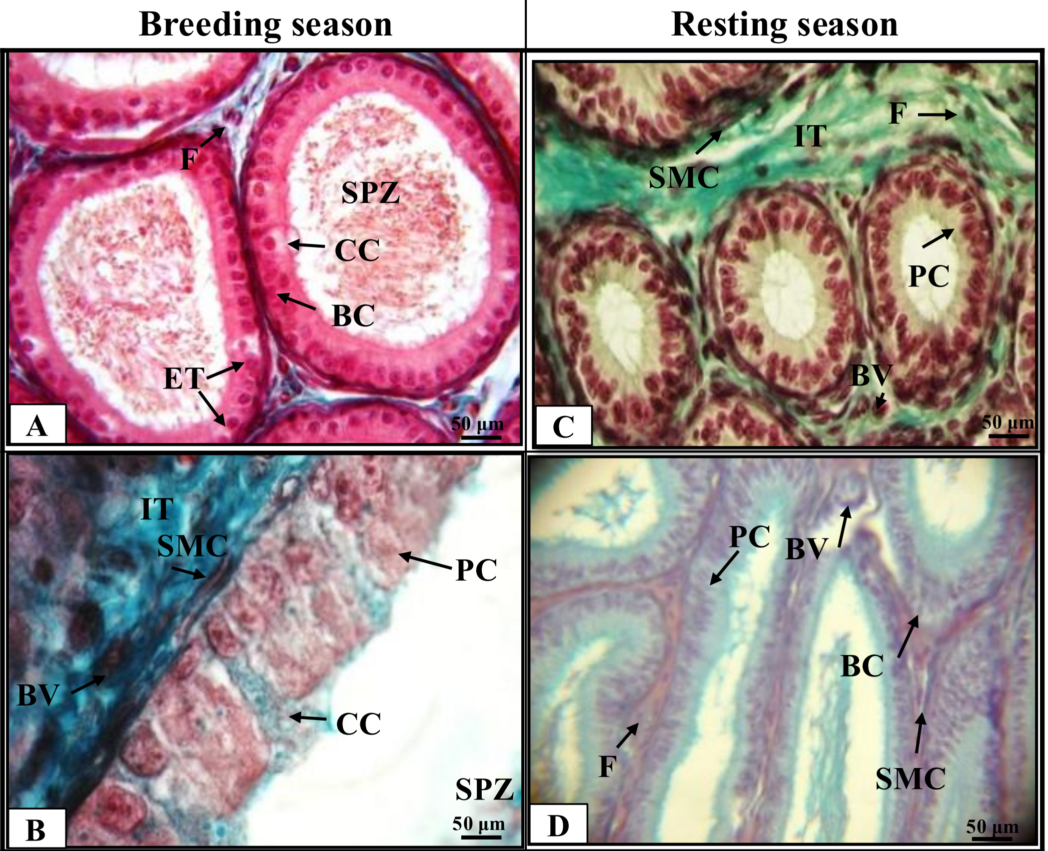


Figure 33: Histology of the distal epididymis of *Psammomys obesus*. Panels A and B during the breeding season, Panels C and D during the resting season, epididymal tubules (ET), principal cells (PC), clear cells (CC), basal cells (BC), spermatozoa (SPZ), interstitial tissue (IT), blood vessels (BV) fibroblasts (F) and smooth muscle cells (SMC). Masson's trichrome stain.

I.C. Immunohistochemistry

All immunohistochemistry results of GPER1 are summarized in **Table 02** for the gerbil *Gerbillus gerbillus* and **Table 03** for the sand rat *Psammomys obesus*.

Organ	Cells		Breeding season	Resting season
Testis	Sertoli cells	N	/	/
		C	+/++	-
	Leydig cells	N	+	/
		C	+++	++
	Spermatogonia	N	/	/
		C	+++	-
	Spermatocyte	N	-	-
		C	++/+++	+++
	Spermatid	N	/	/
		C	++	/
	Spermatozoa	N	++	/
	Peritubular myoid cells	N	+/++	+
	Fibroblast	N	+	+
Efferent ducts	Ciliated cells	N	++	-
		C	++/+++	-/+
	Non ciliated cells	N	+	-
		C	++	-/+
	Basal cells	N	/	/
		C	++	-/+
	Smooth muscle cells	N	++	-
	Fibroblast	N	++	-
Proximal epididymis	Principal cells	N	+	-
		C	++	++
	Basal cells	N	++/-	-
		C	++	+
	Apical cells	N	++	/
		C	++	/
	Spermatozoa	N	+	/
	Smooth muscle cells	N	+/-	-
	Fibroblasts	N	+++	-

Distal epididymis	Principal cells	N	+++	-
		C	+++	++
	Basal cells	N	+++	-/++
		C	+++	-/++
	Clear cells	N	++	/
		C	+	/
	Spermatozoa	N	+++	/
	Smooth muscle cells	N	++	-/+
	Fibroblast	N	+++	-/+

Table 02: Immunolocalization of GPER1 in the testis, the efferent ducts, the proximal epididymis, and the distal epididymis of *Gerbillus gerbillus*. Abbreviations: N nuclei, C cytoplasm, - absence of staining, + weak staining, ++ moderate staining, +++ strong staining, / not mentioned.

Organ	Cells		Breeding season	Resting season
Testis	Sertoli cells	N	-	-
		C	-	-
	Leydig cells	N	+++	+++
		C	+++	+++
	Spermatogonia	N	+++/-	-
		C	-	-
	Spermatocyte	N	-	+++/-
		C	+++	+++
	Spermatid	N	++/-	/
		C	++/-	/
	Spermatozoa	N	++	/
	Peritubular myoid cells	N	++/-	-
	Fibroblast	N	/	/
Efferent ducts	Ciliated cells	N	+++/-	+++/-
		C	+++/-	+++/-
	Non ciliated cells	N	-	+++/-
		C	+/-	+++/-

	Basal cells	N	++/+	++
		C	++/+	++
	Smooth muscle cells	N	+++	+++
	Fibroblast	N	+++/-	+++/-
Proximal epididymis	Principal cells	N	-/+	-
		C	+++	++
	Basal cells	N	+++	-
		C	+++	++
	Apical cells	N	+++	/
		C	+++	/
	Spermatozoa	N	+	/
	Smooth muscle cells	N	++/-	+/-
	Fibroblasts	N	++	+/-
Distal epididymis	Principal cells	N	-	+/-
		C	+++	+
	Basal cells	N	+++/-	-/+
		C	+++	+
	Clear cells	N	++	/
		C	+	/
	Spermatozoa	N	+++	/
	Smooth muscle cells	N	+	+
	Fibroblast	N	-	+

Table 03: Immunolocalization of GPER1 in the testis, the efferent ducts, the proximal epididymis and the distal epididymis of *Psammomys obesus*. Abbreviations: N nuclei, C cytoplasm, - absence of staining, + weak staining, ++ moderate staining, +++ strong staining, / not mentioned.

I.C.1. The testis: In the gerbil *Gerbillus gerbillus*, during the breeding season, GPER1 was found in all the cells that compose the testis. In the seminiferous tubules, Sertoli cells and spermatid had moderate cytoplasmic immunohistochemical staining, spermatozoa had average nuclear signal **(Figure 34, Panel A)**. however, spermatogonia and spermatocytes had intense

cytoplasmic signal, but their nuclei were not marked. In the interstitial compartment, Leydig cells strongly expressed GPER1 in their cytoplasm but weakly expressed it in their nuclei. Peritubular myoid cells and fibroblast were moderately immunostained **(Figure 34, Panel B)**.

During the resting season, as spermatogenesis was blocked in the stage of spermatocytes, it was noticed that these cells still strongly express GPER1 even in the resting season **(Figure 34, Panel C)**. In addition, Leydig cells were moderately immune-positive, peritubular myoid cells and fibroblast were poorly stained. Interestingly, Sertoli cells and spermatogonia did not reveal any immunohistochemical signal **(Figure 34, Panel D)**.

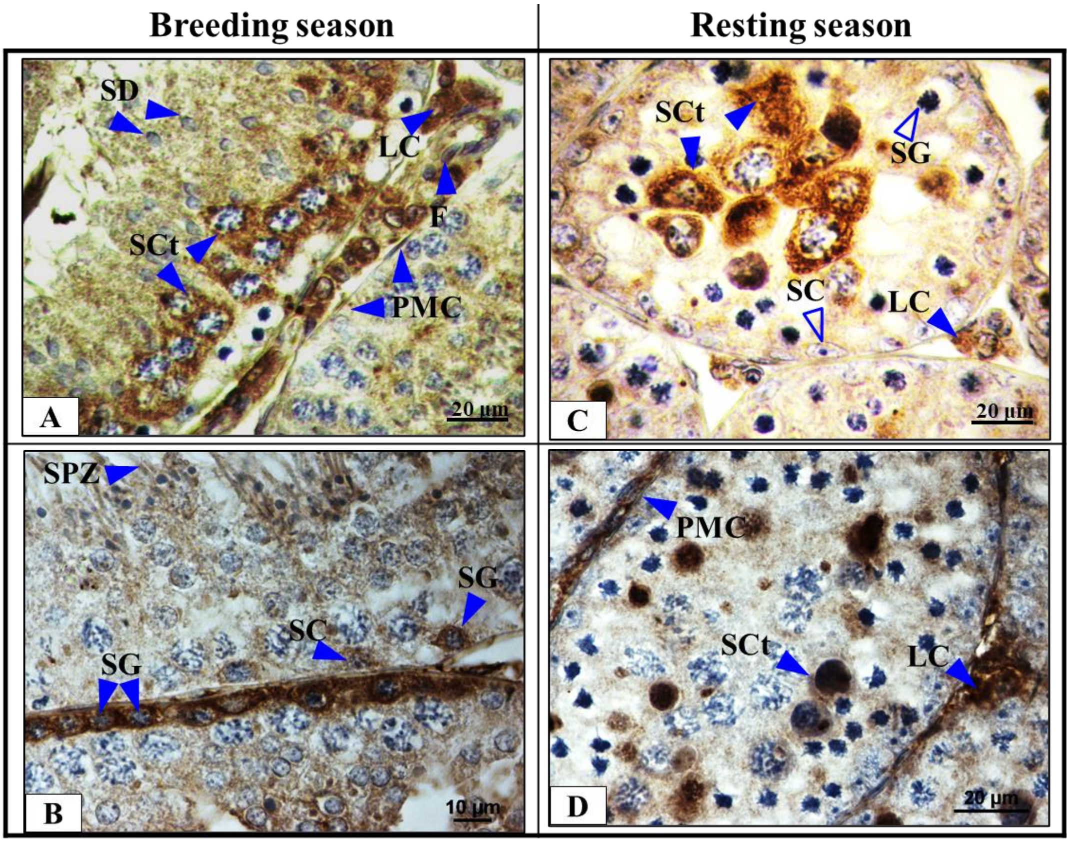


Figure 34: Immunolocalization of GPER1 in the testis of *Gerbillus gerbillus*. Panels A and B during the breeding season, Panels C and D during the resting season. Spermatogonia (SG), spermatocytes (SCt), spermatids (SD), spermatozoa (SPZ), Leydig cells (LC), Sertoli cells (SC), fibroblasts (F) and peritubular myoid cells (PMC). Empty arrows: absence of immunohistochemical staining. Full arrows: presence of immunohistochemical staining.

In the *Psammomys obesus*, Sertoli cells did not express GPER1 during both season in contrast to Leydig cells which was immunoreactive during the hole year **(Figure 35, Panel A)**. In addition, all germ cells were marked during the breeding season **(Figure 35, Panel B)**, but only spermatocyte that were stained during the resting season **(Figure 35, Panel C)**. Peritubular myoid cells expressed GPER1 only during the breeding season **(Figure 35, Panel D)**.

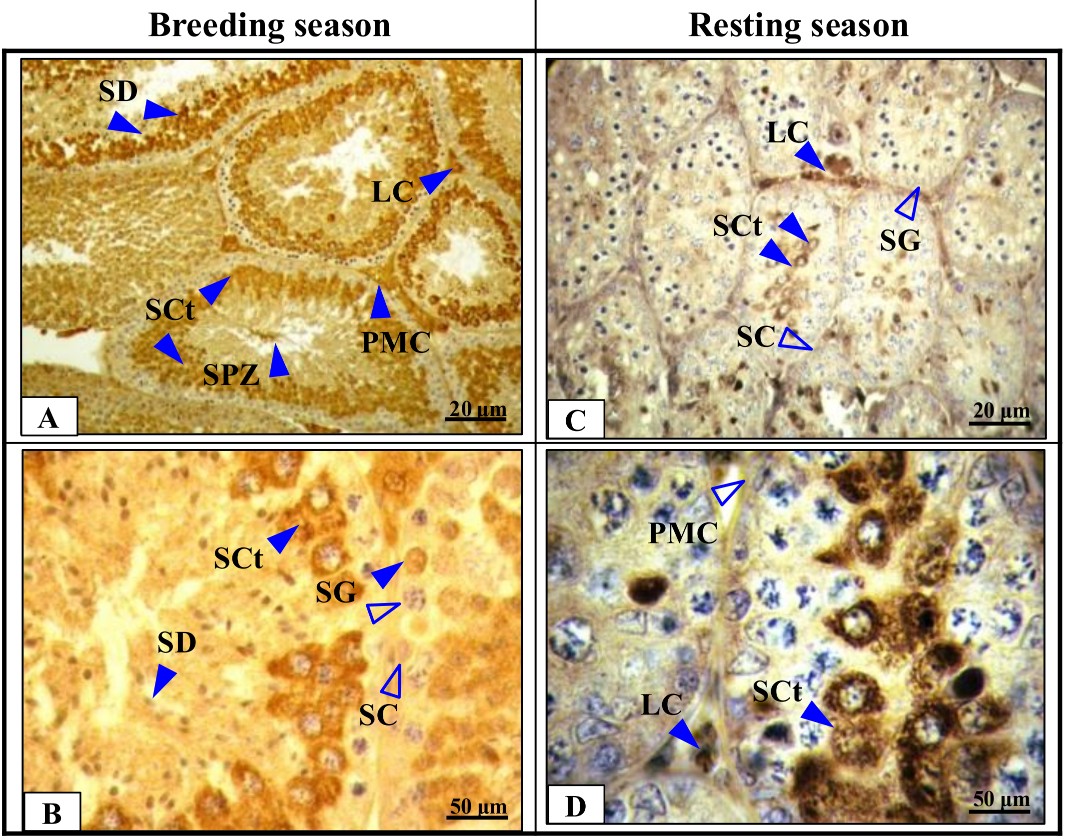


Figure 35: Immunolocalization of GPER1 in the testis of *Psammomys obesus*. Panels A and B during the breeding season, Panels C and D during the resting season. Spermatogonia (SG), spermatocytes (SCt), spermatids (SD), spermatozoa (SPZ), Leydig cells (LC), Sertoli cells (SC), fibroblasts (F) and peritubular myoid cells (PMC). Empty arrows: absence of immunohistochemical staining. Full arrows: presence of immunohistochemical staining.

I.C.2. The efferent ducts: During the breeding season, in both species, GPER1 was predominantly found in the efferent ducts. Ciliated cells had strong cytoplasmic immunostaining and moderate nuclear immunoreactivity **(Figure 36, Panel A; Figure 37,**

Panel A). Meanwhile, non-ciliated cells, basal cells, smooth muscle cells and fibroblast showed average immunohistochemical signal **(Figure 36, Panel B; Figure 37, Panel B)**.

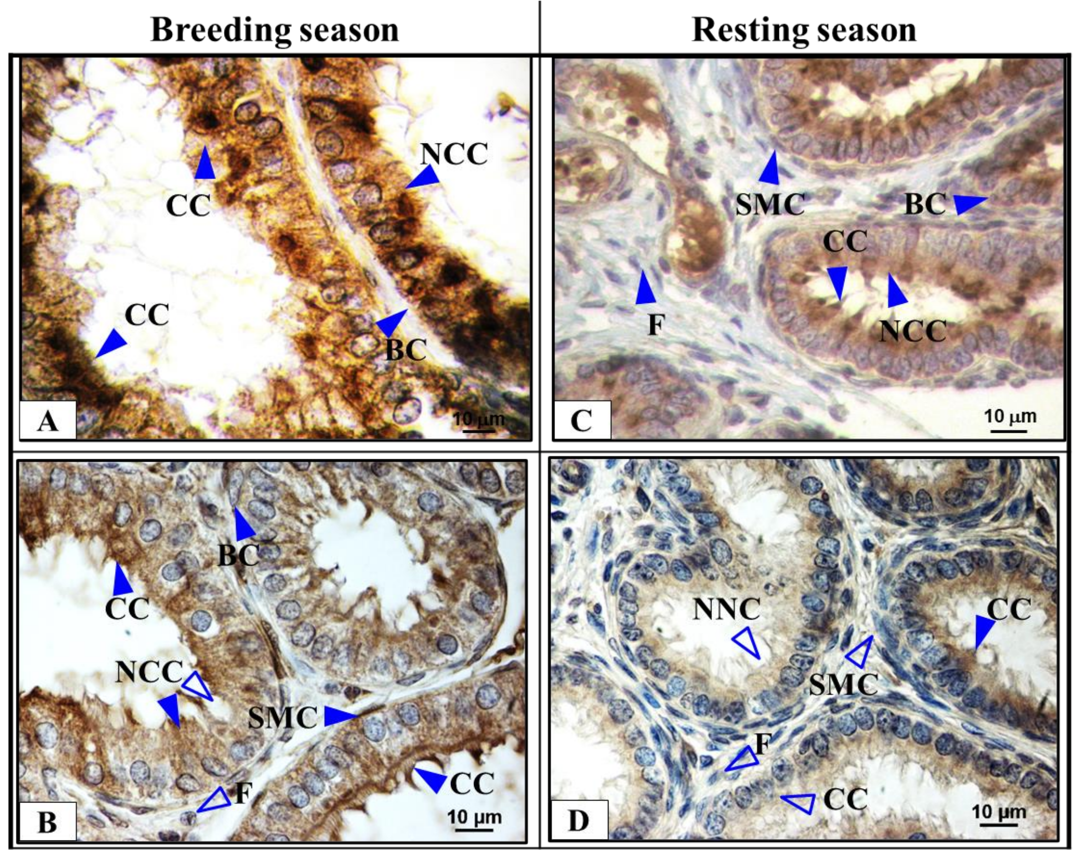


Figure 36: Immunolocalization of GPER1 in the efferent ducts of *Gerbillus gerbillus*. Panels A and B during the breeding season, Panels C and D during the resting season, ciliated cells (CC), non-ciliated cells (NCC), basal cells (BC), fibroblasts (F) and smooth muscle cells (SMC). Empty arrows: absence of immunohistochemical staining. Full arrows: presence of immunohistochemical staining.

During the resting season, in the gerbil *Gerbillus gerbillus*, GPER1 immuoexpression was markedly attenuated in the efferent ducts **(Figure 36, Panel C)**. Some ciliated cells, non-ciliated cells, and basal cells weakly expressed GPER1 **(Figure 36, Panel D)**. In contrast, the expression of the GPER1 in the sand rat *Psammomys obesus* has not remarkably changed during the resting season **(Figure 37, Panel C)**. Ciliated cells, nonciliated cells, basal cells, fibroblast as well as the smooth muscle cells were strongly immunoreactive **(Figure 37, Panel D)**.

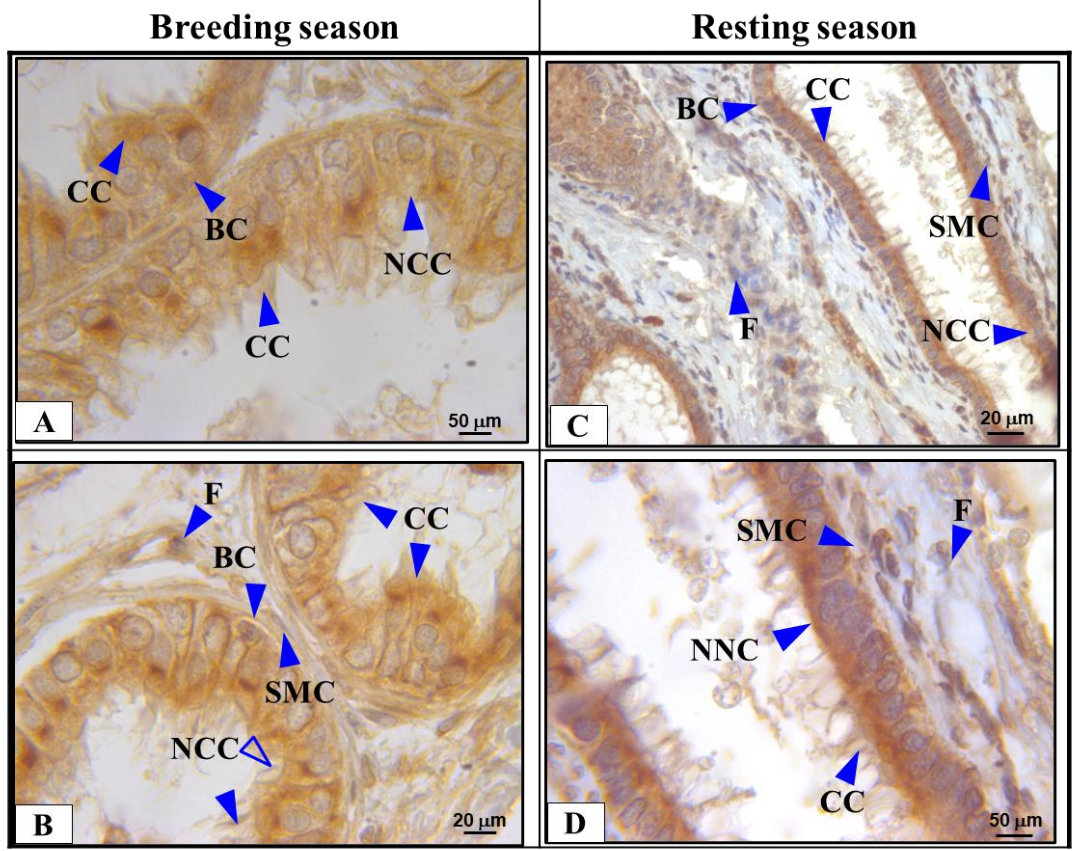


Figure 37: Immunolocalization of GPER1 in the efferent ducts of *Psammomys obesus*. Panels A and B during the breeding season, Panels C and D during the resting season, ciliated cells (CC), non-ciliated cells (NCC), basal cells (BC), fibroblasts (F) and smooth muscle cells (SMC). Empty arrows: absence of immunohistochemical staining. Full arrows: presence of immunohistochemical staining.

I.C.3. The proximal epididymis: In the gerbil *gerbillus gerbillus*, during the breeding season, GPER1 was ubiquitously found in the proximal epididymis. Principal cells moderately expressed GPER1 in their cytoplasm and weakly express it in their nuclei, while basal cells as well as apical cells had moderately marked cytoplasm and nuclei **(Figure 38, Panel A)**. Furthermore, smooth muscle cells and spermatozoa were weakly stained, unlike fibroblasts, which expressed strong immunohistochemical signal **(Figure 38, Panel B)**.

During the resting season, GPER1 was absent in the apical cells, smooth muscle cells and fibroblast **(Figure 38, Panel C)**. Moreover, the nuclei of the principal cells and basal cells

were not marked, but their cytoplasm exhibited weak/moderate immunohistochemical signal **(Figure 38, Panel D)**.

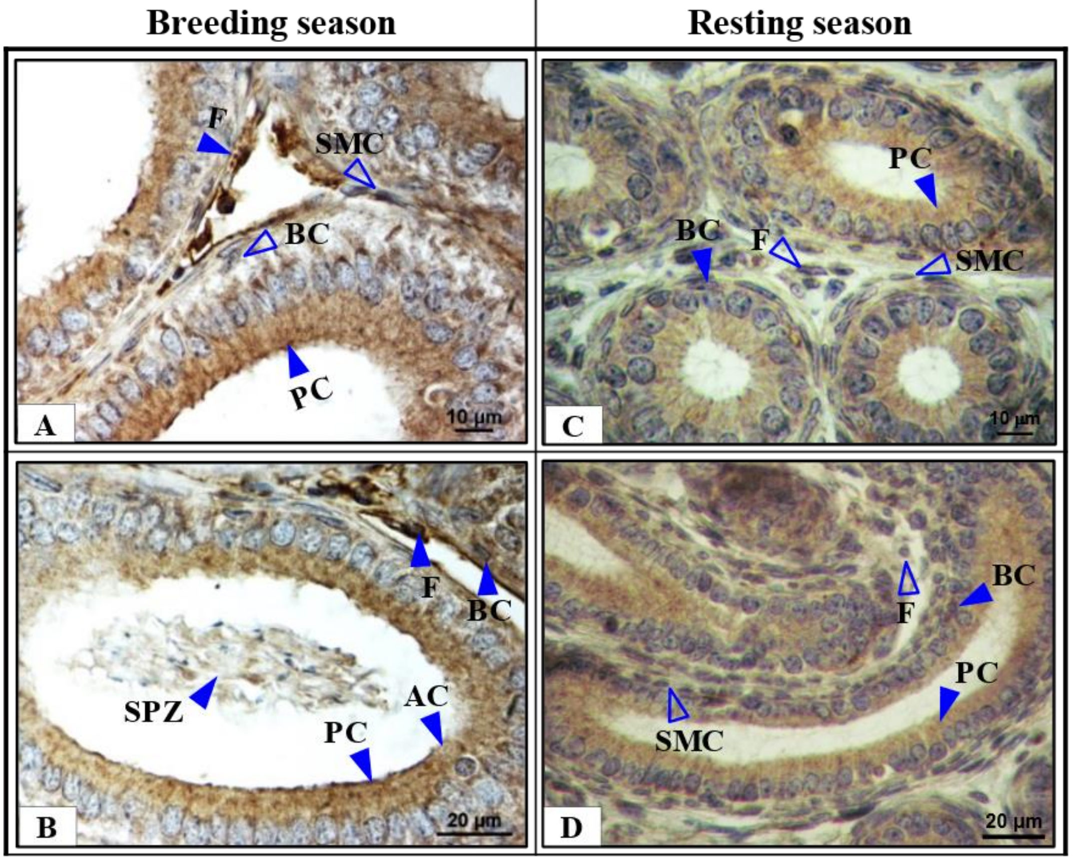


Figure 38: Immunolocalization of GPER1 in the proximal epididymis of *Gerbillus gerbillus*. Panels A and B during the breeding season, Panels C and D during the resting season, principal cells (PC), apical cells (AC), basal cells (BC), spermatozoa (SPZ), fibroblasts (F) and smooth muscle cells (SMC). Empty arrows: absence of immunohistochemical staining. Full arrows: presence of immunohistochemical staining.

In the sand rat *Psammomys obesus*, during the breeding season GPER1 was present in all the cell types. Principal cells strongly expressed GPER1 in their cytoplasm, while their nuclei were weakly marked **(Figure 39, Panel A).** Basal cells, apical cells, spermatozoa, smooth muscle cells and fibroblast were moderately stained **(Figure 39, Panel B).** The resting season affected GPER1 presence in the interstitial tissue as some fibroblasts and smooth muscle

cells were not stained **(Figure 39, Panel C)**, while in the epithelium, principal cells and basal cells were moderately marked **(Figure 39, Panel D).**

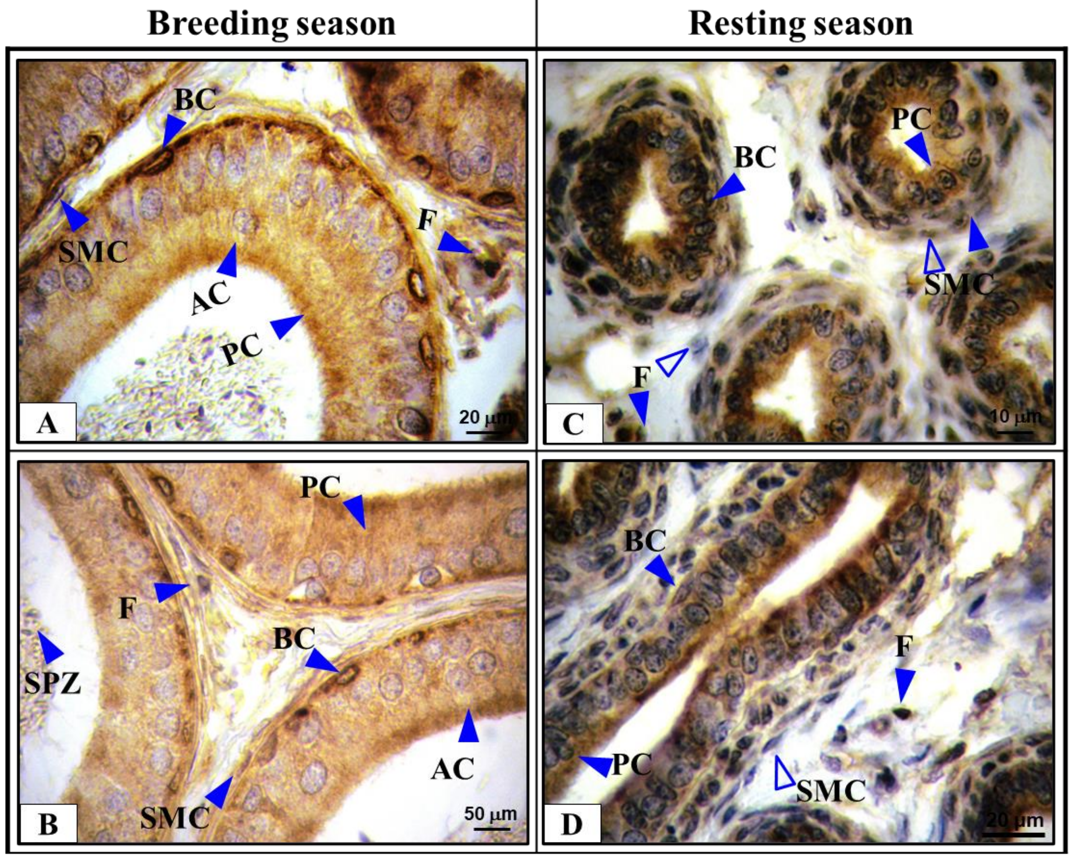


Figure 39: Immunolocalization of GPER1 in the proximal epididymis of *Psammomys obesus*. Panels A and B during the breeding season, Panels C and D during the resting season, principal cells (PC), apical cells (AC), basal cells (BC), spermatozoa (SPZ), fibroblasts (F) and smooth muscle cells (SMC). Empty arrows: absence of immunohistochemical staining. Full arrows: presence of immunohistochemical staining.

I.C.4. The distal epididymis: During the breeding season, in the gerbil *Gerbillus gerbillus*, GPER1 immunoreaction was strongly and obviously apparent in the distal epididymis. Principal cells, clear cells, as well as basal cells showed the higher signal intensity of GPER1 in their nuclei and cytoplasm **(Figure 40, Panel A)**. On the contrary, in the clear cells and smooth muscle cells the immunoreaction was negative, while spermatozoa and fibroblast were accurately stained **(Figure 40, Panel B)**.

During the resting season, a withdraw of GPER1 immunohistochemical staining was noticed. Some basal cells, smooth muscle cells and fibroblast did not express GPER1, while others had weak staining **(Figure 40, Panel C)**. However, principal cells were moderately immunoreactive **(Figure 40, Panel D)**.

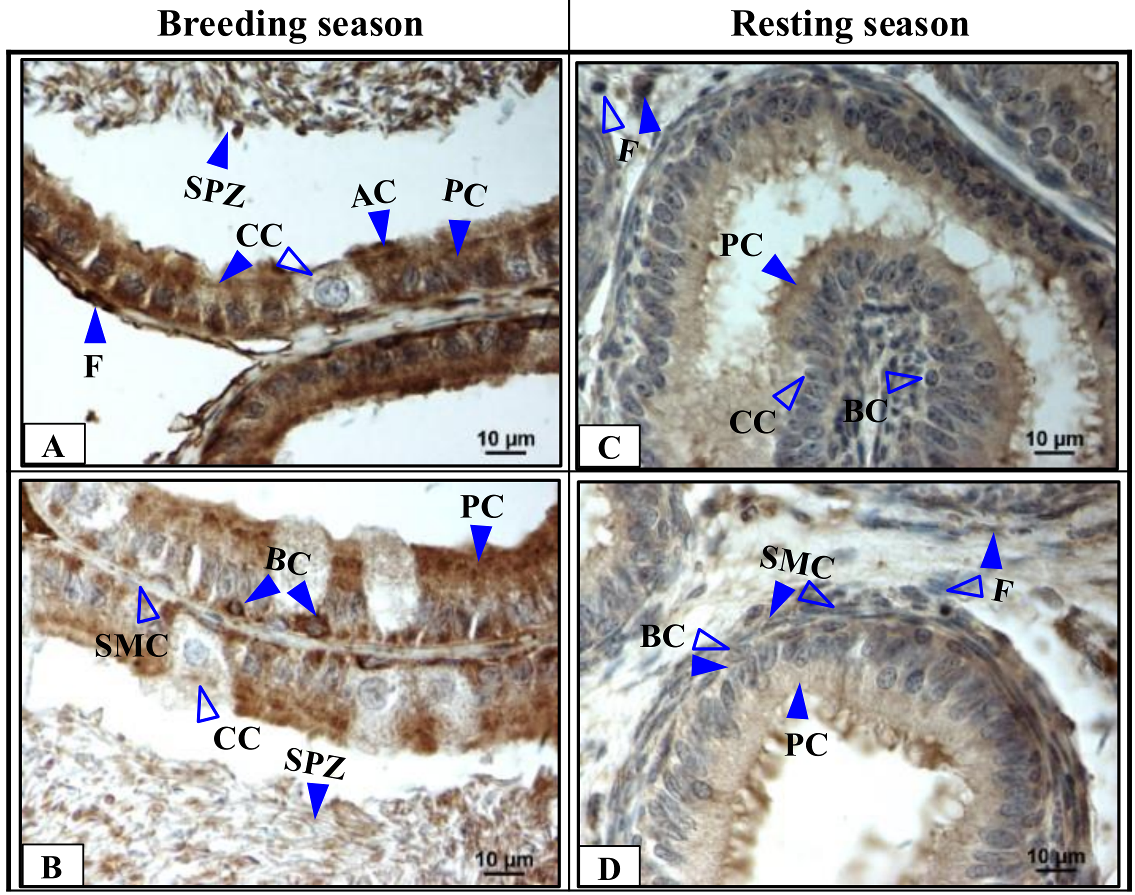


Figure 40: Immunolocalization of GPER1 in the distal epididymis of *Gerbillus gerbillus*. Panels A and B during the breeding season, Panels C and D during the resting season, principal cells (PC), clear cells (CC), apical cytoplasm (AC), basal cells (BC), spermatozoa (SPZ), fibroblasts (F) and smooth muscle cells (SMC). Empty arrows: absence of immunohistochemical staining. Full arrows: presence of immunohistochemical staining.

In the *Psammomys obesus*, during the breeding season, GPER1 was all over the epididymal epithelium as principal cells, basal cells and clear cells were strongly immunoreactive **(Figure 41, Panel A)**. In the interstitial tissue fibroblast did not show any reactivity **(Figure 41, Panel B)**. During the resting season, the immunomarquage of the GPER1

persisted in the distal epididymis **(Figure 41, Panel C)**, but with moderate staining **(Figure 41, Panel D)**.

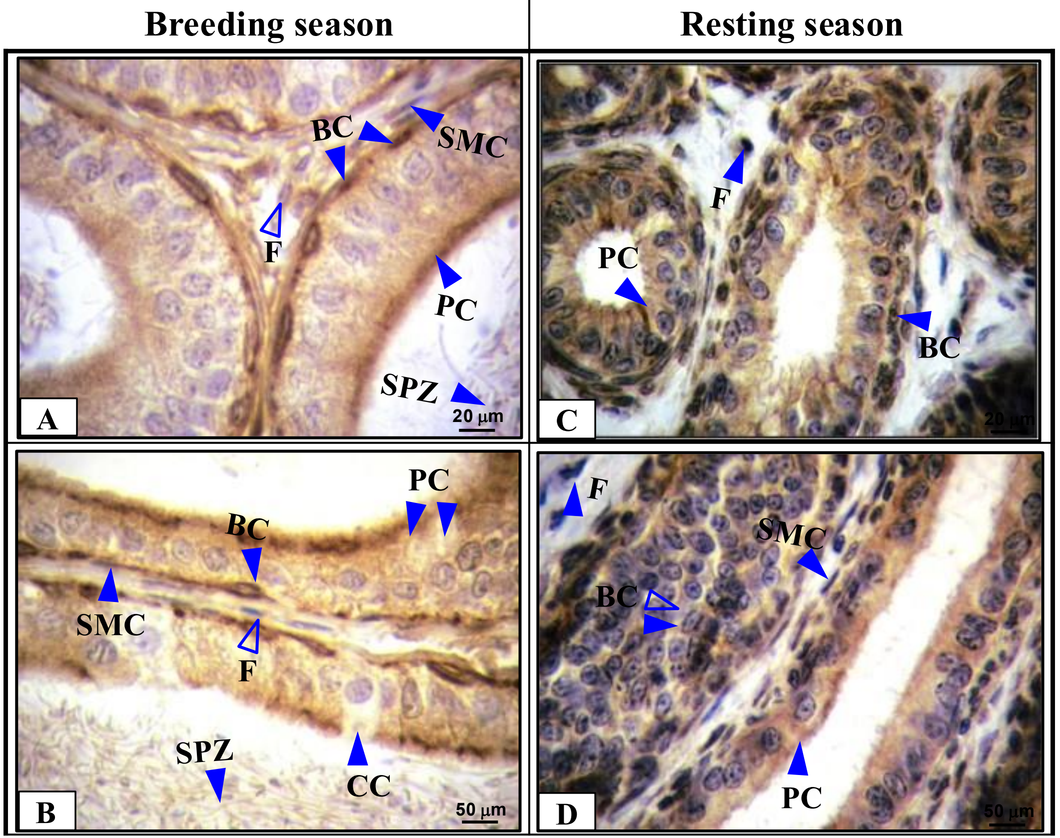


Figure 41: Immunolocalization of GPER1 in the distal epididymis of *Psammomys obesus*. Panels A and B during the breeding season, Panels C and D during the resting season, principal cells (PC), clear cells (CC), apical cytoplasm (AC), basal cells (BC), spermatozoa (SPZ), fibroblasts (F) and smooth muscle cells (SMC). Empty arrows: absence of immunohistochemical staining. Full arrows: presence of immunohistochemical staining.

II. *In silico* study

All results of molecular docking of GPER1 with distinct types of ligands are summarized in tables below, while interactions between them are shown in figures below.

II.A. Known ligands: The molecular docking results of the GPER1 with the know ligands are shown in **Table 04**. For each ligand, the Free binding energy, the inhibition constant Ki, the interactions, the amino acids involved, and the distances of the bonds are mentioned. E2, the classical ligand, exhibited lower ΔG of -6.67 kcal/mol and Ki of 13.00 uM, interacted

with the GPER1 using one hydrogen bond (SER177), five hydrophobic alkyl bonds (ILE181, ALA184, VAL225, PRO226, ILE229) and one hydrophobic Pi-alkyl bond (PHE146), as E2 was the first ligand identified to the GPER1 **(Figure 42, Panels A and B)**.

Moreover, Fulvestrant, famous SERDs, showed ΔG of -2.66 kcal/mol and Ki of 11.22 mM, linked GPER1 utilising one halogen bond (LEU64), Five hydrophobic alkyl bonds (LEU322, ILE323, LEU319, LEU64, LEU69) and one hydrophobic Pi-alkyl bond (PHE362) **(Figure 42, Panels C and D)**. In addition, Tamoxifen, the commonly used SERMs, manifested ΔG of -5.46 kcal/mol and Ki of 100.27 uM, one hydrophobic Pi-Sigma bond (PHE146), one hydrophobic Pi-Pi stacked bond (TRP150), one hydrophobic alkyl bond (ALA184) and three hydrophobic Pi-alkyl bonds (ILE229, ILE181, PRO226) **(Figure 42, Panels E and F)**.

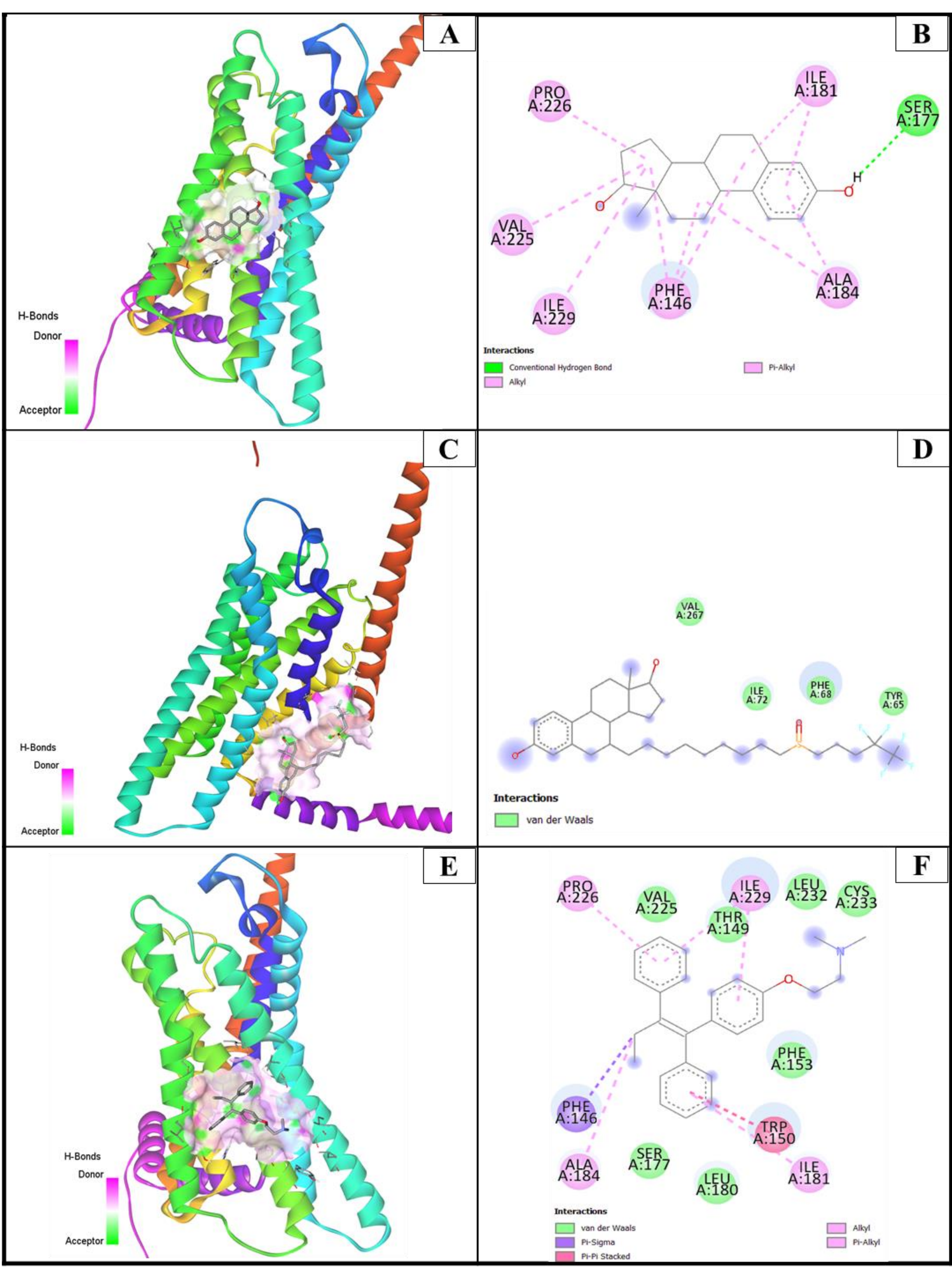
Nevertheless, the synthetic agonist G1 displayed ΔG of -6.43 kcal/mol and Ki of 19.30 uM, two hydrophobic Pi-Sigma bonds (THR149, PHE146), three hydrophobic alkyl bonds (VAL225, PRO226, ILE229) and four hydrophobic Pi-alkyl bonds (ILE229, ALA184, PHE153, PHE146) **(Figure 42, Panels G and H)**. Furthermore, the selective antagonist G36, has predicted the formation of one hydrophobic Pi-Sigma bond (THR149), one hydrophobic Pi-Pi stacked bond (PHE146), five hydrophobic alkyl bonds (ALA188, VAL225, PRO226, ILE229, LEU221) and four hydrophobic Pi-alkyl bonds (PHE146, PHE153, ALA184,ILE229), all having ΔG of -6.66 kcal/mol and Ki of 13.16 uM **(Figure 42, Panels I and J)**. E2,G1 and G36 had comparable affinities toward GPER1 and similar binding features.

Type	Name	RMSD (A°)	Binding Energy	Ki	Interactions	Distance
Known natural and synthetic ligands	E2	19.107 A	-6.67 kcal/mol	13.00 uM	Conventional Hydrogen Bond: E2:H Donor-SER177:O H Acceptor	2,34023 A°
					Hydrophobic Alkyl : ILE181 : Alkyl- E2 : Alkyl	4,98168 A°
					ALA184: Alkyl- E2: Alkyl	5,00403 A°
					VAL225: Alkyl- E2: Alkyl	5,49857 A°
					PRO226: Alkyl- E2: Alkyl	4,87058 A°
					ILE229: Alkyl- E2: Alkyl	5,40199 A°
					Hydrophobic Pi-Alkyl: PHE146: Pi-Orbitals- E2: Alkyl	4,54451 A°
						3,88335 A°
						5,39559 A°
	Fulvestrant	14.770 A	-2.66 kcal/mol	11.22 mM	Halogen: LEU64:O Acceptor- Fulvestrant: F Halogen (Fluorine)	2,78069 A°
					Hydrophobic Alkyl: LEU322 : Alkyl- Fulvestrant: Alkyl	5,23914 A°
						5,06283 A°
						5,01693 A°
						4,85199 A°
					ILE323: Alkyl- Fulvestrant: Alkyl	4,95238 A°
					LEU319: Alkyl- Fulvestrant: Alkyl	4,61377 A°
					LEU64: Alkyl- Fulvestrant: Alkyl	5,14016 A°
					LEU69: Alkyl- Fulvestrant: Alkyl	4,64685 A°
					Hydrophobic Pi-Alkyl: PHE326: Pi-Orbitals- Fulvestrant: Alkyl	4,71419 A°
						4,9481 A°

	Tamoxifen	18.932 A	-5.46 kcal/mol	100.27 uM	Hydrophobic Pi-Sigma: Tamoxifen: CH- PHE146 Pi-Orbitals	3,67586 A°
					Hydrophobic Pi-Pi Stacked: Tamoxifen Pi-Orbitals - TRP150 Pi-Orbitals	5,24127 A°
					Hydrophobic Alkyl: ALA184: Alkyl- Tamoxifen: Alkyl	4,44907 A°
					Hydrophobic Pi-Alkyl: ILE229 : Pi-Orbitals- Tamoxifen: Alkyl	4,41724 A°
						4,78071 A°
					ILE181: Pi-Orbitals- Tamoxifen: Alkyl	5,32514 A°
					PRO226: Pi-Orbitals- Tamoxifen: Alkyl	5,03621 A°
						5,0442 A°
	G1	18.716 A	-6.43 kcal/mol	19.30 uM	Hydrophobic Pi-Sigma: THR149: CH - G1 Pi-Orbitals	3,82086 A°
					PHE146 Pi-Orbitals - G1 Pi-Orbitals	4,08458 A°
					Hydrophobic Alkyl: VAL225: Alkyl- G1: Alkyl	4,86088 A°
					PRO226: Alkyl- G1: Alkyl	4,23412 A°
					ILE229: Alkyl- G1: Alkyl	5,25037 A°
					Hydrophobic Pi-Alkyl: PHE146: Pi-Orbitals- G1: Alkyl	4,72704 A°
						4,34843 A°
					PHE153: Pi-Orbitals- G1: Alkyl	3,62818 A°
					ALA184: Pi-Orbitals- G1: Alkyl	4,45084 A°
					ILE229: Pi-Orbitals- G1: Alkyl	5,04575 A°
	G36	18.683 A	-6.66 kcal/mol	13.16 uM	Hydrophobic Pi-Sigma: THR149: CH- G36 Pi-Orbitals	3,66632 A°
					Hydrophobic Pi-Pi Stacked: PHE146 Pi-Orbitals - G36 Pi-Orbitals	3,94167 A°

					Hydrophobic Alkyl: ALA188: Alkyl- G36: Alkyl	4,10337 A°
					VAL225: Alkyl- G36: Alkyl	4,93481 A°
					PRO226: Alkyl- G36: Alkyl	4,06968 A°
					ILE229: Alkyl- G36: Alkyl	4,98668 A°
					LEU221: Alkyl- G36: Alkyl	4,44125 A°
					Hydrophobic Pi-Alkyl: PHE146 : Pi-Orbitals- G36: Alkyl	4,69801 A°
						4,31625 A°
						4,85109 A°
					PHE153 : Pi-Orbitals- G36: Alkyl	3,6925 A°
					ALA184 : Pi-Orbitals- G36: Alkyl	4,44584 A°
					ILE229 : Pi-Orbitals- G36: Alkyl	4,71528 A°

Table 04: Molecular docking results of GPER1 with the known ligands.


A
H-Bonds
Donor
Acceptor
B
PRO A:226
ILE A:181
SER A:177
VAL A:225
ILE A:229
PHE A:146
ALA A:184
Interactions
Conventional Hydrogen Bond
Alkyl
Pi-Alkyl
C
H-Bonds
Donor
Acceptor
D
VAL A:267
ILE A:72
PHE A:68
TYR A:65
Interactions
van der Waals
E
H-Bonds
Donor
Acceptor
F
PRO A:226
VAL A:225
ILE A:229
LEU A:232
CYS A:233
THR A:149
PHE A:153
PHE A:146
TRP A:150
ALA A:184
SER A:177
LEU A:180
ILE A:181
Interactions
van der Waals
Pi-Sigma
Pi-Pi Stacked
Alkyl
Pi-Alkyl

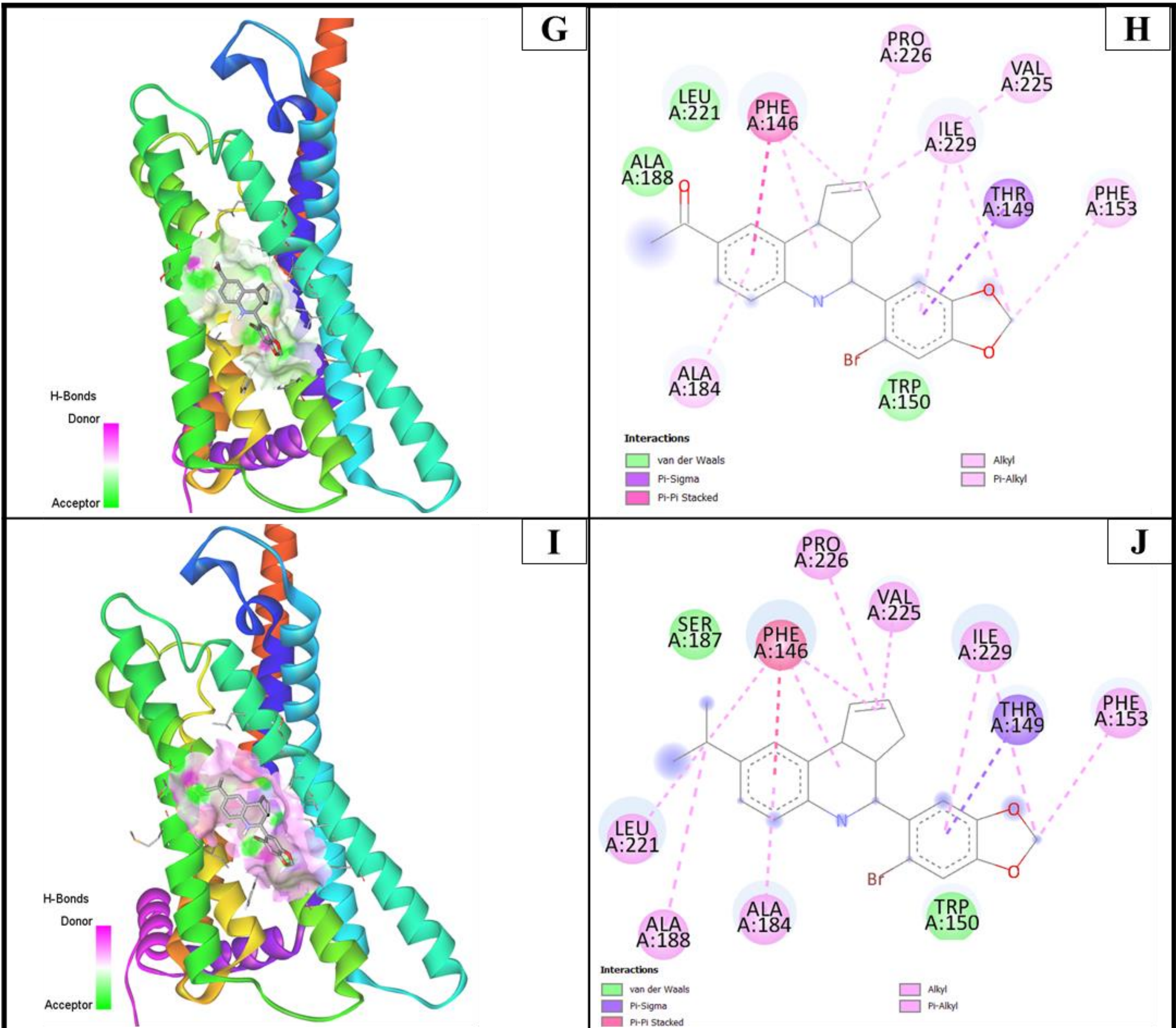


Figure 42: Predicted binding sites of known ligands in the GPER1 pocket (left panels), as well as the 2D representation of the amino acids involved in the interactions between the GPER1 and the known ligands (right panels). Panels A and B E2, Panels C and D Fulvestrant, Panels E and F Tamoxifen, Panels G and H G1, Panels I and J G36.

II.B. Phytochemicals: Phytochemicals tested in this study exhibited strong interaction with the GPER1 as shown in **Table 05**.

Type	Name	RMSD (A°)	Binding Energy	Ki	Interactions	Distance
Phytoestrogens	Anethole	15.548 A	-5.44 kcal/mol	102.29 uM	Hydrophobic Amide-Pi: A:GLY306:C,O;HIS307:N amide- Anethole: Pi-Orbitals	4,82519 A°
					Hydrophobic Alkyl: Anethole: Alkyl- LEU119: Alkyl	4,22012 A°
					Anethole: Alkyl- ARG299: Alkyl	4,35263 A°
	Genistein	14.553 A	-8.47 kcal/mol	617.07 nM	Conventional Hydrogen Bond: Genistein: H-Donor- CYS207: H-Acceptor	2,31989 A°
					Genistein: H-Donor - GLU121: H-Acceptor	
					Genistein: H-Donor - LEU119: H-Acceptor	2,29868 A°
					ARG299: H-Donor - Genistein: H-Acceptor	2,37699 A°
					Electrostatic Pi-Anion: GLU115: Negative- Genistein: Pi-Orbitals	3,15985 A°
					Hydrophobic Pi-Sigma: HIS307: CH- Genistein: Pi-Orbitals	3,55456 A°
					Hydrophobic Pi-Pi Stacked: GLY306: Amide- Genistein: Pi-Orbitals	3,89191 A°
						4,96718 A°
					Hydrophobic Pi-Alkyl: Genistein: Pi-Orbitals- CYS207: Alkyl	4,9665 A°
						4,9046 A°

	Biochanin A	14.002 A	-8.33 kcal/mol	789.35 nM	Conventional Hydrogen Bond: THR92: H-Donor- Biochanin A: H-Acceptor	1,86623 A°
					GLU329: H-Donor- Biochanin A: H-Acceptor	2,13002 A°
					GLY328: H-Donor- Biochanin A: H-Acceptor	3,28751 A°
					Hydrophobic Pi-Sigma: ALA257: CH- Biochanin A: Pi-Orbitals	3,93995 A°
					Hydrophobic Alkyl: Biochanin A: Alkyl- ILE93: Alkyl	3,87484 A°
					Hydrophobic Pi-Alkyl: Biochanin A: Pi-Orbitals- PRO94: Alkyl	5,41718 A°
						4,92453 A°
					Biochanin A: Pi-Orbitals- ALA257: Alkyl	4,44342 A°
					Biochanin A: Pi-Orbitals- MET260: Alkyl	5,20502 A°
	Resveratrol	14.969 A	-7.55 kcal/mol	2.94 uM	Conventional Hydrogen Bond: GLY58: H-Donor- Resveratrol: H-Acceptor	2,54862 A°
					Resveratrol: H-Donor- GLU115: H-Acceptor	2,3488 A°
					Hydrophobic Pi-Sigma: PRO303: CH- Resveratrol: Pi-Orbitals	3,8292 A°
					Hydrophobic Pi-Alkyl: Resveratrol: Pi-Orbitals- LEU119: Alkyl	4,82353 A°
	Eugenol	15.349 A	-5.53 kcal/mol	87.84 uM	Conventional Hydrogen Bond: HIS307: H-Donor- Eugenol: H-Acceptor	2,63739 A°
					Eugenol: H-Donor- GLU115 : H-Acceptor	3,32803 A°
					Eugenol: H-Donor- ASN118 : H-Acceptor	3,7351 A°
					Hydrophobic Amide-Pi: A:GLY306:C,O;HIS307:N amide- Eugenol: Pi-Orbitals	4,70973 A°
					Hydrophobic Alkyl : Eugenol: Alkyl- LEU119: Alkyl	4,07099 A°
					Hydrophobic Pi-Alkyl: HIS307: Pi-Orbitals- Eugenol: Alkyl	4,86004 A°

	Coumestrol	14.389 A	-9.03 kcal/mol	241.1 8 nM	Conventional Hydrogen Bond: ASN118: H-Donor- Coumestrol: H-Acceptor	1,89886 A°
					Coumestrol: H-Donor- CYS207 : H-Acceptor	2,15991 A°
					Coumestrol: H-Donor- PRO303 : H-Acceptor	1,87809 A°
					Electrostatic Pi-Anion: GLU115: Negative- Coumestrol: Pi-Orbitals	3,4854 A°
						3,53992 A°
					CYS207: Negative- Coumestrol: Pi-Orbitals	3,96257 A°
					TYR124: Negative- Coumestrol: Pi-Orbitals	2,8547 A°
					Hydrophobic Pi-Pi T Shaped: TYR124: Amide- Coumestrol: Pi-Orbitals	5,1787 A°
					Coumestrol: Amide- TYR124: Pi-Orbitals	5,59177 A°
					Hydrophobic Pi-Alkyl: Coumestrol: Pi-Orbitals- CYS207: Alkyl	5,44677 A°
					Coumestrol: Pi-Orbitals- ARG299: Alkyl	5,28066 A°
	Sesamol	26.114 A	-6.22 kcal/mol	27.73 uM	Conventional Hydrogen Bond: CYS205: H-Donor- Sesamol: H-Acceptor	1,7734 A°
					Sesamol: H-Donor- ASP210: H-Acceptor	2,18574 A°
					Sesamol: H-Donor- PHE206: H-Acceptor	2,73043 A°
					Hydrophobic Pi-Pi T Shaped: PHE206: Amide- Sesamol: Pi-Orbitals	4,68675 A°
					Hydrophobic Amide-Pi: A:GLN199:C,O;HIS200:N amide- Sesamol: Pi-Orbitals	5,46935 A°
					Hydrophobic Alkyl: CYS205: Alkyl- Sesamol: Alkyl	4,87533 A°
					Hydrophobic Pi-Alkyl: HIS200: Pi-Orbitals- Sesamol: Alkyl	5,01354 A°
					PHE206: Pi-Orbitals- Sesamol: Alkyl	4,86235 A°
					Sesamol: Pi-Orbitals- CYS205:Alkyl	4,63806 A°

	Lupalbigenin	20.162 A	-8.20 kcal/mol	982.02 nM	Conventional Hydrogen Bond: Lupalbigenin: H-Donor- SER177: H-Acceptor	2,09075 A°
					Hydrophobic Pi-Sigma : LEU180 : CH- Lupalbigenin : Pi-Orbitals	3,80933 A°
					Hydrophobic Pi-Pi Stacked : TRP150 : Amide- Lupalbigenin : Pi-Orbitals	4,66895 A° 5,2808 A° 4,40023 A°
					Hydrophobic Pi-Alkyl : PHE146 : Pi-Orbitals- Lupalbigenin : Alkyl	4,98058 A°
					TRP150 : Pi-Orbitals- Lupalbigenin : Alkyl	5,08575 A°
					PHE168 : Pi-Orbitals- Lupalbigenin : Alkyl	4,05918 A°
					HIS173 : Pi-Orbitals- Lupalbigenin : Alkyl	4,70968 A° 5,30827 A°
					Lupalbigenin : Pi-Orbitals- LEU180 : Alkyl	4,69079 A°
					Lupalbigenin : Pi-Orbitals- ILE181 : Alkyl	5,22779 A°
					Lupalbigenin: Pi-Orbitals- ALA184: Alkyl	

	Glabren	18.484 A	-8.19 kcal/mol	992.9 4 nM	Conventional Hydrogen Bond: Glabren: H-Donor- SER177: H-Acceptor	2,56882 A°
					Glabren: H-Donor- LEU221: H-Acceptor	2,5065 A°
					Hydrophobic Pi-Pi Stacked: PHE146: Amide- Glabren: Pi-Orbitals	3,96834 A°
					TRP150: Amide- Glabren: Pi-Orbitals	5,6751 A°
						4,87912 A°
					Hydrophobic Alkyl: ILE181: Alkyl- Glabren: Alkyl	5,19466 A°
					ALA184: Alkyl- Glabren: Alkyl	3,35433 A°
					LEU221: Alkyl- Glabren: Alkyl	5,14682 A°
					Hydrophobic Pi-Alkyl: PHE146: Pi-Orbitals- Glabren: Alkyl	4,16452 A°
						4,29455 A°
						5,29287 A°
					Glabren: Pi-Orbitals- PRO226: Alkyl	5,27749 A°
					Glabren: Pi-Orbitals- LEU180: Alkyl	5,46713 A°
					Glabren: Pi-Orbitals- ILE181: Alkyl	5,00954 A°

	Enterodiol	7.387 A	-5.18 kcal/mol	160.10 uM	Conventional Hydrogen Bond: Enterodiol: H-Donor- LEU61: H-Acceptor Hydrophobic Pi-Pi stacked: PHE68: Pi-Orbitals- Enterodiol: Pi-Orbitals Hydrophobic Amide-Pi: A:LEU311:C,O;ALA312:N amide- Enterodiol: Pi-Orbitals Hydrophobic Alkyl : Enterodiol : Alkyl- LEU64 : Alkyl Enterodiol : Alkyl- LEU69 : Alkyl Hydrophobic Pi-Alkyl : Enterodiol : Pi-Orbitals- LEU64 : Alkyl Enterodiol : Pi-Orbitals- ILE308 : Alkyl Enterodiol : Pi-Orbitals- LEU311 : Alkyl Enterodiol: Pi-Orbitals- ALA312: Alkyl	2,26098 A° 4,71257 A° 4,22079 A° 5,34926 A° 4,65196 A° 4,96285 A° 5,41356 A° 4,69932 A° 4,60491 A°
	Piceatannol	15.003 A	-7.89 kcal/mol	1.65 uM	Conventional Hydrogen Bond: Piceatannol: H-Donor- GLN54: H-Acceptor ARG299: H-Donor- Piceatannol: H-Acceptor Hydrophobic Pi-Sigma: PRO303: CH- Piceatannol: Pi-Orbitals Hydrophobic Amide-Pi: A:PRO303:C,O;LEU304:N amide- Piceatannol: Pi-Orbitals Hydrophobic Pi-Alkyl : Piceatannol: Pi-Orbitals- LEU119: Alkyl	2,24797 A° 3,33727 A° 3,76471 A° 3,78098 A° 4,78303 A°

	Wedelolactone	23.722 A	-7.33 kcal/mol	4.20 uM	Conventional Hydrogen Bond: TYR97:H-Donor- Wedelolactone: H-Acceptor	2,3898 A°
					Wedelolactone: H-Donor- SER177: H-Acceptor	2,12923 A°
					Wedelolactone: H-Donor- ASP154: H-Acceptor	2,19072 A°
						1,99154 A°
					Hydrophobic Pi-Sigma: HIS173: CH- Wedelolactone: Pi-Orbitals	3,98033 A°
					Hydrophobic Pi-Pi T shaped: TRP150: Pi-Orbitals- Wedelolactone: Pi-Orbitals	4,90266 A°
					Wedelolactone: Pi-Orbitals - TRP150: Pi-Orbitals	4,60547 A°
					Hydrophobic Alkyl : Wedelolactone : Alkyl- LEU176 : Alkyl	4,85041 A°
					Wedelolactone : Alkyl- LEU180 : Alkyl	5,04608 A°
					Hydrophobic Pi-Alkyl: Wedelolactone: Pi-Orbitals- LEU176: Alkyl	45,2769 A°

Table 05: Molecular docking results of GPER1 with phytochemicals.

For instance, Anethole presented relatively low ΔG of -5.44 kcal/mol and Ki of 102.29 uM, one hydrophobic amide bond (GLY306, HIS307), two hydrophobic alkyl bonds (LEU119, ARG299) **(Figure 43, Panels A and B)**.

Moreover, Genistein displayed a low binding energy ΔG of -8.47 kcal/mol and Ki of 617.07 nM with four hydrogen bonds (CYS207, GLU121, LEU119, ARG299), one electrostatic Pi-Anion bond (GLU115), one hydrophobic Pi-Sigma bond (HIS307), one hydrophobic Pi-Pi stacked bond (GLY306) and one hydrophobic Pi-alkyl bond (CYS207) **(Figure 43, Panels C and D)**. In addition, Biochanin A had a lower binding energy ΔG of -8.33 kcal/mol and Ki of 789.3 nM, with three hydrogen bonds (TYR92, GLU329, GLY328), on hydrophobic Pi sigma bond (ALA257), one hydrophobic alkyl bond (ILE93) and three hydrophobic Pi alkyl bonds (PRO94, ALA257, MET206) **(Figure 43, Panels E and F)**.

Meanwhile, Resveratrol manifested low binding energy ΔG of -7.55 kcal/mol and Ki of 2.94 uM, two hydrogen bonds (GLY58, CLU115), one hydrophobic Pi-Sigma bond (PRO303) and one hydrophobic Pi-alkyl bond (LEU119) **(Figure 43, Panels G and H)**. In contrast, Eugenol showed relatively high ΔG of -5.53 kcal/mol and Ki of 87.84 uM, three hydrogen bonds (HIS307, GLU115, ASN118), one hydrophobic amide bond (GLY306, HIS307), one hydrophobic Pi-alkyl bond (LEU119) and one hydrophobic Pi alkyl bond (HIS307) **(Figure 43, Panels I and J)**.

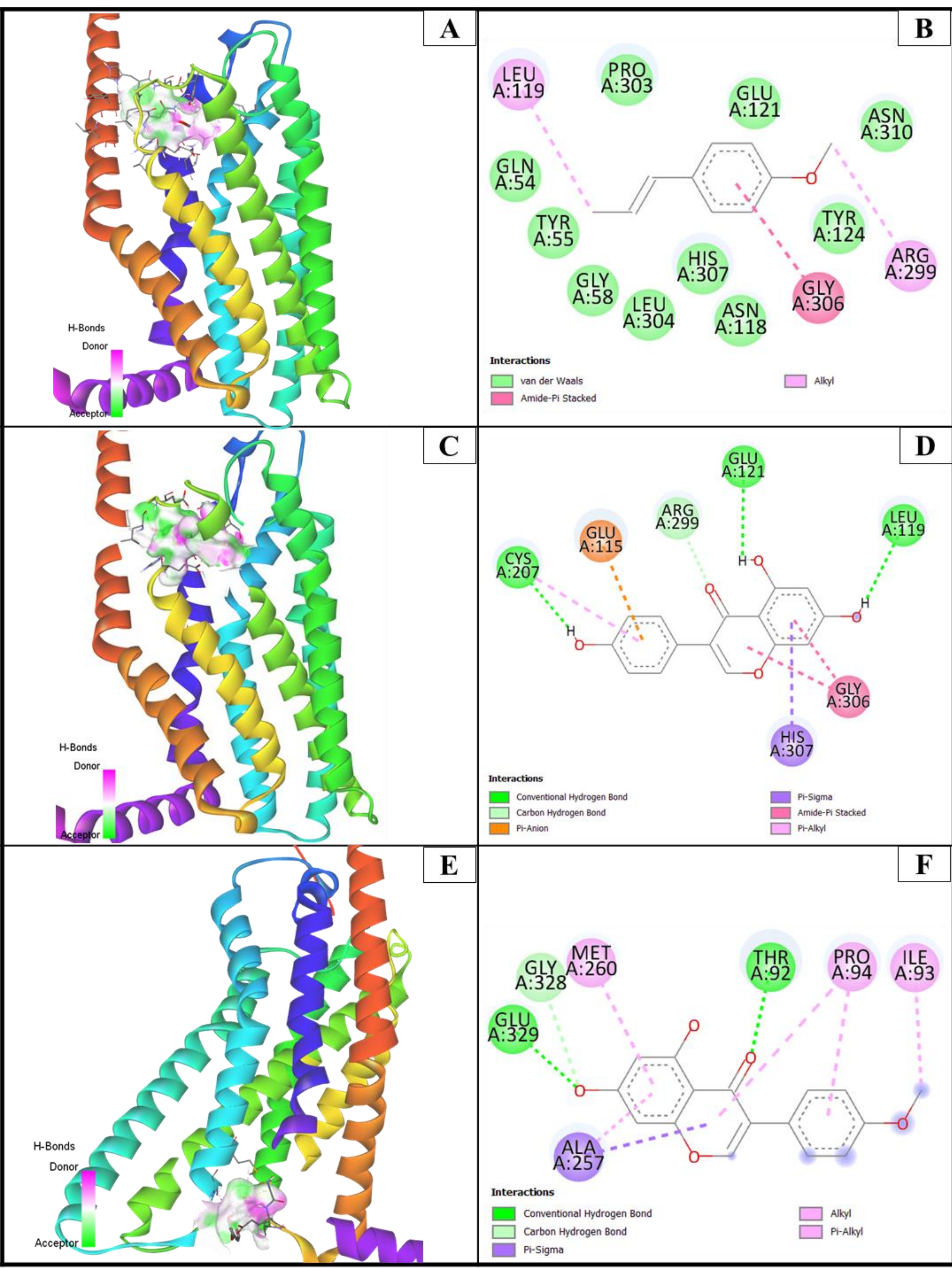
Interestingly, Coumestrol exhibited the lowest binding energy ΔG of -9.03 kcal/mol and Ki of 241.18 nM, with three hydrogen bonds (ASN118, CYS207, PRO303), three electrostatic Pi anion bonds (GLU115, CYS207, TYR124), two hydrophobic Pi T-shaped bonds (TYR124) and two hydrophobic Pi alkyl bonds (CYS207, ARG299) **(Figure 43, Panels K and L)**. In the same manner, Sesamol showed low binding energy ΔG of -6.22 kcal/mol and Ki of 27.73 uM, with three hydrogen bonds (CYS205, ASP210, PHE206), one hydrophobic Pi T-shaped bond

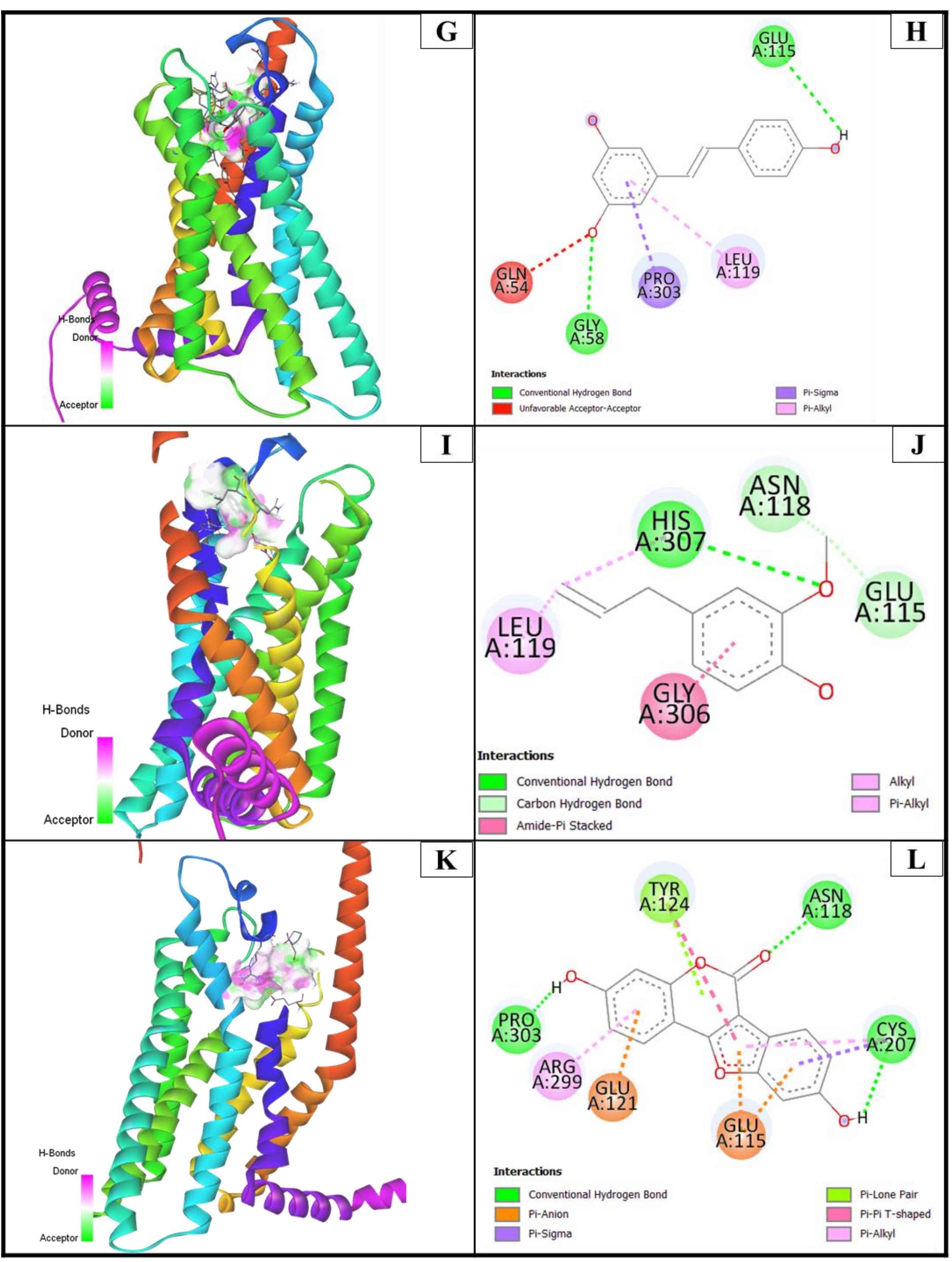
(PHE206), one hydrophobic amide bond (GLN199, HIS200), one hydrophobic alkyl bond (CYS205) and three hydrophobic Pi alkyl bonds (HIS200, PHE206, CYS205) **(Figure 43, Panels M and N)**.

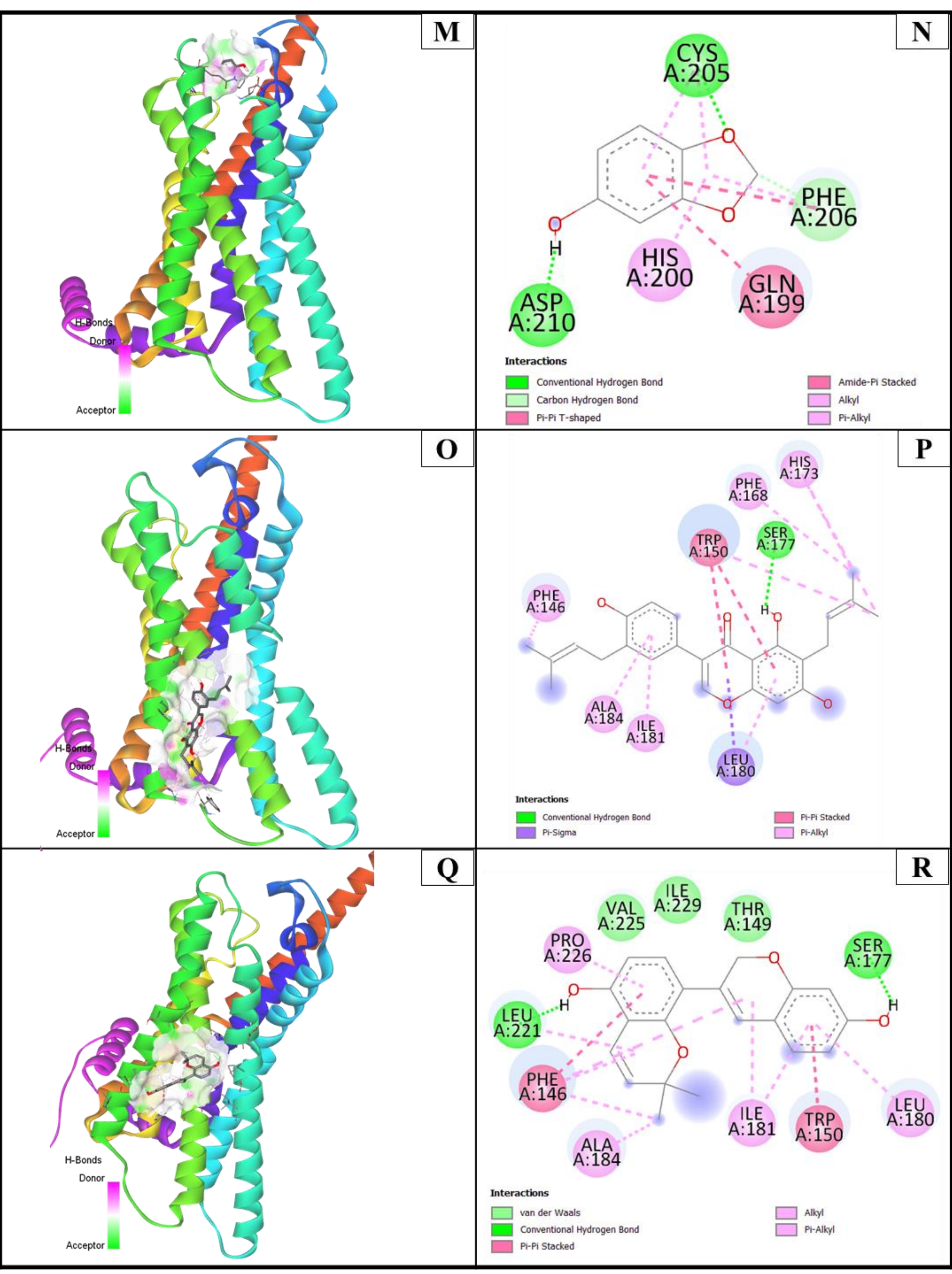
Nevertheless, Lupalbigenin displayed low binding energy ΔG of -8.20 kcal/mol and Ki of 982.02 nM, with one hydrogen bond (SER177), one hydrophobic Pi sigma bond (LEU180), one hydrophobic Pi stacked bond (TRP150), seven hydrophobic Pi alkyl bonds (PHE146, TRP150, PHE168, HIS173, LEU180, ILE181, ALA184) **(Figure 43, Panels O and P)**. Besides, Glabrene presented low binding energy ΔG of -8.19 kcal/mol and Ki of 992.94 nM, with two hydrogen bonds (SER177, LEU221), two hydrophobic Pi stacked bonds (PHE146, TRP150), three hydrophobic alkyl bonds (ILE181, ALA184, LEU221) and four hydrophobic Pi alkyl bonds (PHE146, PRO226, LEU180, ILE181) **(Figure 43, Panels Q and R)**.

Furthermore, Enterodiol had highest binding energy ΔG of -5.18 kcal/mol and Ki of 160.10 uM, with one hydrogen bond (LEU61), one hydrophobic Pi stacked bond (PHE68), one hydrophobic amide bond (LEU311), two hydrophobic alky bonds (LEU64, LEU69), four hydrophobic Pi alkyl bonds (LEU64, ILE308, LEU311; ALA312) **(Figure 43, Panels S and T)**. In addition, Piceatannol also had low binding energy ΔG of -7.89 kcal/mol and Ki of 1.65 uM, with two hydrogen bonds (GLN54, ARG299), one hydrophobic Pi sigma bond (PRO303), one hydrophobic amide bond (PRO303) and one hydrophobic Pi alkyl bond (LEU119) **(Figure 43, Panels U and V)**.

Finally, Wedelolactone displayed low binding energy ΔG of -7.33 kcal/mol and Ki of 4.20 uM, with three hydrogen bonds (TYR97, SER177, ASP154), one hydrophobic Pi sigma bond (HIS173), two hydrophobic Pi T-shaped bonds (TRP150), two hydrophobic alkyl bonds (LEU176, LEU180) and one hydrophobic Pi alkyl bond (LEU17) **(Figure 43, Panels W and X)**.


A
H-Bonds
Donor
Acceptor
B
LEU A:119
PRO A:303
GLU A:121
ASN A:310
GLN A:54
TYR A:55
TYR A:124
HIS A:307
ARG A:299
GLY A:58
LEU A:304
ASN A:118
GLY A:306
Interactions
van der Waals
Amide-Pi Stacked
Alkyl
C
H-Bonds
Donor
Acceptor
D
GLU A:121
ARG A:299
LEU A:119
GLU A:115
CYS A:207
GLY A:306
HIS A:307
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Anion
Pi-Sigma
Amide-Pi Stacked
Pi-Alkyl
E
H-Bonds
Donor
Acceptor
F
MET A:260
GLY A:328
THR A:92
PRO A:94
ILE A:93
GLU A:329
ALA A:257
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Alkyl
Pi-Alkyl


G
H-Bonds
Donor
Acceptor
H
GLU A:115
GLN A:54
PRO A:303
LEU A:119
GLY A:58
Interactions
Conventional Hydrogen Bond
Unfavorable Acceptor-Acceptor
Pi-Sigma
Pi-Alkyl
I
H-Bonds
Donor
Acceptor
J
ASN A:118
HIS A:307
GLU A:115
LEU A:119
GLY A:306
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Amide-Pi Stacked
Alkyl
Pi-Alkyl
K
H-Bonds
Donor
Acceptor
L
TYR A:124
ASN A:118
PRO A:303
CYS A:207
ARG A:299
GLU A:121
GLU A:115
Interactions
Conventional Hydrogen Bond
Pi-Anion
Pi-Sigma
Pi-Lone Pair
Pi-Pi T-shaped
Pi-Alkyl


M
N
CYS A:205
PHE A:206
HIS A:200
GLN A:199
ASP A:210
H-Bonds
Donor
Acceptor
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Pi T-shaped
Amide-Pi Stacked
Alkyl
Pi-Alkyl
O
P
HIS A:173
PHE A:168
TRP A:150
SER A:177
PHE A:146
ALA A:184
ILE A:181
LEU A:180
Interactions
Conventional Hydrogen Bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl
Q
R
ILE A:229
VAL A:225
THR A:149
PRO A:226
SER A:177
LEU A:221
PHE A:146
ILE A:181
TRP A:150
LEU A:180
ALA A:184
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Pi Stacked
Alkyl
Pi-Alkyl

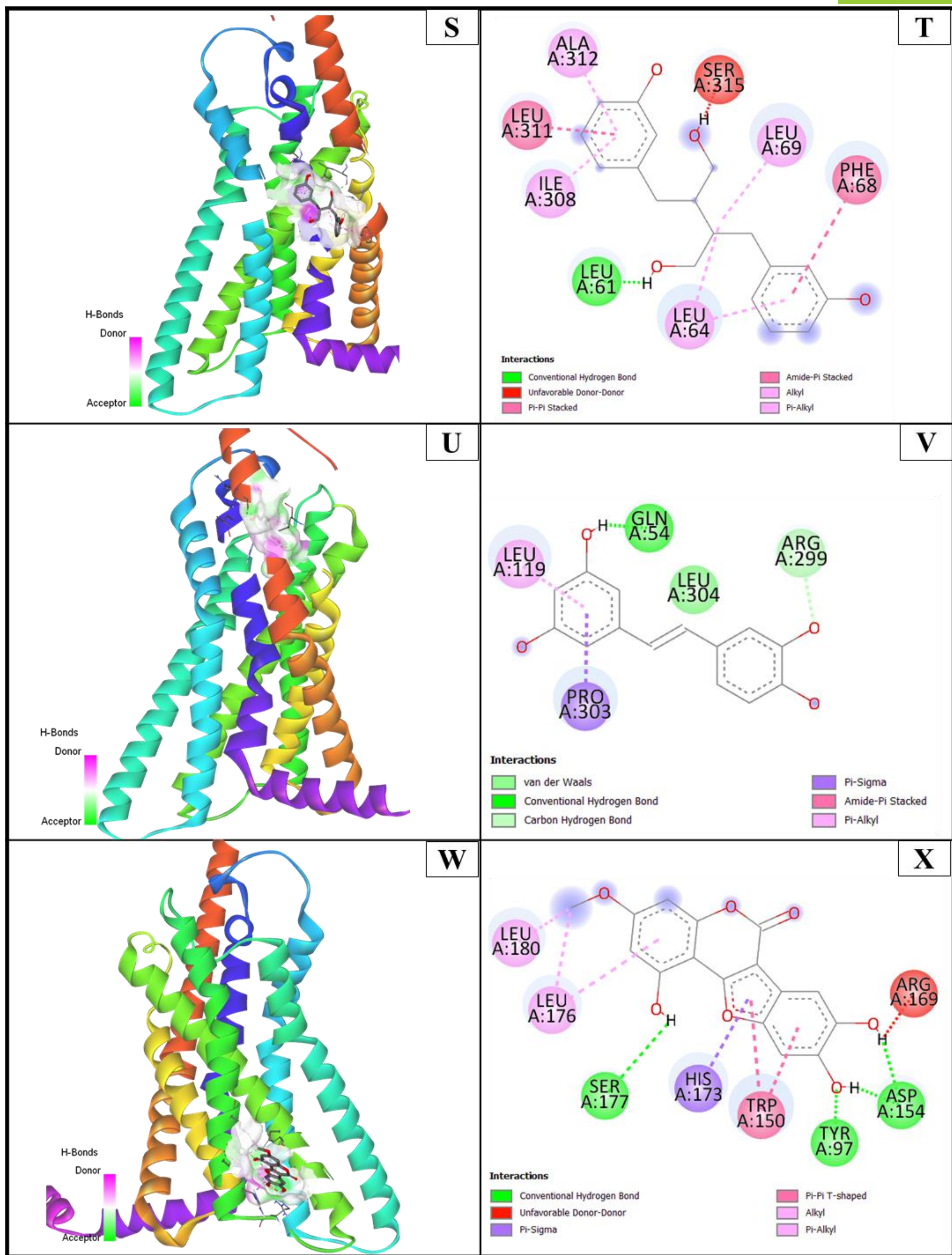


Figure 43: Predicted binding sites of phytoestrogens in the GPER1 pocket (left panels), as well as the 2D representation of the amino acids involved in the interactions between the phytochemicals and the GPER1 (right panels). Panels A and B Anethole, Panels C and D

Genistein, Panels E and F Biochanin A, Panels G and H Resveratrol, Panels I and J Eugenol, Panels K and L Coumestrol, Panels M and N Sesamol, Panels O and P Lupalbigenin, Panels Q and R Glabren, Panels S and T Enterodiol, Panels U and V Piceatannol, Panels W and X Wedelolactone.

II.C. Heavy metals: Heavy metals are widely spread, can easily penetrate in the living organisms, and cause harmful effects. The famous heavy metals: lead acetate, cadmium chloride and Dimethyl mercury have been tested in our study and predicated to have high affinity for the GPER1, **Table 06**.

Notably, Cadmium chloride showed binding energy ΔG of -3.09 kcal/mol and Ki of 5.44 mM with one hydrophobic Pi alkyl bond (HIS200) **(Figure 44, Panels A and B)**, lead acetate exhibited significantly low binding energy ΔG of -4.12 kcal/mol and Ki of 956.17 uM and two hydrogen bonds (CYS205,PHE206) **(Figure 44, Panels C and D)**, while Dimethyl mercury presented a binding energy ΔG of -2.45 kcal/mol and Ki of 16.07 mM respectively with one hydrophobic Pi sigma bond (HIS200) **(Figure 44, Panels E and F)**.

Type	Name	RMSD (A°)	Binding Energy	Ki	Interactions	Distance
Heavy metals	Lead Acetate	25.109 A	-4.12 kcal/mol	956.17 uM	Conventional Hydrogen Bond: CYS205:H-Donor- Lead acetate H-Acceptor Lead acetate: H-Donor- PHE206: H-Acceptor	2,18871 A° 1,72193 A°
	Cadmium Chloride	24.393 A	-3.09 kcal/mol	5.44 mM	Hydrophobic Pi-Alkyl: HIS200: Pi-Orbitals- Cadmium Chloride: Alkyl	3,75258 A°
	Dimethyl mercury	24.397 A	-2.45 kcal/mol	16.07 mM	Hydrophobic Pi-Sigma: Dimethylmercury: CH- HIS200: Pi-Orbitals	3,71731 A°

Table 06: Molecular docking results of GPER1 with heavy metals.

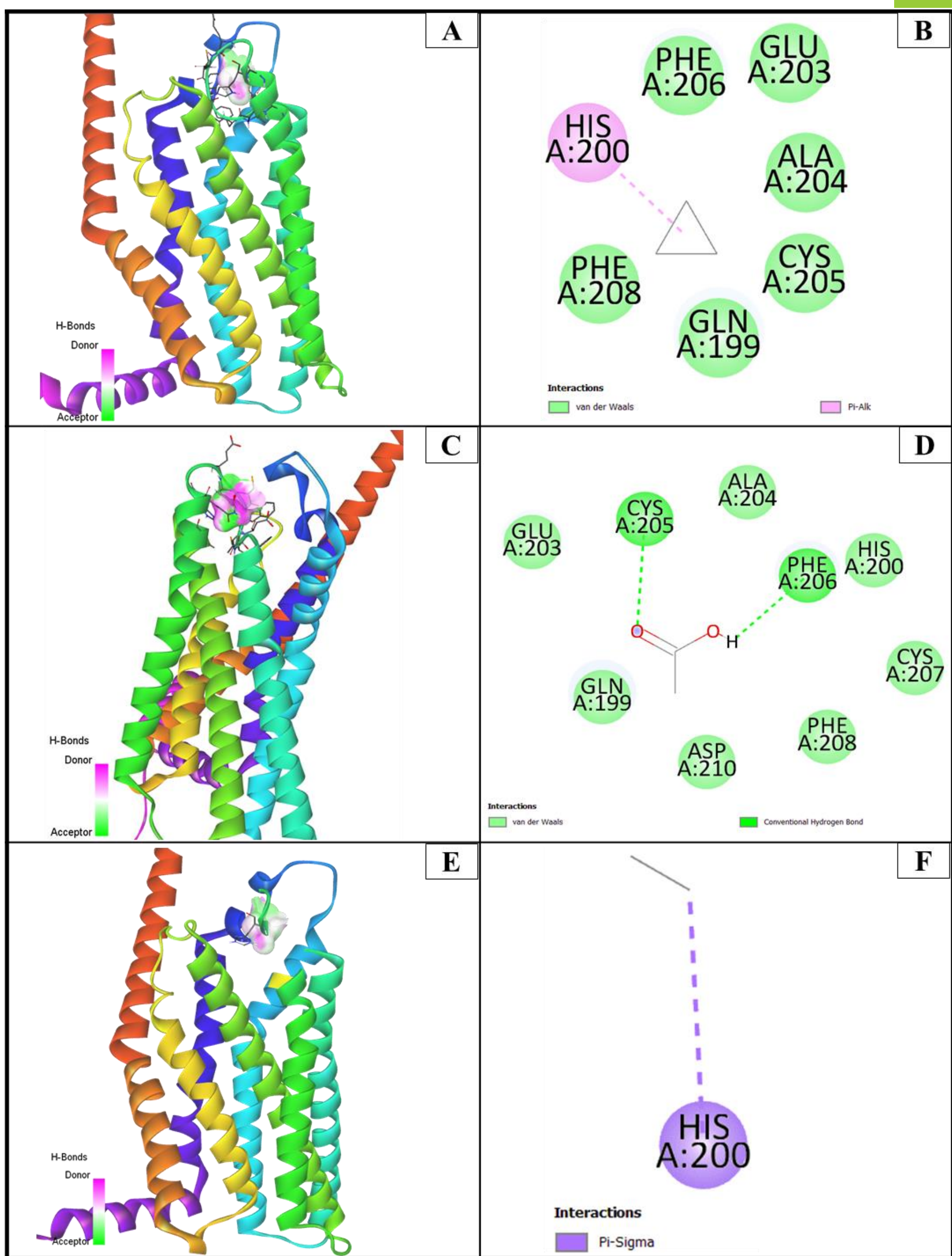


Figure 44: Predicted binding sites of heavy metals in the GPER1 pocket (left panels), as well as the 2D representation of the amino acids involved in the interactions between heavy metals and the GPER1 (right panels). Panels A and B Cadmium chloride, Panels C and D Lead acetate, Panels E and F Dimethyl Mercury .

II.D. Pesticides: The analyse of pesticides interactions with the GPER1 revealed interesting results presented in **Table 07**.

Type	Name	RMSD (A°)	Binding Energy	Ki	Interactions	Distance
Pesticides	Captan	19.910 A	-6.38 kcal/mol	20.92 uM	Electrostatic Pi-Sulfur: Captan: S- TRP150: Pi-Orbitals	5,06724 A°
						4,90244 A°
					Hydrophobic Alkyl : ILE181 : Alkyl- Captan : Alkyl	5,43118 A°
					ALA184: Alkyl- Captan: Alkyl	3,74818 A°
					Hydrophobic Pi-Alkyl: PHE146: Pi-Orbitals- Captan: Alkyl	5,04146 A°
					TRP150: Pi-Orbitals- Captan: Alkyl	5,16394 A°
	DDT	18.525 A	-5.93 kcal/mol	45.13 uM	Hydrophobic Pi-Sigma: THR149: CH- DDT: Pi-Orbitals	3,93245 A°
					Hydrophobic Pi-Pi Stacked: PHE153: Amide- DDT: Pi-Orbitals	5,76003 A°
					Hydrophobic Alkyl : DDT : Alkyl- LEU221 : Alkyl	4,13765 A°
					DDT : Alkyl- VAL225 : Alkyl	4,00488 A°
					Hydrophobic Pi-Alkyl : PHE153 : Pi-Orbitals- DDT : Alkyl	3,25977 A°
					ILE229: Pi-Orbitals- DDT: Alkyl	5,14901 A°
	Fenvalerate	19.596 A	-6.48 kcal/mol	17.72 uM	Hydrophobic Pi-Pi Stacked: PHE146: Pi-Orbitals- Fenvalerate: Pi-Orbitals	3,94411 A°
					Hydrophobic Alkyl: Fenvalerate: Alkyl- LEU180: Alkyl	3,92951 A°
					Hydrophobic Pi-Alkyl: TRP150: Pi-Orbitals- Fenvalerate: Alkyl	4,73233 A°
					PHE153: Pi-Orbitals- Fenvalerate: Alkyl	4,87235 A°
					Fenvalerate: Pi-Orbitals-ALA184 : Alkyl	4,37585 A°
					Fenvalerate: Pi-Orbitals-ALA188 : Alkyl	4,42136 A°
					Fenvalerate : Pi-Orbitals- LEU221 : Alkyl	5,33865 A°

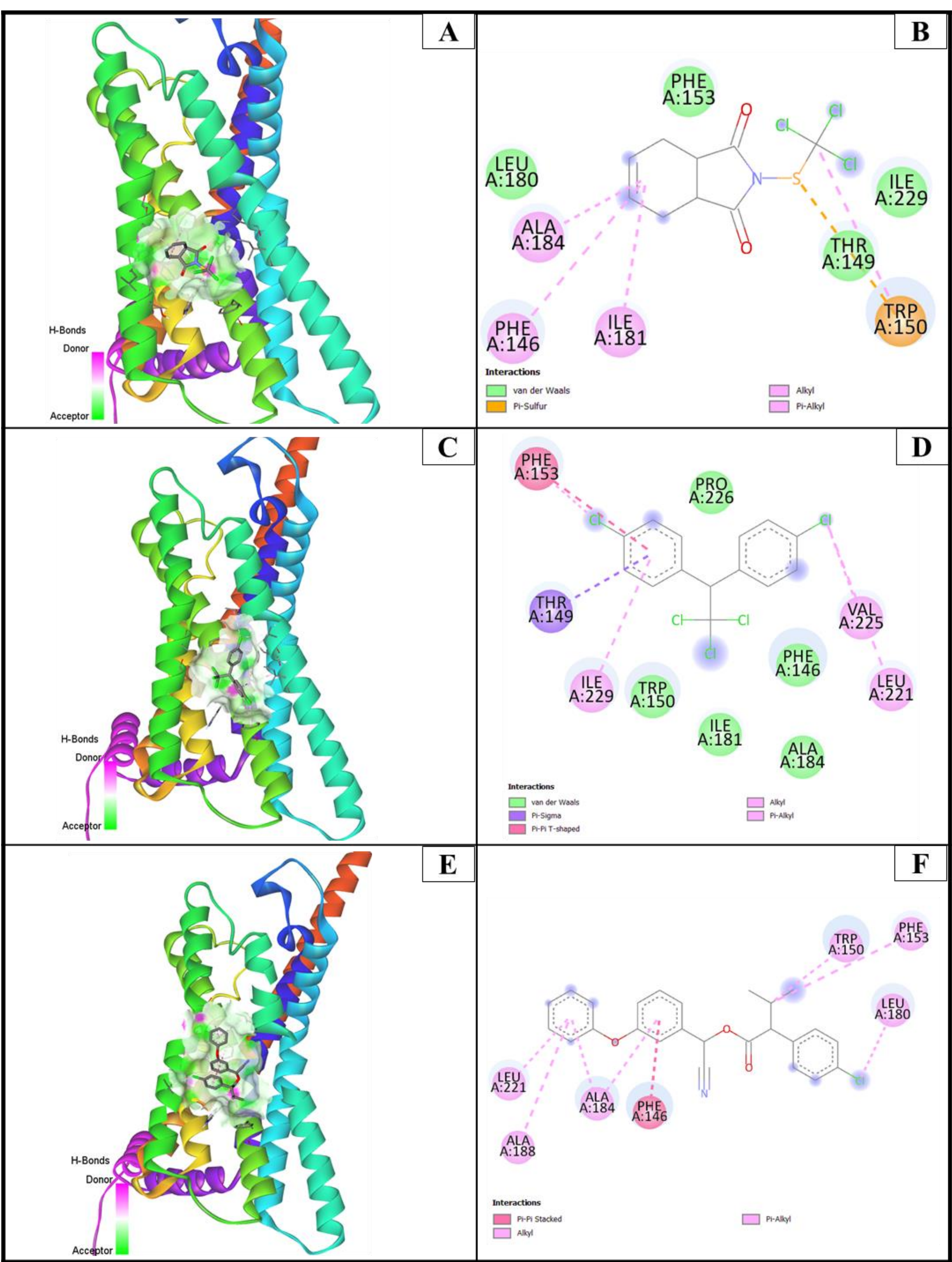
	Propoxur	27.469 A	-5.87 kcal/mol	50.06 uM	Conventional Hydrogen Bond: Propoxur: H-Donor- ASP210:H-Acceptor	3,03909 A°
						2,37189 A°
					Propoxur: H-Donor- GLU203:H-Acceptor	3,17064 A°
					Hydrophobic Pi-Pi T-Shaped: HIS200: Pi-Orbitals- Propoxur: Pi-Orbitals	5,91493 A°
					PHE206: Pi-Orbitals- Propoxur: Pi-Orbitals	5,03355 A°
					Hydrophobic Alkyl: Propoxur: Alkyl- PRO293: Alkyl	4,55028 A°
					Propoxur: Alkyl- CYS294: Alkyl	5,08463 A°
					Hydrophobic Pi-Alkyl: Propoxur: Pi-Orbitals- CYS205: Alkyl	5,09222 A°
	Acetamiprid	21.925 A	-6.37 kcal/mol	21.27 uM	Conventional Hydrogen Bond: HIS300: H-Donor- Acetamiprid: H-Acceptor	2,23309 A°
					Hydrophobic Pi-Sigma : Acetamiprid: CH- HIS120 : Pi-Orbitals	3,74503 A°
					Hydrophobic Amide-Pi: A:GLN54:C,O;TYR55:N amide- Acetamiprid: Pi-Orbitals	3,78178 A°
						3,83215 A°
					Hydrophobic Alkyl : Acetamiprid : Alkyl- LEU119 : Alkyl	3,99624 A°
					Acetamiprid : Alkyl- LEU304 : Alkyl	5,12731 A°
					Hydrophobic Pi-Alkyl : Acetamiprid : Pi-Orbitals- PRO303 : Alkyl	4,70062 A°

Table 07: Molecular docking results of GPER1 with pesticides.

For example, Captan showed binding energy ΔG of -6.38 kcal/mol and Ki of 20.92 uM, one electrostatic Pi-sulfur bond (TRP150), two hydrophobic alkyl bonds (ALA181, ALA184) and two hydrophobic Pi-alkyl bonds (TRP150, PHE146) **(Figure 45, Panels A and B)**. In addition, DDT had a binding energy ΔG of -5.93 kcal/mol and Ki of 45.13 uM, one hydrophobic Pi-Sigma bond (THR149), one hydrophobic Pi-Pi Stacked bond (PHE153), two hydrophobic alkyl bonds (LEU221, VAL225) and two hydrophobic Pi-alkyl bonds (PHE153, ILE229) **(Figure 45, Panels C and D)**, which were the same interactions exhibited by E2, G1 and G36.

Moreover, the insecticide Fenvalerate displayed lowest binding energy among the tested pesticides, ΔG of -6.48 kcal/mol and Ki of 17.72 uM, one hydrophobic Pi-Pi Stacked bond (PHE146), one hydrophobic alkyl bond (LEU180) and five hydrophobic Pi-alkyl bonds (TRP150, PHE153, ALA184, ALA188, LEU221) **(Figure 45, Panels E and F)**. Nevertheless, Propoxur manifested ΔG of -5.87 kcal/mol and Ki of 50.06 uM, two hydrogen bonds (ASP210, GLU203), two hydrophobic Pi T-shaped bonds (HIS200, PHE206), two hydrophobic alkyl bonds (PRO293, CYS294) and one hydrophobic Pi-alkyl bond (CYS205) **(Figure 45, Panels G and H)**.

Finaly, Acetamiprid revealed low binding energy ΔG of -6.37 kcal/mol, Ki of 21.27 uM, with one hydrogen bond (HIS300), one hydrophobic Pi-Sigma bond (HIS120), one hydrophobic amide bond (GLN54, TYR55), two hydrophobic alkyl bonds (LEU119, LEU304) and one hydrophobic Pi-alkyl bond (PRO303) **(Figure 45, Panels I and J)**.


A
B
C
D
E
F
H-Bonds
Donor
Acceptor
PHE A:153
LEU A:180
ALA A:184
PHE A:146
ILE A:181
ILE A:229
THR A:149
TRP A:150
Interactions
van der Waals
Pi-Sulfur
Alkyl
Pi-Alkyl
PRO A:226
VAL A:225
PHE A:146
LEU A:221
ALA A:184
Pi-Sigma
Pi-Pi T-shaped
LEU A:221
ALA A:188
PHE A:146
Pi-Pi Stacked

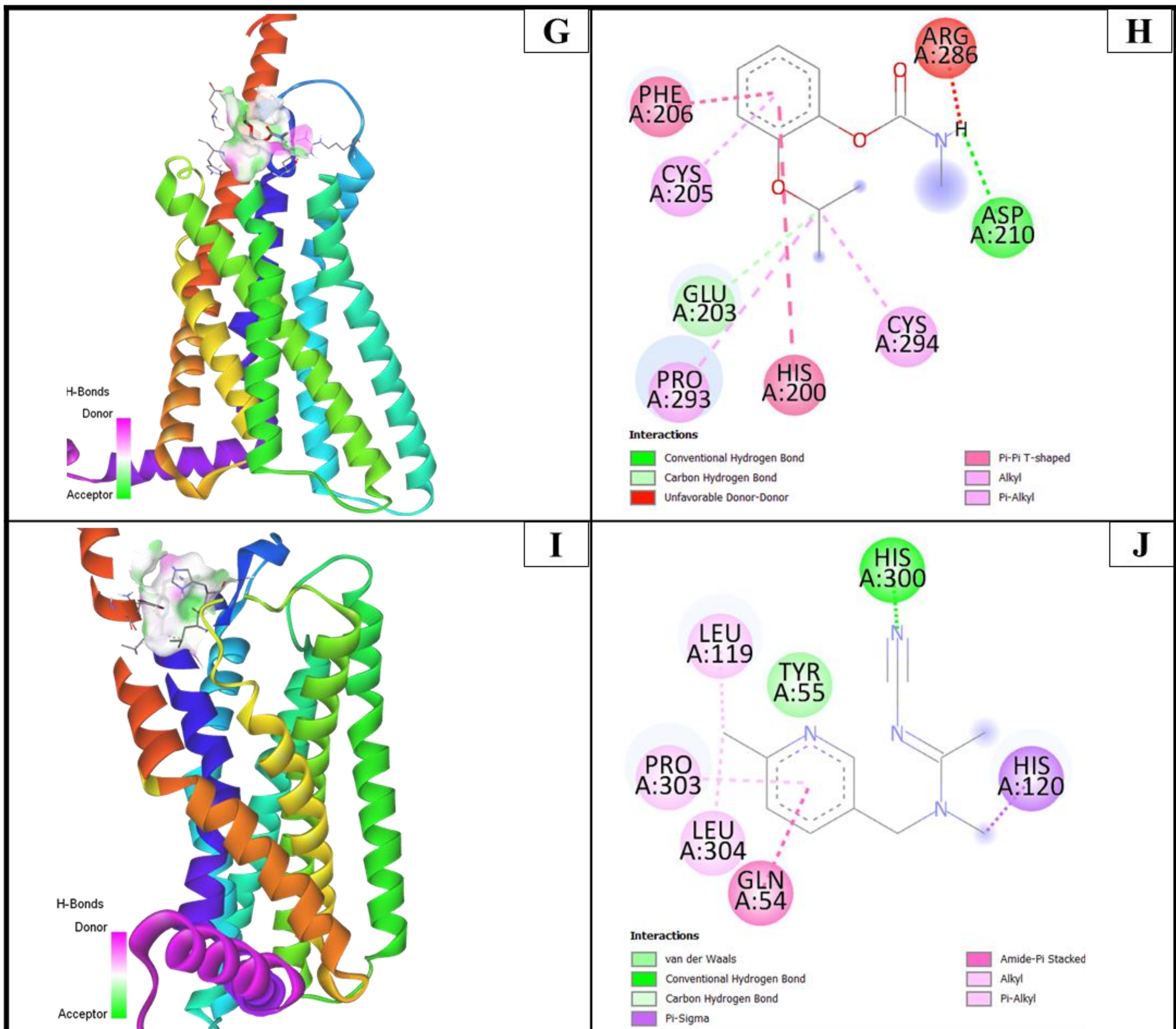


Figure 45: Predicted binding sites of pesticides in the GPER1 pocket (left panels), as well as the 2D representation of the amino acids involved in the interactions between the pesticides and the GPER1 (right panels). Panels A and B Captan, Panels C and D DDT, Panels E and F Fenvalerate, Panels G and H Propoxur, Panels I and J Acetamiprid.

II.E. Food additives : Molecular docking results revealed interesting information about the interactions between the GPER1 and food additives as shown in **Table 08**.

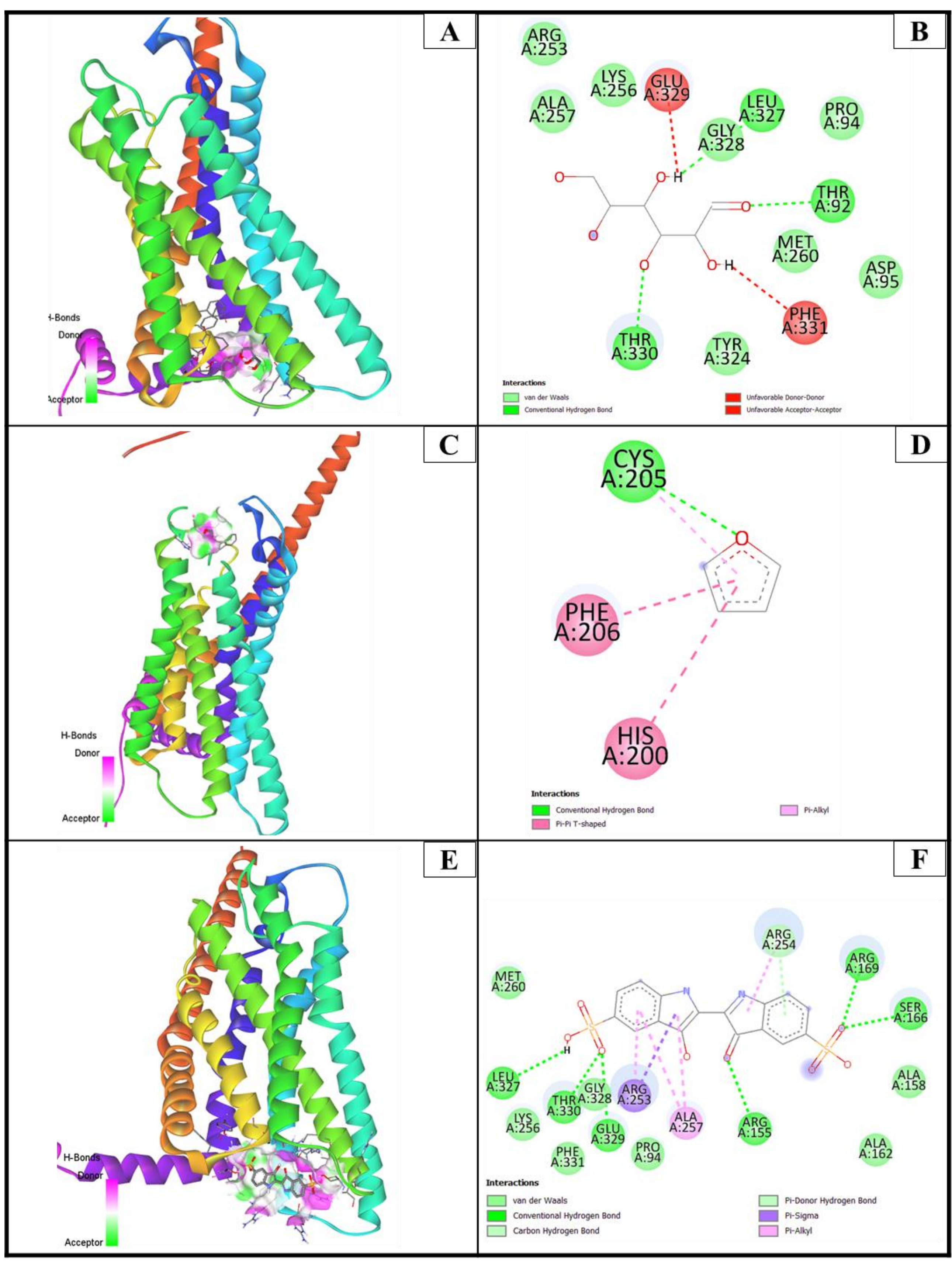
Type	Name	RMSD (A°)	Binding Energy	Ki	Interactions	Distance
Food additives	Carboxymethyl cellulose	18.276 A	-4.90 kcal/mol	257.52 uM	Conventional Hydrogen Bond: THR92: H-Donor- Carboxymethyl cellulose: H-Acceptor	2,20815 A°
					THR330: H-Donor- Carboxymethyl cellulose: H-Acceptor	1,84226 A°
					Carboxymethyl cellulose: H-Donor- LEU327: H-Acceptor	2,62181 A°
	Furan	25.849 A	-3.69 kcal/mol	1.99 mM	Conventional Hydrogen Bond: CYS205: H-Donor- Furan: H-Acceptor	2,2271 A°
					Hydrophobic Pi-Pi T-Shaped: HIS200: Pi-Orbitals- Furan: Pi-Orbitals	5,44833 A°
					Furan: Pi-Orbitals- PHE206: Pi-Orbitals	4,95948 A°
					Hydrophobic Pi-Alkyl: Furan: Pi-Orbitals- CYS205: Alkyl	4,8973 A°
	Indigo carmine	21.351 A	-8.00 kcal/mol	1.36 uM	Conventional Hydrogen Bond: ARG155: H-Donor- Indigo carmin: H-Acceptor	2,65799 A°
					SER166: H-Donor- Indigo carmin: H-Acceptor	2,25134 A°
					ARG169: H-Donor- Indigo carmin: H-Acceptor	2,1381 A°
						3,79953 A°
					GLU329: H-Donor- Indigo carmin: H-Acceptor	2,19681 A°
					THR330: H-Donor- Indigo carmin: H-Acceptor	1,92188 A°
					Indigo carmin: H-Donor- LEU327: H-Acceptor	2,36876 A°
					ARG254: H-Donor- Indigo carmin: Pi-Orbitals	3,08794 A°
					Hydrophobic Pi-Sigma: ARG253: CH- Indigo carmin: Pi-Orbitals	3,9682 A°
					Hydrophobic Pi-Alkyl: Indigo carmin: Pi-Orbitals- ALA257: Alkyl	4,43323 A°
					Indigo carmin: Pi-Orbitals- ARG253: Alkyl	4,55023 A°
					Indigo carmin: Pi-Orbitals- ARG254: Alkyl	5,07632 A°

	Monosodium glutamate	15.232 A	-5.40 kcal/mol	110.1 uM	Conventional Hydrogen Bond: HIS307: H-Donor- Monosodium glutamate: H-Acceptor	2,41506 A°
					ASN310: H-Donor- Monosodium glutamate: H-Acceptor	2,56808 A°
					Monosodium glutamate: H-Donor- PRO303: H-Acceptor	2,30385 A°
					Monosodium glutamate: H-Donor- ASN118: H-Acceptor	2,4351 A°
					PRO303: H-Donor- Monosodium glutamate: H-Acceptor	2,9941 A°
	Quinoline yellow	8.004 A	-7.00 kcal/mol	7.37 uM	Conventional Hydrogen Bond: SER315: H-Donor- Quinoline yellow: H-Acceptor	2,48937 A°
					Hydrophobic Pi-Sigma: LEU311: CH- Quinoline yellow: Pi-Orbitals	3,9334 A°
					Hydrophobic Pi-Pi Stacked: PHE68: CH- Quinoline yellow: Pi-Orbitals	4,75995 A°
					Hydrophobic Amide-Pi: PHE68: amide- Quinoline yellow: Pi-Orbitals	4,60505 A°
					Hydrophobic Pi-Alkyl: Quinoline yellow: Pi-Orbitals- LEU61: Alkyl	4,75512 A°
					Quinoline yellow: Pi-Orbitals- ILE308: Alkyl	5,46727 A°
					Quinoline yellow: Pi-Orbitals- LEU64: Alkyl	4,51047 A°
					Quinoline yellow: Pi-Orbitals- LEU69: Alkyl	5,35604 A°
						5,45134 A°

Table 08: Molecular docking results of GPER1 with food additives.

For instance, Carboxymethyl cellulose exhibited rather low binding energy ΔG of -4.90 kcal/mol and Ki of 257.52 uM, with three hydrogen bonds (THR92,THR330,THR327) **(Figure 46, Panels A and B)**. In addition, Furan also had slightly low binding energy ΔG of -3.69 kcal/mol and Ki of 1.99 mM, with one hydrogen bond (CYS205), two hydrophobic Pi-Pi T-shaped bonds (HIS200,PHE206) and one Hydrophobic Pi-Alkyl bond (CYS205) **(Figure 46, Panels C and D)**. Interestingly, Indigo Carmin displayed the lowest binding energy among food additives with ΔG of -8.00 kcal/mol and Ki of 1.36 uM, seven hydrogen bonds (ARG155, SER166, ARG169, GLU329, THR330, LEU327, ARG254), one hydrophobic Pi-sigma bond (ARG253) and three Hydrophobic Pi-Alkyl bonds (ALA257, ARG253, ARG254) **(Figure 46, Panels E and F)**. Meanwhile, Monosodium Glutamate manifested moderately low binding energy ΔG of -5.40 kcal/mol, Ki of 110.1 uM and five hydrogen bonds (HIS307, ASN310, PRO303, ASN118, PRO303) **(Figure 46, Panels G and H)**.

Nevertheless, Quinoline Yellow significantly lower binding energy ΔG of -7.00 kcal/mol, Ki of 7.37 uM, one hydrogen bond (SER315), one hydrophobic Pi-sigma bond (LEU311), one hydrophobic Pi-Pi stacked bond (PHE68), one hydrophobic amide-Pi bond (PHE68) and four hydrophobic Pi-Alkyl bonds (LEU61, ILE308, LEU64, LEU69) **(Figure 46, Panels I and J)**.


A
B
C
D
E
F
H-Bonds
Donor
Acceptor
ARG A:253
LYS A:256
GLU A:329
ALA A:257
LEU A:327
PRO A:94
GLY A:328
THR A:92
MET A:260
ASP A:95
PHE A:331
THR A:330
TYR A:324
Interactions
van der Waals
Conventional Hydrogen Bond
Unfavorable Donor-Donor
Unfavorable Acceptor-Acceptor
CYS A:205
PHE A:206
HIS A:200
Pi-Pi T-shaped
Pi-Alkyl
ARG A:254
ARG A:169
SER A:166
ALA A:158
ALA A:162
ARG A:155
ALA A:257
ARG A:253
GLY A:328
THR A:330
LEU A:327
LYS A:256
GLU A:329
PHE A:331
PRO A:94
MET A:260
Carbon Hydrogen Bond
Pi-Donor Hydrogen Bond
Pi-Sigma

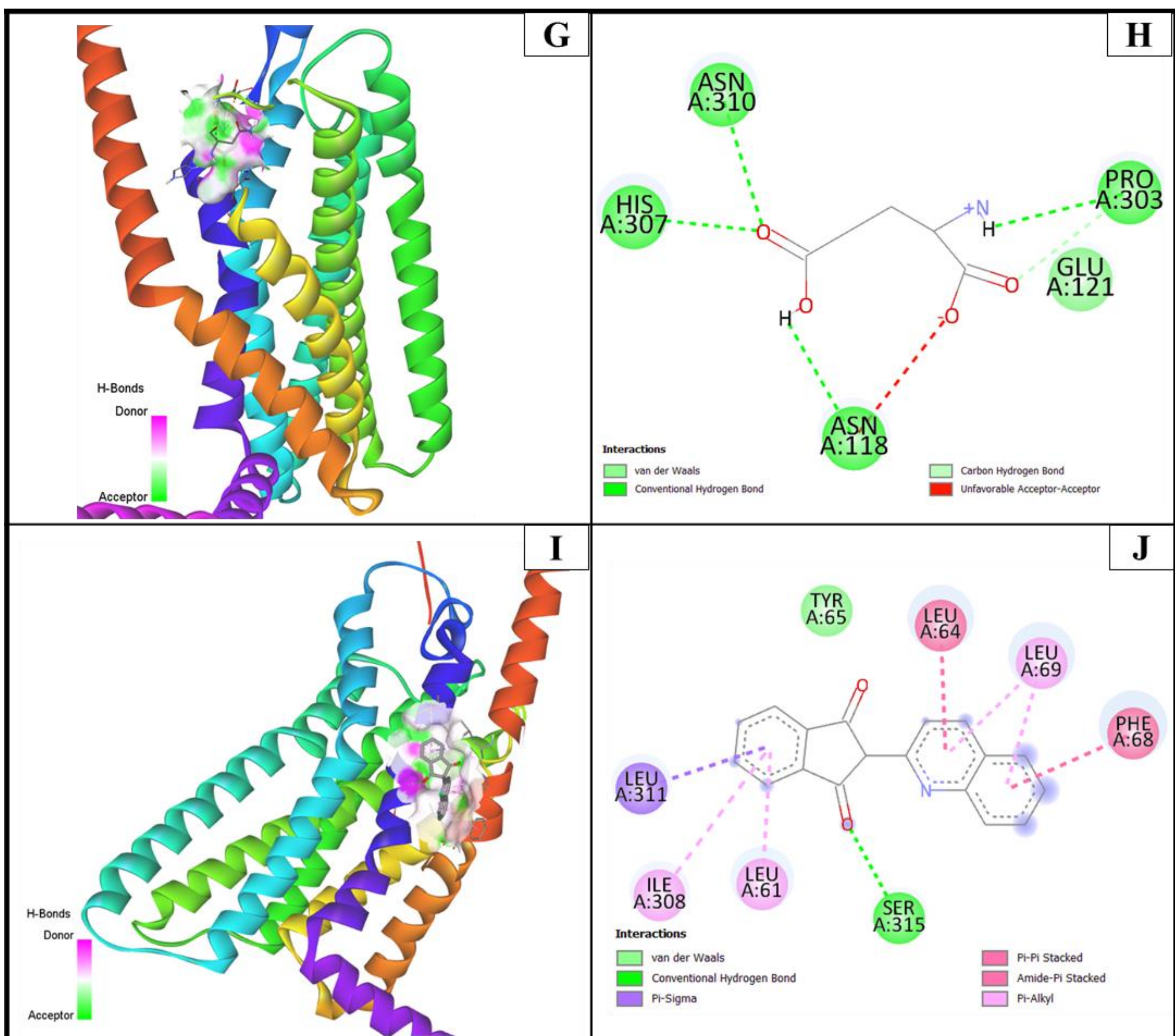


Figure 46: Predicted binding sites of food additives in the GPER1 pocket (left panels), as well as the 2D representation of the amino acids involved in the interactions between the food additives and the GPER1 (right panels). Panels A and B Carboxymethyl cellulose, Panels C and D Furan, Panels E and F Indigo Carmine, Panels G and H Monosodium Glutamate, Panels I and J Quinoline Yellow.

II.F. Personal care chemicals: Interaction between personal care chemicals and GPER1 have been evaluated and summarised in **Table 09**.

Type	Name	RMSD (A°)	Binding Energy	Ki	Interactions	Distance
Personal care	Dimethyl Phthalate	24.813 A	-6.32 kcal/mol	23.50 uM	Conventional Hydrogen Bond: TYR97: H-Donor- Dimethyl phthalate: H-Acceptor	2,26646 A°
					TRP150: H-Donor- Dimethyl phthalate: H-Acceptor	2,08095 A°
					SER177: H-Donor- Dimethyl phthalate: H-Acceptor	2,49827 A°
					Dimethyl phthalate: H-Donor- ASP154: H-Acceptor	3,43061 A°
					Hydrophobic Pi-Sigma: Dimethyl phthalate: CH- TRP150: Pi-Orbitals	3,97669 A°
					Hydrophobic Pi-Pi T-shaped: TRP150: CH- Dimethyl phthalate: Pi-Orbitals	4,93206 A°
					Hydrophobic Alkyl: ALA174: Alkyl- Dimethyl phthalate: Alkyl	4,15217 A°
					Dimethyl phthalate: Alkyl- ILE93: Alkyl	4,03407 A°
					Dimethyl phthalate: Alkyl- ARG169: Alkyl	4,05268 A°
	Homosalate	20.230 A	-6.55 kcal/mol	15.83 uM	Conventional Hydrogen Bond: TRP150: H-Donor- Homosalate: H-Acceptor	3,04668 A°
					Hydrophobic Pi-Sigma: Homosalate: CH- PHE146: Pi-Orbitals	3,87172 A°
					Hydrophobic Alkyl: Homosalate: Alkyl- VAL225: Alkyl	4,86056 A°
					Homosalate: Alkyl- PRO226: Alkyl	3,54837 A°
					Homosalate: Alkyl- ILE229: Alkyl	4,30123 A°
					Hydrophobic Pi-Alkyl: PHE146: Pi-Orbitals- Homosalate: Alkyl	4,67381 A°
					Homosalate: Pi-Orbitals- LEU180: Alkyl	5,49395 A°
					Homosalate: Pi-Orbitals- ILE181: Alkyl	4,93163 A°
					Homosalate: Pi-Orbitals- ILE184: Alkyl	5,32592 A°

	Methyl paraben	15.043 A	-6.21 kcal/mol	28.03 uM	Conventional Hydrogen Bond: ASN118: H-Donor- Methyl paraben: H-Acceptor	2,93527 A°
					HIS307: H-Donor- Methyl paraben: H-Acceptor	2,02673 A°
					Methyl paraben: H-Donor- PRO303: H-Acceptor	2,5686 A°
					Methyl paraben: H-Donor- GLU115: H-Acceptor	3,58506 A°
					Electrostatic Pi-Anion: GLU121: Negative- Methyl paraben: Pi-Orbitals	4,1406 A°
					Hydrophobic Amide-Pi Stacked: GLY306: amide- Methyl paraben: Pi-Orbitals	4,20942 A°
					Hydrophobic Pi-Alkyl: HIS307: Pi-Orbitals- Methyl paraben: Alkyl	4,68467 A°
					PHE314: Pi-Orbitals- Methyl paraben: Alkyl	5,46668 A°
					Methyl paraben: Pi-Orbitals- ARG299: Alkyl	5,29602 A°
	Resorcinol	26.056 A	-5.30 kcal/mol	130.27 uM	Conventional Hydrogen Bond: Resorcinol: H-Donor- GLU203: H-Acceptor	2,21551 A°
					Resorcinol: H-Donor- PHE206: H-Acceptor	1,70585 A°
					Hydrophobic Pi-Pi T-shaped: HIS200: Pi-Orbitals - Resorcinol: Pi-Orbitals	5,95455 A°
					PHE206: Pi-Orbitals - Resorcinol: Pi-Orbitals	4,88731 A°
					Hydrophobic Pi-Alkyl: Resorcinol: Pi-Orbitals- CYS205: Alkyl	4,95237 A°
	Tricresyl phosphate	18.685 A	-6.71 kcal/mol	12.16 uM	Hydrophobic Pi-Sigma: THR149: CH- Tricresyl phosphate: Pi-Orbitals	3,74959 A°
					Hydrophobic Pi-Pi Stacked: PHE146: Pi-Orbitals - Tricresyl phosphate: Pi-Orbitals	3,82951 A°
					Hydrophobic Pi-Pi T-shaped: PHE153: Pi-Orbitals - Tricresyl phosphate: Pi-Orbitals	5,35718 A°

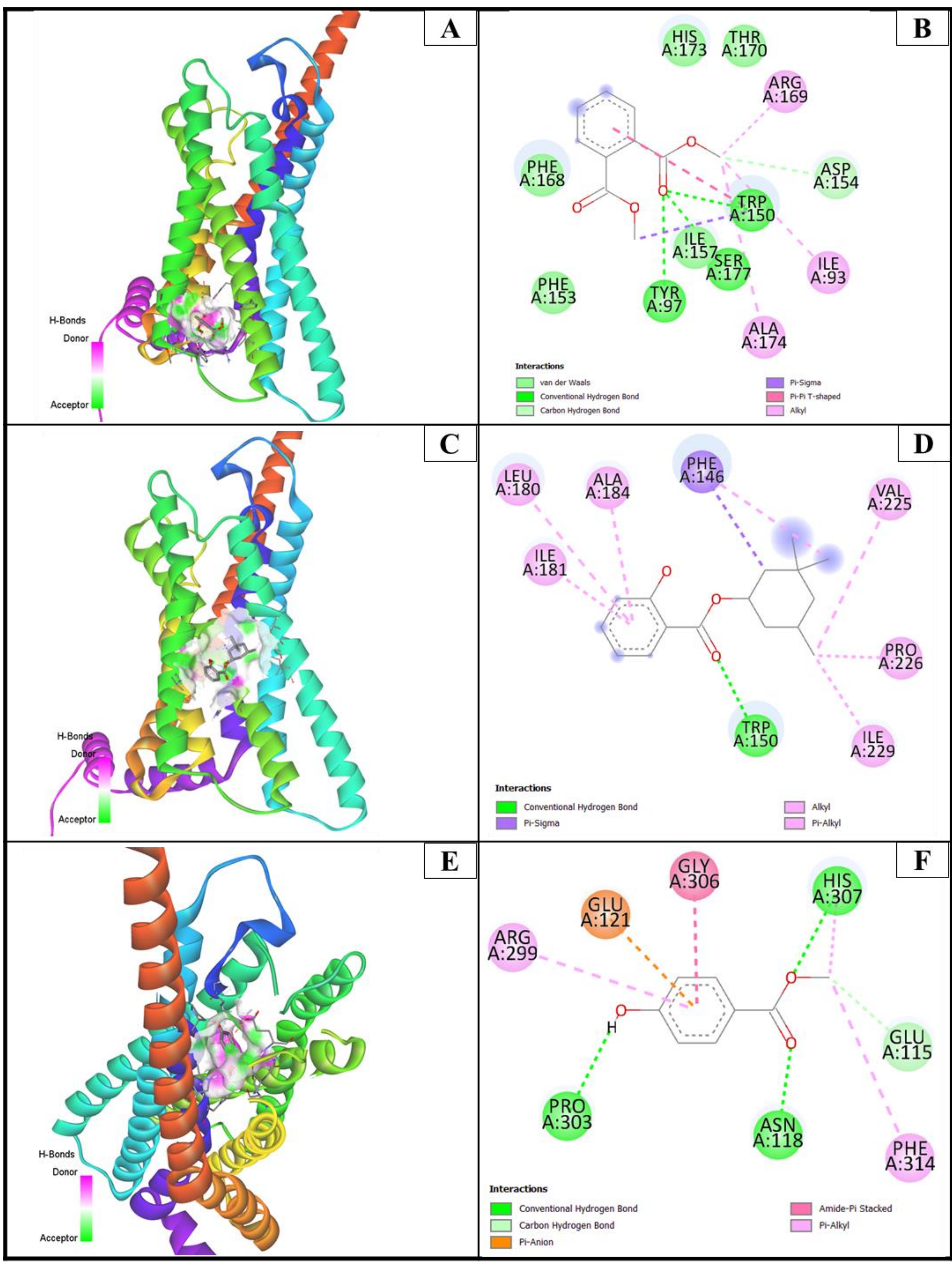
					Hydrophobic Alkyl: Tricresyl phosphate: Alkyl- ILE229: Alkyl	4,46956 A°
					Tricresyl phosphate: Alkyl- LEU180: Alkyl	5,08492 A°
					Tricresyl phosphate: Alkyl- LEU221: Alkyl	5,04364 A°
					Tricresyl phosphate: Alkyl- VAL225: Alkyl	4,79573 A°
					Hydrophobic Pi-Alkyl: PHE146: Pi-Orbitals- Tricresyl phosphate: Alkyl	5,03505 A°
					PHE153: Pi-Orbitals- Tricresyl phosphate: Alkyl	4,53706 A°
					Tricresyl phosphate: Pi-Orbitals- ILE229: Alkyl	5,00671 A°

Table 09: Molecular docking results of GPER1 with personal care chemicals.

In fact, Dimethyl Phthalate showed lower binding energy ΔG of -6.32 kcal/mol, Ki of 23.50 uM, four hydrogen bonds (TYR97, TRP150, SER177, ASP154), one hydrophobic Pi-sigma bond (TRP150), one hydrophobic Pi-Pi T-shaped bond (TRP150) and three hydrophobic alkyl bonds (ALA174, ILE93, ARG169) **(Figure 47, Panels A and B)**.

Moreover, Homosalate exhibited low binding energy ΔG of -6.55 kcal/mol, Ki of 15.83 uM, one hydrogen bond (TRP150), one hydrophobic Pi-sigma bond (PHE146), three hydrophobic alkyl bonds (VAL225, PRO226, OLE229) and four hydrophobic Pi-alkyl bonds (PHE146, LEU180, ILE181, ILE184) **(Figure 47, Panels C and D)**. Moreover, Methyl Paraben also displayed low binding energy -6.21 kcal/mol, Ki of 28.03 uM, four hydrogen bonds (ASN118, HIS307, PRO303, GLU115), one electrostatic Pi-anion bond (GLU121), one hydrophobic amide Pi-stacked bond (GLY306) and three hydrophobic Pi-alkyl bonds (HIS307, PHE314, ARG299) **(Figure 47, Panels E and F)**.

Furthermore, Resorcinol manifested ΔG of -5.30 kcal/mol, Ki of 130.27 uM, two hydrogen bonds (GLU203, PHE206), two hydrophobic Pi-Pi T-shaped bonds (HIS200, PHE206) and one hydrophobic Pi-alkyl bond (CYS205) **(Figure 47, Panels G and H)**. Nevertheless, Tricresyl Phosphate has predicated the formation of one hydrophobic Pi-sigma bond (THR149), one hydrophobic Pi-Pi stacked bond (PHE146), one hydrophobic Pi-Pi T-shaped bond (PHE153), four hydrophobic alkyl bonds (ILE229, LEU180, LEU221, VAL225) and three hydrophobic Pi-alkyl bonds (PHE146, PHE153, ILE229), all had a binding energy ΔG of -6.71 kcal/mol and Ki of 12.16 uM **(Figure 47, Panels I and J)**.


A
B
C
D
E
F
H-Bonds
Donor
Acceptor
HIS A:173
THR A:170
ARG A:169
PHE A:168
ASP A:154
TRP A:150
ILE A:157
SER A:177
ILE A:93
PHE A:153
TYR A:97
ALA A:174
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Pi-Pi T-shaped
Alkyl
LEU A:180
ALA A:184
PHE A:146
VAL A:225
ILE A:181
PRO A:226
TRP A:150
ILE A:229
Conventional Hydrogen Bond
Pi-Sigma
Alkyl
Pi-Alkyl
GLY A:306
HIS A:307
GLU A:121
ARG A:299
GLU A:115
PRO A:303
ASN A:118
PHE A:314
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Anion
Amide-Pi Stacked
Pi-Alkyl

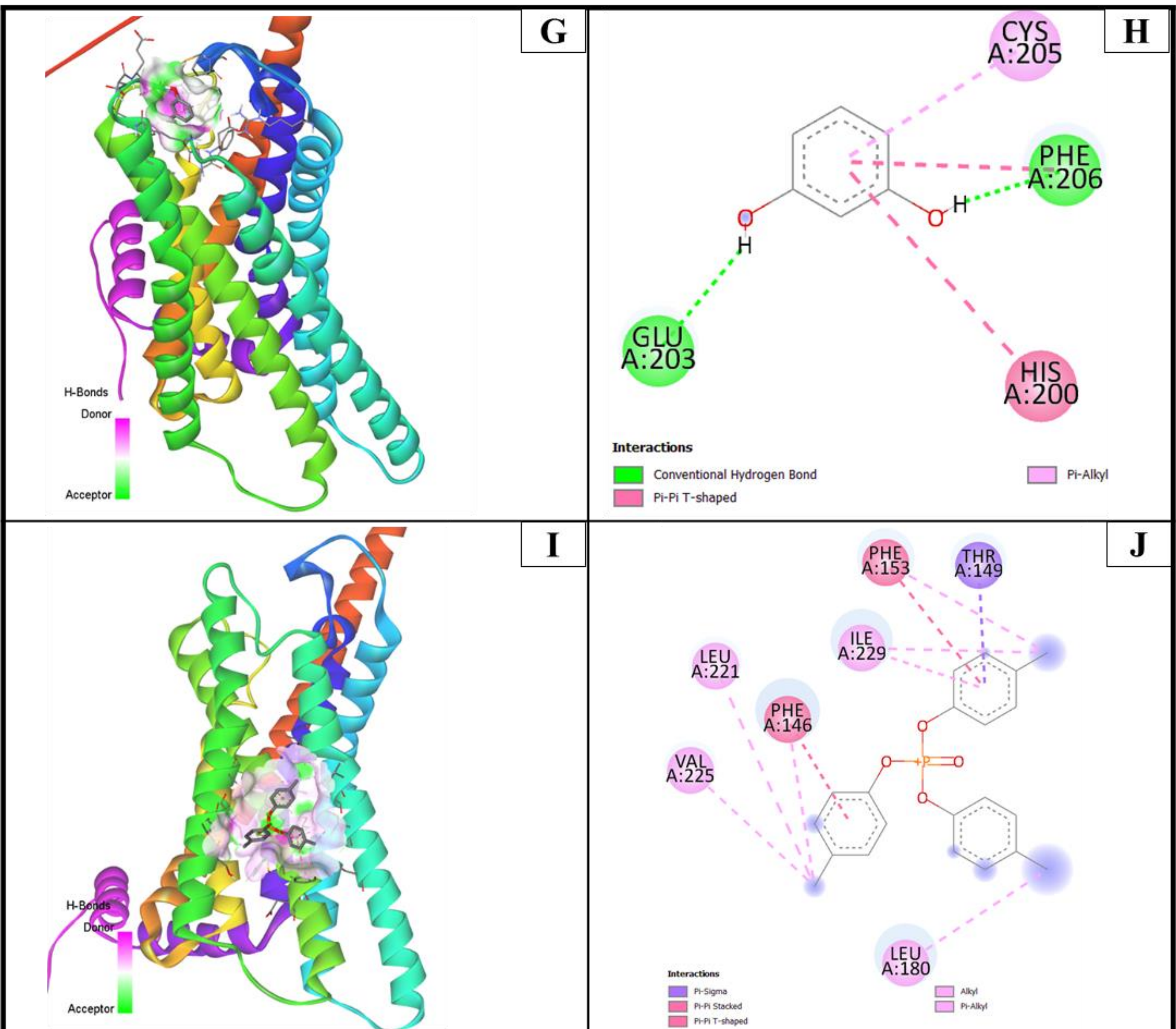


Figure 47: Predicted binding sites of Personal care chemicals in the GPER1 pocket (left panels), as well as the 2D representation of the amino acids involved in the interactions between the personal care chemicals and the GPER1 (right panels). Panels A and B Dimethyl Phthalate, Panel C and D Homosalate, Panels E and F Methyl Paraben, Panels G and H Resorcinol, Panels I and J Tricresyl Phosphate.

II.G. Plasticisers and flame retardants: Molecular docking revealed important details about the interactions between the plasticizers and flame retardants with the GPER1 as shown in **Table 10**.

Type	Name	RMSD (A°)	Binding Energy	Ki	Interactions	Distance
Plasticizers and flame retardant	Bisphenol A (BPA)	18.853 A	-16.47 kcal/mol	851.51 fM	Hydrophobic Alkyl: LEU69: Alkyl - Bisphenol A: Alkyl	4,80399 A°
					CYS318: Alkyl - Bisphenol A: Alkyl	4,8888 A°
					Bisphenol A: Alkyl - VAL109: Alkyl	3,86488 A°
					Bisphenol A: Alkyl - CYS318: Alkyl	3,65029 A°
					Hydrophobic Pi-Alkyl: TYR65: Pi-Orbitals- Bisphenol A: Alkyl	4,67477 A°
	Acrylamide	25.035 A	-8.78 kcal/mol	364.26 nM	Hydrophobic Alkyl: Acrylamide: Alkyl - CYS318: Alkyl	3,98583 A°
					Hydrophobic Pi-Alkyl: TYR65: Pi-Orbitals- Acrylamide: Alkyl	5,25394 A°
	Decabromodiphenyl oxide (DBDPO)	19.016 A	-7.00 kcal/mol	7.45 uM	Hydrophobic Alkyl: DBDPO: Alkyl -VAL225: Alkyl	5,17068 A°
					DBDPO: Alkyl - PRO226: Alkyl	3,94927 A°
					DBDPO: Alkyl - ILE229: Alkyl	4,40013 A°
					DBDPO: Alkyl - LEU221: Alkyl	4,33356 A°
						4,15447 A°
						4,38574 A°
						4,61096 A°
					Hydrophobic Pi-Alkyl: PHE146: Pi-Orbitals- DBDPO: Alkyl	5,3597 A°
						4,5874 A°
						4,40093 A°

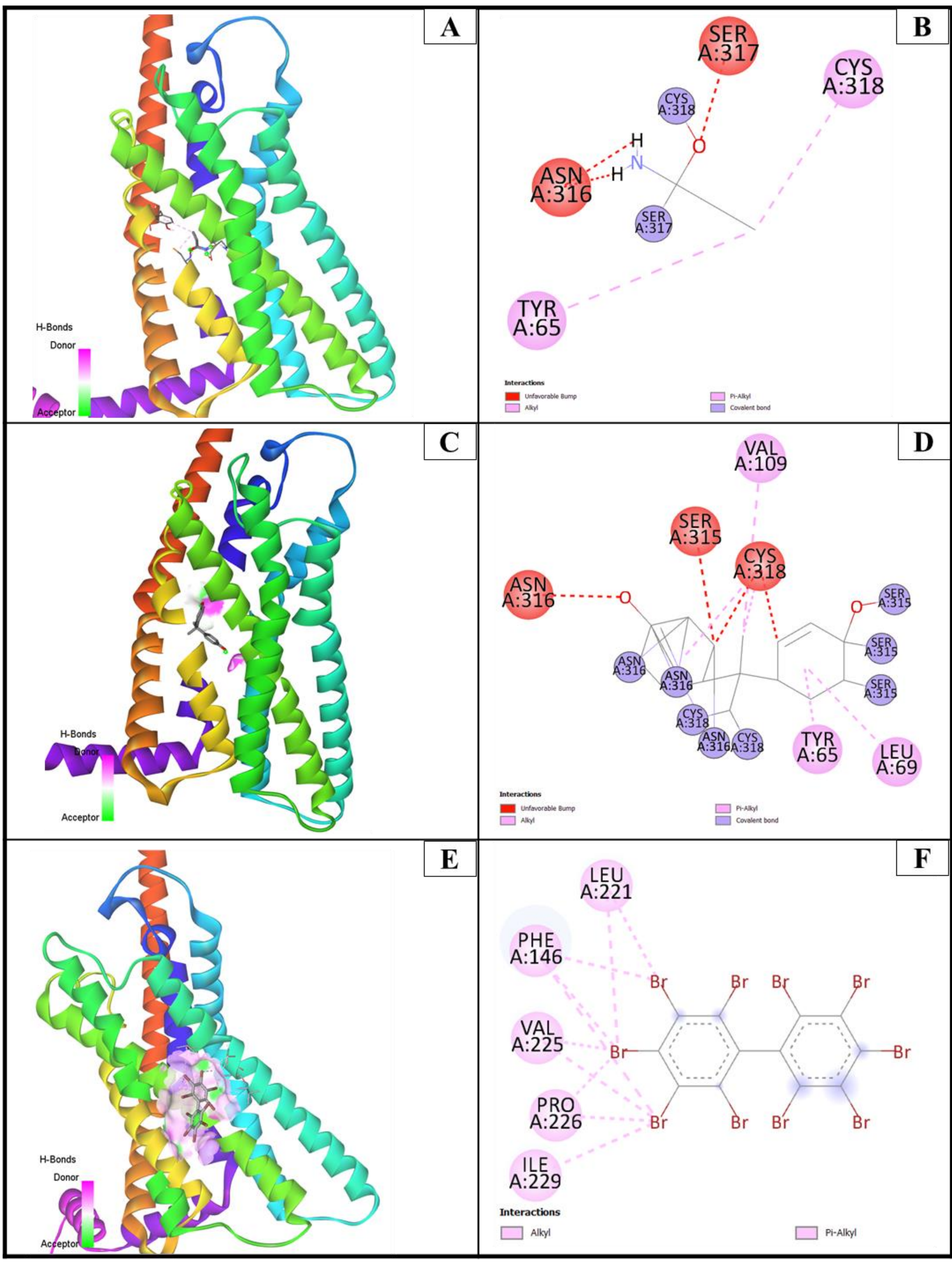
	Polychlorinated biphenyls (PCBs)	18.755 A	-10.35 kcal/mol	25.93 nM	Conventional Hydrogen Bond: PCB: H-Donor- ASN140: H-Acceptor	3,5281 A°
					Hydrophobic Alkyl: CYS318: Alkyl - PCB: Alkyl	4,7849 A°
					PCB: Alkyl - CYS271: Alkyl	4,45458 A°
					PCB: Alkyl - LEU69: Alkyl	4,11994 A°
					Hydrophobic Pi-Alkyl: TYR65: Pi-Orbitals- PCB: Alkyl	3,60723 A°
					TRP272: Pi-Orbitals- PCB: Alkyl	5,26915 A°
	Perfluorooctanesulfonamide (PFSA)	19.808 A	-5.85 kcal/mol	51.32 uM	Halogen: PHE146:O Acceptor- PFSA: F Halogen (Fluorine)	3,18185 A°
						3,12127 A°
					THR149:O Acceptor- PFSA: F Halogen (Fluorine)	3,26152 A°
						2,86977 A°
					Hydrophobic Pi-Alkyl: PHE153: Pi-Orbitals- PFSA: Alkyl	3,91339 A°

Table 10: Molecular docking results of GPER1 with plasticizers and flame retardants.

For instance, Acrylamid also exhibited low binding energy ΔG of -8.78 kcal/mol, Ki of 364.26 nM, one hydrophobic alky bond (CYS318) and one hydrophobic Pi-alkyl bond (TYR65) **(Figure 48, Panels A and B)**. Interestingly, Bisphenol A displayed the lowest binding energy among all the tested chemicals with ΔG of -16.47 kcal/mol, Ki of 851.51 fM, four hydrophobic alkyl bonds (LEU69, CYS318,VAL109,CYS318) and one hydrophobic Pi-alkyl bond (TYR65) **(Figure 48, Panels C and D)**.

Moreover, Decabromodiphenyl oxide manifested low binding energy ΔG of -7.00 kcal/mol, Ki of 7.45 uM, four hydrophobic alkyl bonds (VAL225, PRO226,ILE229, ILE221) and one hydrophobic Pi-alkyl bond (PHE146) **(Figure 48, Panels E and F)**. In addition, the Perfluorooctane Sulfonamide had quite low binding energy of ΔG of -5.85 kcal/mol, Ki of 51.32 uM, two halogen bonds (PHE146, THR149) and one hydrophobic Pi-alkyl bond (PHE153) **(Figure 48, Panels G and H)**.

Meanwhile, Polychlorinated Biphenyls showed significantly lower binding energy of ΔG of -10.35 kcal/mol, Ki of 25.93 nM, one hydrogen bond (ASN140), three hydrophobic alkyl bonds (CYS318, CYS271, LEU69) and two hydrophobic Pi-alkyl bonds (TYR65, TRP272) **(Figure 48, Panels I and J)**.


A
H-Bonds
Donor
Acceptor
B
SER A:317
CYS A:318
CYS A:318
ASN A:316
SER A:317
TYR A:65
Interactions
Unfavorable Bump
Alkyl
Pi-Alkyl
Covalent bond
C
H-Bonds
Donor
Acceptor
D
VAL A:109
SER A:315
CYS A:318
ASN A:316
TYR A:65
LEU A:69
Interactions
Unfavorable Bump
Alkyl
Pi-Alkyl
Covalent bond
E
H-Bonds
Donor
Acceptor
F
LEU A:221
PHE A:146
VAL A:225
PRO A:226
ILE A:229
Interactions
Alkyl
Pi-Alkyl

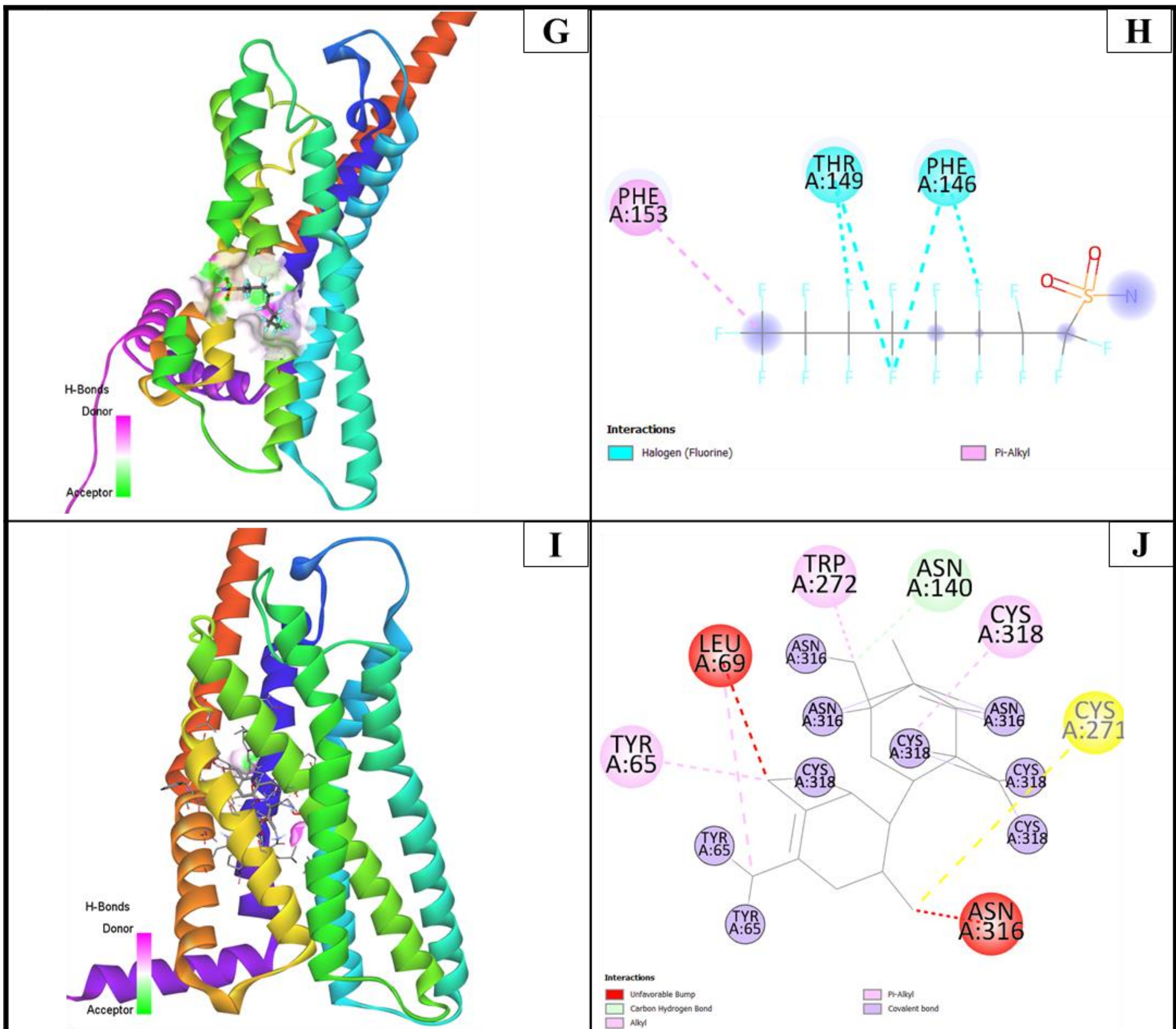


Figure 48: Predicted binding sites of plasticizers and flame retardants in the GPER1 pocket (left panels), as well as the 2D representation of the amino acids involved in the interactions between the plasticizers and flame retardants and the GPER1 (right panels). Panels A and B Acrylamide, Panels C and D BPA, Panels E and F Decabromodiphenyl oxide, Panels G and H PFSA, Panels I and J PCB.

II.H. Chemotherapy: Chemotherapeutic agents trigger different biological processes, so we used them in our study to investigate their effects on the GPER1 as presented in **Table 11**.

Type	Name	RMSD (A°)	Binding Energy	Ki	Interactions	Distance
Chemotherapy	Estramustine phosphate	19.926 A	-7.45 kcal/mol	3.45 uM	Hydrophobic Pi-Sigma: THR149: CH- Estramustine phosphate: Pi-Orbitals	3,649 A°
					Estramustine phosphate: CH- PHE153: Pi-Orbitals	3,95104 A°
					Hydrophobic Pi-Pi T-shaped: PHE153: Pi-Orbitals - Estramustine phosphate: Pi-Orbitals	5,34418 A°
					Hydrophobic Alkyl : ALA184 : Alkyl- Estramustine phosphate : Alkyl	4,32153 A°
					ILE229 : Alkyl- Estramustine phosphate : Alkyl	4,68694 A°
						4,46876 A°
					Estramustine phosphate : Alkyl- ILE229 : Alkyl	4,11609 A°
						5,34802 A°
					Estramustine phosphate : Alkyl- LEU232 : Alkyl	5,26102 A°
					Hydrophobic Pi-Alkyl: PHE146: Pi-Orbitals- Estramustine phosphate: Alkyl	4,72884 A°
						4,83667 A°
					Estramustine phosphate : Pi-Orbitals- ILE229 : Alkyl	4,93291 A°
	Cyclophosphamide	20.405 A	-5.26 kcal/mol	140.24 uM	Conventional Hydrogen Bond: ILE181: H-Donor- Cyclophosphamide: H-Acceptor	3,18155 A°
					Cyclophosphamide: H-Donor- LEU180: H-Acceptor	3,5555 A°
					Halogen: SER177:O Acceptor- Cyclophosphamide: CL Halogen (chlor)	3,05524 A°
					Hydrophobic Alkyl : ALA184: Alkyl- Cyclophosphamide : Alkyl	3,62521 A°
					Cyclophosphamide : Alkyl- ILE181 : Alkyl	5,26501 A°
						5,34515 A°

					Cyclophosphamide : Alkyl- LEU180 : Alkyl	4,32812 A°
					Hydrophobic Pi-Alkyl: TRP150: Pi-Orbitals- Cyclophosphamide: Alkyl	5,45112 A°
						4,65862 A°
	Doxorubicin	5.270 A	-6.31 kcal/mol	23.88 uM	Conventional Hydrogen Bond: CYS271: H-Donor- Doxorubicin: H-Acceptor	3,53461 A°
					Doxorubicin: H-Donor- CYS271: H-Acceptor	2,58165 A°
					Doxorubicin: H-Donor- ILE308: H-Acceptor	2,30073 A°
					Hydrophobic Pi-Sigma : LEU319 : CH- Doxorubicin : Pi-Orbitals	3,86817 A°
					Hydrophobic Alkyl : Doxorubicin : Alkyl- LEU311 : Alkyl	3,44209 A°
					Hydrophobic Pi-Alkyl : TYR65 : Pi-Orbitals- Doxorubicin : Alkyl	5,01356 A°
					Doxorubicin: Pi-Orbitals- VAL267: Alkyl	5,28758 A°
					Doxorubicin: Pi-Orbitals- LEU319: Alkyl	4,91329 A°
	Colchicine	18.714 A	-7.52 kcal/mol	3.05 uM	Conventional Hydrogen Bond: Colchicine: H-Donor- SER177: H-Acceptor	3,36177 A°
					Hydrophobic Pi-Sigma: Colchicine: CH- TRP150: Pi-Orbitals	3,68216 A°
					Hydrophobic Pi-Pi Stacked: TRP150: Pi-Orbitals - Colchicine: Pi-Orbitals	5,37007 A°
					Hydrophobic Alkyl : ILE181: Alkyl- Colchicine: Alkyl	5,45903 A°
					Colchicine: Alkyl- LEU180: Alkyl	4,35579 A°
					Colchicine : Alkyl- ILE181: Alkyl	5,04671 A°
					Hydrophobic Pi-Alkyl : PHE146: Pi-Orbitals- Colchicine : Alkyl	5,20353 A°
					TRP150: Pi-Orbitals- Colchicine: Alkyl	4,35175 A°
					Colchicine: Pi-Orbitals- ILE181: Alkyl	5,35103 A°

	Methotrexate	24.392 A	-2.33 kcal/mol	19.46 mM	Conventional Hydrogen Bond: Methotrexate: H-Donor- ASP125: H-Acceptor	1,85948 A°
					Methotrexate: H-Donor- CYS294: H-Acceptor	2,12909 A°
					Methotrexate: H-Donor- LYS295: H-Acceptor	2,77651 A°
					ALA301: H-Donor- Methotrexate: H-Acceptor	2,35147 A°
					Methotrexate: H-Donor- HIS300: H-Acceptor	3,71927 A°
						3,01648 A°
					Electrostatic Pi-Anion: GLU121: Negative- Methotrexate: Pi-Orbitals	4,13889 A°
					Hydrophobic Pi-Pi Stacked: HIS300: Pi-Orbitals - Methotrexate: Pi-Orbitals	5,04247 A°
					Hydrophobic Pi-Alkyl : Methotrexate: Pi-Orbitals- ALA301 : Alkyl	4,7403 A°
	Carboplatin	15.751 A	-5.77 kcal/mol	58.70 uM	Conventional Hydrogen Bond: ARG299: H-Donor- Carboplatin: H-Acceptor	2,30357 A°
					ASN310: H-Donor- Carboplatin: H-Acceptor	3,09643 A°
					Carboplatin: H-Donor- ASN118: H-Acceptor	1,99775 A°
					Carboplatin: H-Donor- PRO303: H-Acceptor	2,38437 A°
					ARG299: H-Donor- Carboplatin: H-Acceptor	3,09944 A°

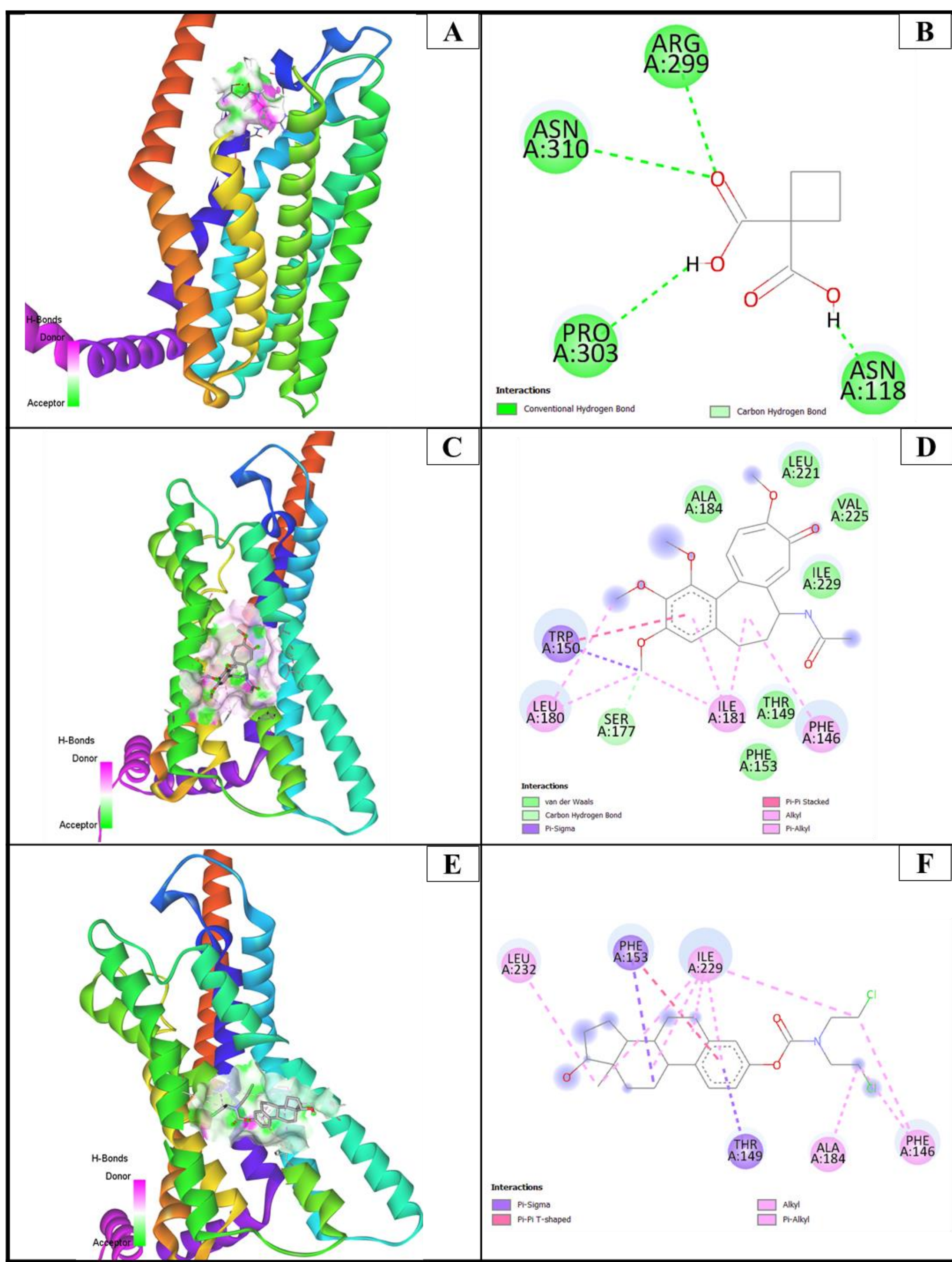
Table 11: Molecular docking results of GPER1 with chemotherapy.

For instance, Carboplatin displayed quite low binding energy ΔG of -5.77 kcal/mol and Ki of 58.70 uM with five hydrogen bonds (ARG299, ASN310, ASN118, PRO303,ARG299) **(Figure 49, Panels A and B)**. Moreover, Colchicine also had lower binding energy ΔG of -7.52 kcal/mol and Ki of 3.05 uM with one hydrogen bond (SER177), one hydrophobic Pi-sigma bond (TRP150), one hydrophobic Pi-Pi stacked bond (TRP150), three hydrophobic alkyl bonds (ILE181, LEU180, ILE181) and three hydrophobic Pi-alkyl bonds (PHE146, TRP150, ILE181) **(Figure 49, Panels C and D)**.

Interestingly, Estramustine phosphate displayed the lowest binding energy ΔG of -7.45 kcal/mol and Ki of 3.45 uM with two hydrophobic Pi-sigma bonds (THR149, PHE153), one hydrophobic Pi-Pi T-shaped bond (PHE153), four hydrophobic alkyl bonds (ALA184, ILE229, LEU232, ILE229) and two hydrophobic Pi-alkyl bonds (PHE146, ILE229) **(Figure 49, Panels E and F)**. Meanwhile, Cyclophosphamide exhibited rather low binding energy of ΔG of -5.26 kcal/mol and Ki of 140.24 uM with two hydrogen bonds (ILE181, LEU180), one halogen bond (SER177), three hydrophobic alkyl bonds (ALA184, ILE181, LEU180) and one hydrophobic Pi-alkyl bond (ERP150) **(Figure 49, Panels G and H)**.

In addition, Doxorubicin manifested low binding energy ΔG of -6.31 kcal/mol and Ki of 23.88 uM with three hydrogen bonds (CYS271, ILE308, CYS271), one hydrophobic Pi-sigma bond (LEU319), one hydrophobic alkyl bond (LEU311) and three hydrophobic Pi-alkyl bonds (TYR65, WAL267, LEU319) **(Figure 49, Panels I and J)**.

Finaly, Methotrexate presented significantly rather high binding energy ΔG of -2.33 kcal/mol and Ki of 19.46 mM with five hydrogen bonds (ASP125, CYS294, LYS295, ALA301, HIS300), one electrostatic Pi-anion bond (GLU121), one hydrophobic Pi-Pi stacked bond (HIS300) and one hydrophobic Pi-alkyl bond (ALA301) **(Figure 49, Panels K and L)**.


A
H-Bonds
Donor
Acceptor
B
ARG A:299
ASN A:310
PRO A:303
ASN A:118
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
C
H-Bonds
Donor
Acceptor
D
LEU A:221
ALA A:184
VAL A:225
ILE A:229
TRP A:150
LEU A:180
SER A:177
ILE A:181
THR A:149
PHE A:146
PHE A:153
Interactions
van der Waals
Carbon Hydrogen Bond
Pi-Sigma
Pi-Pi Stacked
Alkyl
Pi-Alkyl
E
H-Bonds
Donor
Acceptor
F
LEU A:232
PHE A:153
ILE A:229
THR A:149
ALA A:184
PHE A:146
Interactions
Pi-Sigma
Pi-Pi T-shaped
Alkyl
Pi-Alkyl

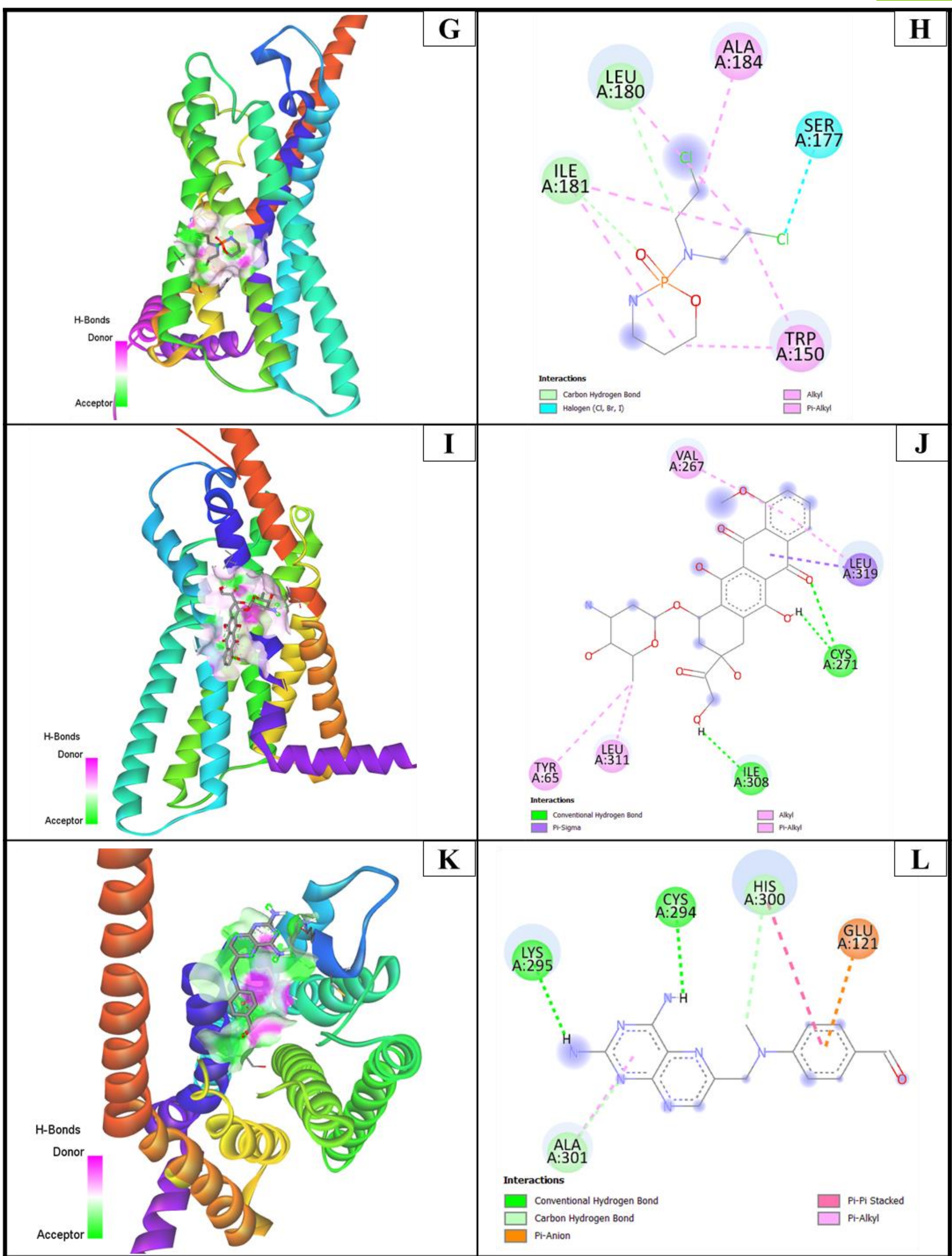


Figure 49: Predicted binding sites of chemotherapy in the GPER1 pocket (left panels), as well as the 2D representation of the amino acids involved in the interactions between the chemotherapy and the GPER1 (right panels). Panels A and B Carboplatin, Panels C and D Colchicine, Panels E and F Estramustin, Panels G and H Cyclophosphamide, Panels I and J Doxorubicin, Panels K and L Methotrexate.

Discussion

The purpose of this work is to clarify the importance of the GPER1 as an oestrogen receptor in the male reproductive system, as well as in promoting various physiological and physio pathological process under the modulation of distinct types of chemicals.

Our animal models, the gerbil *Gerbillus gerbillus* and the sand rat *Psammomys obesus* rodents from North Africa, undergoes extreme environmental variations that influence their reproductive behaviour. For instance, the gerbil *Gerbillus gerbillus* has seasonal reproduction cycle, with a breeding season starting in winter (December to January) and reaching its summit in April, and a resting season starting late spring (May - June) and continues until autumn **(Zaina Amirat *et al.,* 1977)**. Several desert rodents manifest same seasonality including: *Meriones libycus, Meriones crassus, Massouteria mzabi, Jaculus orientalis, Mesocricetus auratus,* and *Phodopus sungorus* **(Hoffman & Reiter, 1965; Hoffmann, 1973; Cheniti Lamine, 1974; LeBerre & Chevallier, 1990; Belhocine & Gernigon-Spychalowicz, 1994; Belhocine *et al.*, 2001; Boufermes *et al.*, 2021)**, while others exert it in different periods of the year. Indeed, the sand rat (*Psammomys obesus*) breeds from autumn to early spring **(Khammar, 1987)**. The small Gerboise (*Jaculus jaculus*) breeds in Fall-Winter **(Ghobrial & Hodieb, 1973)**, the Dwarf Gerbil (*Gerbillus nanus*) in winter **(LeBerre & Chevallier, 1990)**, the Saharian Goundi (*Ctenodactylus gundi*) in winter-spring **(Gouat, 1985)**, and the white-bellied Gerbil (*Gerbilliscus leucogaster*) in autumn-winter-spring **(Muteka *et al.,* 2018)**. Thus, they are great model to study the hormonal fluctuations as well as the changes that occur in the reproductive system.

Our work provides a good insight regarding histological changes and GPER1 distribution pattern in the reproductive system of the sand rat *Psamomys obesus* and the gerbil *Gerbillus gerbillus* during the reproductive cycle. For instance, the weight of the testis of the gerbil *Gerbillus gerbillus* encountered a significant decrease in the resting season comparing to the breeding season similarly to what was observed in the *Gerbillus gerbillus* **(Zaina Amirat**

***et al.*, 1977)**, the sand rat *Psammomys obesus* **(Gernigon-Spychalowicz, 1992b)**, *Gerbillus tarabuli* **(Hamidatou Khati & Hammouche, 2021)** and *Gerbilliscus leucogaster* **(Muteka *et al.*, 2018)**.

Moreover, the testis anatomy underwent noticeable alterations, the seminiferous tubules seemed atrophied, and had narrow lumen without sperm, as spermatogenesis was blocked at the stage of spermatocyte **(Figure 50)**. These observations are in line with the modifications noted in *Psammomys obesus, Gerbillus tarabuli*, and *Gerbilliscus leucogaster* **(Khammar, 1987; Gernigon-Spychalowicz, 1992b; Menad et al., 2017c; Muteka et al., 2018; Hamidatou Khati & Hammouche, 2021)**. Nevertheless, the changes observed in the Leydig cells of *Gerbillus gerbillus* can be explained by the inhibition of Leydig cell's activity leading to an elevation in nucleo-cytoplasmic ratio and the depletion of cytoplasmic volume during the resting season as reported in *Psammomys obesus* and *Gerbillus tarabuli* **(Gernigon-Spychalowicz, 1992b; Hamidatou Khati & Hammouche, 2021)**.

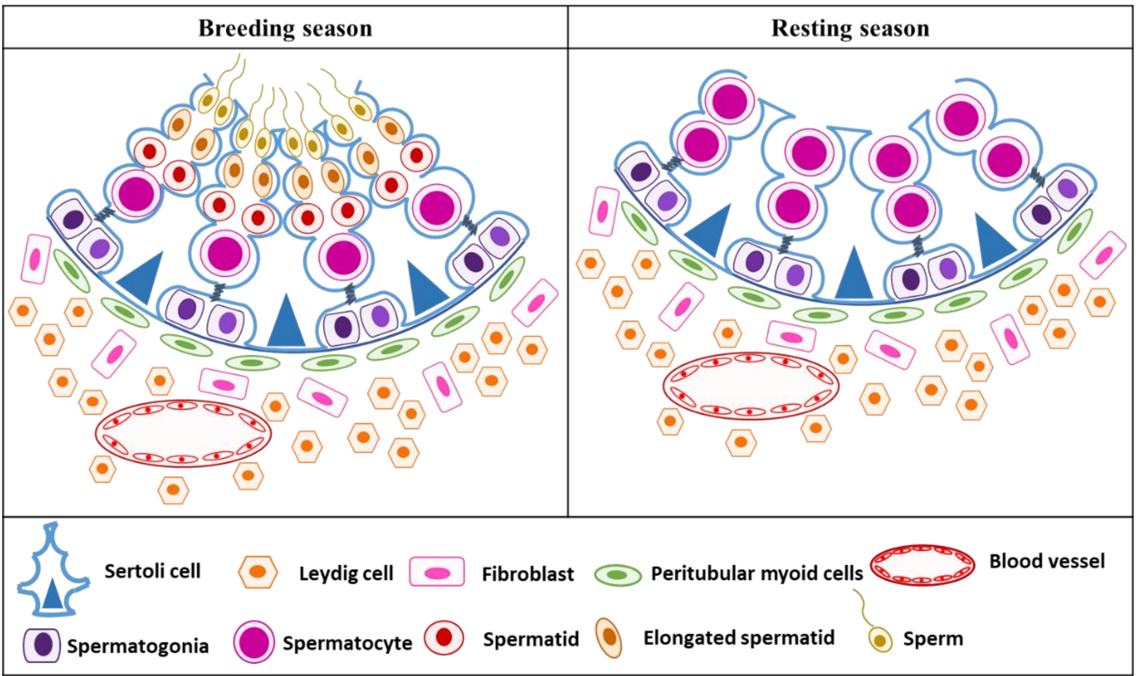


Figure 50: Schematic representation showing the histological changes of the testis in *Gerbillus gerbillus* and *Psammomys obesus* during the breeding season and the resting season.

In our study the interstitial compartment looked looser than it was during the breeding season. Interestingly, our results broaden the knowledge about seasonal variations of testicular structure and function that has been previously recorded in *Psammomys obesus*, *Gerbillus tarabuli*, and *Gerbilliscus leucogaster* **(Zaina Amirat et al., 1977; Khammar, 1987; Khammar & Brudieux, 1987; Gernigon-Spychalowicz, 1992b; Boufermes *et al.*, 2013; Muteka et al., 2018; Hamidatou Khati & Hammouche, 2021)**.

Nevertheless, Sperm leaving the testis passes through the efferent ducts. In the sand rat *Psammomys obesus* as well as in the gerbil *Gerbillus gerbillus*, they are formed of pseudostratified cubic epithelium mainly made of ciliated cells, non-ciliated cells, and basal cells, surrounded with three layers of smooth muscle cells and a connective tissue **(Figure 51)**.

However, the remodelling of the efferent ducts epithelium noticed during the resting season, mirrors that obtained by Oliveira *et al.* in the bat *Artibeus lituratus* **(Oliveira *et al.*, 2012)** and that obtained by our previous study in the *Psammomys obesus* **(Menad *et al.*, 2020b)**.

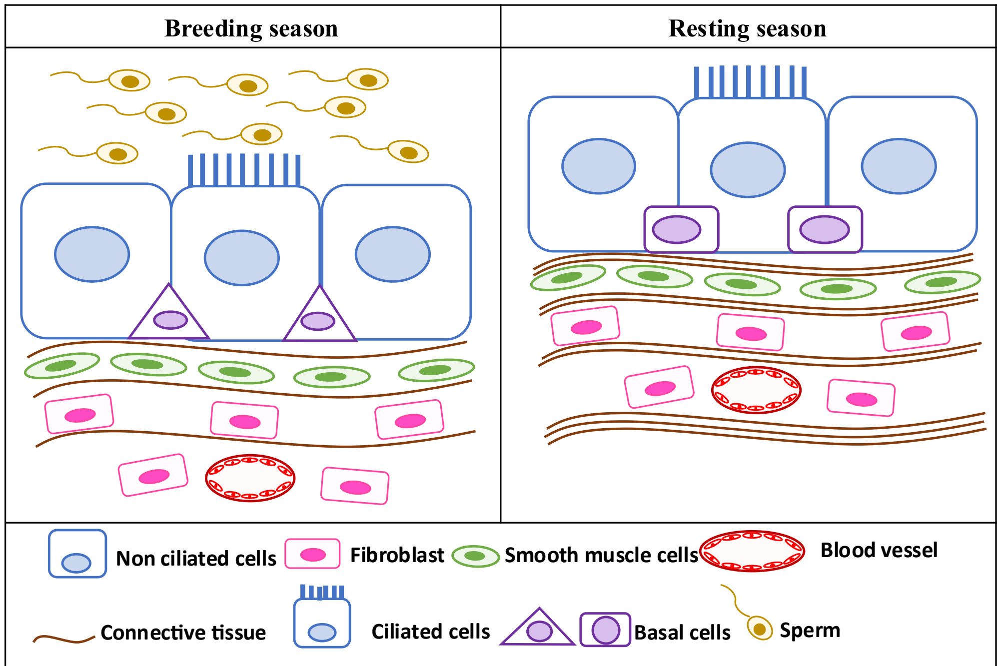


Figure 51: Schematic representation showing the histological changes of the efferent ducts in *Gerbillus gerbillus* and *Psammomys obesus* during the breeding season and the resting season.

Furthermore, the epididymis is considered as the site of sperm maturation **(Cornwall, 2009; James et al., 2020; Barrachina *et al.*, 2022)**, it is formed of several tubular section surrounded by interstitial compartment and a layer of smooth muscle cells that is thought to be responsible for sperm passage through the epididymis **(Sullivan & Belleannée, 2017)**. During the resting season, the epididymis went through drastic alteration **(Figure 52)**.

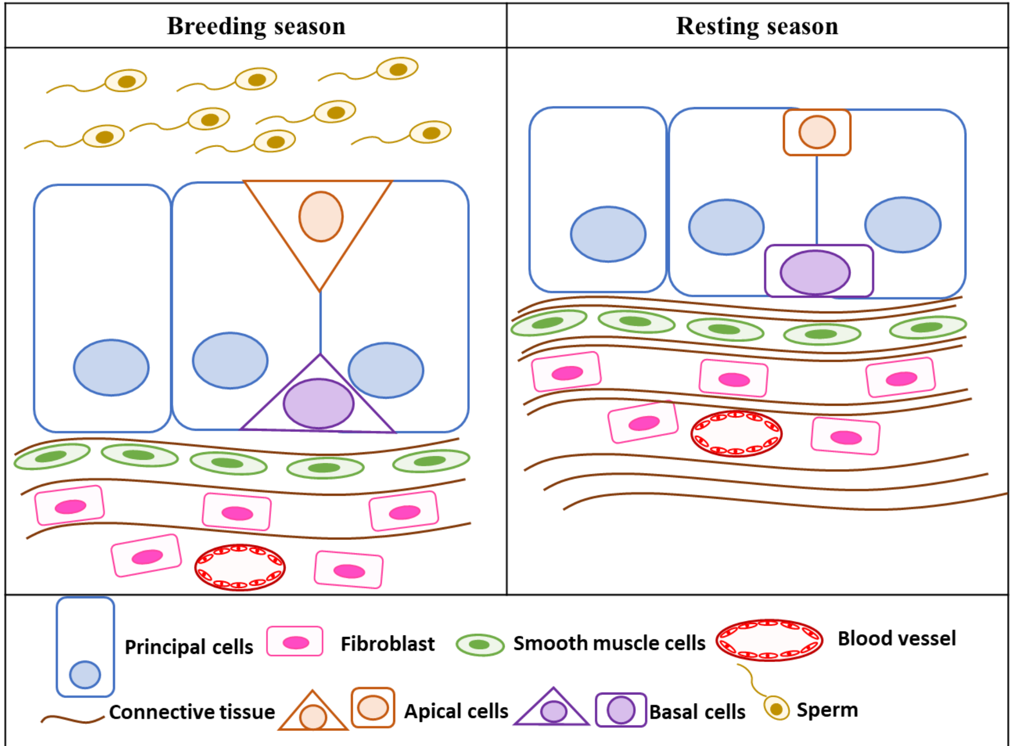


Figure 52: Schematic representation showing the histological changes of the proximal epididymis in *Gerbillus gerbillus* and *Psammomys obesus* during the breeding season and the resting season.

Both caput and cauda epididymis had disorganized epithelium with the absences of the cells that characterises each section , while the interstitial compartment and the layer of the smooth muscle cells appeared thicker **(Figure 53)**. Previous study on other species reported the same observations in *Psammomys obesus* **(Gernigon *et al.,* 1991; Menad *et al.,* 2017a)**, in mice **(Abou-Haila, 1987)**, *Meriones crassus* and *merione libycus* **(Belhocine, 1998)**.

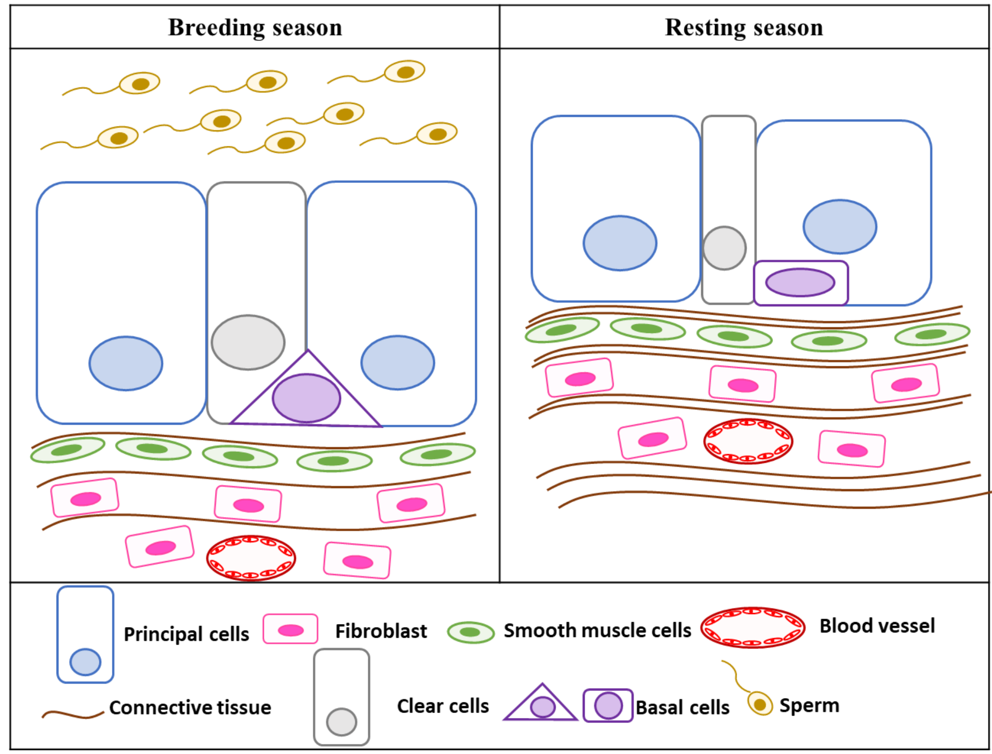


Figure 53: Schematic representation showing the histological changes of the distal epididymis in *Gerbillus gerbillus* and *Psammomys obesus* during the breeding season and the resting season.

Androgens are the main actors in male reproductive physiology, they control its functions and development **(Patrão *et al.*, 2009a; Murashima *et al.*, 2015)**, as androgen receptors are ubiquitously found in the testis, the epididymis, and the efferent ducts **(Danzo, 1997; Goyal *et al.*, 1997; Zhou et al., 2002; Matsuyama & DeFalco, 2024)**. Indeed, our previous work on the sand rat *Psammomys obesus* reported the presence of androgen receptor (AR) in the reproductive system and highlighted the impact of seasonality on this receptor as well as others such as ESR1, ESR2, Aromatase and E2 **(Menad *et al.*, 2017b; Menad *et al.*, 2020a; Menad *et al.*, 2021)**. Additionally, the group of Amirat *et al.* documented seasonal changes in plasma concentration, testicular content of testosterone and androstenedione in *Gerbillus gerbillus*, as they mark high levels in April and low levels in autumn **(Zaina Amirat *et al.*, 1977)**, similarly with what was reported in other rodent species such as : *Psammomys obesus*, *Gerbillus tarabuli*, and *Gerbilliscus leucogaster*, where testosterone decreases during

the resting season **(Gernigon-Spychalowicz, 1992b; Boufermes et al., 2014; Muteka et al., 2018; Hamidatou Khati & Hammouche, 2021)**. This androgen fluctuations during the season may be responsible for the structural reshuffle in the male reproductive system, for instance Belhocine *et al.* reported an increase in collagen I and III during the resting season, that is likely because of testosterone regulation of the MMPs and their inhibitors TIMPS in the extracellular matrix of the seminal vesicle and the prostate of *Meriones libycus* **(Belhocine *et al.,* 2010)**. Moreover, Occludins, Zonulaoccludens 1, and E-cadherin are reported to be androgen dependent **(Cyr *et al.,* 1993; Levy & Robaire, 1999)**.

Besides androgen, oestrogens play significant role in regulating male reproduction **(Hess & Cooke, 2018)**. While, oestrogen's main source is local conversion of androgen using the cytochrome 450 aromatase, which is found all over the testis **(Levallet *et al.,* 1998; Oliveira *et al.,* 2012)**, the epididymis **(Janulis *et al.*, 1996; Pereyra-Martinez *et al.*, 2001; Carpino *et al.*, 2004; Hejmej *et al.*, 2005; Shayu & Rao, 2006; Menad, 2008; Menad et al., 2017a)**, and the efferent ducts **(Oliveira *et al.,* 2012)**. Oestrogens control Sertoli cells, Leydig cells and germ cells functions, thus modulate the hypothalamic-pituitary-testicular axis **(Hess *et al.,* 1997; O'Donnell *et al.,* 2001)**. Furthermore, oestrogens control spermatogenesis, stem cells division and survival **(Miura *et al.*, 1999; Ebling *et al.*, 2000; Pentikäinen *et al.*, 2000)**. In the epididymis, oestrogens maintain epithelial structure and regulates epididymal functions **(Kobayashi & Behringer, 2003; França *et al.*, 2005; Hess *et al.*, 2011)**. Moreover, testicular oestrogens could be involved in the spermatocyte–spermatid transition in the testis, and in the acquisition of sperm motility in the epididymis according a study performed recently on rabbit **(Dewaele *et al.*, 2022)**. Indeed, knockout mice had altered spermatogenesis and fertility **(Dupont, 2000)** as well as a modified epididymal morphology **(Hess *et al.,* 2000)**. In the efferent ducts, oestrogens manage efferent duct morphology and functions as they directly influence epithelial cells **(Joseph *et al.,* 2011)**.

Oestrogens exert their effects through the classical nuclear receptors (ESR1 and ESR2) to alter gene transcription and result in genomic response **(Kuiper *et al.*, 1997; Cooke et al., 2017)**. These receptors are widely expressed in the testis, epididymis, and efferent ducts **(Hess et al., 1997; Rosenfeld *et al.*, 1998; Saunders *et al.*, 2001; Nie *et al.*, 2002; Menad et al., 2017c; Chimento *et al.*, 2020; Liguori *et al.*, 2024)**. In addition, oestrogens can trigger rapid non-genomic cellular responses via their new membrane receptor GPER1 **(Prossnitz *et al.*, 2007a)**, and which is poorly documented in the reproductive system especially in free-ranging animals. Thus, our work aims to investigate GPER1 distribution in the reproductive system of *Psammomys obesus* and *Gerbillus gerbillus* during the seasonal reproductive cycle. For this purpose, we performed immunohistochemical analysis of the testis; the efferent ducts and the epididymis using selective antibodies.

The current investigation, about the gerbil *Gerbillus gerbillus*, found that GPER1 was expressed in all the cell types of the testis during the breeding season but was only found in the Leydig cells and spermatocytes during the resting season **(Figure 54)**. Our results were broadly similar to those reported by Menad *et al.,* in the sand rat *Psammomys obesus* **(Menad et al., 2017c)**. The presence of GPER1 in the nuclei and the cytoplasm of Leydig cells may be due to its localisation in the endoplasmic reticulum, Golgi apparatus **(Prossnitz *et al.,* 2007a)** and in the plasma membrane **(Filardo *et al.,* 2007)**. In the sand rat, Leydig cells did not only expressed GPER1 but also ESRs and aromatase **(Menad et al., 2017c)**, which accentuate the fact that these cells are regulated by E2 **(Saunders *et al.*, 1998; Pelletier & El-Alfy, 2000)**, in addition isolated Leydig cells treated with E2 and GPER1 antagonist (G1) secreted less testosterone compared to non-treated cells **(Vaucher *et al.,* 2014)**, which could be related to the decrease in testosterone levels observed during the resting season in *Gerbillus gerbillus* and in *Psammomys obesus* **(Khammar & Brudieux, 1987)**. According to a previous study, the blockage of GPER1 downregulated the expression of ESRs but upregulated the expression of aromatase, besides no

effects on testis histology was observed but large mitochondria and many lipid droplets were found in the Leydig cells **(Kotula-Balak *et al.,* 2018)**. Therefore, Leydig cells functions are regulated by E2 via GPER1 **(Hess, 2003; Vaucher *et al.,* 2014)**, as also reported in fish gonads **(Thomas *et al.*, 2006; Pang & Thomas, 2010)**.

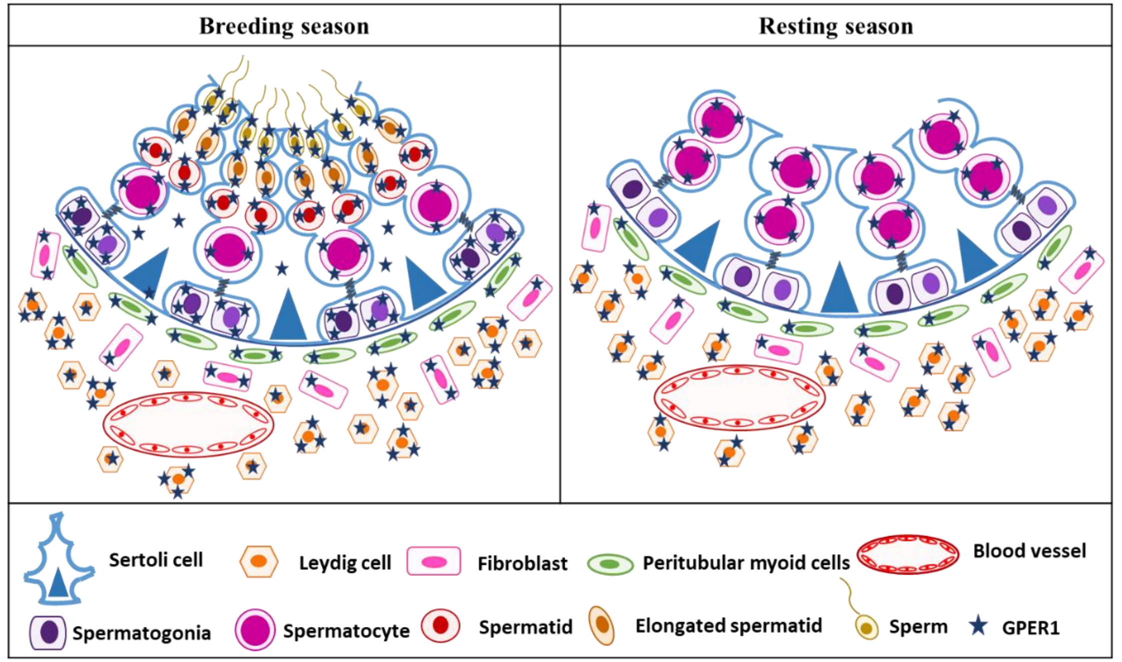


Figure 54: Schematic representation of the GPER1 distribution in the testis of *Gerbillus gerbillus* during the breeding season and the resting season.

Sertoli cells main functions are maintaining germ cells and supervising spermatogenesis **(Jeégou & Rolland, 2018)** under control of androgen and E2 **(Hess & França, 2005)**. In our study, Sertoli cells only expressed GPER1 during the breeding season in the gerbil *Gerbillus gerbillus* but GPER1 was absent in this cell during the hole season in the sand rat *Psammomys obesus*, in contrast to the Sertoli cells in human and rat, in which other receptors such as : Ars and ESRs also were present. **(Lucas *et al.*, 2008; Filipiak *et al.*, 2012; Menad et al., 2017c; Guercio *et al.*, 2020)**. Moreover, in Sertoli cells, GPER1 stimulation promotes gene expression and anti-apoptotic effect by activating EGFR and phosphorylating MAPK3/1 **(Lucas *et al.*, 2010; Lucas *et al.*, 2011; Royer *et al.*, 2012)**, while E2 induces cell proliferation via GPER1, by activating Src/PI3K/Akt pathway **(Yang *et al.*, 2017)**.

Another finding that emerges from the results reported in this work is that all germ cells expressed GPER1 during the breeding season, but as spermatogenesis was blocked at the spermatocyte stage, spermatocytes were the only germ cells expressing GPER1 during the resting season **(Figure 55)**. In Several species, it was stated that E2 blocked spermatogenesis during resting season **(Gancarczyk *et al.*, 2004; Schön & Blottner, 2008; Zhang *et al.*, 2010; Li *et al.*, 2015; Gautier *et al.*, 2016)** as it exhibit an apoptotic effect **(Scaia *et al.*, 2015)**, besides, the cross talk between ESRs and GPER1 activates the EGFR/ERK/fos/cyclin D1 in mice spermatogonia and promotes division of spermatogonia **(Sirianni *et al.*, 2008; Pierantoni *et al.*, 2009)**, decreases the expression of cyclin A1 and B1 mRNA and increases the pro-apoptotic factor Bax in pachytene spermatocytes **(Sirianni et al., 2008; Chimento *et al.*, 2010)**. During the breeding season, E2 stimulates spermatid differentiation **(Robertson *et al.*, 2002)**, it moderates EGFR/ERK cyclin B1 to balance between apoptosis and differentiation **(Chimento *et al.*, 2011)**. In addition, the implications of E2 through the GPER1 was documented to be responsible for spermatocyte and sperm maturation **(Müller *et al.*, 2012; Arkoun *et al.*, 2014; O'Shaughnessy, 2014; Smith *et al.*, 2015; Gautier et al., 2016)**, and mobility **(Aquila & De Amicis, 2014)**. Moreover, ERKO mice did not display severe testicular changes and spermatogenesis was not entirely affected compared to ArKO mice **(Murata *et al.*, 2002)**, consequently, E2 triggers GPER1 as alternative receptor through which it can exerts its effects. Therefore, the presences of GPER1 in germ cells in *Gerbillus gerbillus*, may be a strong indicator of E2 modulation of spermatogenesis through the GPER1. These findings match those observed by earlier studies in the sand rat *Psammomys obesus* , where it was reported a co-localisation of ESRs and GPER1 in germ cells **(Menad et al., 2017c)**.

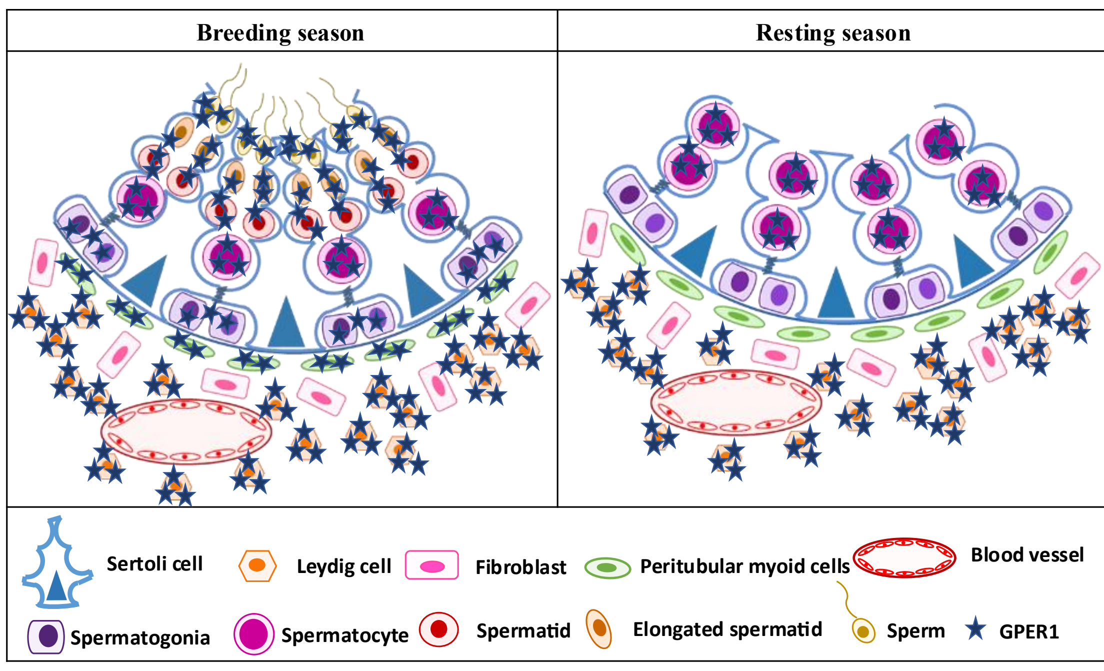


Figure 55: Schematic representation of the GPER1 distribution in the testis of *Psammomys obesus* during the breeding season and the resting season.

Another important results in our work were that the peritubular myoid cells express GPER1 during both season, similar results were found in the sand rat *Psammomys obesus*, where it did not only express GPER1 but also ESRs **(Menad et al., 2017c)**, In addition, it was reported that GPER1 is located in human and non-human primates peritubular myoid cells and is linked to male fertility. Moreover, animal testis expressed GPER1 in the peritubular myoid cells from birth to puberty and a decrease in GPER1 expression in peritubular myoid cells was documented in men with fertility problems **(Sandner *et al.*, 2014)**. These findings support the idea that E2 regulates peritubular myoid cells, especially through the GPER1, this later controls vascular functions and tone **(Elvira Haas *et al.*, 2009; Prabhushankar *et al.*, 2013)**, responsible for sperm transport through the seminiferous tubules **(Shughrue *et al.*, 1996)**. Furthermore, the human peritubular myoid cells regulates spermatogonial cell niche by secreting regulating factors such as GDNF **(Spinnler *et al.*, 2010)**.

In the efferent ducts of the gerbil *Gerbillus gerbillus*, we found strong expression of GPER1 in the ciliated cells, moderate expression in all the rest of type cells during the breeding

season, while during the resting season a withdraw of GPER1 signal was observed, as only rare ciliated cells, non-ciliated cells and basal cells were marked **(Figure 56)**. As was reported in our previous study, in the sand rat *Psammomys obesus* **(Figure 57)**, the seasonality did not much affect the presence of the GPER1 but rather impacted the intensity of its expression, but the co-located ESRs were fully influenced by the season **(Menad et al., 2020a)**. Besides, ESRs are also located in monkey, hamster, and mouse efferent ducts **(Joseph *et al.*, 2011)**.

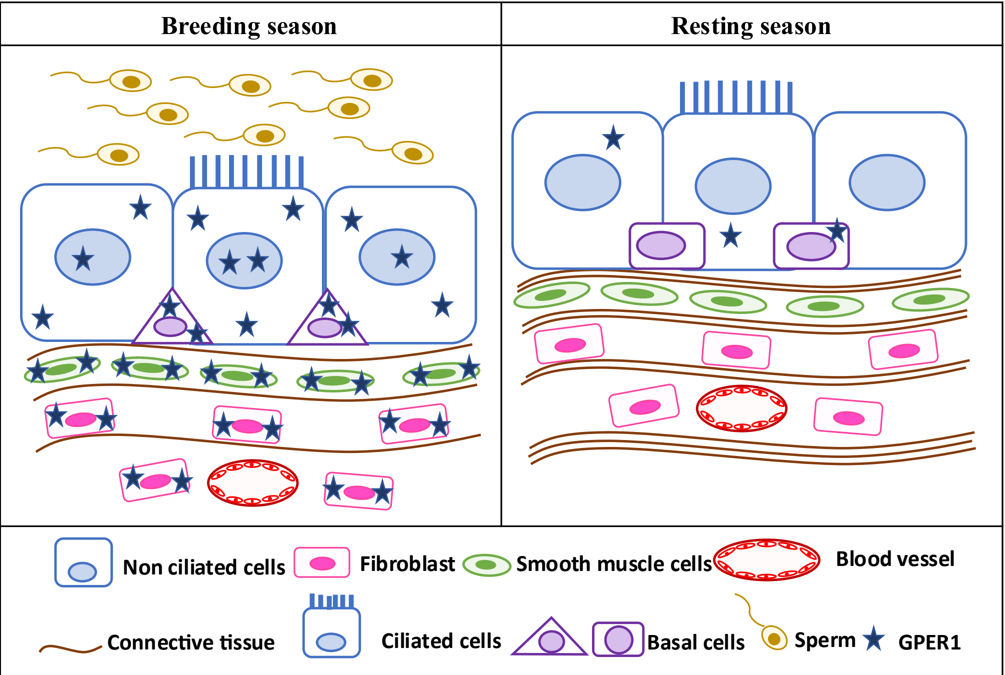


Figure 56: Schematic representation of the GPER1 distribution in the efferent ducts of *Gerbillus gerbillus* during the breeding season and the resting season.

These findings suggest a cross talk between these receptors to mediate estrogenic effects in the efferent ducts. In fact, E2 is essential for fluid reabsorption in the efferent ducts, blocking ESRs inhibited this process **(Hess & Cooke, 2018)**. Even more, the cytoplasmic expression of the GPER1 mirrors its cellular distribution in the Golgi apparatus and endoplasmic reticulum as well as in the plasma membrane **(Revankar *et al.*, 2005)**. Interestingly, smooth muscle cells in our study in *Gerbillus gerbillus* expressed GPER1 similarly to the muscle cells in the testis and the epididymis, while , our study on the sand rat *Psammomys obesus* stated same results

(Menad et al., 2017a; Menad et al., 2017c; Menad et al., 2020a), supporting the fact that E2 controls tubules contractility by acting on oxytocin **(Filippi *et al.*, 2002)**.

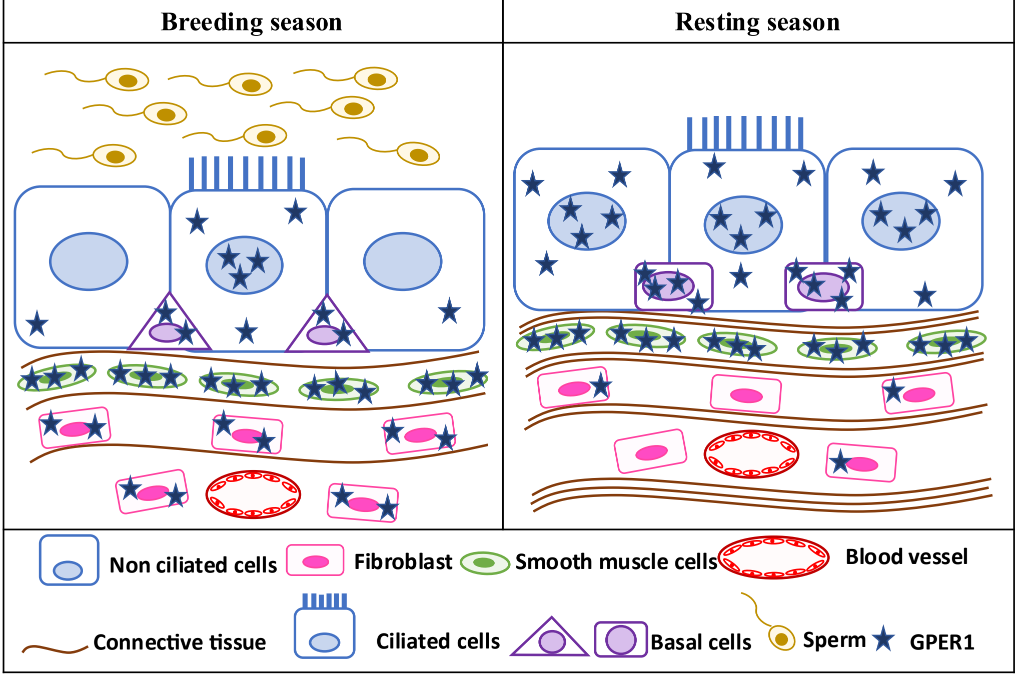


Figure 57: Schematic representation of the GPER1 distribution in the efferent ducts of *Psammomys obesus* during the breeding season and the resting season.

The proximal epididymis in our study was largely stained but exhibited moderate staining **(Figure 58)**, all cell types expressed GPER1 during the breeding season, but only principal cells and basal expressed GPER1 during the resting season. However, the findings of the current study do not matches with the previous research performed in the sand rat *Psammomys obesus*, where smooth muscle cells and fibroblast also presented GPER1 during the resting season **(Menad *et al.*, 2017a)**. Furthermore, the distal epididymis in our study showed stronger immunohistochemical staining during the breeding season compared to the proximal epididymis, principal cells were moderately marked during the resting season, while some basal cells , fibroblast and smooth muscle cells showed weak staining. In the sand rat *Psammomys obesus*, the signal presented same intensity during the breeding season and all cell types were weakly marked during the resting season **(Menad *et al.*, 2017a)**. In the same manner,

the withdraw of GPER1 expression reported during the resting season **(Figure 59)**, suggests that this receptor is highly influenced by the hormonal fluctuations during the season, such as the decline in testosterone levels observed during the resting season **(Zaïna Amirat *et al.*, 1977)** as well as other hormones **(Amirat *et al.*, 1980; Boufermes *et al.*, 2014)**.

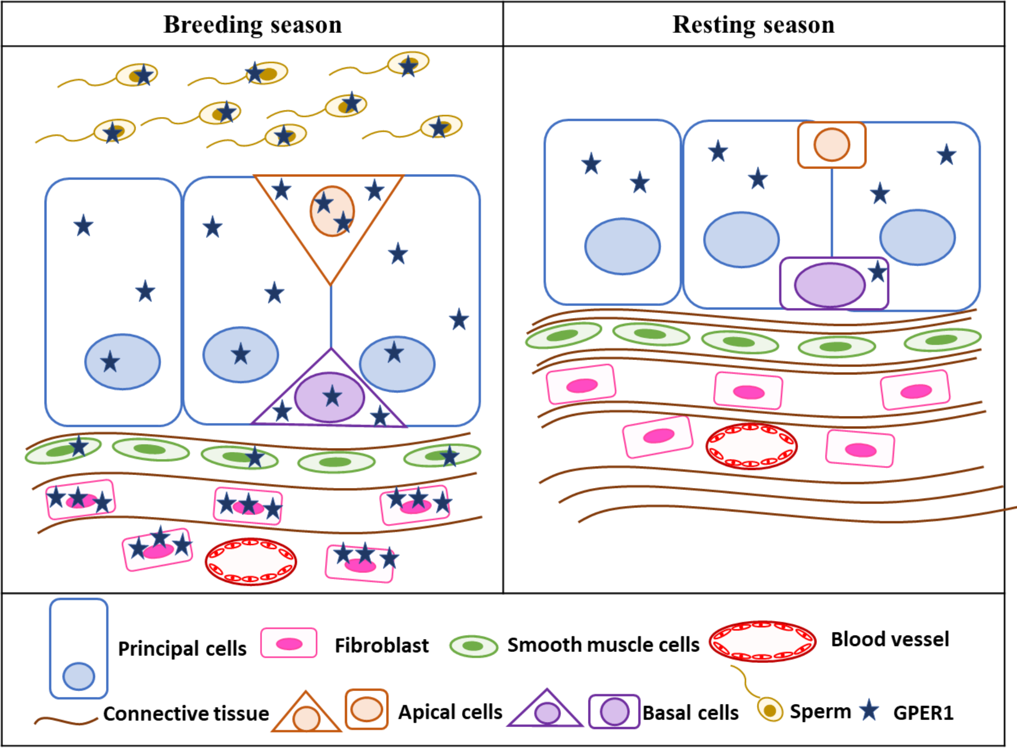


Figure 58: Schematic representation of the GPER1 distribution in the proximal epididymis of *Gerbillus gerbillus* during the breeding season and the resting season.

Regarding the difference in GPER1 expression between the caput and the cauda epididymis, similarly to *Gerbillus gerbillus* **(Figure 60)**, Brown Norway and Wistar rat showed a strong expression of GPER1 in the cauda epididymis compared to caput epididymis, in contrast to Sprague Dawley rat **(Martínez-Traverso & Pearl, 2015)**, who had the opposite , intense signal in the caput compared to the cauda, while *Psammomys obesus* presented the same pattern in both region **(Menad *et al.*, 2017a) (Figure 61)**, this may be due to distinct responsiveness between the segments and species **(Pereira *et al.*, 2014)**.

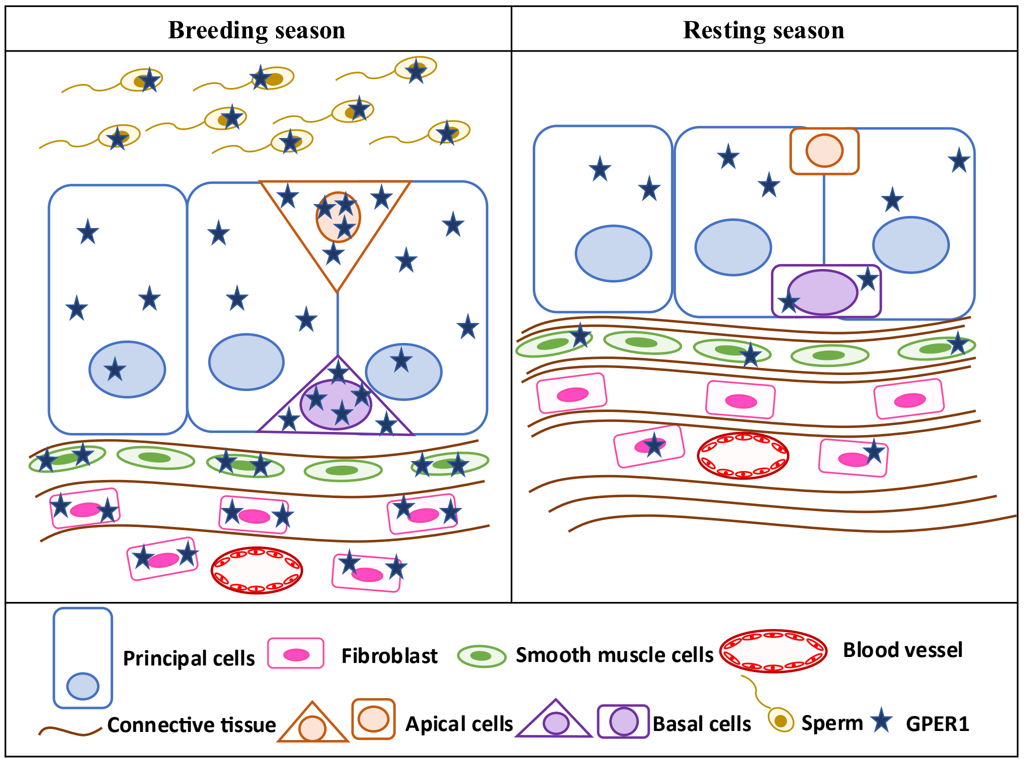


Figure 59: Schematic representation of the GPER1 distribution in the proximal epididymis of *Psammomys obesus* during the breeding season and the resting season.

Another interesting finding was the different cellular localisation of the GPER1 among cells and seasons. For instance, the cytoplasmic distribution might be explained by the fact that GPER1 is mostly expressed on the surface of Golgi apparatus and endoplasmic reticulum **(Revankar et al., 2005; Prossnitz et al., 2008a; Liverman *et al.*, 2009; Filardo & Thomas, 2012)**, as well as on the cell surface **(S. B. Cheng *et al.,* 2011)**. In addition, GPER1 is found to be colocalized with the receptor activity-modifying protein 3 (RAMP3) that down regulates the GPER1 from the plasma membrane to the intracellular membranes and the Golgi apparatus **(S. B. Cheng et al., 2011; Bouschet *et al.*, 2012; Lenhart *et al.*, 2013)**. This aspect is important for GPER1 functioning and metabolism, for example, GPER1 stimulates β pancreatic cells to release insulin **(Sharma & Prossnitz, 2011)** and promotes lipid metabolism **(Santolla *et al.,* 2012)**. In addition, the nuclear localisation of the GPER1 reported in our study about the gerbil *Gerbillus gerbillus* confirm what was stated in the sand rat *Psammomy obesus* **(Menad *et al.,***

2017a), even more, the nuclear localisation of the GPER1 in cancerous fibroblasts mediates, cell proliferation and survival **(Bologa *et al.,* 2006; Madeo & Maggiolini, 2010)**. In addition, the GPCRs binding with ligand results in the internalisation, rapid recycling, and degradation of the receptor **(Oakley *et al.*, 1999; Marchese *et al.*, 2008)**, more over the endocytosis of GPER1 can lead to intracellular accumulation justifying the absence of the GPER1 in cell surface **(S.-B. Cheng *et al.*, 2011)**.

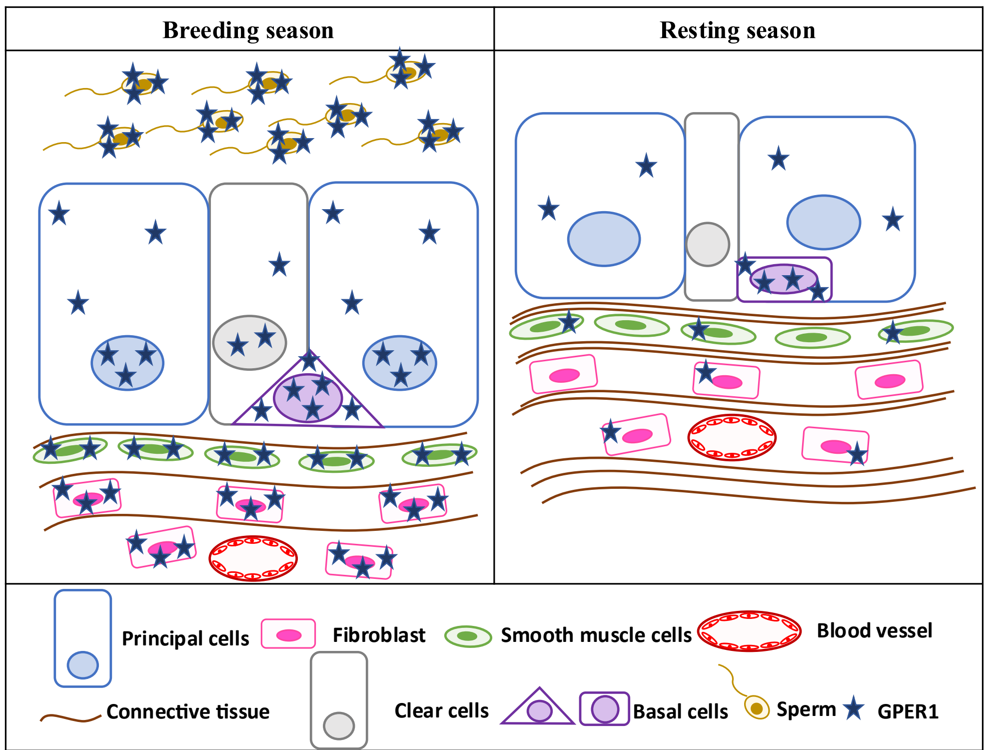


Figure 60: Schematic representation of the GPER1 distribution in the distal epididymis of *Gerbillus gerbillus* during the breeding season and the resting season.

Furthermore, previous studies reported the co-localisation of the GPER1 with the other ESRs **(Menad *et al.,* 2017a)**, supporting the idea of a crosstalk between these receptors to mediate E2 actions in the epididymis, as data in breast cancer cells stated their co-expression for possible interactions to mediates tumour progression **(Filardo *et al.,* 2006; Huang *et al.,* 2010; Prossnitz & Barton, 2011)** or inhibiting cell growth as shown in uterine tumour cells **(Gao *et al.,* 2011)**. In addition, studies revealed that GPER1 exists in anterior pituitary **(Brailoiu**

***et al.*, 2007; Hazell *et al.*, 2009)** and in a subset of LHRH neurons **(Rudolf & Kadokawa, 2013)**, and ESRs antagonism did not block oestradiol action on the gonadotropic pituitary cells **(Demay *et al.*, 2001)**, so there must be another path mediating E2 actions. In line with the previous studies, and the work stating the effect of GPER1 in suppressing GnRH-stimulated LH release in pituitary cell culture **(Rudolf & Kadokawa, 2013)**, GPER1 seems a major player in E2 action on the hypothalamic pituitary axis.

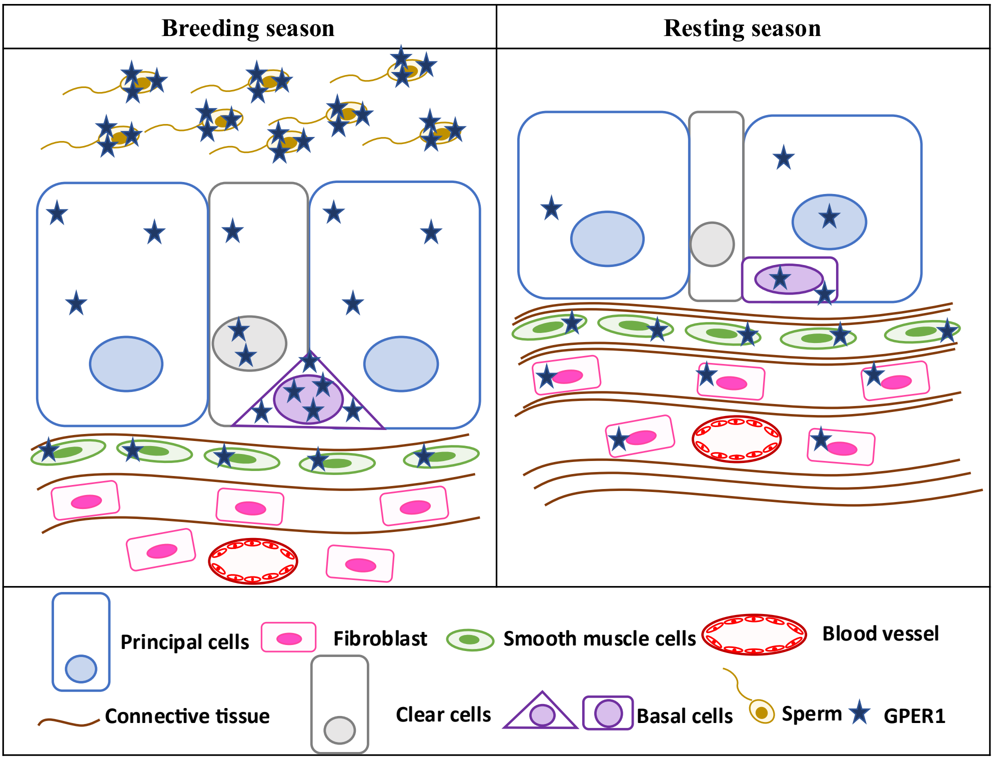


Figure 61: Schematic representation of the GPER1 distribution in the distal epididymis of *Psammomys obesus* during the breeding season and the resting season.

Epididymis is known to be the site of sperm maturation, and its functions are regulated by androgens and oestrogens **(Guo *et al.*, 2009; Robaire & Hinton, 2015b; Singh & Singh, 2017; Ali *et al.*, 2023)**. Oestrogen receptors, including ESRs and GPER1, were largely located in the epididymis of the boar **(Krejčířová *et al.*, 2018)**, rat **(Hess *et al.*, 2011; Martínez-Traverso & Pearl, 2015)**, sheep **(Ge *et al.*, 2020)**, bull **(Antalikova *et al.*, 2020)**, dog **(Liguori et al., 2024)**, pig **(Malivindi *et al.*, 2018)** as well as in the sand rat **(Menad *et al.*, 2017a)**. In

addition, during the process of maturation, spermatozoa bind to protein secreted by the epididymis and their proteins undergo post-translational changes so sperm gain fertilizing ability **(Cornwall, 2018)**. Furthermore, sperm from proximal epididymis express GPER1 in flagellum, while sperm from the distal epididymis express it in the acrosome **(Krejčířová *et al.,* 2018)**, these findings may be an indicator that GPER1 expression in spermatozoa are part of sperm maturation during their transit through the epididymis, in fact, ejaculated and capacitated bull spermatozoa express GPER1 in the post-acrosomal region and in the apical part of the acrosome **(Antalikova *et al.,* 2020)**, which confirms the implication of GPER1 in sperm capacitation and acrosome reaction via calcium mobilization and protein kinase stimulation **(Schwartz *et al.*, 2016; Gao *et al.*, 2022)**. Moreover, the mid-piece of sperm, expressing GPER1, contains large number of mitochondria that are stimulated by calcium mobilisation to produce ATP **(Ho & Suarez, 2003)**, in addition calcium concentration in sperm controls flagella beats **(Darszon *et al.,* 2011)**, meanwhile GPER1 signalling pathway includes intracellular calcium mobilisation **(Prossnitz *et al.,* 2008c)**, which accords with the fact that GPER1 is important for sperm mobility. Interestingly, the group of Gautier et al, stated that GPER1 influence sperm motility, since sperm culture treated with G1 decreases percentage of sperm with total and progressive motility as well as VAP and VCL **(Gautier *et al.,* 2016)**.

Nevertheless, research on the GPER1 revealed its implication in various types of reproductive system pathologies, infertility, and cancers **(Chimento et al., 2020; Walczak-Jędrzejowska *et al.*, 2022)**. For instance, it was found in intratubular germ cell tumours, seminomas, embryonal carcinomas, and teratomas **(Franco *et al.,* 2011; Rago *et al.,* 2011)** as well as benign and neoplastic prostatic cancer **(Rago *et al.,* 2016)**. In testicular germ cell tumour, GPER1 activate PKA/MAPK pathway to induce cell proliferation **(Bouskine *et al.,* 2009; Chevalier *et al.,* 2012)**, yet in testicular stromal neoplasms such as Leydig and Sertoli

cells tumours **(Carpino *et al.,* 2007; Rago *et al.,* 2011)**, the activation of GPER1 using G1 provokes apoptosis by activating ERK1/2 mechanism **(Chimento *et al.,* 2013)**.

Although steroid hormones act classically through nuclear receptors **(Beato & Klug, 2000)**, oestrogens have been reported to act via the membrane receptor GPER1 **(Filardo *et al.,* 2000)**. GPER1 was first documented to mediate estrogenic response in in the early 2000, since then, many studies have been conducted to illustrate its role in normal physiology and in various diseases **(Filardo & Thomas, 2005; Zhang *et al.*, 2020; Prossnitz & Barton, 2023; Liguori et al., 2024)**. In addition, searching for selective ligands for this receptor is crucial to target it in order to treat pathological conditions in which this receptor is involved, it was till 2006-2011 when GPER1 selective agonists and antagonist were synthesized **(Dennis *et al.,* 2009)**. In fact, the group which made these discoveries used *in silico* approach to identify the first GPER1 agonist G1 **(Bologa *et al.,* 2006)**, then screening its analogue to determine two antagonists G15 and G36 **(Dennis *et al.,* 2011a)**.

In addition, *in silico* approaches such as molecular docking are being widely used in different fields such as toxicology, biochemistry and pharmacology, where this approach guide research and predict molecules interactions with protein, consequently, identify potentials treatment targeting specific proteins **(Gupta *et al.,* 2020; Jiao *et al.,* 2021)**, and perhaps the main benefits of this approach are saving time and decreasing costs of research by orientating studies to the potential outcomes, testing compounds before being synthetized and used on animals **(Madan *et al.,* 2013; Raies & Bajic, 2016)**.

For this purpose, the present *in silico* study was designed to determine molecules that interact with GPER1 by comparing their biding pattern with those of known ligands, as an attempt to select compounds that may be used as therapeutic potential of GPER1 to treat male infertility, pathologies of male reproductive system and even cancer. In addition, the tested compounds may act as selective ligands for the GPER1 as they sometimes share similar binding

pattern with those of the synthetic ligands (G1 and G36) **(Figure 62)**, but more work need to be done in order to define the exact behaviour of these chemicals, as they can activates the GPER1 and act as agonists, in some tissues, but deactivates GPER1 and act as antagonists in other tissues **(Figure 63)**.

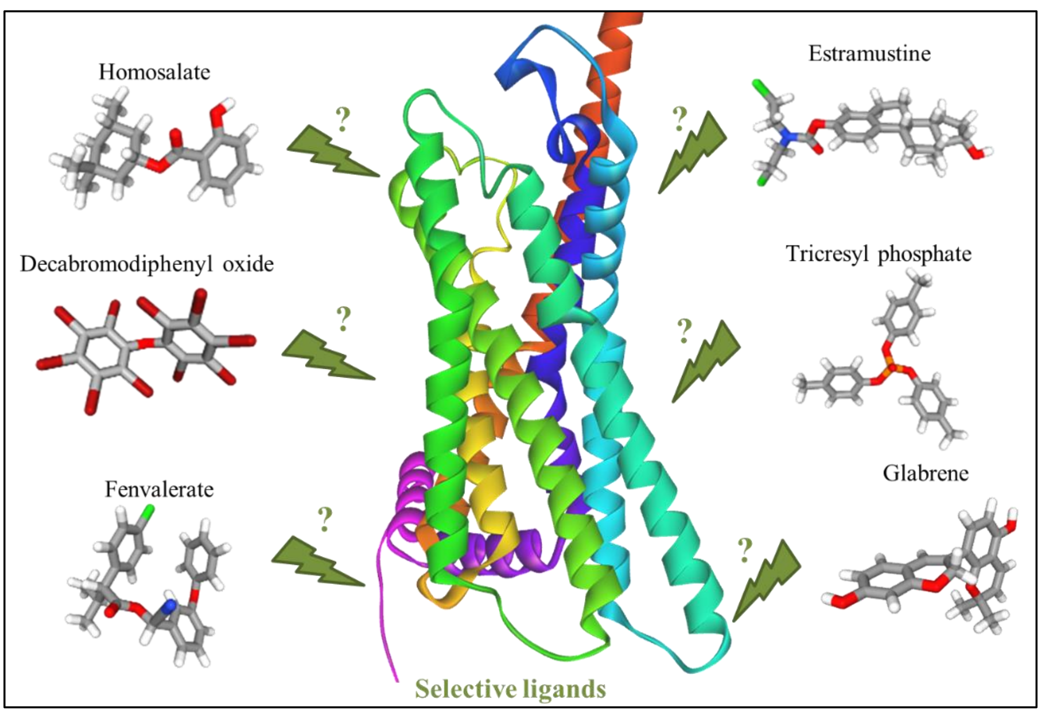


Figure 62: Chemicals that are presumed to be selective ligands for the GPER1.

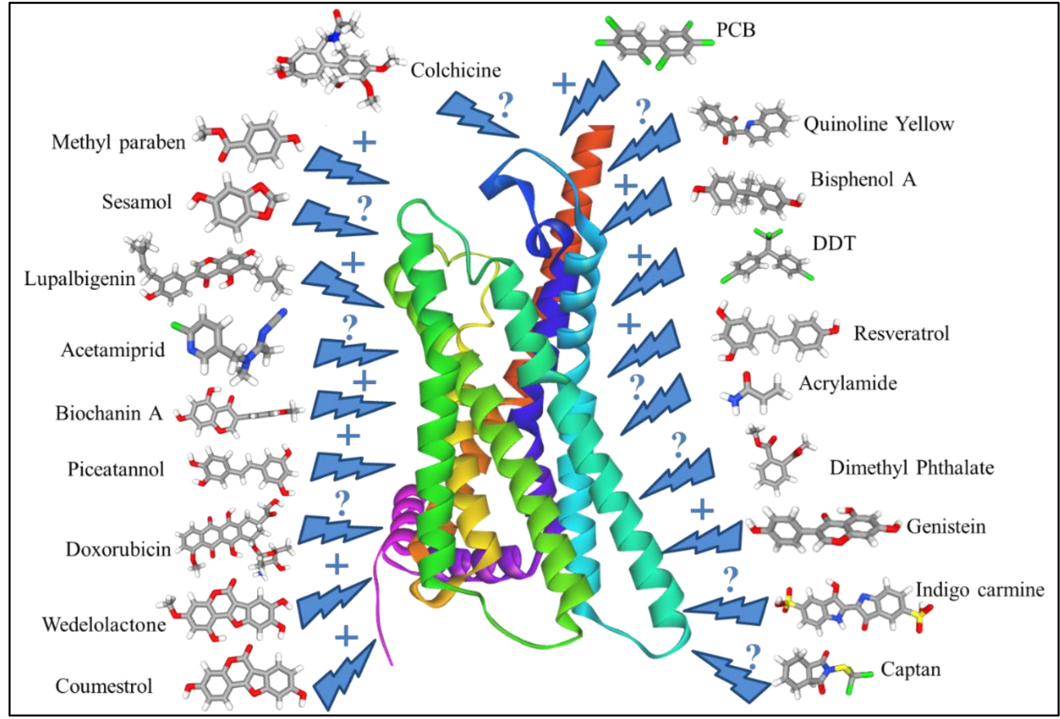


Figure 63: Chemicals that are presumed to be nonselective ligands for the GPER1.

Our results showed that each of the tested compounds exhibits a binding energy (ΔG) and several types of bonds, which gives an insight about the affinity of the GPER1 toward these chemicals **(Meng et al., 2011)**. Indeed, the lower ΔG is the higher affinity and the greater complex (GPER1- ligand) stability **(Pantsar & Poso, 2018)**. Moreover, using the known ligands (E2, G1 and G36) of GPER1 as reference, we organized the tested compounds as shown in **Figure 64**, starting from BPA having the lowest binding energy (ΔG) and ending with Carboxy cellulose having the highest binding energy. In addition, chemicals having binding energy lower than E2, G1 and G36 are represented with green, while those with quite similar binding energy ΔG with E2, G1 and G36 are represented with orange, finally those with higher binding energy compared to E2,G1 and G36 are represented with red.

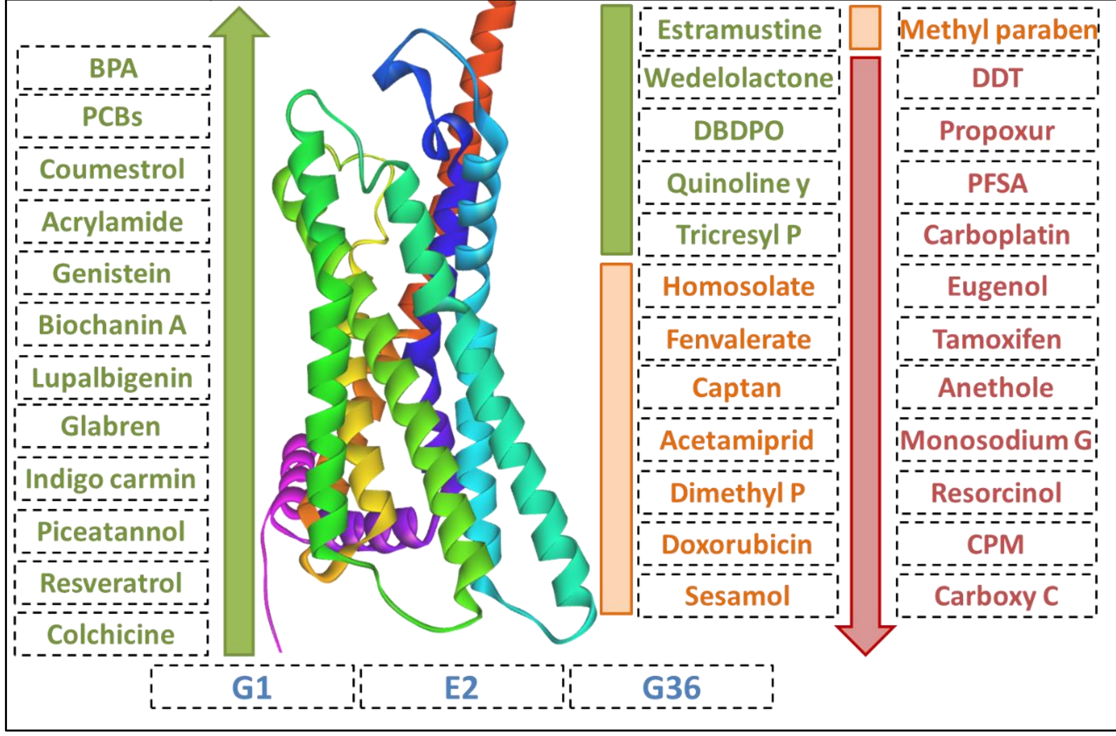


Figure 64: The different tested chemicals classified according to their binding affinity.

Furthermore, E2, Fulvestrant, Tamoxifen, G1 and G36 had binding energies ΔG of -6.67 kcal/mol, -2.66 kcal/mol, -5.46 kcal/mol, -6.43 kcal/mol, -6.66 kcal/mol respectively, while the common amino acids involved in these interactions were: ALA184, PHE146, ILE299

and PRO226. Nonetheless, E2 was the first ligand found to bind with high affinity to GPER1, studies showed that E2 activates cAMP and controls ERK1/2 in MAPK pathway leading to ESRs independent oestrogen cellular response **(Filardo *et al.,* 2000; Revankar *et al.,* 2005; Thomas *et al.,* 2005)**. Later, Tamoxifen (SERM) was reported to activate GPER1, and Fulvestrant (SERD) which is considered as ESRs antagonist also mediates GPER1 estrogenic response **(Meyer *et al.,* 2011a)**. In triple negative breast cancer (TNBC), tamoxifen mediates tumour cell survival and invasion via GPER1, independently from ESRs, as it triggers EGFR/ERK/c-fos pathway **(Du *et al.,* 2012; Schmitz *et al.,* 2022)**. In addition, Fulvestrant selective ERS antagonists trigger GPER1 and activates the same signalling pathway in breast cancer cells **(Filardo *et al.,* 2000)**.

Endocrine disruptors (EDs), the emerging threat of human and non-human health, are increasingly being used in everyday life, in the past decades several reports have been published assessing serious consequences and irreversible damage induced by these chemicals on human health and environment **(Encarnação *et al.,* 2019)**, but it was till 2009, when a scientific statement addressing worries about endocrine disruptors was officially published **(Diamanti-Kandarakis *et al.,* 2009)**, afterward, the WHO released the state of science in 2013 expressing concerns about the harmful effects of EDs **(Bergman *et al.,* 2013)**, but till now there is still no regulatory laws monitoring the usage of these chemicals, and unfortunately, more chemicals are being synthesized and employed subsequently leading to more health problems.

In the present work, we evaluated five major types of EDs: heavy metals, pesticides, food additives, personal care chemicals, plasticizers, and flame retardants. For instance, heavy metals interacted with GPER1 using CYS205, PHE206 and HIS200, but displayed high binding energy compared to known ligands. Studies on breast cancer and ovarian cancer reported the implication of Cadmium in cell proliferation, as it triggers GPER1, increases cAMP levels and activate ERK1/2 pathway leading to tumour progression **(Yu *et al.*, 2010; Hirao-Suzuki *et al.*,**

2020; Sackie, 2023). In male, heavy metals induce fertility problems by altering Leydig cell and Sertoli cell functions, disrupting spermatogenesis and inducing oxidative stress **(López-Botella *et al.,* 2021)**, indeed, the study of **Bentaiba *et al,*** revealed drastic alteration of male reproductive physiology after exposure to Cadmium and Lead **(Bentaiba *et al.,* 2023)**.

Furthermore, Pesticides used in our study are commonly used in agriculture, they exhibited low binding energies with GPER1 and interacted with the same amino acids as the known ligands. Pesticides harmful effects are widely investigated especially on reproductive system, it has been reported that pesticides alter reproductive physiology and cause infertility **(Moreira *et al.*, 2021; Giulioni *et al.*, 2022)**, in fact studies of **Agabi *et al,*** (unpublished data) revealed severe damage in the reproductive system of male rats exposed to pesticides. Besides, DDT, a pesticide having oestrogenic activity, was stated to bind with high affinity to GPER1 **(Peter Thomas & Jing Dong, 2006)**. In the same manner, food additives contained in our daily meals seem to interact with GPER1, our results showed that Indigo Carmin and Quinoline Yellow, dye used in food colouring, had significant lower binding energy in contrast to E2 which is in line with those of previous studies reporting estrogenic activities of food additives **(Bazin *et al.,* 2017)**.

An interesting outcome of our work is the interactions between personal care chemicals and the GPER1. Personal care substances are a family of chemicals used for wellbeing, beauty, and cleaning. Fancy cosmetics, skin products, hair care products, and fragrances, are widely used and can be found almost everywhere in the environment **(Kuchangi *et al.,* 2023)**. Homosalate, Dimethyl Phthalate, Methyl Paraben and Tricresyl Phosphate manifested very low binding energy and shared several amino acid interactions with those of E2, G1 and G36, which may reflect an obvious effect of these chemicals on health via GPER1. In fact, Tricresyl Phosphate was found by the group of **Ji *et al,*** to agonise GPER1 and promotes cAMP production leading to calcium mobilization **(Ji *et al.,* 2020)**. In addition, Paraben derivatives

were declared to have oestrogen like activity **(Nowak *et al.,* 2022)**, also activate GPER1 in breast cancer cells, engage in Erk1/2 pathway and inhibit apoptosis through Akt kinase **(Wróbel & Gregoraszczuk, 2015)**, studies also reported alteration of spermatogenesis manifested by narrow seminiferous tubules and increased germ cell apoptosis **(Alam *et al.,* 2014)**. In humans, a correlation between urinary Paraben concentrations and semen abnormalities including sperm DNA damage, altered morphology and decreased motility was reported **(Jurewicz *et al.,* 2017)**. On the other hand, Phthalates and derivatives promote survival and growth of cervical cancer and breast cancer cells through PI3K-Akt signalling pathway **(Chen & Chien, 2014; Yang *et al.,* 2018)**. In Sertoli cells, Monobutyl Phthalate activated GPER1 and recruited EGFR Erk1/2 signalling pathway leading to changes in Sertoli cell function, while treatment with Panax ginseng metabolite reduced Monobutyl Phthalate actions via GPER1 **(de Freitas *et al.,* 2020)**. Nonetheless, Phthalates exert harmful effects on male reproductive physiology such as testicular dysgenesis syndrome known as Phthalate syndrome, where men manifest hypospadias, cryptorchidism, abnormal testicular development and decreasing anogenital distance **(Sharpe *et al.,* 2008)**, in addition phthalates damage spermatozoa resulting in reduced in semen quality and sterility **(Lyche *et al.*, 2009; Benjamin *et al.*, 2017)**. In a study performed in procreation Clinique, men having sperm abnormalities also had higher urinary levels of phthalates and testosterone/oestrogen imbalance **(Meeker *et al.,* 2009)**. In animal models, phthalates altered seminiferous tubule structure, decreased sperm production and viability **(Fiandanese *et al.,* 2016)**, lowered intratesticular testosterone, down regulated FSH and LH receptors **(Aso *et al.,* 2005)**. What is more surprising is that even males born to exposed mice had reproductive abnormalities and genetic alterations **(Prados *et al.,* 2015)**.

Perhaps the most striking results from those stated above were those of plasticizers and flame retardants. Plasticizers are compounds added in plastic materials to promote softness and flexibility **(Ebnesajjad, 2016)**, while flame retardants are chemicals that inhibits or slow down

fire dissemination by inhibiting chemical reactions in the flame, or by covering the surface with a protective layer **(Speight, 2017)**. Interestingly, Bisphenol A, PCBs and Acrylamide displayed the lowest binding energies among all the tested chemicals, further, these chemicals share some interaction features together but little with E2, G1 G2. Even though BPA has similar structure to E2 **(Brzozowski *et al.,* 1997)** it showed higher affinity to GPER1 and had different binding patterns. Nonetheless, BPA toxic effects have been largely investigated, as it was found to be responsible for several types of cancer, nervous and immune systems dysfunctions, congenital malformations, endocrine disruption, and infertility **(Dueñas-Moreno *et al.,* 2023; Prueitt *et al.,* 2023)**. In fact, BPA triggers GPER1 and several genomic and non-genomic pathways **(Richter *et al.,* 2007)** leading to cancer progression and metastasis, for example in breast cancer as well in testicular cancer cells, BPA activates EGFR/ERK pathway to promote malignant cell proliferation and migration **(Pupo *et al.,* 2012)**, in seminoma cells, BPA stimulates ERK1/2 pathway **(Chevalier *et al.,* 2012)**. Of note, in non-hormonal cancer, BPA via GPER1 induces laryngeal squamous cell carcinoma and lung cancer cells growth **(Li *et al.,* 2017)**. In pancreatic β cells, BPA triggers GPER1 and induces apoptosis in opposition to the anti-apoptotic effect exerted by ERα and Erβ **(Babiloni-Chust *et al.,* 2022)**. While, in Wistar rats, BPA provoke early onset of puberty by modulating GPER1 and Erk1/2 and PI3K/Akt pathways **(Lei *et al.,* 2024)**. In the male reproductive system, BPA was found to be responsible for several alteration, including alteration of the blood-testis barrier as it influences the expression of intercellular junction proteins such as: occludins, zonula occludins, β-catenin, N-cadherin, and F-actin **(Peña-Corona *et al.,* 2021)**. Moreover, BPA initiate GPER/EGFR/ERK1/2 signalling pathway leading to spermatogonia and Sertoli cells proliferation **(Sheng & Zhu, 2011; Ge *et al.*, 2014)**, it also induces spermatocytes apoptosis and inhibits growth via the EGFR-MAPK pathway **(Wang *et al.,* 2017)**. In humans, exposure of BPA is correlated with fertility problems and sperm alteration **(Meeker *et al.,* 2010)**. Nevertheless, exposure to Polychlorinated Biphenyls

induced considerable damage on the male reproductive physiology, after exposure, testis weight decreased, sperm quality was altered, and testosterone levels were lowered **(Cai *et al.,* 2011; Paul *et al.,* 2017; Petersen *et al.,* 2018)**. In addition, acrylamide reduced reproductive organs weight, altered spermatogenesis and Sertoli cell functions **(Wang *et al.,* 2010)**, accentuating on the harmful effects of these chemicals on health.

Cancer treatments often trigger protein or receptors that are implicated in tumour progression and metastasis, thus Estramustin is a combination between oestradiol and nitrogen mustard, exhibited lower biding energy and shared similar binding pattern with E2, G1 an G36, while doxorubicin and colchicine were much similar to E2 rather than to GPER1 selective ligand, in contrast to cyclophosphamide, methotrexate and carboplatin which had higher binding energies.

The involvement of GPER1 in several types of cancer, diseases and infertility makes it a suitable target in treating this health problem, thus in searching for potential therapeutic agents, using phytochemical compounds seems the right choice. Phytochemicals are bioactive compounds extracted from plants, while phytoestrogens are a family of phytochemicals that have estrogenic activity **(Mostrom & Evans, 2011)**, and can be classified into : flavonoids (isoflavones and prenylated flavonoids) and nonflavonoids (lignans, coumestrans, stilbens) **(Murkies & Frydenberg, 2003)**, their estrogenic activity via ESRs has been widely studied **(Sirtori *et al.*, 2005; Sirotkin & Harrath, 2014)**, yet their effect through GPER1 still not fully elucidated. Intriguingly, our results showed high binding affinity between these phytoestrogens and GPER1, while having similar interacting pattern with E2, G1 and G36 as well as other EDs.

For instance, Coumestrol, Genistein, Biochanin A, Resveratrol and Piceatannol showed significantly low binding energy and shared interactions features with each other and with Methyl Parabens, Dimethyl Phthalates, Acetamiprid and Indigo Carmin, thus they could be used to mimic these EDs actions on the GPER1 in a competitive way. Moreover, Glabrene,

Lupalbugenin and Wedelolactone have comparable interactions with each other, but Lupalbugenin and Glabrene appear more like E2 and the selective antagonists (G36), and shares same bindings with Colchicine, Cyclophosphamide, Homosalate and Fenvalerate, so they can be used as drugs that could minimize the effects of these EDs. Therefore, more work needs to be done to assess the possibility of using these phytochemicals to reduce the harmful effects of EDs through GPER1.

Nevertheless, several phytoestrogen have been reported to mediated genomic and non-genomic outcomes through GPER1 **(Watson *et al.,* 2007)**, for example, Genistein is GPER1 agonist that provoke arterial vasodilatation **(Mishra *et al.,* 2000; P. Thomas & J. Dong, 2006)**, moreover in breast cancer cells Genistein increases cAMP, intracellular calcium and activates c-Src as well as PI3K/Akt and ERK1/2MAPK leading to cell apoptosis and cancer regression **(Maggiolini *et al.*, 2004; Lucki & Sewer, 2011)**. In glial cells, isoflavones also stimulate PI3K/FAK/Akt via GPER1 ensuring glial cell migration **(Spinnler et al., 2010)**. Moreover, Coumestrol is found to compete with E2 and exert cancer preventive effect through modulating Akt and ERK1/2 phosphorylation in mammary tumours and breast cancer cells **(Lim *et al.,* 2016)**. Our study provided new insight into chemicals interacting with GPER1 as major player in infertility and carcinogenesis, but despite these interesting results, further work needs to be done to clarify the utility of the phytochemicals against EDs dangerous effects as well to elucidate their mode of usage in preventing and treating cancer and infertility by targeting GPER1.

Conclusion

Conclusion

Since its discovery in male, oestrogens and their functions in male reproductive physiology are largely being studied, but till now, conducted research focused only on the classical oestrogen receptors, despite the crucial role of the GPER1 in mediating oestrogen effects in physiological and physio-pathological processes. Furthermore, the emerging harmful outcomes of endocrine disrupting chemicals on human health led to increase worry about the real process in which these chemicals exert their damage. Consequently, choosing the right animal model to study these issues is important to fully understand them.

The present study was designed to explore the influence of seasonal fluctuations on the expression of the GPER1 in the reproductive system of the sand rat *Psammomys obesus* and the gerbil *Gerbillus gerbillus*, rodents from north Africa. In addition, revealing the interactions between endocrine disrupting chemicals, phytochemicals, and chemotherapeutic agents with the GPER1.

In the testis of the sand rat *Psammomys obesus* and the gerbil *Gerbillus gerbillus*, during the resting season, we noticed disorganization of the germinal epithelium and spermatogenesis was blocked at the stage of spermatocytes, GPER1 that was present all over the testis during the breeding season, could still be found only in Leydig cells and spermatocytes. **These findings suggest that GPER1 may control spermatogenesis via its action on spermatocyte as well as modulating Leydig cells functions during both seasons.**

In the efferent ducts of the sand rat *Psammomys obesus* and the gerbil *Gerbillus gerbillus*, during the resting season the height of the epithelium notably decreased, while the intertubular spaces underwent serious proliferation, GPER1 that was ubiquitously expressed during the breeding season , was absent during the resting season except for some rare ciliated cells, non-ciliated cells, and basal cells. In contrast, the seasonality did not much influence the presence of the GPER1 in the efferent ducts of the sand rat *Psammomys obesus*, but rather the

intensity of GPER1 expression that was affected. **In the efferent ducts, GPER1 could be involved in fluid secretion, homogenization of luminal fluid, as well as reabsorption by ciliated cells and non-ciliated cells.**

In the epididymis of the sand rat *Psammomys obesus* and the gerbil *Gerbillus gerbillus*, the tubular epithelium underwent significant remodelling, in addition to a grave withdraw in GPER1 expression, as it was only detected in principal cells and basal cells during the resting season. Since epididymis main function is sperm maturation, **GPER1 might have a role in regulating principal cells and basal cells activities such as fluid absorption and protein secretion required for sperm maturation.**

The presence of GPER1 in the breeding season and its absence during the resting season signal the influence of the seasonal fluctuations on estrogenic actions via GPER1 and highlight the importance of the GPER1 in normal reproductive physiology of the male.

Since this study was limited to immunohistochemistry and histology, it was not possible to assess the quantity of the GPER1 expressed in each organ during the season, these results may give precise details about the number of oestrogenic effects promoted via the GPER1, and GPER1 regulation influenced by the season.

Interestingly, the endocrine disruptors showed the lowest binding energy among the tested substances, for instance, Bisphenol A, Polychlorinated Biphenyls and Acrylamide had ΔG of -16.47 kcal/mol, -10.35 kcal/mol and -8.78 kcal/mol respectively. **The high binding affinities displayed by endocrine disruptors witness their emerging effects on endocrine system and the need to supervise the use of this harmful chemicals.**

While the phytochemicals having lower binding energies are Coumestrol with ΔG of -9.03 kcal/mol, Genistein with -8.47 kcal/mol and Biochanin A with ΔG of -8.33 kcal/mol, among others with low binding energies. In addition, Estramustin, a chemotherapeutic agent

derived from oestrogen displayed low binding energy ΔG of -7.57 kcal/mol. **The strong interactions between phytochemicals and Estramustin chemotherapeutic agents with GPER1 advocate the possibility of using these chemicals as therapeutic agents targeting the GPER1 in several types of pathologies prompted by the GPER1. Some of chemotherapeutic chemicals as well as familiar used drugs, in addition to their specific actions can triggers GPER1, so they may cause non desirable side effects.**

The generalisability of these results is subject to certain limitations. For instance, tested substances that were found to bind effectively with the GPER1 need to be tested *in situ* using cells cultures, and *in vivo* to assess consequence of modulating the GPER1 by these chemicals.

Our study deserves to be further enlarged using different approaches such as:

- Using *GPER1 knock out* animal models to elucidate the consequence of deactivating this receptor on the male reproductive system.
- *In situ* studies of each substance with normal cells and cancer cells
- *In vivo* studies of the effects of each substance alone and in combination at different doses.
- Testing the different phytochemical substance against various types of physio-pathologies, tumours, and infertility in purpose to develop new treatments.
- Develop new specific chemo therapeutic agents and drugs to selectively trigger specific target molecule without interfering with other process or modulating other proteins.

References

Abou-Haila, A. (1987). *Etude de la régionalisation structurale, métabolique et fonctionnelle de l'épididyme de souris*

Abou-Haïla, A., & Fain-Maurel, M. A. (1984). Regional differences of the proximal part of mouse epididymis: morphological and histochemical characterization. *Anat Rec*, *209*(2), 197-208. https://doi.org/10.1002/ar.1092090207

Acott, T. S., & Carr, D. W. (1984). Inhibition of bovine spermatozoa by caudal epididymal fluid: II. Interaction of pH and a quiescence factor. *Biol Reprod*, *30*(4), 926-935. https://doi.org/10.1095/biolreprod30.4.926

Adamali, H. I., & Hermo, L. (1996). Apical and narrow cells are distinct cell types differing in their structure, distribution, and functions in the adult rat epididymis. *J Androl*, *17*(3), 208-222.

Ahmadian, R., Sheak, J. R., Weise-Cross, L., Jernigan, N. L., Arterburn, J. B., Prossnitz, E. R., . . . Resta, T. C. (2020). GPER Contributes to the Development of Pulmonary Hypertension in Female Rats. *34*(S1), 1-1. https://doi.org/https://doi.org/10.1096/fasebj.2020.34.s1.07087

Alam, M. S., Ohsako, S., Kanai, Y., & Kurohmaru, M. (2014). Single administration of butylparaben induces spermatogenic cell apoptosis in prepubertal rats. *Acta Histochem*, *116*(3), 474-480. https://doi.org/10.1016/j.acthis.2013.10.006

Albanito, L., Madeo, A., Lappano, R., Vivacqua, A., Rago, V., Carpino, A., . . . Maggiolini, M. (2007). G protein-coupled receptor 30 (GPR30) mediates gene expression changes and growth response to 17beta-estradiol and selective GPR30 ligand G-1 in ovarian cancer cells. *Cancer Res*, *67*(4), 1859-1866. https://doi.org/10.1158/0008-5472.Can-06-2909

Albanito, L., Sisci, D., Aquila, S., Brunelli, E., Vivacqua, A., Madeo, A., . . . Maggiolini, M. (2008). Epidermal growth factor induces G protein-coupled receptor 30 expression in estrogen receptor-negative breast cancer cells. *Endocrinology*, *149*(8), 3799-3808. https://doi.org/10.1210/en.2008-0117

Alencar, A. K., Montes, G. C., Montagnoli, T., Silva, A. M., Martinez, S. T., Fraga, A. G., . . . Zapata-Sudo, G. (2017). Activation of GPER ameliorates experimental pulmonary hypertension in male rats. *Eur J Pharm Sci*, *97*, 208-217. https://doi.org/10.1016/j.ejps.2016.11.009

Ali, W., Deng, K., Bian, Y., Liu, Z., & Zou, H. (2023). Spectacular role of epididymis and bio-active cargo of nano-scale exosome in sperm maturation: A review. *Biomedicine &*

Pharmacotherapy, *164*, 114889. https://doi.org/https://doi.org/10.1016/j.biopha.2023.114889

Amirat, Z., Khammar, F., & Brudieux, R. (1977). Variations saisonnières comparées de l'activité sexuelle (données pondérales) chez deux espèces de Rongeurs (Psammomys obesus et Gerbillus gerbillus) du Sahara occidental algérien (*). *Mammalia*, *41*(3), 341-356. https://doi.org/10.1515/MAMM.1977.41.3.341/HTML

Amirat, Z., Khammar, F., & Brudieux, R. (1977). Variations saisonnières comparées de l'activité sexuelle (données pondérales) chez deux espèces de Rongeurs (Psammomys obesus et Gerbillus gerbillus) du Sahara occidental algérien.

Amirat, Z., Khammar, F., & Brudieux, R. (1980). Variations saisonnières comparées du poids de la surrénale chez deux espèces de rongeurs désertiques (Psammomys obesus et Gerbillus gerbillus). *Mammalia*, *44*(3), 399-408. https://doi.org/10.1515/MAMM.1980.44.3.399/HTML

Anchan, D., Gafur, A., Sano, K., Ogawa, S., & Vasudevan, N. (2014). Activation of the GPR30 Receptor Promotes Lordosis in Female Mice. *Neuroendocrinology*, *100*(1), 71-80. https://doi.org/10.1159/000365574 %J Neuroendocrinology

Antalikova, J., Secova, P., Horovska, L., Krejcirova, R., Simonik, O., Jankovicova, J., . . . Manaskova-Postlerova, P. (2020). Missing Information from the Estrogen Receptor Puzzle: Where Are They Localized in Bull Reproductive Tissues and Spermatozoa? , *9*(1), 183. https://www.mdpi.com/2073-4409/9/1/183

Aprea, I., Nöthe-Menchen, T., Dougherty, G. W., Raidt, J., Loges, N. T., Kaiser, T., . . . Omran, H. (2021). Motility of efferent duct cilia aids passage of sperm cells through the male reproductive system. *Mol Hum Reprod*, *27*(3). https://doi.org/10.1093/molehr/gaab009

Apweiler, R., Bairoch, A., Wu, C. H., Barker, W. C., Boeckmann, B., Ferro, S., . . . Yeh, L. S. L. (2004). UniProt: the Universal Protein knowledgebase. *Nucleic Acids Res*, *32*(suppl_1), D115-D119. https://doi.org/10.1093/nar/gkh131 %J Nucleic Acids Research

Aquila, S., & De Amicis, F. (2014). Steroid receptors and their ligands: Effects on male gamete functions. *Experimental Cell Research*, *328*(2), 303-313. https://doi.org/10.1016/J.YEXCR.2014.07.015

Arkoun, B., Gautier, C., Delalande, C., Barrier-Battut, I., Guénon, I., Goux, D., & Bouraïma-Lelong, H. (2014). Stallion spermatozoa: Putative target of estrogens; presence of the estrogen receptors ESR1, ESR2 and identification of the estrogen-

membrane receptor GPER. *GENERAL AND COMPARATIVE ENDOCRINOLOGY*, *200*, 35-43. https://doi.org/10.1016/J.YGCEN.2014.02.016

Aso, S., Ehara, H., Miyata, K., Hosyuyama, S., Shiraishi, K., Umano, T., & Minobe, Y. (2005). A two-generation reproductive toxicity study of butyl benzyl phthalate in rats. *J Toxicol Sci*, *30 Spec No.*, 39-58. https://doi.org/10.2131/jts.30.s39

Babiloni-Chust, I., dos Santos, R. S., Medina-Gali, R. M., Perez-Serna, A. A., Encinar, J.-A., Martinez-Pinna, J., . . . Nadal, A. (2022). G protein-coupled estrogen receptor activation by bisphenol-A disrupts the protection from apoptosis conferred by the estrogen receptors ERα and ERβ in pancreatic beta cells. *Environment International*, *164*, 107250. https://doi.org/https://doi.org/10.1016/j.envint.2022.107250

Bar, Y., Abramsky, Z., & Gutterman, Y. J. J. o. A. E. (1984). Diet of gerbilline rodents in the Israeli desert. *7*(4), 371-376.

Barakat, R., Oakley, O., Kim, H., Jin, J., & Ko, C. J. (2016). Extra-gonadal sites of estrogen biosynthesis and function. *BMB Rep*, *49*(9), 488-496. https://doi.org/10.5483/bmbrep.2016.49.9.141

Barrachina, F., Battistone, M. A., Castillo, J., Mallofré, C., Jodar, M., Breton, S., & Oliva, R. (2022). Sperm acquire epididymis-derived proteins through epididymosomes. *Hum Reprod*, *37*(4), 651-668. https://doi.org/10.1093/humrep/deac015

Barratt, C. L. R., Björndahl, L., De Jonge, C. J., Lamb, D. J., Osorio Martini, F., McLachlan, R., . . . Tournaye, H. (2017). The diagnosis of male infertility: an analysis of the evidence to support the development of global WHO guidance-challenges and future research opportunities. *Hum Reprod Update*, *23*(6), 660-680. https://doi.org/10.1093/humupd/dmx021

Basu, P., & Maier, C. (2018). Phytoestrogens and breast cancer: In vitro anticancer activities of isoflavones, lignans, coumestans, stilbenes and their analogs and derivatives. *Biomed Pharmacother*, *107*, 1648-1666. https://doi.org/10.1016/j.biopha.2018.08.100

Basu, S. (2011). *Male reproductive dysfunction*. JP Medical Ltd.

Bazin, I., Hassine, A. I. H., Mnif, W., Gonzalez, C. J. J. o. E., & Ecography. (2017). YES as a tool for detecting estrogenic activity of some food additives compounds: E 104, E 122, E 124, E 132 and E 171. *3*(03).

Beato, M., & Klug, J. J. H. r. u. (2000). Steroid hormone receptors: an update. *6*(3), 225-236.

Belfiore, C. J., Hawkins, D. E., Wiltbank, M. C., & Niswender, G. D. (1994). Regulation of cytochrome P450scc synthesis and activity in the ovine corpus luteum. *J Steroid Biochem Mol Biol*, *51*(5-6), 283-290. https://doi.org/10.1016/0960-0760(94)90041-8

Belhocine, M. (1998). *Etude histo-cytologique des variations saisonniéres de l'appareil reproducteur male d'un rongeur deserticole nocturne, le {Merion} du desert ({Merion} crasses) et son espece sympatrique*

Belhocine, M., & Gernigon-Spychalowicz, T. (1994). Histologie comparée des variations saisonnières de l'appareil reproducteur male de deux rongeurs sahariens: Psammomys obesus et Meriones crassus. *Bulletin de la Société zoologique de France*, *119*(4), 325-333.

Belhocine, M., Gernigon-Spychalowicz, T., Jacob, M. P., Benazzoug, Y., & Exbrayat, J. M. (2010). Immunoexpression of gelatinase (MMP-2 and MMP-9) in the seminal vesicles and ventral prostate of Libyan jird (Meriones libycus) during the seasonal cycle of reproduction. *Histology and histopathology*, *25*(4), 619-619.

Belhocine, M., Malaprade-Baudoin, D., & Gernigon-Spychalowicz, T. J. B. d. l. S. z. d. F. (2001). Étude ultrastructurale du cycle testiculaire du Mérion de Libye (Meriones Libycus). *126*(1-2), 89-97.

Belleannée, C., Thimon, V., & Sullivan, R. (2012). Region-specific gene expression in the epididymis. *Cell and Tissue Research*, *349*(3), 717-731. https://doi.org/10.1007/s00441-012-1381-0

Benazzou, T., Viegas-Pequignot, E., Prod'Homme, M., Lombard, M., Petter, F., & Dutrillaux, B. (1984). Chromosomal phylogeny of Gerbillidae. III. Species study of the genera Tatera, Taterillus, Psammomys and Pachyuromys. Annales de Génétique,

Benjamin, S., Masai, E., Kamimura, N., Takahashi, K., Anderson, R. C., & Faisal, P. A. (2017). Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. *Journal of Hazardous Materials*, *340*, 360-383. https://doi.org/https://doi.org/10.1016/j.jhazmat.2017.06.036

Bentaiba, K., Belhocine, M., Chougrani, F., Bououdina, M., Mostari, A., Fernini, M., & Bouzouina, M. (2023). Effectiveness of Withania frutescens root extract on testicular damage induced by lead acetate in adult albino rats. *Reproductive Toxicology*, *115*, 102-110. https://doi.org/https://doi.org/10.1016/j.reprotox.2022.12.006

Bergman, Å., Heindel, J. J., Jobling, S., Kidd, K., Zoeller, T. R., & Organization, W. H. (2013). *State of the science of endocrine disrupting chemicals 2012*. World Health Organization.

Bilezikjian, L. M., Blount, A. L., Leal, A. M., Donaldson, C. J., Fischer, W. H., Vale, W. W. J. M., & endocrinology, c. (2004). Autocrine/paracrine regulation of pituitary function by activin, inhibin and follistatin. *225*(1-2), 29-36.

Bologa, C. G., Revankar, C. M., Young, S. M., Edwards, B. S., Arterburn, J. B., Kiselyov, A. S., . . . Prossnitz, E. R. (2006). Virtual and biomolecular screening converge on a selective agonist for GPR30. *Nature Chemical Biology*, *2*(4), 207-212. https://doi.org/10.1038/nchembio775

Bonini, J., Anderson, S., & Steiner, D. (1997). Molecular cloning and tissue expression of a novel orphan G protein-coupled receptor from rat lung. *234*, 190-193.

Boscia, F., Passaro, C., Gigantino, V., Perdonà, S., Franco, R., Portella, G., . . . Chieffi, P. (2015). High Levels of Gpr30 Protein in Human Testicular Carcinoma In Situ and Seminomas Correlate with Low Levels of Estrogen Receptor-Beta and Indicate a Switch in Estrogen Responsiveness. *230*(6), 1290-1297. https://doi.org/https://doi.org/10.1002/jcp.24864

Boufermes, R., Amirat, Z., & Khammar, F. (2013). ETUDE COMPRATIVE DES VARIATIONS SAISONNIERES DES ACTIVITÉS TESTICULAIRE ET THYROIDIENNE CHEZ DEUX ESPÉCES DE RONGEURS DÉSERTICOLES: LA GERBILLE (Gerbillus gerbillus) ET LE RAT DES SABLES (Psammomys obesus). *Sciences & Technologie C*(37), 16-21.

Boufermes, R., Amirat, Z., & Khammar, F. (2014). VARIATIONS SAISONNIÈRES COMPARÉES DES ACTIVITÉS TESTICULAIRE ET THYROIDIENNE CHEZ DEUX ESPÈCES DE RONGEURS DÉSERTICOLES: LA GERBILLE (GERBILLUS GERBILLUS) ET LE RAT DES SABLES (PSAMMOMYS OBESUS). *Sciences & Technologie. C, Biotechnologies*, 9-18.

Boufermes, R., Belhocine, M., Amirat, Z., & Khammar, F. J. A. (2021). Assessment of testicular Lhcgr mRNA expression correlated with testis and seminal vesicle activities in the Libyan jird (Meriones libycus, Rodentia: Muridae) during breeding season compared with nonbreeding season. *11*(2), 320.

Bourque, M., Morissette, M., Côté, M., Soulet, D., & Di, T. (2013). Implication of GPER1 in neuroprotection in a mouse model of Parkinson ' s disease. *NBA*, *34*(3), 887-901. https://doi.org/10.1016/j.neurobiolaging.2012.05.022

Bouschet, T., Martin, S., & Henley, J. M. (2012). Regulation of Calcium Sensing Receptor Trafficking by RAMPs. In W. S. Spielman & N. Parameswaran (Eds.), *RAMPs* (pp. 39-48). Springer US. https://doi.org/10.1007/978-1-4614-2364-5_4

Bouskine, A., Nebout, M., Brücker-Davis, F., Benahmed, M., & Fenichel, P. (2009). Low Doses of Bisphenol A Promote Human Seminoma Cell Proliferation by Activating PKA

and PKG via a Membrane G-Protein–Coupled Estrogen Receptor. *117*(7), 1053-1058. https://doi.org/doi:10.1289/ehp.0800367

Bracke, A., Peeters, K., Punjabi, U., Hoogewijs, D., & Dewilde, S. J. R. b. o. (2018). A search for molecular mechanisms underlying male idiopathic infertility. *36*(3), 327-339.

Brailoiu, E., Dun, S. L., Brailoiu, G. C., Mizuo, K., Sklar, L. A., Oprea, T. I., . . . Dun, N. J. (2007). Distribution and characterization of estrogen receptor G protein-coupled receptor 30 in the rat central nervous system. *J Endocrinol*, *193*(2), 311-321. https://doi.org/10.1677/joe-07-0017

Bromfield, J. J., Ibrahim, L. A., & Rizo, J. A. (2018). Seminal Vesicle Gland—Overview. *Encyclopedia of Reproduction*, 341-343. https://doi.org/10.1016/B978-0-12-801238-3.64598-1

Brunsing, R. L., & Prossnitz, E. R. (2011). Induction of interleukin-10 in the T helper type 17 effector population by the G protein coupled estrogen receptor (GPER) agonist G-1. *Immunology*, *134*(1), 93-106. https://doi.org/10.1111/j.1365-2567.2011.03471.x

Brzozowski, A. M., Pike, A. C. W., Dauter, Z., Hubbard, R. E., Bonn, T., Engström, O., . . . Carlquist, M. (1997). Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature*, *389*(6652), 753-758. https://doi.org/10.1038/39645

Bubb, M., Beyer, A. L., Dasgupta, P., Kaemmerer, D., Sänger, J., Evert, K., . . . Lupp, A. (2022). Assessment of G Protein-Coupled Oestrogen Receptor Expression in Normal and Neoplastic Human Tissues Using a Novel Rabbit Monoclonal Antibody. *Int J Mol Sci*, *23*(9). https://doi.org/10.3390/ijms23095191

Cai, J., Wang, C., Wu, T., Moreno, J. M., Zhong, Y., Huang, X., . . . Zuo, Z. (2011). Disruption of spermatogenesis and differential regulation of testicular estrogen receptor expression in mice after polychlorinated biphenyl exposure. *Toxicology*, *287*(1-3), 21-28. https://doi.org/10.1016/j.tox.2011.05.010

Carlson, B. M. (2019). The Reproductive System. *The Human Body*, 373-396. https://doi.org/10.1016/B978-0-12-804254-0.00014-4

Carmeci, C., Thompson, D. A., Ring, H. Z., Francke, U., & Weigel, R. J. (1997). Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. *Genomics*, *45*(3), 607-617. https://doi.org/10.1006/geno.1997.4972

Carpino, A., Rago, V., Pezzi, V., Carani, C., & Andò, S. (2007). Detection of aromatase and estrogen receptors (ERα, ERβ1, ERβ2) in human Leydig cell tumor. *European Journal*

of Endocrinology, *157*(2), 239-244. https://doi.org/10.1530/EJE-07-0029 %J European Journal of Endocrinology

Carpino, A., Romeo, F., & Rago, V. (2004). Aromatase immunolocalization in human ductuli efferentes and proximal ductus epididymis. *Journal of Anatomy*, *204*(3), 217-220.

Chan, Q. K., Lam, H. M., Ng, C. F., Lee, A. Y., Chan, E. S., Ng, H. K., . . . Lau, K. M. (2010). Activation of GPR30 inhibits the growth of prostate cancer cells through sustained activation of Erk1/2, c-jun/c-fos-dependent upregulation of p21, and induction of G(2) cell-cycle arrest. *Cell Death Differ*, *17*(9), 1511-1523. https://doi.org/10.1038/cdd.2010.20

Chaudhary, K. K., & Mishra, N. J. D. (2016). A review on molecular docking: novel tool for drug discovery. *3*(4), 1029.

Chen, F. P., & Chien, M. H. (2014). Lower concentrations of phthalates induce proliferation in human breast cancer cells. *Climacteric*, *17*(4), 377-384. https://doi.org/10.3109/13697137.2013.865720

Chen, P., Li, B., & Ou-Yang, L. (2022). Role of estrogen receptors in health and disease [Review]. *13*. https://doi.org/10.3389/fendo.2022.839005

Chen, Y.-B. (2023). Cowper gland. *Uropathology*, 10-11. https://doi.org/10.1016/B978-0-323-65395-4.00012-9

Cheng, C. Y., & Mruk, D. D. (2010). The biology of spermatogenesis: the past, present and future. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1546), 1459-1463. https://doi.org/10.1098/RSTB.2010.0024

Cheng, S.-B., Graeber, C. T., Quinn, J. A., & Filardo, E. J. (2011). Retrograde transport of the transmembrane estrogen receptor, G-protein-coupled-receptor-30 (GPR30/GPER) from the plasma membrane towards the nucleus. *Steroids*, *76*(9), 892-896. https://doi.org/https://doi.org/10.1016/j.steroids.2011.02.018

Cheng, S. B., Quinn, J. A., Graeber, C. T., & Filardo, E. J. (2011). Down-modulation of the G-protein-coupled estrogen receptor, GPER, from the cell surface occurs via a trans-Golgi-proteasome pathway. *J Biol Chem*, *286*(25), 22441-22455. https://doi.org/10.1074/jbc.M111.224071

Cheniti Lamine, T. (1974). Cycle testiclaire chez la Gerboise (Jaculus orientalis). *CR Soc. Biol*, *168*, 428-4338.

Cheung, C., Yu, S., Wong, K., Chan, L., Lai, F. M., Wang, X., . . . Metabolism. (2005). Expression and functional study of estrogen receptor-related receptors in human prostatic cells and tissues. *90*(3), 1830-1844.

Chevalier, N., Bouskine, A., & Fenichel, P. (2012). Bisphenol A promotes testicular seminoma cell proliferation through GPER/GPR30. *130*(1), 241-242. https://doi.org/https://doi.org/10.1002/ijc.25972

Chimento, A., Casaburi, I., Bartucci, M., Patrizii, M., Dattilo, R., Avena, P., . . . Sirianni, R. (2013). Selective GPER activation decreases proliferation and activates apoptosis in tumor Leydig cells. *Cell Death & Disease*, *4*(8), e747-e747. https://doi.org/10.1038/cddis.2013.275

Chimento, A., De Luca, A., Nocito, M. C., Avena, P., La Padula, D., Zavaglia, L., & Pezzi, V. (2020). Role of GPER-Mediated Signaling in Testicular Functions and Tumorigenesis. *Cells*, *9*(9). https://doi.org/10.3390/cells9092115

Chimento, A., Sirianni, R., Casaburi, I., & Pezzi, V. (2014). GPER Signaling in Spermatogenesis and Testicular Tumors. *Frontiers in Endocrinology*, *5*(MAR). https://doi.org/10.3389/FENDO.2014.00030

Chimento, A., Sirianni, R., Delalande, C., Silandre, D., Bois, C., Andò, S., . . . Pezzi, V. (2010). Molecular and {Cellular} {Endocrinology} 17 ␤ -{Estradiol} activates rapid signaling pathways involved in rat pachytene spermatocytes apoptosis through {GPR30} and {ER} ␣. *320*, 136-144. https://doi.org/10.1016/j.mce.2010.01.035

Chimento, A., Sirianni, R., Zolea, F., Bois, C., Delalande, C., Andò, S., . . . Pezzi, V. (2011). Gper and ESRs are expressed in rat round spermatids and mediate oestrogen-dependent rapid pathways modulating expression of cyclin B1 and Bax. *34*(5pt1), 420-429. https://doi.org/https://doi.org/10.1111/j.1365-2605.2010.01100.x

Chourasia, T. K., Pang, Y., & Thomas, P. (2015). The catecholestrogen, 2-hydroxyestradiol-17beta, acts as a G protein-coupled estrogen receptor 1 (GPER/GPR30) antagonist to promote the resumption of meiosis in zebrafish oocytes. *Biol Reprod*, *92*(3), 69. https://doi.org/10.1095/biolreprod.114.125674

Clulow, J., Jones, R. C., Hansen, L. A., & Man, S. Y. (1998). Fluid and electrolyte reabsorption in the ductuli efferentes testis. *JOURNAL OF REPRODUCTION AND FERTILITY-SUPPLEMENT-*, 1-14.

Cocuzza, M., Alvarenga, C., & Pagani, R. (2013). The epidemiology and etiology of azoospermia. *Clinics (Sao Paulo)*, *68 Suppl 1*(Suppl 1), 15-26. https://doi.org/10.6061/clinics/2013(sup01)03

Confavreux, C., Hutchinson, M., Hours, M. M., Cortinovis-Tourniaire, P., & Moreau, T. (1998). Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple

Sclerosis Group. *N Engl J Med*, *339*(5), 285-291. https://doi.org/10.1056/nejm199807303390501

Consortium, T. U. (2018). UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res*, *47*(D1), D506-D515. https://doi.org/10.1093/nar/gky1049 %J Nucleic Acids Research

Cook, M. J. (1965). *The anatomy of the laboratory mouse* (Academic Press ed.).

Cooke, P. S., Nanjappa, M. K., Ko, C., Prins, G. S., & Hess, R. A. (2017). Estrogens in Male Physiology. *Physiol Rev*, *97*(3), 995-1043. https://doi.org/10.1152/physrev.00018.2016

Cooper, T. G. (2007). Sperm maturation in the epididymis: A new look at an old problem. *Asian Journal of Andrology*, *9*(4), 533-539. https://doi.org/10.1111/j.1745-7262.2007.00285.x

Cornwall, G. A. (2009). New insights into epididymal biology and function. *Human Reproduction Update*, *15*(2), 213-227. https://doi.org/10.1093/humupd/dmn055

Cornwall, G. A. (2018). Epididymis: Sperm Maturation and Motility. *Encyclopedia of Reproduction*, 292-297. https://doi.org/10.1016/B978-0-12-801238-3.64369-6

Corradi, P. F., Corradi, R. B., & Greene, L. W. (2016). Physiology of the Hypothalamic Pituitary Gonadal Axis in the Male. *Urologic Clinics of North America*, *43*(2), 151-162. https://doi.org/https://doi.org/10.1016/j.ucl.2016.01.001

Creasy, D. M., & Chapin, R. E. (2013). *Chapter 59 – Male Reproductive System* (Third Edit ed.). Elsevier. https://doi.org/10.1016/B978-0-12-415759-0.00059-5

Creasy, D. M., & Chapin, R. E. (2018). Male Reproductive System. *Fundamentals of Toxicologic Pathology: Third Edition*, 459-516. https://doi.org/10.1016/B978-0-12-809841-7.00017-4

Cui, J., Shen, Y., & Li, R. (2013). Estrogen synthesis and signaling pathways during aging: from periphery to brain. *Trends in Molecular Medicine*, *19*(3), 197-209. https://doi.org/10.1016/j.molmed.2012.12.007

Cyr, D. G., Hermo, L., & Robaire, B. (1993). Developmental changes in epithelial cadherin messenger ribonucleic acid and immunocytochemical localization of epithelial cadherin during postnatal epididymal development in the rat. *Endocrinology*, *132*(3), 1115-1124.

da Silva, A. B., Giacomoni, F., Pavot, B., Fillatre, Y., Rothwell, J., Sualdea, B. B., . . . Kopec, R. (2016). PhytoHub V1. 4: A new release for the online database dedicated to food phytochemicals and their human metabolites. The 1. International Conference on Food Bioactives & Health,

Dahia, C. L., Rao, A. J. J. M., & endocrinology, c. (2006). Regulation of FSH receptor, PKIβ, IL-6 and calcium mobilization: possible mediators of differential action of FSH. *247*(1-2), 73-81.

Danzo, B. J. (1997). Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. *Environmental Health Perspectives*, *105*(3), 294-301.

Darszon, A., Nishigaki, T., Beltran, C., & Treviño, C. L. (2011). Calcium channels in the development, maturation, and function of spermatozoa. *Physiol Rev*, *91*(4), 1305-1355. https://doi.org/10.1152/physrev.00028.2010

Davis, K. E., Carstens, E. J., Irani, B. G., Gent, L. M., Hahner, L. M., & Clegg, D. J. (2014). Sexually dimorphic role of G protein-coupled estrogen receptor (GPER) in modulating energy homeostasis. *Horm Behav*, *66*(1), 196-207. https://doi.org/10.1016/j.yhbeh.2014.02.004

De Francesco, E. M., Maggiolini, M., & Musti, A. M. (2018). Crosstalk between Notch, HIF-1α and GPER in Breast Cancer EMT. *Int J Mol Sci*, *19*(7). https://doi.org/10.3390/ijms19072011

de Freitas, A. T. A. G., Pinho, C. F., Aquino, A. M., Domeniconi, R. F., Justulin, L. A., & Scarano, W. R. (2020). Panax ginseng metabolite (GIM-1) modulates the effects of monobutyl phthalate (MBP) on the GPR30/GPER1 canonical pathway in human Sertoli cells. *Reproductive Toxicology*, *96*, 209-215. https://doi.org/https://doi.org/10.1016/j.reprotox.2020.07.004

de Souza, L. O., Machado, G. D. B., de Freitas, B. S., Rodrigues, S. L. C., Severo, M. P. A., Molz, P., . . . Schröder, N. (2021). The G protein-coupled estrogen receptor (GPER) regulates recognition and aversively-motivated memory in male rats. *Neurobiol Learn Mem*, *184*, 107499. https://doi.org/10.1016/j.nlm.2021.107499

Dearden, J. C. (2003). In silico prediction of drug toxicity. *J Comput Aided Mol Des*, *17*(2-4), 119-127. https://doi.org/10.1023/a:1025361621494

DeLeon, C., Wang, H. H., Gunn, J., Wilhelm, M., Cole, A., Arnett, S., . . . Arnatt, C. K. (2020). A novel GPER antagonist protects against the formation of estrogen-induced cholesterol gallstones in female mice. *J Lipid Res*, *61*(5), 767-777. https://doi.org/10.1194/jlr.RA119000592

Demay, F., De Monti, M., Tiffoche, C., Vaillant, C., & Thieulant, M. L. (2001). Steroid-Independent Activation of ER by GnRH in Gonadotrope Pituitary Cells. *Endocrinology*, *142*(8), 3340-3347. https://doi.org/10.1210/endo.142.8.8337 %J Endocrinology

Dennis, M. K., Burai, R., Ramesh, C., Petrie, W. K., Alcon, S. N., Nayak, T. K., . . . Prossnitz, E. R. (2009). In vivo effects of a GPR30 antagonist. *Nature Chemical Biology*, *5*(6), 421-427. https://doi.org/10.1038/nchembio.168

Dennis, M. K., Field, A. S., Burai, R., Ramesh, C., Petrie, W. K., Bologa, C. G., . . . Prossnitz, E. R. (2011a). Identification of a GPER/GPR30 antagonist with improved estrogen receptor counterselectivity. *The Journal of Steroid Biochemistry and Molecular Biology*, *127*(3), 358-366. https://doi.org/https://doi.org/10.1016/j.jsbmb.2011.07.002

Dennis, M. K., Field, A. S., Burai, R., Ramesh, C., Petrie, W. K., Bologa, C. G., . . . Prossnitz, E. R. (2011b). Journal of Steroid Biochemistry and Molecular Biology Identification of a GPER / GPR30 antagonist with improved estrogen receptor counterselectivity. *Journal of Steroid Biochemistry and Molecular Biology*, *127*(3-5), 358-366. https://doi.org/10.1016/j.jsbmb.2011.07.002

Dewaele, A., Dujardin, E., André, M., Albina, A., Jammes, H., Giton, F., . . . Pannetier, M. J. G. (2022). Absence of testicular estrogen leads to defects in spermatogenesis and increased semen abnormalities in male rabbits. *13*(11), 2070.

Diamanti-Kandarakis, E., Bourguignon, J. P., Giudice, L. C., Hauser, R., Prins, G. S., Soto, A. M., . . . Gore, A. C. (2009). Endocrine-disrupting chemicals: An Endocrine Society scientific statement. In (Vol. 30, pp. 293-342): Oxford Academic.

Du, G.-q., Zhou, L., Chen, X.-y., Wan, X.-p., & He, Y.-y. (2012). Biochemical and Biophysical Research Communications The G protein-coupled receptor GPR30 mediates the proliferative and invasive effects induced by hydroxytamoxifen in endometrial cancer cells. *Biochemical and Biophysical Research Communications*, *420*(2), 343-349. https://doi.org/10.1016/j.bbrc.2012.02.161

Duan, R., Xie, W., Burghardt, R. C., & Safe, S. (2001). Estrogen Receptor-mediated Activation of the Serum Response Element in MCF-7 Cells through MAPK-dependent Phosphorylation of Elk-1*. *Journal of Biological Chemistry*, *276*(15), 11590-11598. https://doi.org/https://doi.org/10.1074/jbc.M005492200

Dubé, J. Y., Lesage, R., & Tremblay, R. R. (1976). Androgen and estrogen binding in rat skeletal and perineal muscles. *54*(1), 50-55. https://doi.org/10.1139/o76-008 %M 175903

Dueñas-Moreno, J., Mora, A., Kumar, M., Meng, X.-Z., & Mahlknecht, J. (2023). Worldwide risk assessment of phthalates and bisphenol A in humans: The need for

updating guidelines. *Environment International*, *181*, 108294. https://doi.org/https://doi.org/10.1016/j.envint.2023.108294

Duke, J., & Bogenschutz, M. J. (1994). *Dr. Duke's phytochemical and ethnobotanical databases*. USDA, Agricultural Research Service Washington, DC.

Dupont, S. (2000). *Reproductive phenotypes in ER null mutants*. https://dev.biologists.org/content/127/19/4277.short

Ebling, F. J. P., Brooks, A. N., Cronin, A. S., Ford, H., & Kerr, J. B. (2000). Estrogenic Induction of Spermatogenesis in the Hypogonadal Mouse. *Endocrinology*, *141*(8), 2861-2869. https://doi.org/10.1210/ENDO.141.8.7596

Ebnesajjad, S. (2016). Effect of Chemicals on Plastics. In E. Baur, K. Ruhrberg, & W. Woishnis (Eds.), *Chemical Resistance of Engineering Thermoplastics* (pp. xxvii-xli). William Andrew Publishing. https://doi.org/https://doi.org/10.1016/B978-0-323-47357-6.00022-2

Encarnação, T., Pais, A. A., Campos, M. G., & Burrows, H. D. (2019). Endocrine disrupting chemicals: Impact on human health, wildlife and the environment. *102*(1), 3-42. https://doi.org/10.1177/0036850419826802

Enmark, E., Pelto-Huikko, M., Grandien, K., Lagercrantz, S., Lagercrantz, J., Fried, G., . . . Gustafsson, J. A. (1997). Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern. *J Clin Endocrinol Metab*, *82*(12), 4258-4265. https://doi.org/10.1210/jcem.82.12.4470

Farooq, A. (2015). Structural and Functional Diversity of Estrogen Receptor Ligands. *Curr Top Med Chem*, *15*(14), 1372-1384. https://doi.org/10.2174/1568026615666150413154841

Feldman, R. D., Gros, R., Ding, Q., Hussain, Y., Ban, M. R., McIntyre, A. D., & Hegele, R. A. (2014). A common hypofunctional genetic variant of GPER is associated with increased blood pressure in women. *Br J Clin Pharmacol*, *78*(6), 1441-1452. https://doi.org/10.1111/bcp.12471

Feng, Y., & Gregor, P. (1997). Cloning of a novel member of the G protein-coupled receptor family related to peptide receptors. *231*, 651-654.

Ferlin, A., Raicu, F., Gatta, V., Zuccarello, D., Palka, G., & Foresta, C. (2007). Male infertility: role of genetic background. *Reprod Biomed Online*, *14*(6), 734-745. https://doi.org/10.1016/s1472-6483(10)60677-3

Fiandanese, N., Borromeo, V., Berrini, A., Fischer, B., Schaedlich, K., Schmidt, J. S., . . . Pocar, P. (2016). Maternal exposure to a mixture of di(2-ethylhexyl) phthalate (DEHP)

and polychlorinated biphenyls (PCBs) causes reproductive dysfunction in adult male mouse offspring. *Reprod Toxicol*, *65*, 123-132. https://doi.org/10.1016/j.reprotox.2016.07.004

Filardo, E., Quinn, J., Pang, Y., Graeber, C., Shaw, S., Dong, J., & Thomas, P. (2007). Activation of the Novel Estrogen Receptor G Protein-Coupled Receptor 30 (GPR30) at the Plasma Membrane. *Endocrinology*, *148*(7), 3236-3245. https://doi.org/10.1210/en.2006-1605 %J Endocrinology

Filardo, E. J. (2002). Epidermal growth factor receptor (EGFR) transactivation by estrogen via the G-protein-coupled receptor, GPR30: a novel signaling pathway with potential significance for breast cancer. *The Journal of Steroid Biochemistry and Molecular Biology*, *80*(2), 231-238. https://doi.org/https://doi.org/10.1016/S0960-0760(01)00190-X

Filardo, E. J., Graeber, C. T., Quinn, J. A., Resnick, M. B., Giri, D., DeLellis, R. A., . . . Sabo, E. (2006). Distribution of GPR30, a seven membrane–spanning estrogen receptor, in primary breast cancer and its association with clinicopathologic determinants of tumor progression. *Clinical cancer research*, *12*(21), 6359-6366.

Filardo, E. J., Quinn, J. A., Bland, K. I., & Frackelton, A. R., Jr. (2000). Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. *Mol Endocrinol*, *14*(10), 1649-1660. https://doi.org/10.1210/mend.14.10.0532

Filardo, E. J., Quinn, J. A., Frackelton, A. R., Jr., & Bland, K. I. (2002). Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. *Mol Endocrinol*, *16*(1), 70-84. https://doi.org/10.1210/mend.16.1.0758

Filardo, E. J., & Thomas, P. (2005). GPR30 : a seven-transmembrane- spanning estrogen receptor that triggers EGF release. *16*(8). https://doi.org/10.1016/j.tem.2005.08.005

Filardo, E. J., & Thomas, P. (2012). Minireview: G protein-coupled estrogen receptor-1, GPER-1: its mechanism of action and role in female reproductive cancer, renal and vascular physiology. *Endocrinology*, *153*(7), 2953-2962.

Filipiak, E., Suliborska, D., Laszczynska, M., Walczak-Jedrzejowska, R., Oszukowska, E., Marchlewska, K., . . . Słowikowska-Hilczer, J. (2012). Estrogen receptor alpha localization in the testes of men with normal spermatogenesis. *Folia Histochemica et Cytobiologica*, *50*(3), 340-345. https://doi.org/10.5603/FHC.2012.0046

Filippi, S., Luconi, M., Granchi, S., Vignozzi, L., Bettuzzi, S., Tozzi, P., . . . Maggi, M. (2002). Estrogens, but not androgens, regulate expression and functional activity of oxytocin receptor in rabbit epididymis. *Endocrinology*, *143*(11), 4271-4280. https://doi.org/10.1210/en.2002-220384

Flannigan, R., & Goldstein, M. (2018). Vas Deferens. *Encyclopedia of Reproduction*, 305-308. https://doi.org/10.1016/B978-0-12-801238-3.64371-4

França, L. R., Avelar, G. F., & Almeida, F. F. L. (2005, 2005/1//). Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology,

Franco, R., Boscia, F., Gigantino, V., Marra, L., Esposito, F., Ferrara, D., . . . Chieffi, P. (2011). GPR30 is overexpressed in post-puberal testicular germ cell tumors. *http://dx.doi.org/10.4161/cbt.11.6.14672*, *11*(6), 609-613. https://doi.org/10.4161/CBT.11.6.14672

Fredette, N. C., Meyer, M. R., & Prossnitz, E. R. (2018). Role of GPER in estrogen-dependent nitric oxide formation and vasodilation. *J Steroid Biochem Mol Biol*, *176*, 65-72. https://doi.org/10.1016/j.jsbmb.2017.05.006

Fuentes, N., & Silveyra, P. (2019). Estrogen receptor signaling mechanisms. *Adv Protein Chem Struct Biol*, *116*, 135-170. https://doi.org/10.1016/bs.apcsb.2019.01.001

Gabe, M. (1976). *Histological techniques*. Masson.

Gancarczyk, M., Paziewska-Hejmej, A., Carreau, S., Tabarowski, Z., & Bilińska, B. (2004). Dose- and photoperiod-dependent effects of 17β-estradiol and the anti-estrogen ICI 182,780 on testicular structure, acceleration of spermatogenesis, and aromatase immunoexpression in immature bank voles. *Acta Histochemica*, *106*(4), 269-278. https://doi.org/10.1016/J.ACTHIS.2004.04.002

Gao, D.-D., Lan, C.-F., Cao, X.-N., Chen, L., Lei, T.-L., Peng, L., . . . Sun, Q. J. B. o. R. (2022). G protein-coupled estrogen receptor promotes acrosome reaction via regulation of Ca2+ signaling in mouse sperm. *107*(4), 1026-1034.

Gao, F., Ma, X., Ostmann, A. B., & Das, S. K. (2011). GPR30 activation opposes estrogen-dependent uterine growth via inhibition of stromal ERK1/2 and estrogen receptor alpha (ERα) phosphorylation signals. *Endocrinology*, *152*(4), 1434-1447.

Gauntner, T. D., & Prins, G. S. (2018). Prostate—Cell Biology and Secretion. *Encyclopedia of Reproduction*, 325-333. https://doi.org/10.1016/B978-0-12-801238-3.64372-6

Gautier, C., Barrier-Battut, I., Guénon, I., Goux, D., Delalande, C., & Bouraïma-Lelong, H. (2016). Implication of the estrogen receptors GPER, ESR1, ESR2 in post-testicular

maturations of equine spermatozoa. *GENERAL AND COMPARATIVE ENDOCRINOLOGY*, *233*, 100-108. https://doi.org/10.1016/J.YGCEN.2016.05.022

GBIF, S. (2023). *Gerbillus gerbillus (Olivier, 1800)*. GBIF Backbone Taxonomy. Retrieved 03 January 2024 from GBIF.org

Ge, L.-C., Chen, Z.-J., Liu, H.-Y., Zhang, K.-S., Liu, H., Huang, H.-B., . . . Wang, H.-S. (2014). Involvement of activating ERK1/2 through G protein coupled receptor 30 and estrogen receptor α/β in low doses of bisphenol A promoting growth of Sertoli TM4 cells. *Toxicology Letters*, *226*(1), 81-89. https://doi.org/https://doi.org/10.1016/j.toxlet.2014.01.035

Ge, W., Xiao, L., Duan, H., Jiang, Y., Lv, J., Ding, Z., . . . Zhang, Y. (2020). Androgen receptor, aromatase, oestrogen receptor α/β and G protein-coupled receptor 30 expression in the testes and epididymides of adult sheep. *55*(4), 460-468. https://doi.org/https://doi.org/10.1111/rda.13638

Gernigon-Spychalowicz, T. (1992a). Etudes cytologiques et biochimiques des variationssaisonnières de l'appareil génital mâle d'un rongeur saharien diurne, le rat des sables (Psammomys obesus). In (pp. 180-180).

Gernigon-Spychalowicz, T. (1992b). *Etudes cytologiques et biochimiques des variationssaisonnières de l'appareil génital mâle d'un rongeur saharien diurne, le rat des sables (Psammomys obesus)*

Gernigon, T., Malaprade, D., Mesbah, A., & Lécher, P. (1991). Aspects cytologiques du testicule et de l'épididyme du rat des sables (Psammomys obesus) dans son biotope. *Le Rongeur et l'Espace*, 129-142.

Gervasi, M. G., & Visconti, P. E. (2017). Molecular changes and signaling events occurring in spermatozoa during epididymal maturation. *Andrology*, *5*(2), 204-218. https://doi.org/10.1111/andr.12320

Ghobrial, L. I., & Hodieb, A. S. (1973). Climate and seasonal variations in the breeding of the desert jerboa, Jaculus jaculus, in the Sudan. *Journal of Reproduction and Fertility*, 221–233. https://europepmc.org/article/med/4522375

Giess, M., Lattrich, C., Springwald, A., Goerse, R., Ortmann, O., & Treeck, O. (2010). GPR30 gene polymorphisms are associated with progesterone receptor status and histopathological characteristics of breast cancer patients. *J Steroid Biochem Mol Biol*, *118*(1-2), 7-12. https://doi.org/10.1016/j.jsbmb.2009.09.001

Gilligan, L. C., Rahman, H. P., Hewitt, A. M., Sitch, A. J., Gondal, A., Arvaniti, A., . . . Foster, P. A. (2017). Estrogen Activation by Steroid Sulfatase Increases Colorectal

Cancer Proliferation via GPER. *J Clin Endocrinol Metab*, *102*(12), 4435-4447. https://doi.org/10.1210/jc.2016-3716

Giulioni, C., Maurizi, V., Castellani, D., Scarcella, S., Skrami, E., Balercia, G., & Galosi, A. B. (2022). The environmental and occupational influence of pesticides on male fertility: A systematic review of human studies. *Andrology*, *10*(7), 1250-1271. https://doi.org/10.1111/andr.13228

Gohar, E. Y., Almutlaq, R. N., Daugherty, E. M., Butt, M. K., Jin, C., Pollock, J. S., . . . De Miguel, C. (2021). Activation of G protein-coupled estrogen receptor 1 ameliorates proximal tubular injury and proteinuria in Dahl salt-sensitive female rats. *Am J Physiol Regul Integr Comp Physiol*, *320*(3), R297-r306. https://doi.org/10.1152/ajpregu.00267.2020

Gohar, E. Y., Daugherty, E. M., Aceves, J. O., Sedaka, R., Obi, I. E., Allan, J. M., . . . Pollock, D. M. (2020). Evidence for G-Protein-Coupled Estrogen Receptor as a Pronatriuretic Factor. *J Am Heart Assoc*, *9*(10), e015110. https://doi.org/10.1161/jaha.119.015110

Gosden, J. R., Middleton, P. G., & Rout, D. (1986). Localization of the human oestrogen receptor gene to chromosome 6q24----q27 by in situ hybridization. *Cytogenet Cell Genet*, *43*(3-4), 218-220. https://doi.org/10.1159/000132325

Gouat, J. (1985). Notes sur la reproduction de Ctenodactylus gundi rongeur Ctenodactylidae. *Zeitschrift für Säugetierkunde*, *50*(5), 285-293.

Goyal, H. O., Bartol, F. F., Wiley, A. A., Khalil, M. K., Chiu, J., & Vig, M. M. (1997). Immunolocalization of androgen receptor and estrogen receptor in the developing testis and excurrent ducts of goats. *The Anatomical Record*, *249*(1), 54-62.

Griswold, M. D. (2015). The initiation of spermatogenesis and the cycle of the seminiferous epithelium. *Sertoli Cell Biology*, 233-245. https://doi.org/10.1016/B978-0-12-417047-6.00008-9

Guercio, G., Saraco, N., Costanzo, M., Marino, R., Ramirez, P., Berensztein, E., . . . Belgorosky, A. J. F. i. e. (2020). Estrogens in human male gonadotropin secretion and testicular physiology from infancy to late puberty. *11*, 72.

Guo, C., Diao, H., Lian, Y., Yu, H., Gao, H., Zhang, Y., & Lin, D. (2009). Recombinant expression and characterization of an epididymis-specific antimicrobial peptide BIN1b/SPAG11E. *Journal of biotechnology*, *139*(1), 33—37-33—37. https://doi.org/10.1016/j.jbiotec.2008.10.003

Gupta, M., Sharma, R., & Kumar, A. J. C. B. (2020). Docking techniques in toxicology: an overview. *15*(6), 600-610.

Guyonnet, B., Zabet-Moghaddam, M., SanFrancisco, S., & Cornwall, G. A. (2012). Isolation and proteomic characterization of the mouse sperm acrosomal matrix. *Mol Cell Proteomics*, *11*(9), 758-774. https://doi.org/10.1074/mcp.M112.020339

Haas, E., Bhattacharya, I., Brailoiu, E., Damjanović, M., Brailoiu, G. C., Gao, X., . . . Barton, M. (2009). Regulatory role of G protein-coupled estrogen receptor for vascular function and obesity. *Circ Res*, *104*(3), 288-291. https://doi.org/10.1161/circresaha.108.190892

Haas, E., Bhattacharya, I., Brailoiu, E., Damjanović, M., Brailoiu, G. C., Gao, X., . . . Barton, M. (2009). Regulatory Role of G Protein–Coupled Estrogen Receptor for Vascular Function and Obesity. *104*(3), 288-291. https://doi.org/doi:10.1161/CIRCRESAHA.108.190892

Hadjimarkou, M. M., & Vasudevan, N. (2017). GPER1/GPR30 in the brain: Crosstalk with classical estrogen receptors and implications for behavior. *Journal of Steroid Biochemistry and Molecular Biology*. https://doi.org/10.1016/j.jsbmb.2017.04.012

Haider, S. G. (2004). Cell biology of Leydig cells in the testis. *Int Rev Cytol*, *233*, 181-241. https://doi.org/10.1016/s0074-7696(04)33005-6

Hamidatou Khati, W., & Hammouche, S. (2021). Histomorphometrical study of the testis in the desert Rodent, Gerbillus tarabuli, sexually immature and adult during the annual reproductive cycle. *Anatomia, Histologia, Embryologia*, *50*(2), 324-332. https://doi.org/10.1111/AHE.12633

Hamilton, D. W. (1975). Structure and function of the epithelium lining efferentes, ductus epididymidis and ductus deferens in the rat. *Handbook of physiology, Sect 7*, 259-301.

Hansen, L. A., Clulow, J., & Jones, R. C. (1999). The role of Na+-H+ exchange in fluid and solute transport in the rat efferent ducts. *Experimental physiology*, *84*(3), 521-527.

Harlev, A., Agarwal, A., Gunes, S. O., Shetty, A., & du Plessis, S. S. (2015). Smoking and Male Infertility: An Evidence-Based Review. *World J Mens Health*, *33*(3), 143-160. https://doi.org/10.5534/wjmh.2015.33.3.143

Harrison, D., & Bates, P. J. K., England: Harrison Zoological Museum. (1991). The Mammals of Arabia Sevenoaks. *354*.

Hart, D., Nilges, M., Pollard, K., Lynn, T., Patsos, O., Shiel, C., . . . Vasudevan, N. (2014). Activation of the G-protein coupled receptor 30 (GPR30) has different effects on

anxiety in male and female mice. *Steroids*, *81*, 49-56. https://doi.org/https://doi.org/10.1016/j.steroids.2013.11.004

Hazell, G. G. J., Yao, S. T., Roper, J. A., Prossnitz, E. R., Carroll, A.-m. O., & Lolait, S. J. (2009). Localisation of GPR30 , a novel G protein-coupled oestrogen receptor , suggests multiple functions in rodent brain and peripheral tissues. 223-236. https://doi.org/10.1677/JOE-09-0066

Hejmej, A., Gorazd, M., Kosiniak-Kamysz, K., Wiszniewska, B., Sadowska, J., & Bilińska, B. (2005). Expression of aromatase and oestrogen receptors in reproductive tissues of the stallion and a single cryptorchid visualised by means of immunohistochemistry. *Domestic Animal Endocrinology*, *29*(3), 534-547. https://doi.org/https://doi.org/10.1016/j.domaniend.2005.03.002

Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Hartman, J., . . . Warner, M. (2007a). Estrogen receptors: how do they signal and what are their targets. *Physiological reviews*, *87*(3), 905-931.

Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Hartman, J., . . . Warner, M. J. P. r. (2007b). Estrogen receptors: how do they signal and what are their targets. *87*(3), 905-931.

Hermo, L., & Jacks, D. (2002). Nature's ingenuity: bypassing the classical secretory route via apocrine secretion. *Mol Reprod Dev*, *63*(3), 394-410. https://doi.org/10.1002/mrd.90023

Hermo, L., Oka, R., & Morales, C. R. (1994). Secretion and endocytosis in the male reproductive tract: a role in sperm maturation. In *Int Rev Cytol* (Vol. 154, pp. 105-189). Elsevier.

Hess, R. A. (2002). The efferent ductules: structure and functions. In (pp. 49-80). Springer.

Hess, R. A. (2003). Estrogen in the adult male reproductive tract : A review. *14*, 1-14.

Hess, R. A. (2014). *The Efferent Ductules : Structure and Functions*. https://doi.org/10.1007/978-1-4615-0679-9

Hess, R. A. (2018a). Efferent Ductules: Structure and Function. *Encyclopedia of Reproduction*, 270-278. https://doi.org/10.1016/B978-0-12-801238-3.64593-2

Hess, R. A. (2018b). *Efferent Ductules: Structure and Function* (Second Edi ed., Vol. 1). Elsevier. https://doi.org/10.1016/B978-0-12-801238-3.64593-2

Hess, R. A., Bunick, D., Lubahn, D. B., Zhou, Q., & Bouma, J. (2000). Morphologic {Changes} in {Efferent} {Ductules} and {Epididymis} in {Estrogen} {Receptor}-α {Knockout} {Mice}. *Journal of Andrology*, *21*(1), 107-121.

Hess, R. A., & Cooke, P. S. (2018). Estrogen in the male: a historical perspective†. *Biology of Reproduction*, *99*(1), 27-44. https://doi.org/10.1093/biolre/ioy043 %J Biology of Reproduction

Hess, R. A., Fernandes, S. A. F., Gomes, G. R. O., Oliveira, C. A., Lazari, M. F. M., & Porto, C. S. (2011). Estrogen and its receptors in efferent ductules and epididymis. *Journal of Andrology*, *32*(6), 600-613.

Hess, R. A., & França, L. R. (2005). Structure of the Sertoli cell. *Sertoli Cell Biology*, *40*(1).

Hess, R. A., Sharpe, R. M., & Hinton, B. T. (2021). Estrogens and development of the rete testis, efferent ductules, epididymis and vas deferens. *Differentiation*, *118*, 41-71. https://doi.org/10.1016/j.diff.2020.11.004

Hess, R. A., & Vogl, A. W. (2015). 1 - Sertoli cell anatomy and cytoskeleton. In M. D. Griswold (Ed.), *Sertoli Cell Biology (Second Edition)* (pp. 1-55). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-417047-6.00001-6

Hess, R. E. X. A., Gist, D. H., Bunick, D., Lubahn, D. B., Farrell, A. M. Y., Bahr, J., . . . Greene, G. L. (1997). Estrogen receptor (α and β) expression in the excurrent ducts of the adult male rat reproductive tract. *Journal of Andrology*, *18*(6), 602-611.

Hewitt, S. C., & Korach, K. S. (2018). Estrogen Receptors: New Directions in the New Millennium. *Endocrine Reviews*, *39*(5), 664-675. https://doi.org/10.1210/er.2018-00087 %J Endocrine Reviews

Hirao-Suzuki, M., Takeda, S., Kodama, Y., Takiguchi, M., Toda, A., & Ohara, M. (2020). Metalloestrogenic effects of cadmium are absent in long-term estrogen-deprived MCF-7 cells: Evidence for the involvement of constitutively activated estrogen receptor α and very low expression of G protein-coupled estrogen receptor 1. *Toxicology Letters*, *319*, 22-30. https://doi.org/https://doi.org/10.1016/j.toxlet.2019.10.018

Ho, H. C., & Suarez, S. S. (2003). Characterization of the intracellular calcium store at the base of the sperm flagellum that regulates hyperactivated motility. *Biol Reprod*, *68*(5), 1590-1596. https://doi.org/10.1095/biolreprod.102.011320

Hoffman, R. A., & Reiter, R. J. (1965). Pineal Gland: Influence on Gonads of Male Hamsters. *Science*, *148*(3677), 1609-1611. https://doi.org/10.1126/SCIENCE.148.3677.1609

Hoffmann, K. (1973). The influence of photoperiod and melatonin on testis size, body weight, and pelage colour in the Djungarian hamster (Phodopus sungorus). *Journal of Comparative Physiology*, *85*(3), 267-282. https://doi.org/10.1007/BF00694233/METRICS

Hoque, M., Kim, E. N., Chen, D., Li, F. Q., & Takemaru, K. I. (2022). Essential Roles of Efferent Duct Multicilia in Male Fertility. *Cells*, *11*(3). https://doi.org/10.3390/cells11030341

Huang, G. S., Gunter, M. J., Arend, R. C., Li, M., Arias-Pulido, H., Prossnitz, E. R., . . . Smith, H. O. (2010). Co-expression of GPR30 and ERβ and their association with disease progression in uterine carcinosarcoma. *American journal of obstetrics and gynecology*, *203*(3), 242. e241-242. e245.

Huhtaniemi, I. T. (2018). LH and the Male. *Encyclopedia of Reproduction*, 195-203. https://doi.org/10.1016/B978-0-12-801238-3.64579-8

Ihlenfeldt, W.-D. (2018). PubChem. In *Applied Chemoinformatics* (pp. 245-258). https://doi.org/https://doi.org/10.1002/9783527806539.ch6e

Ilio, K. Y., & Hess, R. A. (1994). Structure and function of the ductuli efferentes: a review. *Microscopy research and technique*, *29*(6), 432-467.

Ivell, R., Agoulnik, A. I., & Anand-Ivell, R. (2017). Relaxin-like peptides in male reproduction - a human perspective. *Br J Pharmacol*, *174*(10), 990-1001. https://doi.org/10.1111/bph.13689

Jacenik, D., Zielińska, M., Mokrowiecka, A., Michlewska, S., Małecka-Panas, E., Kordek, R., . . . Krajewska, W. M. (2019). G protein-coupled estrogen receptor mediates anti-inflammatory action in Crohn's disease. *Sci Rep*, *9*(1), 6749. https://doi.org/10.1038/s41598-019-43233-3

James, E. R., Carrell, D. T., Aston, K. I., Jenkins, T. G., Yeste, M., & Salas-Huetos, A. (2020). The Role of the Epididymis and the Contribution of Epididymosomes to Mammalian Reproduction. *Int J Mol Sci*, *21*(15). https://doi.org/10.3390/ijms21155377

Janulis, L., Hess, R. A., Bunick, D., Nitta, H., Janssen, S., Asawa, Y., & Bahr, J. M. (1996). Mouse Epididymal Sperm Contain Active P450 Aromatase Which Decreases as Sperm Traverse the Epididymis. *17*(2), 111-116. https://doi.org/https://doi.org/10.1002/j.1939-4640.1996.tb01759.x

Jeégou, B., & Rolland, A. D. (2018). Sertoli Cell. *Encyclopedia of Reproduction*, 24-27. https://doi.org/10.1016/B978-0-12-801238-3.64359-3

Jejurikar, B. L., & Rohane, S. H. (2021). Drug designing in discovery studio.

Ji, X., Li, N., Ma, M., Rao, K., Yang, R., & Wang, Z. (2020). Tricresyl phosphate isomers exert estrogenic effects via G protein-coupled estrogen receptor-mediated pathways. *Environmental Pollution*, *264*, 114747. https://doi.org/https://doi.org/10.1016/j.envpol.2020.114747

Jiao, X., Jin, X., Ma, Y., Yang, Y., Li, J., Liang, L., . . . Chemistry. (2021). A comprehensive application: Molecular docking and network pharmacology for the prediction of bioactive constituents and elucidation of mechanisms of action in component-based Chinese medicine. *90*, 107402.

Jiménez-Reina, L., Maartens, P. J., Jimena-Medina, I., Agarwal, A., & du Plessis, S. S. (2016). Overview of the Male Reproductive System. In D. Vaamonde, S. S. du Plessis, & A. Agarwal (Eds.), *Exercise and Human Reproduction: Induced Fertility Disorders and Possible Therapies* (pp. 1-17). Springer New York. https://doi.org/10.1007/978-1-4939-3402-7_1

Johnson, L., Curley, K. O., Graham, J., Welsh, T. H., & Staub, C. (2015). Anatomy and physiology of the male reproductive system and potential targets of toxicants. https://agris.fao.org/agris-search/search.do?recordID=FR20211153481

Joseph, A., Shur, B. D., & Hess, R. A. (2011). Estrogen, efferent ductules, and the epididymis. *Biology of Reproduction*, *84*(2), 207-217.

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., . . . Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature*, *596*(7873), 583-589. https://doi.org/10.1038/s41586-021-03819-2

Jurewicz, J., Radwan, M., Wielgomas, B., Dziewirska, E., Karwacka, A., Klimowska, A., . . . Hanke, W. (2017). Human Semen Quality, Sperm DNA Damage, and the Level of Reproductive Hormones in Relation to Urinary Concentrations of Parabens. *J Occup Environ Med*, *59*(11), 1034-1040. https://doi.org/10.1097/jom.0000000000001106

Kadokawa, H., Pandey, K., Onalenna, K., & Nahar, A. (2018). Reconsidering the roles of endogenous estrogens and xenoestrogens: the membrane estradiol receptor G protein-coupled receptor 30 (GPR30) mediates the effects of various estrogens. *Journal of Reproduction and Development*, *64*(3), 203-208. https://doi.org/10.1262/jrd.2017-153

Kang, K., Lee, S. B., Jung, S. H., Cha, K. H., Park, W. D., Sohn, Y. C., & Nho, C. W. (2009). Tectoridin, a poor ligand of estrogen receptor alpha, exerts its estrogenic effects via an ERK-dependent pathway. *Mol Cells*, *27*(3), 351-357. https://doi.org/10.1007/s10059-009-0045-8

Khammar, F. (1987). *Variations saisonnières de l'activité endocrine de deuxespèces de rongeurs désertiques, le rat des sables (Psammomysobesus) et la gerbille (Gerbillus gerbillus)*

Khammar, F., & Brudieux, R. (1987). Seasonal changes in testicular contents and plasma concentrations of androgens in the desert gerbil (Gerbillus gerbillus). *Journal of Reproduction and Fertility*, *80*(2), 589-594.

Kim, D. E., Chivian, D., & Baker, D. (2004). Protein structure prediction and analysis using the Robetta server. *Nucleic Acids Res*, *32*(suppl_2), W526-W531. https://doi.org/10.1093/nar/gkh468 %J Nucleic Acids Research

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., . . . Bryant, S. H. (2015). PubChem Substance and Compound databases. *Nucleic Acids Res*, *44*(D1), D1202-D1213. https://doi.org/10.1093/nar/gkv951 %J Nucleic Acids Research

Kingdon, J. (2014). *Mammals of Africa: Volume III: Rodents, Hares and Rabbits*. A&C Black.

Klein, J. M., Poulet, A. R., & Orstom, E. S. (1975). Les rongeurs, et en particulier Gerbillus gerbillus. *medecine et parasitologie*, *XIII*(1), 13-28. https://www.cabdirect.org/cabdirect/abstract/19752902912

Klinge, C. M. (2001). Estrogen receptor interaction with estrogen response elements. *Nucleic Acids Res*, *29*(14), 2905-2919. https://doi.org/10.1093/nar/29.14.2905

Kobayashi, A., & Behringer, R. R. (2003). Developmental genetics of the female reproductive tract in mammals. In (Vol. 4, pp. 969-980): Nat Rev Genet.

Kotula-Balak, M., Pawlicki, P., Milon, A., Tworzydlo, W., Sekula, M., Pacwa, A., . . . Galas, J. (2018). The role of G-protein-coupled membrane estrogen receptor in mouse Leydig cell function—in vivo and in vitro evaluation. *Cell and Tissue Research*, *374*(2), 389-412. https://doi.org/10.1007/s00441-018-2861-7

Kowalski, K., & Rzebik-Kowalska, B. (1991). Mammals of algeria.

Krejčířová, R., Maňasová, M., Sommerová, V., Langhamerová, E., Rajmon, R., & Maňásková-Postlerová, P. (2018). G protein-coupled estrogen receptor (GPER) in adult boar testes, epididymis and spermatozoa during epididymal maturation. *International Journal of Biological Macromolecules*, *116*, 113-119. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2018.05.015

Kretser, D. M. (2018). Hypothalamic Pituitary Testis Axis. *Encyclopedia of Reproduction*, 180-183. https://doi.org/10.1016/B978-0-12-801238-3.64576-2

Kuchangi, S. N., Mruthunjayappa, M. H., & Sanna Kotrappanavar, N. (2023). Chapter 4 - An overview of water pollutants in present scenario. In J. K. Pandey, S. Manna, R. K. Patel, & M. Qian (Eds.), *3D Printing Technology for Water Treatment Applications* (pp. 83-105). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-323-99861-1.00001-1

Kuiper, G. G. J. M., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S., & Gustafsson, J. Å. (1997). Comparison of the Ligand Binding Specificity and Transcript Tissue Distribution of Estrogen Receptors α and β. *Endocrinology*, *138*(3), 863-870. https://doi.org/10.1210/ENDO.138.3.4979

Kumar, R., Zakharov, M. N., Khan, S. H., Miki, R., Jang, H., Toraldo, G., . . . Jasuja, R. (2011). The dynamic structure of the estrogen receptor. *J Amino Acids*, *2011*, 812540. https://doi.org/10.4061/2011/812540

Kvingedal, A. M., & Smeland, E. B. (1997). A novel putative G-protein-coupled receptor expressed in lung, heart and lymphoid tissue. *FEBS Letters*, *407*(1), 59-62. https://doi.org/https://doi.org/10.1016/S0014-5793(97)00278-0

Labas, V., Spina, L., Belleannee, C., Teixeira-Gomes, A. P., Gargaros, A., Dacheux, F., & Dacheux, J. L. (2015). Analysis of epididymal sperm maturation by MALDI profiling and top-down mass spectrometry. *J Proteomics*, *113*, 226-243. https://doi.org/10.1016/j.jprot.2014.09.031

Lappano, R., Rosano, C., De Marco, P., De Francesco, E. M., Pezzi, V., & Maggiolini, M. (2010). Estriol acts as a GPR30 antagonist in estrogen receptor-negative breast cancer cells. *Mol Cell Endocrinol*, *320*(1-2), 162-170. https://doi.org/10.1016/j.mce.2010.02.006

Lara, N. L. M., Costa, G. M. J., Avelar, G. F., Lacerda, S. M. S. N., Hess, R. A., & França, L. R. (2018). Testis Physiology—Overview and Histology. *Encyclopedia of Reproduction*, 105-116. https://doi.org/10.1016/B978-0-12-801238-3.64567-1

Le Dily, F., & Beato, M. (2018). Signaling by Steroid Hormones in the 3D Nuclear Space. *Int J Mol Sci*, *19*(2). https://doi.org/10.3390/ijms19020306

Le May, C., Chu, K., Hu, M., Ortega, C. S., Simpson, E. R., Korach, K. S., . . . Mauvais-Jarvis, F. (2006). Estrogens protect pancreatic beta-cells from apoptosis and prevent insulin-deficient diabetes mellitus in mice. *Proc Natl Acad Sci U S A*, *103*(24), 9232-9237. https://doi.org/10.1073/pnas.0602956103

LeBerre, M., & Chevallier, J. (1990). *Faune du Sahara. 2. Mammifères*. Lechevalier.

Lee, C. I., Goodwin, A., & Wilcken, N. (2017). Fulvestrant for hormone-sensitive metastatic breast cancer. *Cochrane Database Syst Rev*, *1*(1), Cd011093. https://doi.org/10.1002/14651858.CD011093.pub2

Lei, B., Yang, Y., Xu, L., Zhang, X., Yu, M., Yu, J., . . . Yu, Y. (2024). Molecular insights into the effects of tetrachlorobisphenol A on puberty initiation in Wistar rats. *Science of*

The Total Environment, *911*, 168643. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.168643

Lenhart, P. M., Broselid, S., Barrick, C. J., Leeb-Lundberg, L. M. F., & Caron, K. M. (2013). G-protein-coupled receptor 30 interacts with receptor activity-modifying protein 3 and confers sex-dependent cardioprotection. *Journal of Molecular Endocrinology*, *51*(1), 191-202. https://doi.org/10.1530/JME-13-0021

Leung, G. P. H., Cheung, K. H., Leung, C. T., Tsang, M. W., & Wong, P. Y. D. (2004). Regulation of epididymal principal cell functions by basal cells: role of transient receptor potential (Trp) proteins and cyclooxygenase-1 (COX-1). *MOLECULAR AND CELLULAR ENDOCRINOLOGY*, *216*(1-2), 5-13.

Levallet, J., Mittre, H., Delarue, B., & Carreau, S. (1998). Alternative splicing events in the coding region of the cytochrome P450 aromatase gene in male rat germ cells. *Journal of Molecular Endocrinology*, *20*(3), 305-312. https://doi.org/10.1677/JME.0.0200305

Levy, S., & Robaire, B. (1999). Segment-specific changes with age in the expression of junctional proteins and the permeability of the blood-epididymis barrier in rats. *Biology of Reproduction*, *60*(6), 1392-1401.

Lew, M., Kunju, L. P., & Pantanowitz, L. (2022). Male Reproductive System. In M. Lew, J. Pang, & L. Pantanowitz (Eds.), *Normal Cytology: An Illustrated, Practical Guide* (pp. 115-124). Springer International Publishing. https://doi.org/10.1007/978-3-031-20336-7_10

LI, J. J., TALLEY, D. J., LI, S. A., & VILLEE, C. A. (1974). An Estrogen Binding Protein in the Renal Cytosol of Intact, Castrated and Estrogenized Golden Hamsters1. *Endocrinology*, *95*(4), 1134-1141. https://doi.org/10.1210/endo-95-4-1134 %J Endocrinology

Li, Q., Zhang, F., Zhang, S., Sheng, X., Han, X., Weng, Q., & Yuan, Z. (2015). Seasonal expression of androgen receptor, aromatase, and estrogen receptor alpha and beta in the testis of the wild ground squirrel (Citellus dauricus Brandt). *European Journal of Histochemistry*, *59*(1). https://doi.org/10.4081/ejh.2015.2456

Li, S., Wang, B., Tang, Q., Liu, J., & Yang, X. (2017). Bisphenol A triggers proliferation and migration of laryngeal squamous cell carcinoma via GPER mediated upregulation of IL-6. *35*(4), 209-216. https://doi.org/https://doi.org/10.1002/cbf.3265

Liang, J., & Shang, Y. (2013). Estrogen and cancer. *Annu Rev Physiol*, *75*, 225-240. https://doi.org/10.1146/annurev-physiol-030212-183708

Liguori, G., Tafuri, S., Pelagalli, A., Ali', S., Russo, M., Mirabella, N., & Squillacioti, C. J. V. S. (2024). G Protein-Coupled Estrogen Receptor (GPER) and ERs Are Modulated in the Testis–Epididymal Complex in the Normal and Cryptorchid Dog. *11*(1), 21.

Lim, W., Jeong, W., & Song, G. (2016). Coumestrol suppresses proliferation of ES2 human epithelial ovarian cancer cells. *J Endocrinol*, *228*(3), 149-160. https://doi.org/10.1530/joe-15-0418

Liu, C., Liao, Y., Fan, S., Fu, X., Xiong, J., Zhou, S., . . . Wang, J. (2019). G-Protein-Coupled Estrogen Receptor Antagonist G15 Decreases Estrogen-Induced Development of Non-Small Cell Lung Cancer. *Oncol Res*, *27*(3), 283-292. https://doi.org/10.3727/096504017x15035795904677

Liverman, C. S., Brown, J. W., Sandhir, R., McCarson, K. E., & Berman, N. E. J. (2009). Role of the oestrogen receptors GPR30 and ERα in peripheral sensitization: relevance to trigeminal pain disorders in women. *Cephalalgia*, *29*(7), 729-741.

Longcope, C., Kato, T., & Horton, R. (1969). Conversion of blood androgens to estrogens in normal adult men and women. *J Clin Invest*, *48*(12), 2191-2201. https://doi.org/10.1172/jci106185

López-Botella, A., Velasco, I., Acién, M., Sáez-Espinosa, P., Todolí-Torró, J. L., Sánchez-Romero, R., & Gómez-Torres, M. J. (2021). Impact of Heavy Metals on Human Male Fertility-An Overview. *Antioxidants (Basel)*, *10*(9). https://doi.org/10.3390/antiox10091473

Lu, D., Wang, X., Feng, C., Liu, D., Liu, Y., Liu, Y., . . . Pang, G. (2023). Study of the Sensing Kinetics of G Protein-Coupled Estrogen Receptor Sensors for Common Estrogens and Estrogen Analogs. *28*(8), 3286. https://www.mdpi.com/1420-3049/28/8/3286

Lucas, T. F. G., Pimenta, M. T., Pisolato, R., Lazari, M. F. M., & Porto, C. S. (2011). 17β-estradiol signaling and regulation of Sertoli cell function. *Spermatogenesis*, *1*(4), 318-324. https://doi.org/10.4161/spmg.1.4.18903

Lucas, T. F. G., Royer, C., Siu, E. R., Lazari, M. F. M., & Porto, C. S. (2010). Expression and signaling of G protein-coupled estrogen receptor 1 (GPER) in rat sertoli cells. *Biology of Reproduction*, *83*(2), 307-317.

Lucas, T. F. G., Siu, E. R., Esteves, C. A., Monteiro, H. P., Oliveira, C. A., Porto, C. S., & Lazari, M. F. M. (2008). 17beta-estradiol induces the translocation of the estrogen receptors {ESR1} and {ESR2} to the cell membrane, {MAPK3}/1 phosphorylation and

proliferation of cultured immature rat {Sertoli} cells. *Biology of Reproduction*, *78*(1), 101-114.

Lucki, N. C., & Sewer, M. B. (2011). Genistein stimulates MCF-7 breast cancer cell growth by inducing acid ceramidase (ASAH1) gene expression. *J Biol Chem*, *286*(22), 19399-19409. https://doi.org/10.1074/jbc.M110.195826

Luo, J., & Liu, D. (2020). Does GPER Really Function as a G Protein-Coupled Estrogen Receptor in vivo? [Mini Review]. *11*. https://doi.org/10.3389/fendo.2020.00148

Luttrell, L. M. (2006). Transmembrane signaling by G protein-coupled receptors. *Methods Mol Biol*, *332*, 3-49. https://doi.org/10.1385/1-59745-048-0:1

Lyche, J. L., Gutleb, A. C., Bergman, Å., Eriksen, G. S., Murk, A. J., Ropstad, E., . . . Skaare, J. U. (2009). Reproductive and Developmental Toxicity of Phthalates. *Journal of Toxicology and Environmental Health, Part B*, *12*(4), 225-249. https://doi.org/10.1080/10937400903094091

Madan, A. K., Bajaj, S., & Dureja, H. (2013). Classification models for safe drug molecules. *Methods Mol Biol*, *930*, 99-124. https://doi.org/10.1007/978-1-62703-059-5_5

Madeo, A., & Maggiolini, M. (2010). Nuclear alternate estrogen receptor GPR30 mediates 17beta-estradiol-induced gene expression and migration in breast cancer-associated fibroblasts. *Cancer Res*, *70*(14), 6036-6046. https://doi.org/10.1158/0008-5472.Can-10-0408

Maggiolini, M., Vivacqua, A., Fasanella, G., Recchia, A. G., Sisci, D., Pezzi, V., . . . Andò, S. (2004). The G protein-coupled receptor GPR30 mediates c-fos up-regulation by 17beta-estradiol and phytoestrogens in breast cancer cells. *J Biol Chem*, *279*(26), 27008-27016. https://doi.org/10.1074/jbc.M403588200

Malivindi, R., Aquila, S., & Rago, V. (2018). Immunolocalization of G Protein-Coupled Estrogen Receptor in the Pig Epididymis [https://doi.org/10.1002/ar.23837]. *The Anatomical Record*, *301*(8), 1467-1473. https://doi.org/https://doi.org/10.1002/ar.23837

Marchese, A., Paing, M. M., Temple, B. R. S., & Trejo, J. (2008). G Protein–Coupled Receptor Sorting to Endosomes and Lysosomes. *48*(1), 601-629. https://doi.org/10.1146/annurev.pharmtox.48.113006.094646

Mårtensson, U. E., Salehi, S. A., Windahl, S., Gomez, M. F., Swärd, K., Daszkiewicz-Nilsson, J., . . . Leeb-Lundberg, L. M. (2009). Deletion of the G protein-coupled receptor 30 impairs glucose tolerance, reduces bone growth, increases blood pressure,

and eliminates estradiol-stimulated insulin release in female mice. *Endocrinology*, *150*(2), 687-698. https://doi.org/10.1210/en.2008-0623

Martínez-Traverso, G. B., & Pearl, C. A. (2015). Immunolocalization of G protein-coupled estrogen receptor in the rat epididymis. *Reproductive Biology and Endocrinology*, *13*(1), 48. https://doi.org/10.1186/s12958-015-0042-z

Martinkovich, S., Shah, D., Planey, S. L., & Arnott, J. A. (2014). Selective estrogen receptor modulators: tissue specificity and clinical utility. *Clin Interv Aging*, *9*, 1437-1452. https://doi.org/10.2147/cia.S66690

Martoja, R., & Martoja-Pierson, M. (1967). Initiation aux techniques de l'histologie animale.

Matsuyama, S., & DeFalco, T. (2024). Steroid hormone signaling: multifaceted support of testicular function [Mini Review]. *11*. https://doi.org/10.3389/fcell.2023.1339385

Meeker, J. D., Calafat, A. M., & Hauser, R. (2009). Urinary metabolites of di(2-ethylhexyl) phthalate are associated with decreased steroid hormone levels in adult men. *J Androl*, *30*(3), 287-297. https://doi.org/10.2164/jandrol.108.006403

Meeker, J. D., Ehrlich, S., Toth, T. L., Wright, D. L., Calafat, A. M., Trisini, A. T., . . . Hauser, R. (2010). Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. *Reprod Toxicol*, *30*(4), 532-539. https://doi.org/10.1016/j.reprotox.2010.07.005

Menad, R. (2008). Régionalisation structurale et fonctionnelle de l'épididyme d'un rongeur déserticole diurne, psammomys obesus CRETZSCHMAR, 1828. http://repository.usthb.dz/bitstream/handle/123456789/1599/resumé.docx?sequence=1&isAllowed=y

Menad, R., Fernini, M., Lakabi, L., Smaï, S., Gernigon-Spychalowicz, T., Farida, K., . . . Exbrayat, J. M. (2021). Androgen and estrogen receptors immunolocalization in the sand rat (Psammomys Obesus) cauda epididymis. *Acta Histochemica*, *123*(2), 151683-151683. https://doi.org/10.1016/J.ACTHIS.2021.151683

Menad, R., Fernini, M., Lakabi, L., Soudani, N., Smaï, S., Bonnet, X., . . . Exbrayat, J. M. (2020a). GPER1, estrogen receptors and androgen receptor in the sand rat (Psammomys obesus) efferent ducts. *Folia Morphologica*, *0*(0). https://doi.org/10.5603/FM.a2020.0053

Menad, R., Fernini, M., Lakabi, L., Soudani, N., Smaï, S., Bonnet, X., . . . Exbrayat, J. M. (2020b). GPER1, estrogen receptors and androgen receptor in the sand rat (Psammomys obesus) efferent ducts. *Folia Morphologica*. https://doi.org/10.5603/fm.a2020.0053

Menad, R., Fernini, M., Smaï, S., Bonnet, X., Gernigon-Spychalowicz, T., Moudilou, E., . . . Exbrayat, J.-M. (2017a). GPER1 in sand rat epididymis: Effects of seasonal variations, castration and efferent ducts ligation. *Animal Reproduction Science*, *183*, 9-20. https://doi.org/https://doi.org/10.1016/j.anireprosci.2017.06.012

Menad, R., Smaï, S., Bonnet, X., Gernigon-Spychalowicz, T., Moudilou, E., Khammar, F., & Exbrayat, J.-M. J. A. h. (2017b). Seasonal variations of aromatase and estrogen receptors expression in the testis of free-ranging sand rats. *119*(4), 382-391.

Menad, R., Smaï, S., Bonnet, X., Gernigon-Spychalowicz, T., Moudilou, E., Khammar, F., & Exbrayat, J. M. (2017c). Seasonal variations of aromatase and estrogen receptors expression in the testis of free-ranging sand rats. *Acta Histochemica*, *119*(4), 382-391. https://doi.org/10.1016/j.acthis.2017.03.008

Meng, X.-Y., Zhang, H.-X., Mezei, M., & Cui, M. J. C. c.-a. d. d. (2011). Molecular docking: a powerful approach for structure-based drug discovery. *7*(2), 146-157.

Mester, J., & Baulieu, E.-E. (1972). Nuclear estrogen receptor of chick liver. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *261*(1), 236-244. https://doi.org/https://doi.org/10.1016/0304-4165(72)90334-0

Métayer, S., Dacheux, F. o., Dacheux, J.-L., & Gatti, J.-L. (2002). Comparison, Characterization, and Identification of Proteases and Protease Inhibitors in Epididymal Fluids of Domestic Mammals. Matrix Metalloproteinases Are Major Fluid Gelatinases1. *Biology of Reproduction*, *66*(5), 1219-1229. https://doi.org/10.1095/biolreprod66.5.1219 %J Biology of Reproduction

Meyer, M. R., Baretella, O., Prossnitz, E. R., & Barton, M. (2010). Dilation of epicardial coronary arteries by the G protein-coupled estrogen receptor agonists G-1 and ICI 182,780. *Pharmacology*, *86*(1), 58-64. https://doi.org/10.1159/000315497

Meyer, M. R., & Barton, M. (2016). *Estrogens and Coronary Artery Disease : New Clinical Perspectives* (1 ed., Vol. 77). Elsevier Inc. https://doi.org/10.1016/bs.apha.2016.05.003

Meyer, M. R., Daniel, C., Fredette, N. C., Amann, K., Barton, M., & Prossnitz, E. R. (2015). Abstract P607: Deletion of GPER Protects from Age-related Renovascular Dysfunction and Tubulo-interstitial Injury. *66*(suppl_1), AP607-AP607. https://doi.org/doi:10.1161/hyp.66.suppl_1.p607

Meyer, M. R., Fredette, N. C., Howard, T. A., Hu, C., Ramesh, C., Daniel, C., . . . Prossnitz, E. R. (2014). G Protein-coupled Estrogen Receptor Protects from Atherosclerosis. *Scientific Reports*, *4*(1), 7564. https://doi.org/10.1038/srep07564

Meyer, M. R., Prossnitz, E. R., & Barton, M. (2011a). The G protein-coupled estrogen receptor GPER / GPR30 as a regulator of cardiovascular function. *Vascular Pharmacology*, *55*(1-3), 17-25. https://doi.org/10.1016/j.vph.2011.06.003

Meyer, M. R., Prossnitz, E. R., & Barton, M. (2011b). The {G} protein-coupled estrogen receptor {GPER} / {GPR30} as a regulator of cardiovascular function. *Vascular Pharmacology*, *55*(1-3), 17-25. https://doi.org/10.1016/j.vph.2011.06.003

Mishra, S. K., Abbot, S. E., Choudhury, Z., Cheng, M., Khatab, N., Maycock, N. J. R., . . . Aaronson, P. I. (2000). Endothelium-dependent relaxation of rat aorta and main pulmonary artery by the phytoestrogens genistein and daidzein. *Cardiovascular Research*, *46*(3), 539-546. https://doi.org/10.1016/S0008-6363(00)00049-3 %J Cardiovascular Research

Miura, T., Miura, C., Ohta, T., Nader, M. R., Todo, T., & Yamauchi, K. (1999). Estradiol-17β Stimulates the Renewal of Spermatogonial Stem Cells in Males. *Biochemical and Biophysical Research Communications*, *264*(1), 230-234. https://doi.org/10.1006/BBRC.1999.1494

Moreira, S., Pereira, S. C., Seco-Rovira, V., Oliveira, P. F., Alves, M. G., & Pereira, M. L. (2021). Pesticides and Male Fertility: A Dangerous Crosstalk. *Metabolites*, *11*(12). https://doi.org/10.3390/metabo11120799

Morris, G. M., & Lim-Wilby, M. J. M. m. o. p. (2008). Molecular docking. 365-382.

Mostrom, M., & Evans, T. J. (2011). Chapter 52 - Phytoestrogens. In R. C. Gupta (Ed.), *Reproductive and Developmental Toxicology* (pp. 707-722). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-382032-7.10052-9

Mruk, D. D., & Cheng, C. Y. (2015). The Mammalian Blood-Testis Barrier: Its Biology and Regulation. *Endocr Rev*, *36*(5), 564-591. https://doi.org/10.1210/er.2014-1101

Müller, G., Martino-Andrade, A. J., Santos, A. S., Reghelin, A. L., Garcia, D. M., Sant'Ana, G. R., . . . Morais, R. N. (2012). Testicular testosterone: Estradiol ratio in domestic cats and its relationship to spermatogenesis and epididymal sperm morphology. *Theriogenology*, *78*(6), 1224-1234. https://doi.org/10.1016/J.THERIOGENOLOGY.2012.05.018

Murashima, A., Kishigami, S., Thomson, A., & Yamada, G. J. B. e. B. A.-G. R. M. (2015). Androgens and mammalian male reproductive tract development. *1849*(2), 163-170.

Murata, Y., Robertson, K. M., Jones, M. E., & Simpson, E. R. (2002). Effect of estrogen deficiency in the male: the ArKO mouse model. *Mol Cell Endocrinol*, *193*(1-2), 7-12. https://doi.org/10.1016/s0303-7207(02)00090-4

Murkies, A. L., & Frydenberg, M. (2003). Phytoestrogens. In H. L. Henry & A. W. Norman (Eds.), *Encyclopedia of Hormones* (pp. 192-201). Academic Press. https://doi.org/https://doi.org/10.1016/B0-12-341103-3/00244-8

Muteka, S. P., Chimimba, C. T., Bastos, A. D., & Bennett, N. C. (2018). The reproductive pattern of the Gerbilliscus cf. leucogaster (Rodentia: Muridae) from Namibia. *https://doi.org/10.1139/cjz-2018-0090*, *97*(1), 57-62. https://doi.org/10.1139/CJZ-2018-0090

Natale, C. A., Li, J., Pitarresi, J. R., Norgard, R. J., Dentchev, T., Capell, B. C., . . . Ridky, T. W. (2020). Pharmacologic Activation of the G Protein-Coupled Estrogen Receptor Inhibits Pancreatic Ductal Adenocarcinoma. *Cell Mol Gastroenterol Hepatol*, *10*(4), 868-880.e861. https://doi.org/10.1016/j.jcmgh.2020.04.016

Naz, M., Kamal, M. J. O. p., & medicine, e. (2017). Classification, causes, diagnosis and treatment of male infertility: a review. *17*, 89-109.

Nie, R., Zhou, Q., Jassim, E., Saunders, P. T. K., & Hess, R. A. (2002). Differential Expression of Estrogen Receptors α and β in the Reproductive Tractsof Adult Male Dogs and Cats. *Biology of Reproduction*, *66*(4), 1161-1168.

Nowak, K., Jabłońska, E., Garley, M., Iwaniuk, A., Radziwon, P., Wołczyński, S., & Ratajczak-Wrona, W. (2022). Investigation of estrogen-like effects of parabens on human neutrophils. *Environmental Research*, *214*, 113893. https://doi.org/https://doi.org/10.1016/j.envres.2022.113893

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, *3*(1), 33. https://doi.org/10.1186/1758-2946-3-33

O'Shaughnessy, P. J. (2014). Hormonal control of germ cell development and spermatogenesis. *Seminars in Cell & Developmental Biology*, *29*, 55-65. https://doi.org/10.1016/J.SEMCDB.2014.02.010

O'Donnell, L., Robertson, K. M., Jones, M. E., & Simpson, E. R. (2001). Estrogen and Spermatogenesis. *Endocrine Reviews*, *22*(3), 289-318. https://doi.org/10.1210/EDRV.22.3.0431

O'Donnell, L., & Stanton, P. G. (2018). Spermiation. In M. K. Skinner (Ed.), *Encyclopedia of Reproduction (Second Edition)* (pp. 145-151). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-801238-3.64571-3

Oakley, R. H., Laporte, S. A., Holt, J. A., Barak, L. S., & Caron, M. G. (1999). Association of β-Arrestin with G Protein-coupled Receptors during Clathrin-mediated

Endocytosis Dictates the Profile of Receptor Resensitization *. *Journal of Biological Chemistry*, *274*(45), 32248-32257. https://doi.org/10.1074/jbc.274.45.32248

Oliveira, R. L., Nogueira, J. C., Mahecha, G. A. B., & Oliveira, C. A. (2012). Seasonal variation in estrogen receptor ERα, but not ERβ, androgen receptor and aromatase, in the efferent ductules and epididymis of the big fruit-eating bat Artibeus lituratus. *GENERAL AND COMPARATIVE ENDOCRINOLOGY*, *179*(1), 1-13.

Orgebin-Crist, M. C. (1967). Sperm maturation in rabbit epididymis. *Nature*, *216*(5117), 816-818. https://doi.org/10.1038/216816a0

Owman, C., Blay, P., Nilsson, C., & Lolait, S. J. (1996). Cloning of Human cDNA Encoding a Novel Heptahelix Receptor Expressed in Burkitt's Lymphoma and Widely Distributed in Brain and Peripheral Tissues. *Biochemical and Biophysical Research Communications*, *228*(2), 285-292. https://doi.org/https://doi.org/10.1006/bbrc.1996.1654

Palmieri, D., Perego, P., & Palombo, D. (2012). Apigenin inhibits the TNFα-induced expression of eNOS and MMP-9 via modulating Akt signalling through oestrogen receptor engagement. *Mol Cell Biochem*, *371*(1-2), 129-136. https://doi.org/10.1007/s11010-012-1429-1

Pang, Y., & Thomas, P. (2010). Role of G protein-coupled estrogen receptor 1, GPER, in inhibition of oocyte maturation by endogenous estrogens in zebrafish. *Dev Biol*, *342*(2), 194-206. https://doi.org/10.1016/j.ydbio.2010.03.027

Pantsar, T., & Poso, A. (2018). Binding Affinity via Docking: Fact and Fiction. *Molecules*, *23*(8). https://doi.org/10.3390/molecules23081899

Patrão, M. T. C. C., Silva, E. J. R., & Avellar, M. C. W. (2009a). Androgens and the male reproductive tract: an overview of classical roles and current perspectives. In (Vol. 53, pp. 934-945): Arq Bras Endocrinol Metabol.

Patrão, M. T. C. C., Silva, E. J. R., & Avellar, M. C. W. (2009b). Androgens and the male reproductive tract: an overview of classical roles and current perspectives. In *Arquivos brasileiros de endocrinologia e metabologia* (Vol. 53, pp. 934-945): Arq Bras Endocrinol Metabol.

Paul, R., Moltó, J., Ortuño, N., Romero, A., Bezos, C., Aizpurua, J., & Gómez-Torres, M. J. (2017). Relationship between serum dioxin-like polychlorinated biphenyls and post-testicular maturation in human sperm. *Reprod Toxicol*, *73*, 312-321. https://doi.org/10.1016/j.reprotox.2017.07.004

Pearl, C. A., Berger, T., & Roser, J. F. (2007). Estrogen and androgen receptor expression in relation to steroid concentrations in the adult boar epididymis. *Domestic Animal Endocrinology*, *33*(4), 451-459. https://doi.org/10.1016/j.domaniend.2006.09.003

Pelletier, G., & El-Alfy, M. (2000). Immunocytochemical Localization of Estrogen Receptors α and β in the Human Reproductive Organs. *The Journal of Clinical Endocrinology & Metabolism*, *85*(12), 4835-4840. https://doi.org/10.1210/jcem.85.12.7029

Peña-Corona, S. I., Vásquez Aguire, W. S., Vargas, D., Juárez, I., & Mendoza-Rodríguez, C. A. (2021). Effects of bisphenols on Blood-Testis Barrier protein expression in vitro: A systematic review and meta-analysis. *Reproductive Toxicology*, *103*, 139-148. https://doi.org/https://doi.org/10.1016/j.reprotox.2021.06.008

Peng, J., Zuo, Y., Huang, L., Okada, T., Liu, S., Zuo, G., . . . Zhang, J. H. (2019). Activation of GPR30 with G1 attenuates neuronal apoptosis via src/EGFR/stat3 signaling pathway after subarachnoid hemorrhage in male rats. *Experimental Neurology*, *320*, 113008-113008. https://doi.org/10.1016/J.EXPNEUROL.2019.113008

Pentikäinen, V., Erkkilä, K., Suomalainen, L., Parvinen, M., & Dunkel, L. (2000). Estradiol Acts as a Germ Cell Survival Factor in the Human Testis in Vitro. *The Journal of Clinical Endocrinology & Metabolism*, *85*(5), 2057-2067. https://doi.org/10.1210/JCEM.85.5.6600

Pereira, M. F. N., Fernandes, S. A. F., Nascimento, A. R., Siu, E. R., Hess, R. A., Oliveira, C. A., . . . Lazari, M. F. M. (2014). Effects of the oestrogen receptor antagonist Fulvestrant on expression of genes that affect organization of the epididymal epithelium. *Andrology*, *2*(4), 559-571.

Pereyra-Martinez, A. C., Roselli, C. E., Stadelman, H. L., & Resko, J. A. (2001). Cytochrome P450 aromatase in testis and epididymis of male rhesus monkeys. *Endocrine*, *16*(1), 15-19.

Petersen, M. S., Halling, J., Jørgensen, N., Nielsen, F., Grandjean, P., Jensen, T. K., & Weihe, P. (2018). Reproductive Function in a Population of Young Faroese Men with Elevated Exposure to Polychlorinated Biphenyls (PCBs) and Perfluorinated Alkylate Substances (PFAS). *Int J Environ Res Public Health*, *15*(9). https://doi.org/10.3390/ijerph15091880

Petter, F. (1962). *Répartition géographique et écologie des Rongeurs désertiques (du Sahara occidental à l'Iran oriental)*. Muséum national d'histoire naturelle, Laboratoire de zoologie des mammifères.

Pierantoni, R., Cobellis, G., Meccariello, R., Cacciola, G., Chianese, R., Chioccarelli, T., & Fasano, S. (2009). Testicular Gonadotropin-releasing Hormone Activity, Progression of Spermatogenesis, and Sperm Transport in Vertebrates. *Annals of the New York Academy of Sciences*, *1163*(1), 279-291. https://doi.org/10.1111/J.1749-6632.2008.03617.X

Pietrement, C., Sun-Wada, G. H., Silva, N. D., McKee, M., Marshansky, V., Brown, D., . . . Breton, S. (2006). Distinct expression patterns of different subunit isoforms of the V-ATPase in the rat epididymis. *Biol Reprod*, *74*(1), 185-194. https://doi.org/10.1095/biolreprod.105.043752

Plöen, L., & Setchell, B. P. (1992). Blood-testis barriers revisited. A homage to Lennart Nicander. *Int J Androl*, *15*(1), 1-4. https://doi.org/10.1111/j.1365-2605.1992.tb01108.x

Poulet, S. (2004). *Les gerbilles: nouveaux rongeurs de compagnie* Thèse de Doctorat. Université Paul-Sebastier, Toulouse. Partie, 2, 150].

Prabhushankar, R., Krueger, C., & Manrique, C. (2013). Membrane Estrogen Receptors: Their Role in Blood Pressure Regulation and Cardiovascular Disease. *Current Hypertension Reports*, *16*(1), 408. https://doi.org/10.1007/s11906-013-0408-6

Prados, J., Stenz, L., Somm, E., Stouder, C., Dayer, A., & Paoloni-Giacobino, A. (2015). Prenatal Exposure to DEHP Affects Spermatogenesis and Sperm DNA Methylation in a Strain-Dependent Manner. *PLoS ONE*, *10*(7), e0132136. https://doi.org/10.1371/journal.pone.0132136

Pratiwi, R. I. A., Widyarti, S., & Sumitro, S. B. J. T. J. o. E. L. S. (2023). Potential of Kesambi Active Compound (Schleichera oleosa) as Antagonist G-Protein Estrogen Receptor 1 (GPER1) by In Silico. *13*(1), 43-51.

Prins, G. S., & Lindgren, M. (2015). Chapter 18 - Accessory Sex Glands in the Male. In T. M. Plant & A. J. Zeleznik (Eds.), *Knobil and Neill's Physiology of Reproduction (Fourth Edition)* (pp. 773-804). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-397175-3.00018-1

Prossnitz, E. R., Arterburn, J. B., & Sklar, L. A. (2007a). GPR30 : A G protein-coupled receptor for estrogen. *266*, 138-142. https://doi.org/10.1016/j.mce.2006.12.010

Prossnitz, E. R., Arterburn, J. B., & Sklar, L. A. (2007b). GPR30: A G protein-coupled receptor for estrogen. In (Vol. 265-266, pp. 138-142).

Prossnitz, E. R., Arterburn, J. B., Smith, H. O., Oprea, T. I., Sklar, L. A., & Hathaway, H. J. (2008a). Estrogen signaling through the transmembrane G protein–coupled receptor GPR30. *Annu. Rev. Physiol.*, *70*, 165-190.

Prossnitz, E. R., Arterburn, J. B., Smith, H. O., Oprea, T. I., Sklar, L. A., & Hathaway, H. J. (2008b). Estrogen Signaling through the Transmembrane G Protein – Coupled Receptor GPR30. https://doi.org/10.1146/annurev.physiol.70.113006.100518

Prossnitz, E. R., & Barton, M. (2009). Prostaglandins and Other Lipid Mediators Signaling , physiological functions and clinical relevance of the G protein-coupled estrogen receptor GPER. *89*, 89-97. https://doi.org/10.1016/j.prostaglandins.2009.05.001

Prossnitz, E. R., & Barton, M. (2011). The G-protein-coupled estrogen receptor GPER in health and disease. *Nature Reviews Endocrinology*, *7*(12), 715-715.

Prossnitz, E. R., & Barton, M. (2014). Estrogen biology: New insights into GPER function and clinical opportunities. In (Vol. 389, pp. 71-83): Elsevier Ireland Ltd.

Prossnitz, E. R., & Barton, M. (2023). The G protein-coupled oestrogen receptor GPER in health and disease: an update. *Nature Reviews Endocrinology*, *19*(7), 407-424. https://doi.org/10.1038/s41574-023-00822-7

Prossnitz, E. R., Oprea, T. I., Sklar, L. A., & Arterburn, J. B. (2008c). The ins and outs of GPR30: A transmembrane estrogen receptor. *The Journal of Steroid Biochemistry and Molecular Biology*, *109*(3-5), 350-353. https://doi.org/10.1016/J.JSBMB.2008.03.006

Prueitt, R. L., Hixon, M. L., Fan, T., Olgun, N. S., Piatos, P., Zhou, J., & Goodman, J. E. (2023). Systematic review of the potential carcinogenicity of bisphenol A in humans. *Regulatory Toxicology and Pharmacology*, *142*, 105414. https://doi.org/https://doi.org/10.1016/j.yrtph.2023.105414

Pupo, M., Pisano, A., Lappano, R., Santolla, M. F., De Francesco, E. M., Abonante, S., . . . Maggiolini, M. (2012). Bisphenol A induces gene expression changes and proliferative effects through GPER in breast cancer cells and cancer-associated fibroblasts. *Environ Health Perspect*, *120*(8), 1177-1182. https://doi.org/10.1289/ehp.1104526

Qiao, C., Ye, W., Li, S., Wang, H., & Ding, X. (2018). Icariin modulates mitochondrial function and apoptosis in high glucose-induced glomerular podocytes through G protein-coupled estrogen receptors. *Mol Cell Endocrinol*, *473*, 146-155. https://doi.org/10.1016/j.mce.2018.01.014

Qumsiyeh, M. B., & Schlitter, D. A. (1991). *Cytogenetic data on the rodent family Gerbillidae*. Texas Tech University Press.

Rago, V., Romeo, F., Giordano, F., Ferraro, A., & Carpino, A. (2016). Identification of the G protein-coupled estrogen receptor (GPER) in human prostate: expression site of the

estrogen receptor in the benign and neoplastic gland. *4*(1), 121-127. https://doi.org/https://doi.org/10.1111/andr.12131

Rago, V., Romeo, F., Giordano, F., Maggiolini, M., & Carpino, A. (2011). Identification of the estrogen receptor GPER in neoplastic and non-neoplastic human testes. *Reproductive Biology and Endocrinology*, *9*(1), 135-135.

Raies, A. B., & Bajic, V. B. (2016). In silico toxicology: computational methods for the prediction of chemical toxicity. *Wiley Interdiscip Rev Comput Mol Sci*, *6*(2), 147-172. https://doi.org/10.1002/wcms.1240

Ravi, L., & Kannabiran, K. J. I. J. o. M. S. (2016). A handbook on protein-ligand docking tool: AutoDock 4. 28-33.

Rejraji, H., Sion, B., Prensier, G., Carreras, M., Motta, C., Frenoux, J. M., . . . Drevet, J. R. (2006). Lipid remodeling of murine epididymosomes and spermatozoa during epididymal maturation. *Biol Reprod*, *74*(6), 1104-1113. https://doi.org/10.1095/biolreprod.105.049304

Revankar, C. M., Cimino, D. F., Sklar, L. A., Arterburn, J. B., & Prossnitz, E. R. (2005). A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science*, *307*(5715), 1625-1630.

Richter, C. A., Birnbaum, L. S., Farabollini, F., Newbold, R. R., Rubin, B. S., Talsness, C. E., . . . vom Saal, F. S. (2007). In vivo effects of bisphenol A in laboratory rodent studies. *Reproductive Toxicology*, *24*(2), 199-224. https://doi.org/https://doi.org/10.1016/j.reprotox.2007.06.004

Robaire, B., & Hamzeh, M. (2011). Androgen action in the epididymis. *J Androl*, *32*(6), 592-599. https://doi.org/10.2164/jandrol.111.014266

Robaire, B., & Hermo, L. (1988). Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. *The physiology of reproduction*, *1*, 999-1080.

Robaire, B., & Hinton, B. T. (2015a). Chapter 17 - The Epididymis. In T. M. Plant & A. J. Zeleznik (Eds.), *Knobil and Neill's Physiology of Reproduction (Fourth Edition)* (pp. 691-771). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-397175-3.00017-X

Robaire, B., & Hinton, B. T. (2015b). *The Epididymis* (Fourth Edi ed., Vol. 1). Elsevier. https://doi.org/10.1016/B978-0-12-397175-3.00017-X

Robertson, K. M., O'Donnell, L., Simpson, E. R., & Jones, M. E. E. (2002). The Phenotype of the Aromatase Knockout Mouse Reveals Dietary Phytoestrogens Impact

Significantly on Testis Function. *Endocrinology*, *143*(8), 2913-2921. https://doi.org/10.1210/ENDO.143.8.8957

Rodenas, M. C., & Cabas, I. (2017a). G {Protein}-{Coupled} {Estrogen} {Receptor} 1 {Regulates} {Human} {Neutrophil} {Functions}. *454981*. https://doi.org/10.1159/000454981

Rodenas, M. C., & Cabas, I. (2017b). G Protein-Coupled Estrogen Receptor 1 Regulates Human Neutrophil Functions. *454981*. https://doi.org/10.1159/000454981

Rodprasert, W., Toppari, J., & Virtanen, H. E. (2023). Environmental toxicants and male fertility. *Best Pract Res Clin Obstet Gynaecol*, *86*, 102298. https://doi.org/10.1016/j.bpobgyn.2022.102298

Ronda, A. C., & Boland, R. L. (2016). Intracellular Distribution and Involvement of GPR30 in the Actions of E2 on C2C12 Cells. *117*(3), 793-805. https://doi.org/https://doi.org/10.1002/jcb.25369

Rønnekleiv, O. K., & Kelly, M. J. J. K. s. i. r. b. (2013). Kisspeptin excitation of GnRH neurons. 113-131.

Rosenfeld, C. S. (2019). Male reproductive tract cilia beat to a different drummer. *Proc Natl Acad Sci U S A*, *116*(9), 3361-3363. https://doi.org/10.1073/pnas.1900112116

Rosenfeld, C. S., Ganjam, V. K., Taylor, J. A., Yuan, X., Stiehr, J. R., Hardy, M. P., & Lubahn, D. B. (1998). Transcription and translation of estrogen receptor-β in the male reproductive tract of estrogen receptor-α knock-out and wild-type mice. *Endocrinology*, *139*(6), 2982-2987.

Rowlands, D. J., Chapple, S., Siow, R. C., & Mann, G. E. (2011). Equol-stimulated mitochondrial reactive oxygen species activate endothelial nitric oxide synthase and redox signaling in endothelial cells: roles for F-actin and GPR30. *Hypertension*, *57*(4), 833-840. https://doi.org/10.1161/hypertensionaha.110.162198

Royer, C., Lucas, T. F. G., Lazari, M. F. M., & Porto, C. S. (2012). 17Beta-Estradiol Signaling and Regulation of Proliferation and Apoptosis of Rat Sertoli Cells1. *Biology of Reproduction*, *86*(4). https://doi.org/10.1095/biolreprod.111.096891

Rudolf, F. O., & Kadokawa, H. (2013). Expression of estradiol receptor, GPR30, in bovine anterior pituitary and effects of GPR30 agonist on GnRH-induced LH secretion. *Anim Reprod Sci*, *139*(1-4), 9-17. https://doi.org/10.1016/j.anireprosci.2013.04.003

Russell, D. W., & Wilson, J. D. (1994). Steroid 5α-reductase: two genes/two enzymes. *J Annual review of biochemistry*, *63*(1), 25-61.

Ryan, K. J. (1959). Biological aromatization of steroids. *J Biol Chem*, *234*(2), 268-272.

Sackie, L. (2023). Role of GPER in Cadmium-Induced Phosphorylation of ERK1/2 in Ovarian Adenocarcinoma.

Samavat, H., & Kurzer, M. S. (2015). Estrogen metabolism and breast cancer. *Cancer Lett*, *356*(2 Pt A), 231-243. https://doi.org/10.1016/j.canlet.2014.04.018

Sandner, F., Welter, H., Schwarzer, J. U., Köhn, F. M., Urbanski, H. F., & Mayerhofer, A. (2014). Expression of the oestrogen receptor GPER by testicular peritubular cells is linked to sexual maturation and male fertility. *2*(5), 695-701. https://doi.org/https://doi.org/10.1111/j.2047-2927.2014.00243.x

Santolla, M. F., Lappano, R., De Marco, P., Pupo, M., Vivacqua, A., Sisci, D., . . . Maggiolini, M. (2012). G protein-coupled estrogen receptor mediates the up-regulation of fatty acid synthase induced by 17β-estradiol in cancer cells and cancer-associated fibroblasts. *J Biol Chem*, *287*(52), 43234-43245. https://doi.org/10.1074/jbc.M112.417303

Sasaki, T., Franco, O. E., & Hayward, S. W. (2018). Prostate—Overview. *Encyclopedia of Reproduction*, 309-314. https://doi.org/10.1016/B978-0-12-801238-3.64595-6

Saunders, P. T. K., Fisher, J. S., …, R. M. S. J. o., & undefined. (1998). Expression of oestrogen receptor beta (ERb) occurs in multiple cell types, including some germ cells, in the rat testis. *researchgate.net*. https://doi.org/10.1677/joe.0.156R013

Saunders, P. T. K., Sharpe, R. M., Williams, K., Macpherson, S., Urquart, H., Irvine, D. S., & Millar, M. R. (2001). Differential expression of oestrogen receptor α and β proteins in the testes and male reproductive system of human and non-human primates. *Molecular Human Reproduction*, *7*(3), 227-236.

Scaia, M. F., Volonteri, M. C., Czuchlej, S. C., & Ceballos, N. R. (2015). Effect of estradiol on apoptosis, proliferation and steroidogenic enzymes in the testes of the toad Rhinella arenarum (Amphibia, Anura). *GENERAL AND COMPARATIVE ENDOCRINOLOGY*, *221*, 244-254. https://doi.org/10.1016/J.YGCEN.2014.12.016

Scaling, A. L., Prossnitz, E. R., & Hathaway, H. J. (2014). GPER mediates estrogen-induced signaling and proliferation in human breast epithelial cells and normal and malignant breast. *Horm Cancer*, *5*(3), 146-160. https://doi.org/10.1007/s12672-014-0174-1

Schjenken, J. E., Sharkey, D. J., & Robertson, S. A. (2018). Seminal Vesicle—Secretion. *Encyclopedia of Reproduction*, 349-354. https://doi.org/10.1016/B978-0-12-801238-3.64600-7

Schmitz, V., Bauerschmitz, G., Gallwas, J., & Gründker, C. (2022). Suppression of G Protein-coupled Estrogen Receptor 1 (GPER1) Enhances the Anti-invasive Efficacy of

Selective ERβ Agonists. *Anticancer Res*, *42*(11), 5187-5194. https://doi.org/10.21873/anticanres.16025

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., . . . Karsch-Mizrachi, I. (2020). NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database (Oxford)*, *2020*. https://doi.org/10.1093/database/baaa062

Schön, J., & Blottner, S. (2008). Estrogens are involved in seasonal regulation of spermatogenesis and sperm maturation in roe deer (Capreolus capreolus). *GENERAL AND COMPARATIVE ENDOCRINOLOGY*, *159*(2-3), 257-263. https://doi.org/10.1016/j.ygcen.2008.09.008

Schulster, M., Bernie, A. M., & Ramasamy, R. (2016). The role of estradiol in male reproductive function. *Asian J Androl*, *18*(3), 435-440. https://doi.org/10.4103/1008-682x.173932

Schwartz, N., Verma, A., Bivens, C. B., Schwartz, Z., & Boyan, B. D. (2016). Rapid steroid hormone actions via membrane receptors. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1863*(9), 2289-2298. https://doi.org/https://doi.org/10.1016/j.bbamcr.2016.06.004

Schweikert, H.-U. (2019). Estrogen in the Male: Nature, Sources, and Biological Effects. *Reference Module in Neuroscience and Biobehavioral Psychology*. https://doi.org/10.1016/B978-0-12-809324-5.23255-0

Serre, V., & Robaire, B. J. B. o. r. (1998). Segment-specific morphological changes in aging Brown Norway rat epididymis. *58*(2), 497-513.

Shang, Y., & Brown, M. (2002). Molecular determinants for the tissue specificity of SERMs. *Science*, *295*(5564), 2465-2468. https://doi.org/10.1126/science.1068537

Sharma, G., Hu, C., Brigman, J. L., Zhu, G., Hathaway, H. J., & Prossnitz, E. R. (2013). GPER deficiency in male mice results in insulin resistance, dyslipidemia, and a proinflammatory state. *Endocrinology*, *154*(11), 4136-4145. https://doi.org/10.1210/en.2013-1357

Sharma, G., & Prossnitz, E. R. (2011). Mechanisms of estradiol-induced insulin secretion by the G protein-coupled estrogen receptor GPR30/GPER in pancreatic beta-cells. *Endocrinology*, *152*(8), 3030-3039. https://doi.org/10.1210/en.2011-0091

Sharpe, R. M., Skakkebaek, N. E. J. F., & sterility. (2008). Testicular dysgenesis syndrome: mechanistic insights and potential new downstream effects. *89*(2), e33-e38.

Shayu, D., & Rao, A. J. (2006). Expression of functional aromatase in the epididymis: role of androgens and {LH} in modulation of expression and activity. *MOLECULAR AND CELLULAR ENDOCRINOLOGY*, *249*(1-2), 40-50.

Sheng, Z.-G., & Zhu, B.-Z. (2011). Low Concentrations of Bisphenol A Induce Mouse Spermatogonial Cell Proliferation by G Protein–Coupled Receptor 30 and Estrogen Receptor-α. *119*(12), 1775-1780. https://doi.org/doi:10.1289/ehp.1103781

Shughrue, P. J., Komm, B., & Merchenthaler, I. (1996). The distribution of estrogen receptor-β mRNA in the rat hypothalamus. *Steroids*, *61*(12), 678-681. https://doi.org/10.1016/S0039-128X(96)00222-X

Simpson, E. R., Misso, M., Hewitt, K. N., Hill, R. A., Boon, W. C., Jones, M. E., . . . Clyne, C. D. (2005). Estrogen—the Good, the Bad, and the Unexpected. *Endocrine Reviews*, *26*(3), 322-330. https://doi.org/10.1210/er.2004-0020 %J Endocrine Reviews

Singh, R., & Singh, K. (2017). Male infertility: Understanding, causes and treatment. *Male Infertility: Understanding, Causes and Treatment*, 1-497. https://doi.org/10.1007/978-981-10-4017-7

Sirianni, R., Chimento, A., Ruggiero, C., De Luca, A., Lappano, R., Andò, S., . . . Pezzi, V. (2008). The novel estrogen receptor, G protein-coupled receptor 30, mediates the proliferative effects induced by 17β-estradiol on mouse spermatogonial GC-1 cell line. *Endocrinology*, *149*(10), 5043-5051.

Sirotkin, A. V., & Harrath, A. H. (2014). Phytoestrogens and their effects. *European Journal of Pharmacology*, *741*, 230-236. https://doi.org/https://doi.org/10.1016/j.ejphar.2014.07.057

Sirtori, C. R., Arnoldi, A., & Johnson, S. K. (2005). Phytoestrogens: End of a tale? *Annals of Medicine*, *37*(6), 423-438. https://doi.org/10.1080/07853890510044586

Smith, H. O., Arias-Pulido, H., Kuo, D. Y., Howard, T., Qualls, C. R., Lee, S. J., . . . Prossnitz, E. R. (2009). GPR30 predicts poor survival for ovarian cancer. *Gynecol Oncol*, *114*(3), 465-471. https://doi.org/10.1016/j.ygyno.2009.05.015

Smith, L. B., Walker, W. H., & O'Donnell, L. (2015). Hormonal regulation of spermatogenesis through Sertoli cells by androgens and estrogens. *Sertoli Cell Biology*, 175-200. https://doi.org/10.1016/B978-0-12-417047-6.00006-5

Soranzo, L., Dadoune, J., & Fain-Maurel, M. J. R., Nutrition, Developpement. (1982). Segmentation of the epididymal duct in mouse: an ultrastructural study. *22*(6), 999-1012.

Sorokina, M., Merseburger, P., Rajan, K., Yirik, M. A., & Steinbeck, C. (2021). COCONUT online: Collection of Open Natural Products database. *Journal of Cheminformatics*, *13*(1), 2. https://doi.org/10.1186/s13321-020-00478-9

Speight, J. G. (2017). Chapter 4 - Sources and Types of Organic Pollutants. In J. G. Speight (Ed.), *Environmental Organic Chemistry for Engineers* (pp. 153-201). Butterworth-Heinemann. https://doi.org/https://doi.org/10.1016/B978-0-12-804492-6.00004-6

Spinnler, K., Köhn, F. M., Schwarzer, U., & Mayerhofer, A. (2010). Glial cell line-derived neurotrophic factor is constitutively produced by human testicular peritubular cells and may contribute to the spermatogonial stem cell niche in man. *Human Reproduction*, *25*(9), 2181-2187. https://doi.org/10.1093/humrep/deq170 %J Human Reproduction

Srivastav, A., Singh, B., Chandra, A., Jamal, F., Khan, M. Y., & Chowdhury, S. R. J. R. (2004). Partial characterization, sperm association and significance of N-and O-linked glycoproteins in epididymal fluid of rhesus monkeys (Macaca mulatta). *127*(3), 343-357.

Steger, K., & Weidner, W. (2011). Anatomy of the Male Reproductive System. *Practical Urology: Essential Principles and Practice*, 57-68. https://doi.org/10.1007/978-1-84882-034-0_4

Sullivan, R., & Belleannée, C. (2017). Role of the epididymis in sperm maturation. *The Sperm Cell: Production, Maturation, Fertilization, Regeneration*(May), 73-87. https://doi.org/10.1017/9781316411124.007

Sullivan, R., Saez, F., Girouard, J., & Frenette, G. (2005). Role of exosomes in sperm maturation during the transit along the male reproductive tract. *35*, 1-10. https://doi.org/10.1016/j.bcmd.2005.03.005

Sun, M., Xie, H. F., Tang, Y., Lin, S. Q., Li, J. M., Sun, S. N., . . . Jian, D. (2017). G protein-coupled estrogen receptor enhances melanogenesis via cAMP-protein kinase (PKA) by upregulating microphthalmia-related transcription factor-tyrosinase in melanoma. *J Steroid Biochem Mol Biol*, *165*(Pt B), 236-246. https://doi.org/10.1016/j.jsbmb.2016.06.012

Tang, H., Zhang, Q., Yang, L., Dong, Y., Khan, M., Yang, F., . . . Wang, R. (2014). Molecular and Cellular Endocrinology GPR30 mediates estrogen rapid signaling and neuroprotection. *MOLECULAR AND CELLULAR ENDOCRINOLOGY*, *387*(1-2), 52-58. https://doi.org/10.1016/j.mce.2014.01.024

Teerds, K. J., & Huhtaniemi, I. T. (2015). Morphological and functional maturation of Leydig cells: from rodent models to primates. *Hum Reprod Update*, *21*(3), 310-328. https://doi.org/10.1093/humupd/dmv008

Thomas, B.-O. (1902). 1. On the Mammals collected during the Whiater Expedition to Tripoli. *72*(3), 2-13. https://doi.org/https://doi.org/10.1111/j.1469-7998.1902.tb08215.x

Thomas, P., & Dong, J. (2006). Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: A potential novel mechanism of endocrine disruption. *The Journal of Steroid Biochemistry and Molecular Biology*, *102*(1), 175-179. https://doi.org/https://doi.org/10.1016/j.jsbmb.2006.09.017

Thomas, P., & Dong, J. (2006). Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. *J Steroid Biochem Mol Biol*, *102*(1-5), 175-179. https://doi.org/10.1016/j.jsbmb.2006.09.017

Thomas, P., Dressing, G., Pang, Y., Berg, H., Tubbs, C., Benninghoff, A., & Doughty, K. (2006). Progestin, estrogen and androgen G-protein coupled receptors in fish gonads. *Steroids*, *71*(4), 310-316. https://doi.org/10.1016/j.steroids.2005.09.015

Thomas, P., Pang, Y., Filardo, E. J., & Dong, J. (2005). Identity of an Estrogen Membrane Receptor Coupled to a G Protein in Human Breast Cancer Cells. *Endocrinology*, *146*(2), 624-632. https://doi.org/10.1210/en.2004-1064 %J Endocrinology

Thompson, R. P., Nilsson, E. E., & Skinner, M. K. (2018). Peritubular Myoid Cells in Testis. *Encyclopedia of Reproduction*, 42-46. https://doi.org/10.1016/B978-0-12-801238-3.64361-1

Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E., & Chambon, P. (1989). The human estrogen receptor has two independent nonacidic transcriptional activation functions. *Cell*, *59*(3), 477-487. https://doi.org/10.1016/0092-8674(89)90031-7

Tortora, G. J., & Derrickson, B. (2014). Principles of Anatomy & Physiology 14th Edition. *Wiley*, 1237-1237.

Triplett, K. D., Pokhrel, S., Castleman, M. J., Daly, S. M., Elmore, B. O., Joyner, J. A., . . . Hall, P. R. (2019). GPER activation protects against epithelial barrier disruption by Staphylococcus aureus α-toxin. *Sci Rep*, *9*(1), 1343. https://doi.org/10.1038/s41598-018-37951-3

Truss, M., & Beato, M. (1993). Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. *Endocr Rev*, *14*(4), 459-479. https://doi.org/10.1210/edrv-14-4-459

Tsai, C. C., Tey, S. L., Chang, L. C., Su, Y. T., Lin, K. J., & Huang, S. C. (2018). Estradiol mediates relaxation of porcine lower esophageal sphincter. *Steroids*, *136*, 56-62. https://doi.org/10.1016/j.steroids.2018.05.001

Usselman, M. C., & Cone, R. A. (1983). Rat Sperm are Mechanically Immobilized in the Caudal Epididymis by "lmmobilin," a High Molecular Weight Glycoprotein. *Biology of Reproduction*, *29*(5), 1241-1253. https://doi.org/10.1095/biolreprod29.5.1241 %J Biology of Reproduction

Vadnais, M. L., Cao, W., Aghajanian, H. K., Haig-Ladewig, L., Lin, A. M., Al-Alao, O., & Gerton, G. L. (2014). Adenine nucleotide metabolism and a role for AMP in modulating flagellar waveforms in mouse sperm. *Biol Reprod*, *90*(6), 128. https://doi.org/10.1095/biolreprod.113.114447

Valdés-Jiménez, A., Peña-Varas, C., Borrego-Muñoz, P., Arrue, L., Alegría-Arcos, M., Nour-Eldin, H., . . . Ramírez, D. J. M. (2021). Psc-db: A structured and searchable 3d-database for plant secondary compounds. *26*(4), 1124.

Varadi, M., Anyango, S., Deshpande, M., Nair, S., Natassia, C., Yordanova, G., . . . Velankar, S. (2021). AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Res*, *50*(D1), D439-D444. https://doi.org/10.1093/nar/gkab1061 %J Nucleic Acids Research

Varadi, M., Bertoni, D., Magana, P., Paramval, U., Pidruchna, I., Radhakrishnan, M., . . . Velankar, S. (2023). AlphaFold Protein Structure Database in 2024: providing structure coverage for over 214 million protein sequences. *Nucleic Acids Res*, *52*(D1), D368-D375. https://doi.org/10.1093/nar/gkad1011 %J Nucleic Acids Research

Vaucher, L., Funaro, M. G., Mehta, A., Mielnik, A., Bolyakov, A., Prossnitz, E. R., . . . Paduch, D. A. (2014). Activation of GPER-1 estradiol receptor downregulates production of testosterone in isolated rat Leydig cells and adult human testis. *PloS one*, *9*(4), e92425-e92425.

Vivacqua, A., Bonofiglio, D., Albanito, L., Madeo, A., Rago, V., Carpino, A., . . . Maggiolini, M. (2006a). 17beta-estradiol, genistein, and 4-hydroxytamoxifen induce the proliferation of thyroid cancer cells through the g protein-coupled receptor GPR30. *Mol Pharmacol*, *70*(4), 1414-1423. https://doi.org/10.1124/mol.106.026344

Vivacqua, A., Bonofiglio, D., Recchia, A. G., Musti, A. M., Picard, D., Andò, S., & Maggiolini, M. (2006b). The G protein-coupled receptor GPR30 mediates the

proliferative effects induced by 17beta-estradiol and hydroxytamoxifen in endometrial cancer cells. *Mol Endocrinol*, *20*(3), 631-646. https://doi.org/10.1210/me.2005-0280

Walczak-Jędrzejowska, R., Forma, E., Oszukowska, E., Bryś, M., Marchlewska, K., Kula, K., & Słowikowska-Hilczer, J. (2022). Expression of G-Protein-Coupled Estrogen Receptor (GPER) in Whole Testicular Tissue and Laser-Capture Microdissected Testicular Compartments of Men with Normal and Aberrant Spermatogenesis. *Biology (Basel)*, *11*(3). https://doi.org/10.3390/biology11030373

Wang, C., Prossnitz, E. R., & Roy, S. K. (2007). Expression of G protein-coupled receptor 30 in the hamster ovary: differential regulation by gonadotropins and steroid hormones. *Endocrinology*, *148*(10), 4853-4864. https://doi.org/10.1210/en.2007-0727

Wang, C., Zhang, J., Li, Q., Zhang, T., & Deng, Z. (2017). Low concentration of {BPA} induces mice spermatocytes apoptosis. *8*(30), 49005-49015.

Wang, H., Huang, P., Lie, T., Li, J., Hutz, R. J., Li, K., & Shi, F. (2010). Reproductive toxicity of acrylamide-treated male rats. *Reproductive Toxicology*, *29*(2), 225-230. https://doi.org/https://doi.org/10.1016/j.reprotox.2009.11.002

Wang, J., Li, H. Y., Shen, S. Y., Zhang, J. R., Liang, L. F., Huang, H. J., . . . Yu, J. (2021). The antidepressant and anxiolytic effect of GPER on translocator protein (TSPO) via protein kinase a (PKA) signaling in menopausal female rats. *J Steroid Biochem Mol Biol*, *207*, 105807. https://doi.org/10.1016/j.jsbmb.2020.105807

Waqas, M. S. (2021). Spermatogenesis. *Bovine Reproduction*, 32-39. https://doi.org/10.1002/9781119602484.CH3

Watson, C. S., Alyea, R. A., Jeng, Y. J., & Kochukov, M. Y. (2007). Nongenomic actions of low concentration estrogens and xenoestrogens on multiple tissues. *MOLECULAR AND CELLULAR ENDOCRINOLOGY*, *274*(1), 1-7. https://doi.org/https://doi.org/10.1016/j.mce.2007.05.011

Wetsel, W. C., Valença, M. M., Merchenthaler, I., Liposits, Z., López, F. J., Weiner, R. I., . . . Negro-Vilar, A. (1992). Intrinsic pulsatile secretory activity of immortalized luteinizing hormone-releasing hormone-secreting neurons. *89*(9), 4149-4153. https://doi.org/doi:10.1073/pnas.89.9.4149

Whitcomb, V., Wauson, E., Christian, D., Clayton, S., Giles, J., & Tran, Q. K. (2020). Regulation of beta adrenoceptor-mediated myocardial contraction and calcium dynamics by the G protein-coupled estrogen receptor 1. *Biochem Pharmacol*, *171*, 113727. https://doi.org/10.1016/j.bcp.2019.113727

Whitney, K. M. (2018). Male Accessory Sex Glands. *Boorman's Pathology of the Rat*, 579-587. https://doi.org/10.1016/B978-0-12-391448-4.00029-0

WHO. (2023). *Infertility prevalence estimates, 1990–2021*. World Health Organization. Retrieved 8 march 2024 from https://www.who.int/publications/i/item/978920068315

Wróbel, A. M., & Gregoraszczuk, E. Ł. (2015). Action of methyl-, propyl- and butylparaben on GPR30 gene and protein expression, cAMP levels and activation of ERK1/2 and PI3K/Akt signaling pathways in MCF-7 breast cancer cells and MCF-10A non-transformed breast epithelial cells. *Toxicology Letters*, *238*(2), 110-116. https://doi.org/https://doi.org/10.1016/j.toxlet.2015.08.001

Yang, W., Tan, W., Zheng, J., Zhang, B., Li, H., & Li, X. (2018). MEHP promotes the proliferation of cervical cancer via GPER mediated activation of Akt. *European Journal of Pharmacology*, *824*, 11-16. https://doi.org/https://doi.org/10.1016/j.ejphar.2018.01.040

Yang, W. R., Zhu, F. W., Zhang, J. J., Wang, Y., Zhang, J. H., Lu, C., & Wang, X. Z. (2017). PI3K/Akt Activated by GPR30 and Src Regulates 17β-Estradiol-Induced Cultured Immature Boar Sertoli Cells Proliferation. *Reprod Sci*, *24*(1), 57-66. https://doi.org/10.1177/1933719116649696

Yohannan, S., Faham, S., Yang, D., Whitelegge, J. P., & Bowie, J. U. (2004). The evolution of transmembrane helix kinks and the structural diversity of G protein-coupled receptors. *Proc Natl Acad Sci U S A*, *101*(4), 959-963. https://doi.org/10.1073/pnas.0306077101

YOUNG, L. S., SPEIGHT, A., CHARLTON, H. M., & CLAYTON, R. N. (1983). Pituitary Gonadotropin-Releasing Hormone Receptor Regulation in the Hypogonadotrophic Hypogonadal (hpg) Mouse*. *Endocrinology*, *113*(1), 55-61. https://doi.org/10.1210/endo-113-1-55 %J Endocrinology

Yu, X., Filardo, E. J., & Shaikh, Z. A. (2010). The membrane estrogen receptor GPR30 mediates cadmium-induced proliferation of breast cancer cells. *Toxicology and Applied Pharmacology*, *245*(1), 83-90. https://doi.org/https://doi.org/10.1016/j.taap.2010.02.005

Yu, X., Ma, H., Barman, S. A., Liu, A. T., Sellers, M., Stallone, J. N., . . . Han, G. (2011). Activation of G protein-coupled estrogen receptor induces endothelium-independent relaxation of coronary artery smooth muscle. *Am J Physiol Endocrinol Metab*, *301*(5), E882-888. https://doi.org/10.1152/ajpendo.00037.2011

Yuan, S., Liu, Y., Peng, H., Tang, C., Hennig, G. W., Wang, Z., . . . Yan, W. (2019). Motile cilia of the male reproductive system require miR-34/miR-449 for development and function to generate luminal turbulence. *Proc Natl Acad Sci U S A*, *116*(9), 3584-3593. https://doi.org/10.1073/pnas.1817018116

Zekas, E., & Prossnitz, E. R. (2015). Estrogen-mediated inactivation of FOXO3a by the G protein-coupled estrogen receptor GPER. *BMC Cancer*, *15*(1), 702. https://doi.org/10.1186/s12885-015-1699-6

Zhang, H., Lu, S., Xu, R., Tang, Y., Liu, J., Li, C., . . . Ma, B. (2020). Mechanisms of Estradiol-induced EGF-like Factor Expression and Oocyte Maturation via G Protein-coupled Estrogen Receptor. *Endocrinology*, *161*(12). https://doi.org/10.1210/endocr/bqaa190

Zhang, H., Sheng, X., Hu, X., Li, X., Zhang, M., Li, B., . . . Taya, K. (2010). Seasonal changes in spermatogenesis and immunolocalization of cytochrome P450 17α-Hydroxylase/c17-20 lyase and cytochrome P450 aromatase in the wild male ground squirrel (Citellus dauricus Brandt). *Journal of Reproduction and Development*, 1002260252-1002260252.

Zheng, Y., Wu, M., Gao, T., Meng, L., Ding, X., Meng, Y., . . . Rong, W. (2020). GPER-Deficient Rats Exhibit Lower Serum Corticosterone Level and Increased Anxiety-Like Behavior. *Neural Plast*, *2020*, 8866187. https://doi.org/10.1155/2020/8866187

Zhou, Q., Nie, R., Prins, G. S., Saunders, P. T. K., Katzenellenbogen, B. S., & Hess, R. E. X. A. (2002). Localization of androgen and estrogen receptors in adult male mouse reproductive tract. *Journal of Andrology*, *23*(6), 870-881.

Zhou, X., Wang, S., Wang, Z., Feng, X., Liu, P., Lv, X. B., . . . Guan, K. L. (2015). Estrogen regulates Hippo signaling via GPER in breast cancer. *J Clin Invest*, *125*(5), 2123-2135. https://doi.org/10.1172/jci79573

Zielińska, M., Fichna, J., Bashashati, M., Habibi, S., Sibaev, A., Timmermans, J.-P., & Storr, M. (2017). G protein-coupled estrogen receptor and estrogen receptor ligands regulate colonic motility and visceral pain. *29*(7), e13025. https://doi.org/https://doi.org/10.1111/nmo.13025

Zondek, B. (1934). Mass Excretion of Œstrogenic Hormone in the Urine of the Stallion. *Nature*, *133*(3354), 209-210. https://doi.org/10.1038/133209a0

Zucchetti, A. E., Barosso, I. R., Boaglio, A. C., Basiglio, C. L., Miszczuk, G., Larocca, M. C., . . . Pozzi, E. J. (2014). G-protein-coupled receptor 30/adenylyl cyclase/protein

kinase A pathway is involved in estradiol 17ß-D-glucuronide-induced cholestasis. *Hepatology*, *59*(3), 1016-1029. https://doi.org/10.1002/hep.26752

•

Appendices

1. Structure of the tested chemicals

Type	Name	Structure	Source
Known natural and synthetic ligands	E2		PubChem CID: 5757
	Fulvestrant		PubChem CID: 104741
	Tamoxifen		PubChem CID: 2733526
	G1		PubChem SID: 481131179
	G36		PubChem CID: 73755224

Type	Name	Structure	Source
Phytoestrogens	Coumestrol		PubChem CID: 5281707
	Sesamol		PubChem CID: 68289
	Genistein		Coconut: CNP0174866
	Resveratrol		Coconut: CNP0247661
	Anethole		PubChem CID: 637563
	Lupalbigenin		PubChem CID: 10001388
	Biochanin A		PubChem CID: 5280373
	Piceatannol		PubChem CID: 667639
	Enterodiol		PubChem CID: 115089

Type	Name	Structure	Source
Phytoestrogens	Wedelolactone		PubChem CID: 5281813
	Eugenol		PubChem CID: 3314
	Glabrene		PubChem CID: 480774
Heavy metals	Lead Acetate		PubChem CID: 9317
	Dimethylmercury	$H_3C-Hg-CH_3$	PubChem CID: 11645
	Cadmium Chloride	Cl–Cd–Cl	PubChem CID: 24947
Pesticides	DDT		PubChem CID: 3036
	Acetamiprid		PubChem CID: 213021

Type	Name	Structure	Source
Pesticides	Fenvalerate		PubChem CID: 3347
	Propoxur		PubChem CID: 4944
	Captan		PubChem CID: 8606
Food additives	Furan		PubChem CID: 8029
	Monosodium glutamate		PubChem CID: 23672308
	Indigo carmin		PubChem CID: 2723854
	Carboxymethyl cellulose		PubChem CID: 24748
	Quinoline Yellow		PubChem CID: 6731

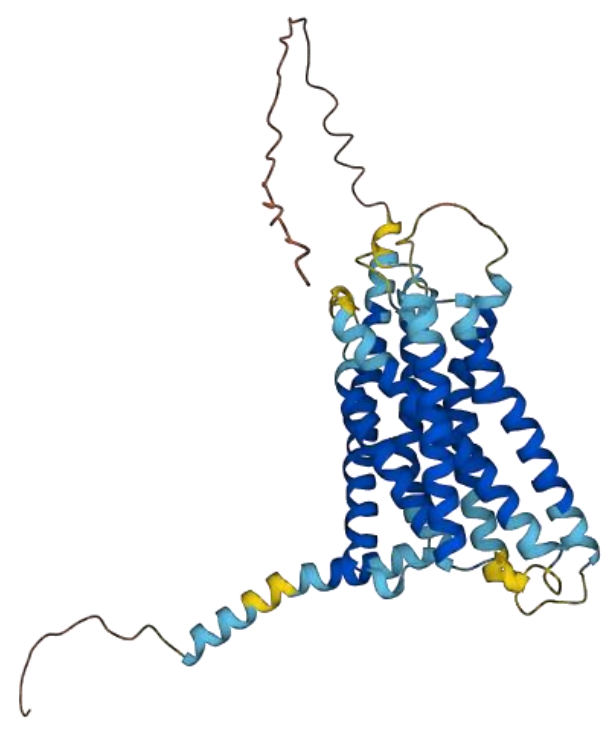
Type	Name	Structure	Source
Personal care chemicals	Dimethyl Phthalate		PubChem CID: 8554
	Homosalate		PubChem CID: 8362
	Methyl paraben		PubChem CID: 7456
	Tricresyl phosphate		PubChem CID: 6529
	Resorcinol		PubChem CID: 5054
Plasticizers and flame retardants	Bisphenol A (BPA)		PubChem CID: 6623
	Acrylamide		PubChem CID: 6579
	Decabromodiphenyl oxide		PubChem CID: 14410

Type	Name	Structure	Source
Plasticizers and flame retardants	Polychlorinated biphenyls (PCBs)		PubChem CID: 40470
	Perfluorooctane sulfonamide PFOSA		PubChem CID: 69785
Chemotherapy	Estramustine phosphate		PubChem CID: 259329
	Cyclophosphamide (CPM)		PubChem CID: 2907
	Doxorubicin		PubChem CID: 31703
	Colchicine		PubChem CID: 6167

Chemotherapy	Methotrexate		PubChem CID: 126941
	Carboplatin		PubChem CID: 426756

2. Structure of the GPER1

AlphaFoldDB **:** AF-O08878-F1 (https://alphafold.ebi.ac.uk/entry/O08878)



3. Fixator and staining preparation

Bouin's fixator

Content

Distillated water 100ml

Neutral copper acetate 2.5g

Picric acid 4g

Formalin 40% 10ml

Acetic acid 15ml

Preparation

Dissolve the cooper acetate in 100ml of distilled water, then add the picric acid while stirring.

After dissolution, filter and add formalin and acetic acid. The fixation duration is 2 to 3 days.

Dehydration after fixation

The pieces are dehydrated using a series of alcohol at increasing concentration:

- 1 bath of 70° alcohol .... 10 minutes
- 2 baths of 90° alcohol ....10 minutes each
- 3 baths of 100° alcohol .... 10 min each

Inclusion and clarification (removal of alcohol using a paraffin solvent)

- 3 toluene baths ....10 minutes each

Waxing: 2 baths of 1 hour each, paraffin melted in the oven at (58-60) °C.

Block making in Leuckart bars.

Masson's trichrome stain

Reagents

- Groat's haematoxylin.

- Fuchsine-Ponceau.

- Orange G-Molybdic.

- Acetic light green.

- 1% aqueous solution of acetic acid.

Operating mode

1- Deparaffinize, hydrate.

2- Stain with Croat's haematoxylin for 2 to 5 minutes.

3- Wash in running water for 5 minutes.

4- Colour with the Fuchsine-Ponceau mixture for 5 minutes.

5- Rinse with acetic water.

6- Colour with G-Molybdic orange for 5 minutes.

7- Rinse with acetic water.

8- Colour with light green for 5 minutes.

9- Wash with acetic water.

10- Dehydrate. To go up.

Results:

The nuclei are coloured black, the acidophilic cytoplasm and the nucleoli pink, the secretions are either red or green. The muscles are red and the collagen fibres green.

Preparation of reagents:

1- Groat's haematoxylin (3-month storage)

- Concentrated sulfuric acid .... 0.8 ml

- Iron alum 1g

- Distilled water .... 50 ml
- Haematoxylin ....0.5 g
- Alcohol 95° .... 50 ml

After dissolution, mix solutions and leave for 1 hour and filter.

2- Fuchsine-Ponceau (unlimited storage)

- Acid fuchsin 0.1g
- Ponceau 0.2g
- Distilled water .... 300 ml

After dissolution add: - Acetic acid... 0.6 ml.

3- Orange G-Molybdic (unlimited storage)

- Orange G ....2 g
- Distilled water .... 100 ml
- Phosphomolybdic acid ....1 g

4- Acetic light green (unlimited conservation)

- Light green ....0.1 g
- Distilled water .... 100 ml
- Acetic acid .... 2 ml

Scientific communications

National conference papers:

- **Meriem Fernin**i, Mansouria Belhocine, Rafik Menad. The interactions of some environmental pollutants with the human g-coupled oestrogen receptor 1: *in silico* approach. 1er Webinar National Sante & Environnement SENASE, November 2022. University of Mhamed Bougara Boumerdes.
- **Meriem Fernin**i, Kamilia Zineb Mahloul, Zineb Loughreit, Yasmine Trad, Rafik Menad. Le GPER1 constitue-t-il véritablement un récepteur d'œstrogènes ? Approche par docking moléculaire. The Second National Webinar on Biodiversity, Biochemistry & Environmental Microbiology, October 2021. University of Amar Thelidji Laghouat.

International conference papers:

- **Meriem Fernin**i, Mansouria Belhocine, Rafik Menad. *In silico* approach to predict the interactions between some plant's active compounds and the human g-coupled oestrogen receptor 1. The first International Seminar on Bioresources, Nutrition, and Health (ISBNH, 2022), November 2022. University of Abdelhamid Ibn Badis Mostaganem.

Research articles:

- **Fernini, M.,** Menad, R., Belhocine, M., Lakabi, L., Smaï, S., Gernigon-Spychalowicz, T., Khammar, F., Bonnet, X., Exbrayat, J. M., Moudilou, E. (2023). Seasonal variations of testis anatomy and of G-coupled oestrogen receptor 1 expression in *Gerbillus gerbillus*. Anatomia, Histologia, Embryologia, 52(6), 1016-1028. DOI: 10.1111/ahe.12962
- **Fernini, M.,** Menad, R., Belhocine, M., Medjdoub-Bensaad, F., Lakabi, L., Smaï, S., Gernigon-Spychalowicz, T., Exbrayat, J. M., Moudilou, E. (2023, October). Seasonal variation of gper1 expression in efferent ducts of *Gerbillus gerbillus*. In Annales d'Endocrinologie (Vol. 84, No. 5, p. 618). Elsevier Masson. DOI: 10.1016/j.ando.2023.07.314

- Menad, R., **Fernini, M.**, Lakabi, L., Soudani, N., Smaï, S., Bonnet, X., ... & Exbrayat, J. M. (2020). G protein-coupled oestrogen receptor 1, oestrogen receptors and androgen receptors in the sand rat (Psammomys obesus) efferent ducts. *Folia morphologica*, *79*(4), 756-766.
- Menad, R., Lakabi, L., **Fernini, M.**, Smaï, S., Gernigon Spychalowicz, T., Khammar, F., ... & Exbrayat, J. M. (2021). L'apoptose dans l'épididyme du rat des sables Psammomys obesus, Cretzschmar, 1828 : effets des variations saisonnières, castration et ligature des canaux efférents.
- Menad, R., **Fernini, M.**, Lakabi, L., Smaï, S., Gernigon-Spychalowicz, T., Farida, K., ... & Exbrayat, J. M. (2021). Androgen and estrogen receptors immunolocalization in the sand rat (Psammomys Obesus) cauda epididymis. *Acta Histochemica*, *123*(2), 151683.