Endocrinology of male and female sex development and reproduction

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Introduction

The development and function of the hypothalamic-pituitary-gonadal (HPG) axis and gonads underpin biological sex, influence gender identity, and are major determinants of our general health, quality of life (QoL) and reproductive capacity. Over 25 million people, or an estimated 1 out of 7 couples in the EU are affected by infertility, which is due to congenital or acquired conditions of the genital organ structures or function, reproductive hormone imbalances, or a combination of these [10.1016/j.clinbiochem.2018.03.012]. Lifestyle factors such as smoking, excessive alcohol intake and obesity can affect fertility and the HPG axis. Exposure to environmental pollutants and toxins can be directly toxic to gametes (eggs and sperm), resulting in their decreased numbers and poor quality, and can affect the embryonic development of reproductive organs and/or the development or function of the HPG axis [10.1007/s00204-016-1849-x][10.1016/j.envres.2021.112040]. In addition, many individuals remain involuntarily childless for non-medical reasons.

The disease burden resulting from these conditions is high. Worldwide, age-standardised, disability-adjusted life-years (DALYs) of infertility have increased from 1990 until 2017 by 0.396% per year for women and by 0.296% per year for men, with the highest increase in women living in high-income countries [10.18632/aging.102497]. Infertility and its treatments reinforce gender inequalities and work capacity [10.1016/S0140-6736(20)32667-2] .

Human sex development and variations in sex development

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Epidemiology, societal impact and research state of the art

Human sex development is driven by genetic, epigenetic and hormonal factors that are only partially understood. Numerical variations of the sex chromosomes are relatively frequent: around 1/500 male fetuses have a 47,XXY karyotype (Klinefelter syndrome, KS), many of which remain undiagnosed [10.1210/er.2017-00212][10.1007/s40618-016-0541-6] ; around 1/2000 women have (partial) loss of an X chromosome (Turner syndrome, TS) [10.1530/EJE-16-0582]. More rare differences/disorders of sex development (DSD), estimated at 1/4000 live births, have been associated with the congenital absence of a uterus (Mayer-Rokitanski-Küster-Hauser (MRKH) syndrome), and with variations in multiple genes that result in disrupted gonadal differentiation into either a testis or ovary, with disrupted secondary sex organ differentiation due to sex steroid production defects, or with defects in androgen signaling [10.1136/adc.2006.098319][10.1038/s41585-023-00754-x]. Intra-uterine growth retardation due to placental insufficiency is associated with a 30% risk of genital variation and decreased reproductive capacity in males [10.1016/j.ebiom.2022.104119]. Environmental factors and endocrine disrupting chemicals (EDCs) have a major impact on both male and female sex development, mini-puberty, puberty and fertility, and are addressed in the chapter Environmental endocrinology.

Many individuals who have conditions that negatively affect sex development need lifelong hormone replacement therapy and medical care for secondary conditions resulting from lack of sex steroid effects on peripheral target tissues, e.g. the cardiovascular and musculoskeletal systems, and the brain [10.1016/S0140-6736(20)32667-2][10.1210/endrev/bnab043]. Mental health problems are reported by 80%, and most experience a need for receiving psychosocial support. Lack of self-esteem may interfere with their social relationships.

Current research on conditions affecting sex development is focused on understanding the broad range of (epi-)genetic and environmental etiologies, on advancing medically assisted reproduction (MAR) technologies, and on improving care, including psychosocial aspects for affected individuals.

Future research priorities

Etiology of DSD

1. The diagnostic yield in DSD remains relatively low. More research is needed to further unravel the (epi)genetic factors influencing male and female sex development. This requires extension of genetic analyses for DSD to non-coding regions via whole genome sequencing, including long range sequencing with concomitant assessment of epigenetics [10.1016/bs.ctdb.2019.01.005].
2. There is emerging evidence that hormone dysregulation during embryonic development influences postnatal maturation of male gonadal Leydig and Sertoli cells, and hence adult steroid hormone synthesis and fertility [10.1093/ejendo/lvad007]. Thus, hormone-dependent sex development needs further research, i.p. the hormonal activation of genes that drive sex differentiation, including oestrogen action, which is still poorly understood.
3. The foetal-placental-maternal unit is pivotal in metabolising steroid hormones produced by the fetus and mother to safeguard hormonal balance, especially with respect to circulating androgens [10.1007/s11154-022-09756-3]. This system is out of balance in utero in some virilising conditions, shifting excess androgens towards recently proposed alternative pathways that involve androgen-production or metabolisation and that play a role in health

and disease. These pathways may affect pubertal development, gonadal function and fertility [10.1093/ejendo/lvad047][10.1038/s41574-020-0336-x].

4. The gene regulatory networks governing gonad development and function are not evolutionarily conserved across all model organisms used in research and humans, marking a bottleneck in research on etiology and mechanism(s) of disease progression in DSD. One way forward is the use of ex vivo, patient-derived cellular and organoid models [10.1530/EJE-18-0861], which would also pave the way for personalized medicine approaches. Working towards this, successful cellular reprogramming of mouse and human gonadal cells has been achieved [10.1126/sciadv.abn9793] but their relevance to the clinical heterogeneity encountered in practice needs further exploration.

DSD and the human brain

1. Both clinical and neuroimaging data obtained in patients with altered androgen levels/actions suggest an important role of androgens in inducing male-typical neural and psychosexual characteristics [10.1111/jne.13050]. Studies investigating the neural and psychosexual functioning in DSD characterized by low/absent estradiol action can shed light on the role of estrogens in the development of female-typical neural and psychosexual characteristics, including gender identity.

Co-morbidities and risk factors for reduced QoL and long-term outcomes

1. Congenital extra-genital anomalies are often present in individuals who have a DSD [10.1210/jc.2013-2918][10.1038/s41574-018-0010-8], as well as co-morbidities such as reduced bone mineral density [10.1111/cen.14149][10.3389/fendo.2022.939897], early cardiovascular disease [10.1093/eurheartj/ehac112][OBJ][OBJ], cancer [10.3390/ijms20205017] neuropsychiatric disorders, developmental delay and additional[OBJ][OBJ]10.1016/j.jpeds.2021.08.066][10.1159/000447958]. Some of these may be secondary to hormone deficiencies and some may be independent. Integrating results from lifelong in-depth clinical phenotyping studies and tissue-specific multi-omics research that provides mechanistic understanding is critical to reduce morbidity and mortality, provide realistic expectations and improve QoL via appropriate risk analyses.
2. Individuals with DSD and a Y chromosome are at increased risk for developing gonadal germ cell tumors (GCT) [10.1007/s00431-005-0056-1]. Prophylactic gonadectomy to prevent GCT development should be targeted to individuals who have the highest GCT risk and lowest chances for hormone production and fertility. Research on stratification of risk profiles has been descriptive so far [10.1016/j.eururo.2014.07.011]. Molecular genetic profiling of gonads and cell types is needed to understand the pathobiology of GCT in DSD and to develop specific biomarkers for detection of premalignant cells. Such biomarkers are also required to support surveillance programs for preserved high-risk gonads [10.1038/s41568-019-0178-9.
3. The case for standardized data collection across geographical borders is particularly strong in controversial areas such as DSD where the marked regional and sociocultural variation in care is based on minimal evidence of benefit (DOI: 10.1530/EJE-19-0363). Registries collecting real-world data such as the I-dsd registry will increasingly become important tools for pragmatic trials, and for this, there will be a need for greater interaction with regulators and health care policy makers. These trials should focus on:
4. Optimization of puberty induction and sex hormone replacement therapy in DSD, e.g. improved formulations, route of administration, pubertal timing and progression, optimization of hormonal effects in conditions with reduced sex steroid end organ sensitivity and long-term outcomes for general health issues [10.1210/endrev/bnab043][10.1530/EJE-22-0073]. Especially during adulthood, it is not clear which types of substitution with sex hormones is most efficacious [10.1210/clinem/dgac167][10.1016/S2213-8587(18)30197-9].

5. Optimization of fertility capacity and technologies [10.1007/s12020-021-02618-z]
6. Optimization of sexual function, at all ages, considering functionality of genitalia, eventual genital surgery, hormonal status, psychological features, sociocultural background, and type of DSD [10.1080/0092623X.2019.1610123][10.1210/er.2019-00049].
7. Development of effective psychosocial interventions in DSD, aiming to facilitate acceptance, increase self-esteem, optimise social participation and improve QoL of affected individuals [10.1159/000536296]. Strategies should also target their caregivers, as empowered caregivers will pass over their coping abilities to their children [10.1186/s12902-022-01079-3]. In parallel, measures to increase societal awareness of DSD are urgently needed to reduce stigma [10.1007/s10508-016-0862-8][10.1111/cen.15002].

Impact of living with atypical genitals on (gender) identity development, (gender) well-being and QoL

1. With childhood genital surgery becoming strongly restricted following EU resolution 2191, an increasing number of children grow up nowadays with a clear genital difference. Also, in some European countries, intersex or non-binary sex registration has recently become an option. Scientific evidence for successful outcomes with these approaches is currently not available. Systematic follow-up of the concerned individuals is urgently required to understand the influence of genital ambiguity and non-binary sex registration on gender identity, gender comfort, and gender and body contentedness [10.1159/000536296].

Reproductive options for individuals who have a DSD

2. The natural history of the deterioration of gonadal function in DSD is not understood and needs to be studied longitudinally through the collection of standardised data [10.1530/EJE-19-0363], from fetal life onwards, and with a focus on genomics and proteomics.
3. Expected cryopreservation options need to be integrated with the above research to be successful in DSD [10.1016/j.fertnstert.2023.08.004]. Efficient strategies for fertility preservation need to be developed for individuals with (expected) limited gametogenesis.
4. The applicability of future MAR options for these individuals needs to be explored, including the ethical, social and moral acceptability of these methods [10.1186/s13619-023-00176-5][10.3390/jcm12093305][https://doi.org/10.1057/s41599-023-01674-2].
5. The technique of uterus transplantation for individuals with absent Müllerian structures has not gained wide uptake yet, in spite of its first application more than 10 years ago, and remaining barriers should be urgently addressed [10.1093/humupd/dmad012].

Anticipated impact of future research

Improved diagnostic capacity will allow precision medicine, tailored to the needs of each individual, while at the same time avoiding unnecessary treatments, preventing co-morbidities, and promoting healthy aging. Research on effective psychosocial therapies to address stigma and the burden of infertility, combined with societal research on ways to promote openness towards genital variation, and a better understanding of how gender identity development is influenced by variations in sex characteristics will have a major impact on QoL of affected individuals, and will reduce the need of controversial types of surgery, i.p. genital surgery. The expansion of MAR options, including uterus transplantation, to individuals who have a DSD has repeatedly been stated a top priority by affected individuals.

HPG regulation across the lifetime

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Epidemiology, societal impact, research state of the art

Incidence, economic and healthcare burden

The Hypothalamic-Pituitary-Gonadal (HPG) axis plays a critical role in regulating reproductive function across the lifetime. Alterations in HPG axis function can lead to a range of conditions, including precocious puberty, hypogonadotropic hypogonadism and infertility, each with unique epidemiological profiles, economic and healthcare burdens, and wider societal impacts. Observations from diverse geographic regions, including Denmark, Korea, and Italy, indicate a concerning rise in the incidence of precocious puberty in recent decades. This trend brings with it a host of associated sequelae, impacting not only metabolic health but also fertility and psychosocial well-being. The burgeoning incidence underscores a pressing need for effective prevention strategies, genomic discovery, and improved therapeutics to mitigate its broad-ranging consequences. Central Hypogonadotropic Hypogonadism (CHH) and Primary Ovarian Insufficiency (POI), impacting 1% of women, stand as poignant examples of conditions underscored by their genetic foundations and profound effects on fertility and overall health. Understanding the intricate genetic pathways underlying these conditions is pivotal for targeted interventions and improved patient outcomes.

The economic and healthcare burdens of these conditions are substantial. Direct costs, from diagnostic tests to ongoing management, place significant pressure on healthcare systems and families alike. These expenses are compounded by indirect costs, including loss of productivity and the profound psychosocial impacts experienced by those affected. Infertility, often driven by conditions like Primary Ovarian Insufficiency in women, presents its own set of challenges. Expensive interventions such as IVF and HRT add layers to the financial burden. Meanwhile, the emotional toll of coping with infertility further compounds the strain on individuals and families, amplifying the need for comprehensive support systems.

Wider societal impact

The societal impact of these conditions extend beyond the immediate healthcare implications. Early or delayed puberty can affect psychological development, social interactions, and academic performance, with potential long-term consequences on individual life courses. Recent Mendelian Randomization (MR) studies indicate a causal link between pubertal timing and reproductive- and general health including incidence of type 2 diabetes (T2D) and cardiovascular disease. Additionally, the psychosocial stress on families and caregivers, coupled with societal stigma, can exacerbate the challenges faced by affected individuals. Further, infertility brings its own set of complex social and emotional challenges, often leading to significant distress and societal pressure.

Research state of the art

Current research directions are multifaceted, focusing on genomic discovery, clinical diagnosis, and therapeutic innovation.

Gonadal function is governed by the hypothalamic-pituitary-gonadal (HPG) axis. This neuro-hormonal system integrates three main groups of signals arising from (i) the hypothalamus, where a scattered population of neurons produce the decapeptide, gonadotropin-releasing hormone (GnRH); (ii) the pituitary, where gonadotroph cells, under the stimulus of GnRH, synthesize and release both gonadotropins, LH and FSH, and (iii) the gonads, i.e., testis and ovary, which respond to gonadotropins, by promoting gametogenesis and releasing hormones, including sex steroids. These elements are connected by feed-back loops to ensure the dynamic regulation of the HPG axis along the lifespan.

While the main elements of the HPG axis are established before birth, the dynamic function of the axis and their inter-regulation undergo dramatic changes during pre- and postnatal maturation, heralded by specific features at mini-puberty, puberty, and adulthood, which display divergent traits depending on the sex and nutritional status, and are under the influence of a myriad of regulatory cues.

The quest to understand the triggers of puberty and the genetic landscape of CHH and POI has led to significant advances, though many questions remain unanswered. Pathogenic variants in key genes and the exploration of novel diagnostic and treatment approaches, including the use of gonadotropins and kisspeptin testing, represent the cutting edge of research in the field.

The importance of gonadotropins for gonadal function have been known for decades and are used today clinically to stimulate gametogenesis and or modify gonadal hormone production in both sexes. The potency of FSH in stimulating spermatogenesis and folliculogenesis have been demonstrated in multiple studies and today FSH is still used during hyperstimulation of women undergoing assisted reproductive techniques and are also currently being tested as a novel treatment option for idiopathic male infertility as previous attempts using antiestrogens, SERM, aromatase inhibitors and other agents have not been successful.

The effect of endocrine signals outside of the HPG-axis have also been investigated but so far none of these factors are used routinely clinically. For example, the influence of stress and the adrenal homeostasis has only recently been noted. Although in recent years more research focus has been on factors influencing mineral homeostasis including Vitamin D, calcium and RANKL inhibitors, skeletal function, and glucose homeostasis in functional hypogonadism due to obesity and their possible role in infertility.

Moreover, environmental factors including nutrition or endocrine-disrupting chemicals (EDCs) play a significant role in modulating the HPG axis. Conditions such as nutritional stress, including anorexia, can suppress pubertal maturation and gonadal function by inhibiting excitatory inputs to the HPG axis. Conversely, obesity seems to operate in a biphasic manner, initially over-activating the HPG axis, which can ultimately lead to suppressed gonadal function, particularly in males. This phenomenon, known as secondary hypogonadism due to obesity or obesity-induced hypogonadism, highlights the intricate interplay between metabolic and reproductive processes and underscores the importance of understanding external influences on the HPG axis for optimizing fertility and reproductive health.

However, despite these advancements, disparities in the availability of genetic testing and the need for consensus on best practice management underscore the ongoing challenges in optimizing care for populations affected by disorders of the HPG axis.

Future research priorities

1. Diagnostic challenges and the importance of early recognition

One of the foremost challenges in the field is the early recognition of clinical phenotypes associated with disorders of the HPG axis. The ability of general practitioners and pediatricians to identify these conditions in infancy and childhood is crucial for facilitating early diagnosis and management as early intervention can significantly improve long-term health outcomes, psychological well-being, and fertility. Thus, dedicated research towards developing pragmatic investigative tools and pathways for these conditions is warranted.

Concerning adult patients, there is a need for stratification of patients experiencing infertility. Particularly in male infertility, where not all men can be treated with the same therapy, the selection must be based on careful clinical phenotyping, preferably in combination with genotyping for relevant variants modulating treatment response or specific factors measured in body fluids. Given the complexity of the regulation of the reproductive function, there is a paucity of controlled clinical trials testing different endocrine pathways apart from androgens and gonadotrophins, and their response to treatment.

2. Mini-puberty: A critical window

Minipuberty, a transient surge in the activity of the hypothalamic–pituitary–gonadal (HPG) axis during early infancy, represents a key period for growth and development, particularly in males. It is during this time that significant gonadal processes occur, impacting future fertility potential. Conditions such as congenital hypogonadotropic hypogonadism (CHH) disrupt this critical period, and may theoretically lead to several negative outcomes if left untreated. Questions regarding the timely identification of affected infants, the mimicking of mini-puberty physiology through hormone replacement, and the long-term outcomes on puberty, adult fertility and psychological well-being demand attention. The relevance of minipuberty for female fertility is much less clear. Also the exposure with EDCs during minipuberty has to be addressed. Such research could not only improve the QoL for individuals with disordered mini-puberty but also offer insights into the broader implications of hormonal deficiencies during developmental windows.

3. Unraveling genetic and environmental influences on pubertal timing

Puberty marks the transitional period resulting in the attainment of full adult reproductive capacity. Pubertal timing, influenced by genetic and environmental factors, poses a complex puzzle. While specific genetic variants are being identified, understanding their interactions with environmental factors like nutrition and EDCs remains elusive. Future research aims to clarify these interactions, identifying modifiable factors to mitigate adverse outcomes associated with early or delayed puberty.

4. Genetic diagnosis and precision medicine

High-throughput sequencing technologies have revolutionized genetic diagnosis for conditions like CHH and POI, yet a significant proportion of patients still lack a molecular diagnosis. Integrating genetic diagnostics with clinical management could greatly enhance patient outcomes, particularly in fertility and mental health. Precision medicine approaches are crucial, considering the diverse genetic forms of hypogonadism and the need for tailored therapies.

5. Advancing fertility preservation in CHH

Fertility preservation remains a critical concern for patients with CHH. Current treatments for CHH during adolescence and young adulthood, such as testosterone therapy and gonadotropin replacement, offer some benefits but also highlight the need for further research into more effective and physiologically congruent options. The potential for earlier diagnosis and intervention, e.g. physiological replacement of mini-puberty, coupled with advancements in therapeutic strategies, underscores the importance of fertility as an outcome measure in research.

6. Exploring broader implications and emerging research areas

Beyond the direct impact on fertility and developmental outcomes, conditions affecting mini-puberty and puberty have broader implications for cognitive function, metabolic diseases and overall health. The interrelation of reproductive and neurocognitive pathways, as demonstrated in studies involving Down Syndrome and GnRH replacement, presents an intriguing area for further investigation. Additionally, the effects of environmental factors, such EDCs and the impact of prematurity on developmental outcomes, warrant closer examination.

Moreover, there is an urgent need to collect prospective data on outcomes and side effects from replacement therapies in both infants and adolescents. This could be achieved by integrating data collection within international electronic registries, such as the existing SDM and EuRRECa registries. Prioritizing research in these areas will not only address current gaps in knowledge but also contribute to the advancement of science, environmental understanding, and societal well-being.

Anticipated impact of future research

Impact on disease management and healthcare

The trajectory of future research into HPG axis disorders holds promising prospects for transformative impacts across multiple dimensions of healthcare, societal well-being, and environmental health. Targeted screening strategies for evaluating mini-puberty in male infants with bilateral maldescended testes or micropenis could significantly improve early detection and intervention strategies. This precision in early-life diagnosis and management has the potential to substantially alter treatment outcomes, particularly in fertility for males with CHH, by ensuring timely and physiologically appropriate hormonal replacement and timely gonadal development and maturation. Future research will unravel genetic, epigenetic, life style and environmental factors responsible for the ongoing secular trend towards earlier pubertal onset. Identification of such risk factors, especially identification of the most deleterious EDCs, will enable future preventive initiatives.

Revolutionizing fertility outcomes

Recent advances include the use of gonadotropin replacement therapy during critical developmental windows such as mini-puberty and puberty to more closely mimic physiological development in males with CHH. This approach has demonstrated potential in improving long-term fertility in males with CHH but lacks robust evaluation and optimization.

Understanding novel endocrine signaling pathways and the clinical relevance of their defects has the potential to develop evidence-based novel treatment options with the ultimate advantage of a more efficient personalized therapy. This would increase the understanding of infertility in both sexes and ensure that women were less susceptible to be treated with assisted reproductive techniques when the male have impaired semen quality.

Improvement in non-reproductive health outcomes

Beyond its effects on fertility, hormonal replacement therapy during key developmental periods is anticipated to have a wide-ranging impact on general health outcomes. Specifically, the establishment of a causal link between pubertal timing and the risk of developing type 2 diabetes (T2D) and cardiovascular diseases underscores the importance of correct pubertal management. Early intervention in cases of abnormal pubertal timing could potentially mitigate these risks, highlighting the need for comprehensive treatment plans that consider both reproductive and non-reproductive health outcomes. Improved understanding and management of these links could revolutionize patient care, leading to better prevention strategies and health outcomes for individuals with disorders of pubertal timing.

Societal effects

Improving psychosocial and psychosexual development challenges faced by individuals with pubertal disorders can enhance social integration, relationship formation, and overall QoL, particularly during critical stages like adolescence and young adulthood. By addressing these challenges through improved management and potential therapeutic interventions, future research stands to significantly enhance societal well-being and individual fulfillment.

Building supportive networks

The establishment of networks of centers in geographically diverse locations for the equitable capture of patients and powering of studies underscores the importance of collaborative research efforts. Such networks can facilitate comprehensive care and support for patients and their families, contributing to a more inclusive healthcare system. Additionally, the diverse origins and wide-ranging impact of disorders of reproductive function require broad collaboration between researchers across endocrinology, fertility, developmental pediatrics, metabolic health, genetics, and psychology.

Rationale for selected priorities

The prioritization of these research areas is driven by the need to address the comprehensive health challenges posed by HPG axis disorders. The potential to significantly improve patient outcomes, enhance quality of life, and reduce the healthcare and societal burdens associated with these conditions underscores the urgency and importance of these research directions.

Potential benefits of the research

Future research holds the promise of delivering profound benefits, including:

1. A substantially different, life-course informed approach to the management of individuals with CHH and related disorders to improve fertility and reproductive health outcomes.
2. Significant advancements in the management of non-reproductive health outcomes linked to HPG axis activity dynamics, contributing to improved cognitive, psychosocial, and mental health.
3. The establishment of a more holistic and inclusive healthcare system through the development of supportive networks and collaborative research efforts.
4. Contributions to environmental health through the identification of environmental determinants of HPG axis disorders and the promotion of sustainable healthcare practices.

In conclusion, the anticipated impact of future research into HPG axis disorders extends far beyond the immediate realm of healthcare, offering potential benefits that encompass societal well-being and environmental health. As we advance our understanding and treatment of these conditions, we move closer to a future where individuals with HPG axis disorders can lead fuller, healthier lives, supported by a more inclusive and sustainable healthcare system.

Female reproduction

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Female reproduction: Epidemiology, societal impact and research state of the art

During reproductive years, the hypothalamic-pituitary-ovarian (HPO) axis might be affected by various diseases leading to the suppression of estrogen secretion, development of chronic anovulation, and/or onset of hyperandrogenism. Hypoestrogenicity is often associated with the presence of hypothalamic amenorrhea (HA), which might be functional, organic, or drug-induced (PMID: 28368518). Functional HA (FHA) affects approximately 1-3% of the mature female population or, more specifically, 25%-35% of women with secondary amenorrhea (PMID: 36103784). Furthermore, hyperandrogenic conditions, such as polycystic ovarian syndrome (PCOS) and non-classic forms of congenital adrenal hyperplasia (NCCAH), are common in adolescents and young women. They might be associated not only with chronic anovulation and reproductive failure but also with lifelong metabolic complications, e.g., metabolic-associated fatty liver disease (MAFLD) (PMID: 14711538, PMID: 18950759, PMID: 27804265). PCOS is the most prevalent cause of hyperandrogenism (70%) and the most common female endocrinopathy, affecting up to 13% of women of reproductive age (PMID: 27664216). Conversely, NCCAH occurs in 1 in 200-1000 in the Caucasian population but is often misdiagnosed as PCOS, especially in women with long-lasting hirsutism (PMID 36891552, PMID: 15598692).

Diseases of the female reproductive system could harm both the individual and society because of their high prevalence and long-term effects on metabolism, fertility, well-being, work capacity, morbidity, and mortality. Chronic hypoestrogenism associated with FHA might damage the skeletal system, cardiovascular system, nervous system, sexual function, and mental health (PMID: 36103784). Similarly, PCOS represents a major socioeconomic burden, considering its high prevalence and severe short- and long-term outcomes. Metabolic dysfunction is particularly common in PCOS women with excess weight and shows differences according to the PCOS phenotype (PMID: 22777527; PMID: 37930586). Androgen excess may be partly responsible for the development of metabolic disorders in PCOS, and in turn, insulin resistance and metabolic status affect androgen synthesis and action. Moreover, PCOS women are more susceptible to deterioration of oxidative stress, development of low-grade inflammation, subclinical cardiovascular disease, and metabolic-associated fatty liver disease (PMID: 21667435; PMID: 25878664). The usual treatment with combined oral contraceptives (COCPs) improves androgen excess and elicits regular menses but does not target the root cause and is off-label for adolescent patients. (PMID: 37440702; PMID: 28712591; PMID: 32342022). Thus, the effects of different dietary and medication approaches, e.g., insulin sensitizers and Spiomet, should be investigated in various age groups.

Obesity and hyperandrogenism might be important factors predisposing to pregnancy complications. Gestational diabetes mellitus (GDM) is the most common medical condition in pregnant women (PMID: 33343512), and a diagnosis of GDM is linked to adverse maternal and offspring outcomes during pregnancy and in the future (PMID: 33343512). In mothers, GDM is the best predictor of T2D, as women with previous GDM have a sevenfold-increased risk of T2D compared to women without GDM (PMID: 33343512). Other diseases are also more common after a GDM pregnancy, e.g., cardiovascular disease (CVD) (PMID: 36085031), chronic kidney disease (PMID: 38100751), as well as psychiatry-related disorders (PMID: 36928320). Offsprings of mothers with GDM have an increased risk of type 2 diabetes (T2D) in adulthood (PMID: 18000174), and they also have more cognitive deficits compared to pregnancies without GDM (PMID: 18000174). Furthermore, there is an established risk between GDM and obesity of offspring (PMID: 23000698), a path probably regulated through epigenetic mechanisms (36870961). The prevalence of GDM varies greatly worldwide from 1 % to above 30 % (PMID: 31296866), depending on the characteristics of the population, that is, BMI, age, ethnicity, polycystic ovary syndrome (PCOS) status (PMID: 37402524) and genetics (PMID: 33343512). An increase in blood pressure (BP) during pregnancy, within the pre-hypertension interval, doubles the risk of hypertension later in life (PMID: 28074637). Trajectories of maternal blood pressure might be associated with glucose levels in overweight women with GDM (PMID: 35792096). We treat pregnant women to reduce adverse obstetric outcomes as well as long-term health risks for mother and child, and the reduction of glucose levels is the main focus. However, minimizing the adverse outcomes of GDM must start early on, in combination with therapy during pregnancy and prevention of future disease in women and offspring with the highest risk.

Opposite to reproductive diseases, menopause is an inevitable change in every female individual, and on average, European women would spend 30% to 40% of their lifespan in menopause. However, the development of complications could lead to significant disability-adjusted life years (DALYs) losses, reaching 7.8 in patients with severe postmenopausal osteoporosis and prior bone fracture (PMID: 25880810). Recently recognized pathologic entities, such as menopause-related overweight and obesity, as well as metabolic derangements (i.e., increased insulin resistance, hyperlipidemia), necessitate extra efforts of understanding and management. Menopausal hormonal therapy (MHT) has been recommended as a first-line option for vasomotor and vulvovaginal symptoms, osteoporosis prevention, and treatment of hypoestrogenism in hypogonadal women by multiple guidelines (PMID: 35797481; PMID: 37188372; PMID: 35717745). New therapies, e.g., neurokinin-3 receptor antagonists, are also coming (PMID: 37076317; PMID: 37252752). However, despite the abundance of therapeutic opportunities, the undertreatment of symptomatic women is common, even in the case of early surgically induced menopause (PMID: 37252752; PMID: 37667667). The undertreatment of peri- and

postmenopausal women might be associated not only with pronounced individual clinical symptoms and complications but also with a substantial socioeconomic impact. Direct costs of the menopausal transition are related to medical consultations, laboratory analyses, and over-the-counter and prescription drugs for multiple complaints, while indirect costs reflect decreased work productivity and motivation, prolonged sickness absence, and reduced work hours (PMID: 23532198; PMID: 28975014; PMID: 34274202; PMID: 37542726).

Female reproduction: Future research perspectives

Research priorities in Hypothalamic amenorrhea:

In Pathophysiology and diagnosis:

1. To study the CNS in women with FHA through functional MRI and possible correlates with metabolic and psychological parameters, and if hypothalamic inflammation (activation of microglia) could be of potential linkage to these associative processes (PMID: 33562540).
2. To study the genetic and epigenetic signatures of patients with FHA, markers for prediction reversal of FHA (GnRH-induced LH pulse patterns and sleep patterns) as well molecular imaging targeting opioid receptors, serotonin receptors, etc.
3. To further study the co-existing FHA and PCOS in the same patients and the role of GnRH pump therapy for a short period to unravel underlying PCOS phenotypes.
4. To establish diagnostic criteria for biomarkers of the HPO axis function (dynamic tests of kisspeptin, GnRH and FSH, LH, and estradiol response) as well as markers of ovarian reserve for the study of fertility outcomes.

In Clinical management:

1. To investigate optimal combinations of different nutritional, exercise, and psychological intervention modalities in managing FHA.
2. To study potential treatment modalities with molecules such as kisspeptin and various cytokines/adipokines for restoration of reproductive function in women with FHA (PMID: 15342807, PMID 21464293), proper estrogenization (replacement therapy) in drug-induced hypothalamic amenorrhea, the use on anti-prolactin treatments as well fertility treatment strategies including GnRH pulsatile therapy and relevance of IVF treatment in FHA.
3. To investigate hypoestrogenic condition on peak bone mass and bone turnover markers in FHA (PMID: 28498239), cardiovascular long-term complications, hip fractures, and overall deleterious effects of FHA in society.

Research priorities in Hyperandrogenism and metabolic outcomes

In Pathophysiology, definition and diagnosis:

1. To define early specific and sensitive biological and clinical biomarkers of insulin resistance at the liver and skeletal muscle level, adipose tissue dysfunction, and association of specific metabolomic signatures encompassing alterations in ceramides, bile acids, short-chain fatty acids, branched-chain amino acids and gut-derived metabolic pathways, with defined PCOS phenotypes to predict the metabolic risk and to identify novel pharmacologic targets.
2. To set up large-scale studies using modern LC-MS/MS assays for validating the steroid markers in different settings of PCOS and NCCAH and for determining harmonized diagnostic cut-offs for both ovarian and adrenal steroids. To identify and validate novel steroid markers dissecting the peripheral contribute to steroid activation and inactivation (ie. in hypertrophic and dysfunctional adipose tissue). Determination of diagnostic cut-off levels for both ovarian and adrenal steroids.

In Clinical management:

1. To perform RCTs to establish the specific short and long-term impact of different dietary interventions as related to different PCOS phenotypes and metabolic comorbidities.
2. To perform RCTs to estimate and validate the short and long-term effectiveness of different medications, either approved, such as the GLP-1 agonists, or under development, such as low-dose Spiomet for specific subpopulations of patients with PCOS.

Research priorities in Gestational diabetes mellitus, gestational and offspring outcomes

1. To establish which maternal factors matter regarding risk of future diabetes in mothers and children after a GDM pregnancy (i.e. maternal age, pre-pregnancy BMI, PCOS, GDM severity (PMID: 38100751), other pregnancy-related factors as well factors in offspring (i.e. birth weight, abdominal circumference, fat mass).
2. To investigate established and novel therapeutic approaches for GDM including but not limited to metformin, sulfonylureas, insulin analogs; GLP1-analogs, and related new drugs (PMID: 38300523).
3. To set up prospective studies to define the ideal normal cut-offs of systolic and diastolic blood pressure during every pregnancy trimester, based on ambulatory and continuous measurements adjusted for age and BMI in women with GDM, to investigate for optimal pharmacologic interventions, and to identify risk biomarkers of probable preeclampsia development in women with GDM.
4. To assess the long-term effects on offspring regarding obesity development as well long-term neurological and psychological outcomes (neurodevelopment, IQ evolution, autism, attention-deficit/hyperactivity disorder) related to the glycemic control through pregnancy.
5. Application of cutting-edge methods like AI, to characterize distinct phenotypes of GDM and their relation to fetal life development as well as to offspring evolution, role of mobile health (mHealth) in GDM pregnancy and during follow-up of long-term outcomes, and to evaluate the role of Ecological Momentary Assessment (EMA) (PMID: 38115555) in GDM and outcomes.

Research priorities in Menopause

In pathophysiology and diagnosis

1. Additional research on postmenopausal neuroendocrine regulation to develop new non-hormonal drug candidates beyond the serotonin and noradrenaline reuptake inhibitors, GABA modulators, and neurokinin receptor antagonists (PMID: 37076317).
2. Developing early biomarkers for ovarian functional capacity and implementing screening programs are crucial for fertility preservation in young women at risk including those with premature ovarian follicular exhaustion due to genetic, immunologic and other causes (PMID: 27008889), or planning oncology treatments (PMID: 26728489; PMID: 31783875).

In clinical management

1. Clinical studies among healthy postmenopausal women and patients with specific comorbidities to find the optimal dose, time for initiation, type of progestins and estrogens, route of administration, and appropriate duration of MHT treatment (PMID: 28093732; PMID: 33244065; PMID: 30705938). More data are needed to enlighten the optimal approach for MHT discontinuation and follow-up (PMID: 35717745) as well the role of endogenous androgens, and exogenous testosterone and dehydroepiandrosterone supplementation for sexual function, metabolism, and bone health in the postmenopause (PMID: 30488972).

2. Long-term studies with support of AI should target the efficacy and safety of non-hormonal therapies compared to hormonal drugs or placebo for vasomotor and other hypoestrogenic symptoms, as well as their additional influence on cardiovascular, neurological, and bone health of postmenopausal women (PMID: 37188372).

Female reproduction: Anticipated impact of future research

Future research focused on female hypoestrogenic and hyperandrogenic diseases would stimulate society to recognize these pathologic entities and their harmful impact on young women's lives. The establishment of units of FHA management with other specialties (i.e., psychiatrists and ergophysiologists) might help develop extensive epidemiological and physiopathological studies regarding the role of stress and excessive exercise in the development of the disease and, at the same time, provide multidisciplinary, organized care of these patients. Early diagnosis of the FHA and promotion of etiologic rather than symptomatic treatment approaches are also crucial.

So far, clinical studies failed to identify clinical parameters able to differentiate between NCCAH and PCOS. The proposed research priorities could help in differentiating among the most common hyperandrogenic disorders as well as better clinical phenotyping for PCOS and NCCAH. Nonetheless, the differential diagnosis, which relies on the laboratory, is necessary to set the appropriate therapy. Steroid profiling can guide the opportunity of performing genetic tests to confirm the presence of 21-hydroxylase deficit [PMID: 26195035]. The broad implementation of LC/MS techniques will guide the diagnostic process and molecular testing through genome sequencing, or the application of miRNAs would confirm the diagnosis. From the therapeutic side, research outcomes could lead to targeted and tailored metabolic therapies for specific PCOS populations. However, effectiveness, tolerability, and safety are currently under investigation. RCTs on anti-obesity drugs, such as glucagon-like peptide 1 receptor agonists, are needed.

GDM is not a disease but a risk condition, which is an obstetric threat as well as a future maternal and offspring health risk. The steep increase in BMI worldwide is an important factor for hyperglycemia in pregnancy, but there are additionally correctable risk factors. Identifying and characterizing these factors may improve pre-gestational and obstetric healthcare, which may reduce the incidence of GDM or ameliorate the negative consequences of GDM for mother and child. Detailed knowledge regarding optimal therapy of hyperglycemia, blood pressure, and prevention of preeclampsia may have a substantial social and economic impact on society. Both mothers and offspring are resources for society and potentially contribute significantly. Potential new therapies in pregnancy, like GLP1 analogs, need to be examined in large European RCTs, as GLP1 analogs have the potential to improve both hyperglycemia in pregnancy as well as future obesity and comorbidity in mother and child and thus improve most adverse outcomes. However, the safety of these drugs needs to be established through independent RCTs. The use of mHealth and EMA may reach individuals who are unwilling to engage in standard follow-up programs. Hopefully, we can also optimize their health outcomes, both GDM and related adverse outcomes, through information and treatment. Still, we need large clinical studies in Europe to establish the utility of these new tools.

Menopause is a natural physiological process; nevertheless, the transition from reproductive to post-reproductive years is accompanied by health and social challenges for many women. Expanding knowledge about the pathophysiology and prevalence of menopausal symptoms as well as postmenopausal disease burden might be beneficial for the adequate counseling and support of aging women. The launch of interdisciplinary long-term pan-European clinical trials is paramount to exploring best practices regarding peri- and postmenopausal women, considering their risk profile and genetic and sociocultural background. The AI-assisted extensive dataset analyses could help develop individualized menopausal therapies and tools to alleviate personal symptoms and reduce the impact of recently recognized pathologic entities. Timely diagnosis and use of new biomarkers and algorithms for early

identification of women prone to developing premature ovarian insufficiency is crucial for the implementation of targeted European programs for the prevention and treatment of infertility.

In conclusion, the development of new diagnostic and therapeutic approaches for the management of reproductive disorders and menopausal transition, as well as the construction of a supportive work environment deprived of neglect and stigma, could improve the physical well-being, quality of life as well as productivity of women, who will remain an essential part of the European workforce in the coming years.

Male reproduction, couple infertility and offspring following medically-assisted reproduction

[redacted]

Epidemiology, societal impact, research state of the art

Infertility concerns approximately 15% of couples worldwide, with a similar contribution from both genders. Both female and male infertility has a largely unexplored complex multifactorial origin. The two extreme infertile phenotypes are azoospermia (absence of spermatozoa in the ejaculate) in the male and premature ovarian insufficiency (POI) in the female, both showing an incidence of 1% in the general population. Besides these extreme infertile phenotypes, many different infertile conditions are known (e.g. altered semen parameters, impaired ovarian reserve, PCOS).

After a comprehensive diagnostic work-up, in about 50% of cases of male infertility the etiology remains unknown and we refer to this condition as “idiopathic” infertility (PMID: 27395770). It implies, that only limited numbers of infertile patients benefit from personalized rational treatment while the majority will undergo MAR, mainly in vitro fertilization. These techniques present a significant challenge with their relatively low success rates, forcing couples to undergo multiple treatment cycles. This imposes a heavy psychological and economic burden on the affected individuals and on the National Health services. In azoospermic men, testicular sperm retrieval through testis biopsy (TESE) precedes in-vitro fertilization. TESE in an invasive procedure, with an average 50% success rate, and with no clinical/hormonal parameters able to predict its outcome.

A significant proportion of idiopathic infertility cases is predicted to be due to yet unidentified genetic anomalies (mainly monogenic model) or to gene-environmental interaction (polygenic model). While recent research efforts, based on massive parallel sequencing, have led to the discovery of several novel infertility-related genes, their clinical implications remain largely unexplored (REF PMID: 29622783, PMID: 34498060). Some of the newly discovered genes are involved in DNA repair mechanisms pointing out that mutations in these genes not only affect reproductive health but also elevate the risk for cancer development. These findings support epidemiological studies, which have drawn connections between severe spermatogenic failure and higher risk for cancer development in azoospermic men (ref PMID: 23790640)

In parallel with the growing prevalence of couple infertility, the number of children conceived through MAR is steadily increasing, reaching 5-10% of total newborns in many EU countries. In half of the cases, in vitro fertilization is performed in men with quantitative and/or qualitative impairment of

spermatogenesis (Figure 2). Spermatozoa carrying genetic or epigenetic defects may lead to potential intergenerational inheritance of infertility and even to somatic health defects of offspring.

There is a big knowledge gap concerning the health status of MAR children. An increased risk of adverse perinatal outcomes and congenital malformations in children conceived using in vitro fertilization was observed compared with naturally conceived children. It is still not clear whether this is due to the in vitro manipulation of gametes and embryos or more likely to the genetic/epigenetic quality of the gametes used or the MAR techniques used, e.g. in vitro culture. Data on the sperm parameters of ICSI children are based on a few small cohorts and results are controversial (PMID: 30870183). Some studies have suggested poorer cardiovascular and metabolic profiles in ICSI children compared to those conceived naturally, but long-term data on large cohorts are missing, hence the long-term health consequences of MAR into adulthood remain unknown.

Fertility options for transgender and gender diverse people is an emerging issue. Many transgender and gender diverse people (TGDP) remain involuntarily childless [10.1080/26895269.2022.2035883]. Those who (aim to) participate in existing fertility programs or pursue MAR, may experience multiple challenges [10.1080/26895269.2022.2100644]. Use of gender affirming hormone therapies (GAHT) induce histopathological changes (testicular hyalinisation, ovarian collagenization and cyst formation) in gonads of TGDP and impact gametogenesis [10.1210/endocr/bqaa014][10.1093/humrep/deab240].

Like infertility, birth control is major component of public health within the EU. Despite the wide spectrum of contraceptive methods developed for women, the development of male contraceptive methods is lagging and consequently, the burden of contraception falls primarily on women. Global surveys have reported that both women and men of different culture, religion, social status support the development of reversible male contraceptives (doi10.1093/humrep/deh574; 10.1016/j.contraception.2010.03.016) In the last decades, research has focused on the development of hormonal and non-hormonal methods. The androgen-progestin combinations (PMID: 15634716) have provided highly encouraging results in terms of sperm suppression and safety. Most recently, the preliminary results of an ongoing phase IIb trial have suggested that the combination gel containing testosterone and segesterone acetate could provide rapid and profound sperm suppression and be highly acceptable to men PMID: 38745531 A number of promising novel testosterone molecules have also been developed and will be tested in combination with progestins in future clinical trials for male contraception.. An alternative to hormonal contraceptives are those compounds, which interrupt steps of male germ cell development required for fertility maintenance, including spermatogenesis, spermiation, and sperm motility. Several potential agents are in development however, they may cause off-target effects that have hampered the development of some of these molecules. Therefore, each molecule will have to demonstrate not only effectiveness as contraceptive but also safety and reversibility. The development of these molecules is still largely in the preclinical phase.

Future research priorities

Male factor infertility and the role of genetic factors involved in spermatogenic disturbances

1. Given the expected elevated number of genes involved in infertility, there is an urgent need for large scale genomic studies with the ultimate aim to provide precision molecular diagnosis and future successful targeted treatments (PMID: 29622783)
2. Standardized data collection and data sharing within large international consortia would substantially accelerate the translation of novel findings into clinical practice: i) improving diagnostic capacity; ii) allowing personalized treatments (e.g. predicting TESE outcome and therefore avoiding invasive procedures; predicting fertilization failure avoiding ICSI)
3. Integration of genomic data with other “omics” (transcriptome and proteomics) and epigenome sequencing data is necessary for advancing our understanding of basic

reproductive physiology and pathophysiology and the risk of transmission of genetic/epigenetic alterations

4. Comprehensive analysis of genetic variants in multiple genes (polygenic model) and their relationship with developmental, environmental and life style factors for preventive purposes
5. Multicentre efforts are needed in order to collect long-term follow-up data (especially morbidity and mortality), on large groups of genetically well-characterized infertile patients in order to identify those genetic anomalies which have implications also on general health.

Health status of children born following in vitro fertilization

1. To increase the health status of children born after MAR, identification of the underlying cause of infertility is needed, with specific focus on transmissible genetic defects. This implies a parallel improvement of the diagnostic capacity of genetic tests (see above). Reliable and easy-to-perform methodologies need to be developed to analyze the (epi-)genomic integrity of spermatozoa of ICSI fathers.
2. Large, multicenter database is needed, containing standardized clinical and experimental information on the parents (etiology of the infertility, age, life style, environmental exposure, sperm genome/epigenome integrity) and their children conceived using in vitro fertilization: i) perinatal outcome (malformations) ii) growth and development in childhood-puberty; iii) reproductive (e.g. semen parameters, reproductive success) and endocrine parameters; iv) general health status (with specific focus on neurocognitive pathologies and cancer)

Fertility options for transgender and gender diverse people

1. A dedicated research program is needed in order to widen reproductive options for TGDP and make existing programs more accessible for them.
2. Fertility preservation strategies, such as follicle culture, cytoplasm rejuvenation, and in vitro maturation of immature cryopreserved stem cells need to be optimised in TGDP using GAHT. Gamete quality and embryo development in TGDP that have used GAHT need to be assessed [10.1016/j.mayocp.2019.10.040].
3. Dedicated research is needed to identify (personal, institutional and societal) barriers to fertility preservation that TGDP people may experience and to develop strategies to lower these barriers.
4. Particularly challenging is how to include the impact on fertility in the decision-making process to initiate medical treatments in TGD adolescents and young adults [10.1016/j.jadohealth.2020.01.007][10.1080/26895269.2023.2257062]. The development of tools to discuss the impact of their trajectory on fertility, assessing their level of understanding and capacity for informed consent, and the creation of age-adjusted decisional aids to support future-oriented reproductive decisions need to be prioritised.
5. Long-term health outcomes and QoL of both children, born by use of preserved gametes, and their TGD parents need to be assessed [10.1001/jama.2023.16208][10.1001/jama.2023.7688]

Male contraception

1. The objectives in this field are to achieve a uniform sperm suppression in all men; a rapid suppression of spermatogenesis; and a rapid recovery of spermatogenesis after interruption of the treatment. For hormonal combinations that have provided the most promising results, larger efficacy trials need to be set up. Also, new molecules need to be developed, more potent in terms of sperm suppression but devoid of side effects. A few of these molecules are already being tested in small pilot studies.
2. Trials in men with subnormal sperm concentrations are also needed. Since hormonal male contraceptives will need to be available for all men, information on efficacy and reversibility in men with subnormal semen parameters are of fundamental importance.

Understanding the physiology of spermatogenesis and sperm function

1. Studies on basic physiological mechanisms of spermatogenesis and sperm function are urgently needed in on in vitro cellular models and testicular organoids. These studies will help to develop : i) novel treatment options for patients with impaired spermatogenesis ; ii) optimal hormonal and non-hormonal male contraceptive (REF PMID: 37727884). The same models will be useful also in TGDP subjects to mechanistically study eventual germ cell pool loss and damage induced by GAHT, along with the impact of clinical modifiers, e.g. age at start, duration, type and dose of GAHT as stand-alone therapy or in combination with gonadal hormone-suppressing medications.

Anticipated impact of future research

Large collaborative genomic studies would allow a rapid advancement in the discovery of novel genetic factors and their translation into clinical practice. Integration of "omics" has the potential to provide a comprehensive evaluation of the fundamental mechanisms underpinning male fertility. The identification of genetic defects and their molecular consequences in the field of infertility would have a substantial impact on developing personalized treatment options with an expected reduction of the psychological and economical burden on the concerned individuals and the society. Understanding how specific genetic variations predispose to infertility and how they interact with environmental factors will enable early detection of individuals at risk and the actuation of targeted actions (personalized counselling on lifestyle factors; raising public awareness about environmental toxicants). Identifying genetic anomalies affecting not only spermatogenesis but also general health (especially cancer predisposition), will allow timely actuation of preventive measures in the mutation carriers and their future descendants.

While registries reported malformation rates in newborns, the long-term consequences of in vitro fertilization are largely unexplored. Large-scale, prospective well-controlled follow-up studies comprehensive studies will allow a realistic prediction of the consequences of different etiologies of infertility on the health of children conceived using MAR, with adjustment for parental confounders. It is expected that genetic/epigenetic alterations causing infertility may also harm somatic health both in the father and his children. If such a correlation will be demonstrated, timely intervention and prevention would represent one of the major benefits of these studies both at the individual and the society level. These studies could identify specific individual lifestyle and environmental factors interfering with the sperm epigenome and able to influence health outcome of MAR children. Improving our knowledge on this relationship, would allow the actuation of preventive measures prior to MAR, with an expected higher probability of a healthy child.

The development of new male contraceptives could help bridge the gender gap in contraception. It will help achieve socially, ethically, and economically important goals such as reestablishing gender equity within the couple and providing men with the opportunity to contribute equally to family planning. It will help achieve the still unmet goal of preventing unwanted pregnancies and slow down population growth and its burden on the environment (61, 62).