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Metabolomic analysis of infertility biomarkers in follicular fluid by solid-phase microextraction and gas chromatography-mass spectrometry

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Dedicatória

Para mim.

“멈춰서도 괜찮아
아무 이유도 모르는 채 달릴 필요 없어
꿈이 없어도 괜찮아
잠시 행복을 느낄 네 순간들이 있다면” - 방탄소년단

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Resumo Alargado

A infertilidade é definida como a incapacidade de gerar uma gravidez clínica após seis meses a um ano de atividade sexual desprotegida. Cerca de 186 milhões de pessoas são afetadas globalmente por esta condição clínica, sendo reconhecida como uma doença social pela Organização Mundial de Saúde. A infertilidade pode ser causada por fatores femininos, masculinos, ou intrínsecos a ambos os elementos do casal. Relativamente aos fatores femininos, entre os mais prevalentes encontram-se a síndrome de ovários poliquístico (SOP), a endometriose e a falência ovárica prematura (FOP). A SOP, do inglês *polycystic ovarian syndrome* (PCOS), é um desequilíbrio ginecológico, endócrino e metabólico, predominante entre mulheres em idade reprodutiva. De acordo com o Consenso de Roterdão sobre Critérios de Diagnóstico para PCOS, esta doença afeta 5-20% das mulheres em todo o mundo. A endometriose é uma doença complexa que afeta 10-15 % das mulheres em idade reprodutiva, gerando infertilidade e dor pélvica crónica. A FOP, do inglês *premature ovarian faillure* (POF) ocorre aquando do esgotamento do número de folículos em idades precoces, muito antes da idade da menopausa. Pode ser acompanhado por danos autoimunes nos ovários e pode haver predisposição genética, desenvolvendo-se em cerca de 1% das mulheres.

O fluido folicular (FF) é uma matriz biológica complexa que permite a comunicação entre células germinativas e células somáticas, sendo constituído por uma vasta gama de metabolitos produzidos por células da granulosa e da teca, da parede folicular, transudado de plasma e difusão de soro, permitindo assim várias reações essenciais ao desenvolvimento do oócito. Assim, torna-se imperativo o estudo aprofundado do FF, uma vez que se trata de uma matriz que reflete as alterações no microambiente de condições como PCOS, endometriose e falha ovariana precoce.

A metabolómica permite detetar vários compostos, em diversas matrizes biológicas, referentes a mudanças dinâmicas e perturbação do organismo, possibilitando a identificação e quantificação de diferentes biomarcadores. A volatilómica, um subgrupo da metabolómica, abrange todos os compostos orgânicos voláteis (COV), do inglês *volatile organic compounds* (VOC), que derivam de fontes exógenas, nomeadamente da nutrição, de fármacos, e da exposição ambiental, bem como fontes endógenas, provenientes de processos metabólicos e bioquímicos. Especificamente, os estudos volatilómicos podem aplicar-se à análise orientada de um número restrito de

metabolitos ligados a uma via biológica específica ou à recolha de impressões digitais de uma fração significativa de metabolitos. Neste estudo em particular, foi necessária uma abordagem não direcionada para detetar e identificar possíveis alterações no FF de mulheres inférteis.

Relativamente às metodologias analíticas utilizadas para a deteção e quantificação de VOCs, a técnica de eleição para a preparação da amostra foi a microextracção em fase sólida (SPME), em modo *headspace*, e a análise qualitativa através da Cromatografia Gasosa Acoplada com Espectrometria de Massa (GC-MS).

No desenvolvimento da dissertação, foram analisadas 52 amostras de FF de modo a determinar o padrão volatilómico e identificar potenciais biomarcadores de PCOS, endometriose e POF. Especificamente, foram detetados 136 VOCs, sendo que um total de 37 (27%) estavam presentes em pelo menos duas amostras. Os resultados revelaram perfis bioquímicos alterados, bem como o comprometimento de algumas vias metabólicas nas várias doenças. Os compostos que apresentaram maior representatividade foram o ftalato de dietila, o 4-metil-2,4-bis(4-hidroxifenil)pent-1-eno, e o tetradecametilciclohepta siloxano. De entre as amostras de POF foi possível identificar a presença de 1-dodecanol e do 4,6-dimetildodecano, adicionalmente nas amostras de endometriose foram determinados o tetradecametilhexasiloxano e o hexadecametilheptasiloxano, e nas amostras de PCOS o tetradecametilciclohepta siloxano, o 1-etil-2,3-dimetilbenzeno e o docosano. Assim, as metodologias de alto rendimento aplicadas no desenvolvimento deste trabalho sugerem a possibilidade de utilizar este tipo de identificação metabólica na determinação de potenciais biomarcadores de infertilidade, favorecendo-se as ferramentas clínicas de diagnóstico atualmente disponíveis.

Palavras-chave

Infertilidade;síndrome de ovários poliquísticos;endometriose;falência ovárica prematura;compostos orgânicos voláteis;microextracção em fase sólida;GC-MS.

Abstract

Infertility has become a prominent public health issue, posing a significant challenge to modern reproductive medicine. Even though infertility relates to the couple rather than an individual, owing to social constructs, women are the predominant individuals seeking medical assistance. Some clinical conditions that lead to female infertility include polycystic ovary syndrome (PCOS), endometriosis, and premature ovarian failure (POF). Follicular fluid (FF) allows the study of clinical conditions related to women's infertility. This biological matrix is the only one in direct contact with the oocyte and can, therefore, predict its quality. Volatilomics, a specific field of metabolomics, has emerged as a non-invasive, straightforward, affordable, and simple method of profiling various diseases as well as the effectiveness of their current therapies. In this study, 52 samples of FF were analysed to determine the volatomic pattern and find potential biomarkers of PCOS, endometriosis, and POF. The technique chosen for the sample preparation was solid-phase microextraction (SPME), followed by the analytes isolation and qualitative analysis techniques through chromatography-mass spectrometry (GC-MS). 136 VOCs were detected, and 37 (27%) were present in at least two samples. The findings point to specific metabolite patterns as potential biomarkers for the diseases in the study. These open the door to further research into the relevant metabolomic pathways to enhance infertility knowledge and diagnostic tools.

Keywords

Infertility;polycystic ovary syndrome;endometriosis; premature ovarian failure;follicular fluid;volatile organic compounds;SPME;GC-MS .

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AFC	Antral follicle count
AMH	Anti-Müllerian hormone
AR	Androgen receptor
ART	Assisted reproductive technology
BMI	Body mass index
BPA	Bisphenol A
BTEX	Benzene, toluene, ethylbenzene, xylene
COV	Compostos orgânicos voláteis
DIE	Infiltrating endometriosis
E2	Estradiol
FF	Follicular fluid
FOP	Falência ovárica prematura
FSH	Follicle stimulating hormone
GC	Gas chromatography
GC-MS	Gas chromatography coupled with mass spectrometry
GCs	Granulosa cells
GnRH	Gonadotropin-releasing hormone
HA	Hyperandrogenism
HDL-C	High-density lipoprotein cholesterol
HPLC	High-performance liquid chromatography
HS-SPME	Headspace solid-phase microextraction
LC-MS	Liquid chromatography coupled with mass spectrometry
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LH	Luteinizing hormone
IR	Insulin resistance
IUI	Intrauterine insemination
IVF	In vitro fertilization
MBP	4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene
METs	Microextraction techniques
MS	Mass spectrometry

NMR	Nuclear magnetic resonance
OGTT	Oral glucose tolerance test
OMA	Ovarian endometrioma
PCOS	Polycystic ovarian syndrome
PDMS	Polydimethylsiloxane
POF	Premature ovarian failure
POI	Premature ovarian insufficiency
PR	Progesterone receptor
SOP	Síndrome de ovários poliquísticos
SPME	Solid-phase microextraction
SUP	Superficial endometriosis
TG	Triglycerides
UHPLC-MS	Ultra-high performance liquid chromatography coupled with mass spectrometry
UHPLC-MS/MS	Ultra-high performance liquid chromatography with tandem mass spectrometry
VOC	Volatile organic compounds

Chapter I

Introduction

1 Infertility: an overview

Infertility or impaired fecundity are described as the inability to establish a clinical pregnancy to term after six months (women over 35 years old) or one year (women below 35 years old) of unprotected sexual intercourse [1,2]. According to the Universal Declaration of Human Rights, the desire to start a family is a natural human need, and infertility poses a significant challenge to modern reproductive medicine. Due to its prevalence, the World Health Organization classified infertility as a social disease [3,4], and it is , therefore, becoming a growing public health concern [5,6]. Recent sources have reported that infertility affects as many as 186 million people worldwide [3,7].

Individually, both elements of the couple can be in the best of physical and mental health, but as a couple their combined characteristics may not provide a favourable breeding environment, leading to infertility [3,4]. As a result, infertility relates to the couple rather than an individual [1,5,8,9]. Owing to current social constructs women are more likely to seek medical attention and establish reproductive health evaluation at an earlier age [1]. It is estimated that the malefactor plays a role in 50% of infertile couples, with a solo contribution in 20% of cases [1,3,7]. About 1.2 million women/year are seen in fertility clinics, whereas only 20% of partners (approximately 240 000 men) undergo a fertility evaluation. It is, therefore, decisive to ensure appropriate investigations of both partners to rule out potentially reversible causes of infertility to improve their chances of natural fecundity [1]. From another sociological perspective, the gender disparity in infertility workup is in the biology of reproduction itself. Women are the carriers of conception and pregnancy and are traditionally seen as the primary site of the reproductive process. So, the early research on assisted reproductive technologies (ART) focuses on the female factor [1,10]. Urban myths also flourished that women are the usual cause of infertility [1,11], where men are secondary participants, with masculinity strongly tied to fertility [1,10].

Infertility can be divided into primary and secondary infertility. The former refers to a couple that has never been able to conceive, whereas the latter categorizes a couple that has had at least one prior successful conception. Statistics reveal that one in seven couples are infertile in developed countries and one in four in developing countries [1,12]. It is worth noting that some cases of infertility remain unexplained [5,6], reflecting the uncertain causal relationship

between abnormalities in infertility testing and the actual cause of infertility, as well as some couples' lack of information and interest [5].

The therapy of infertility must precisely target the diagnosed cause of infertility, applying methods which guarantee the highest chance for pregnancy and delivery [3]. Procedures implemented to mitigate infertility include diagnostic of the cause, surgical therapy (preferably endoscopic techniques), initiation of pharmacological therapy (ovulation-inducing medications), artificial insemination (intrauterine insemination (IUI)), and ART such as in vitro fertilization (IVF) and intracytoplasmic sperm injection [1,3].

Fertility is affected by multiple factors: age, acute or chronic conditions, environmental toxins, occupational exposures, general lifestyle issues, infectious diseases, genetic conditions, and specific reproductive disorders that can affect the man or the woman [5]. Age is one of the most significant factors associated with female infertility. Fecundability, the ability to achieve pregnancy per cycle, declines as women age. Indeed, the number of oocytes decreases throughout reproductive years as their quality, generating an increased incidence of chromosomal abnormalities and spontaneous abortions [5]. Women also face an increased risk for disorders contributing to infertility as they age, such as endometriosis, leiomyomata, or tubal disease [5,13]. For this reason, women aged 35 years or older are determined to be infertile after only six months or less of frequent, unprotected sexual intercourse and should be evaluated for infertility issues earlier than healthy women under 35 years [5,13]. The most common cause of female factor infertility is ovulatory dysfunction. Women affected by oligoovulation or anovulation have difficulty becoming pregnant because an oocyte is not available monthly for fertilization. The most frequent cause of anovulation is polycystic ovarian syndrome (PCOS) [14]. Also, ovulatory dysfunction can occur because of any disturbance in the hypothalamic-pituitary axis. Triggers of hypothalamic-pituitary axis dysfunction include intense exercise, eating disorders, extreme stress, hyperprolactinemia, pituitary adenomas, or autoimmune disease [5,15]. It is relevant to notice that certain medications might be associated with ovulatory dysfunction, these including antidepressants, antipsychotics, corticosteroids, and chemotherapeutic agents [16].

Although the prevalence of infertility has remained relatively stable in the past several decades, the demand for infertility services has increased substantially partially due to delayed childbearing trends, combined with advances in ART [5,8,9].

1.1 Female infertility causing disorder

1.1.1 Polycystic ovary syndrome (PCOS)

PCOS is one of the prevailing gynecological, endocrine, and metabolic disorder among women of reproductive age, representing one of the leading causes of anovulatory infertility [17–20]. According to the Rotterdam Consensus on Diagnostic Criteria for PCOS, this is a multifaceted pathology that encompasses many clinical manifestations. It is necessary to present two out of

three of these characteristics in order to be diagnosed with PCOS: oligoanovulation [21]; clinical and/or biochemical hyperandrogenism (HA) [22]; polycystic ovarian morphology that does not encompass other abnormalities, such as Cushing's syndrome, congenital adrenal hyperplasia, and androgen-secreting tumors [23]. According to the criteria in practice, this disease affects 5% to 20% of women worldwide [17–20].

To be considered a woman with polycystic ovary morphology, it is necessary to present at least one ovary with "12 or more follicles, measuring 2–9 mm in diameter, and/or increased ovarian volume, namely above 10 mL" [24–28]. This definition, however, does not apply to women who use the oral contraceptive pill because its use alters ovarian morphology [24,29]. Any evidence of ovarian asymmetry or an abnormal cyst requires a more in-depth inquiry, as well as asymptomatic polycystic ovarian morphology women, since they do not present ovulatory disorder or HA [24,30–32].

Metabolic abnormalities are also common, even if they are not considered to classify a PCOS woman. Three out of the following five qualify for metabolic syndrome: blood pressure [24,33,34]; abdominal obesity (waist circumference) [24,33,35]; high levels of triglycerides (TG) [24,33]; fasting and two-hours glucose from oral glucose tolerance test; low levels of high-density lipoprotein cholesterol (HDL-C). These might further generate an increased risk of type 2 diabetes [33,36,37], insulin resistance [33,38,39], and cardiovascular diseases [33,40–44], and they might disturb the body mass index (BMI) [24,33].

Because of the variety of clinical and biochemical manifestations of PCOS, two of the hallmarks of these women, which heavily influence the disease phonotype, are overweight and obesity, with only 30–50% of PCOS patients exhibiting an average weight. Such conditions may result in IR and metabolic syndrome [18]. Glucose intolerance and an oral glucose tolerance test should be used to screen obese women with PCOS for metabolic syndrome. IR is described as the decrease in the use of glucose mediated by insulin. This metabolic abnormality occurs in more than 50% of PCOS cases and leads to reproductive complications. Improving the lifestyle and recovering with pharmacological intervention can help mitigate further irregularities [24,45]. Insulin can keep lipid metabolism in check by blocking the release of free fatty acids from adipose tissue, and in patients with IR, inhibition of the lipid oxidation rate is weakened, resulting in an increase in the concentration of free fatty acids in follicular fluid (FF) [20,46,47]. Some criteria for defining a metabolic syndrome were developed. These include components associated with the IR syndrome, such as centripetal obesity, hypertension, fasting hyperglycemia, and dyslipidemia [33,36,37,48]. It was proposed to add an oral glucose tolerance test (OGTT) to the fasting blood tests. The 2-h glucose level after a 75-g oral glucose challenge for glucose intolerance could be evaluated in this manner. Within obese PCOS women, impaired glucose tolerance and type 2 diabetes are two common features, both being diagnosed by OGTT [24,37]. IR, common in PCOS patients, increases the risk of metabolic syndrome and cardiovascular morbidity, and it generates higher glucose concentrations [19,49]. PCOS women that present obesity, a family history of type 2 diabetes, IR, or beta-cell dysfunction have high

probabilities of developing diabetes [36–38,50,51]. Apart from these metabolic abnormalities, it is also suggested that these woman present an increased risk of having strokes and cardiovascular diseases [24,40,52]. Other characteristics may be considered as additional risk factors, such as a family history of diabetes and excessive weight or BMI [24]. Clinical studies have revealed that PCOS patients with weight and BMI reduction were often associated with menstrual bleedings, the return of ovulation, and the normalization of other metabolic parameters [20,53].

One of the key features of PCOS is the presence of clinical and/or biochemical androgen excess, aside from other diseases [24,54]. Some clinical features of HA encompass the presence of hirsutism, acne, and androgenic alopecia when coupled with oligoovulation [24,54–56]. In addition, some studies pointed out that the circulating androgen levels, corresponding to the biochemical fraction, might represent an inherited marker for androgen excess in some women [57–62]. However, the quantification of the different androgens is challenging [24,63–65]. Due to the wide variety of the population, the values of the hormones may differ more than expected, and, therefore, control limits have not yet been set [24,44,66].

Due to an increase in the amplitude and frequency of luteinizing hormone (LH) pulses, LH concentrations and their relationship to follicle-stimulating hormone (FSH) levels are immensely elevated in PCOS women [24,67–69]. These levels may be influenced by the timing of ovulation, BMI (being lower in PCOS women with a higher BMI), and the analytical method used. The effects of LH on human reproduction are highly debated, with some studies suggesting that high levels of this hormone may reduce oocyte maturation and fertilization, resulting in higher miscarriage rates [24,68,70,71]. Others concluded that abnormal LH concentrations did not affect oocyte and embryo quality, implantation, fertilization, or pregnancy outcomes [24,72,73]. Many studies show gonadotropin-releasing hormone (GnRH) being used to reduce endogenous LH. However, some studies suggested that this practice reduced miscarriage rates [24,74], while others questioned its therapeutic effect [24,75,76]. Nonetheless, LH can be used as a secondary parameter, particularly in women who are not overweight [24].

Until today, both the subsequent etiology and pathophysiology of PCOS remain unclear, and several studies were conducted to facilitate the diagnosis and treatments [17,77]. Patients with PCOS that undergo ART might present a poor to exaggerated response, low oocyte quality, ovarian hyperstimulation syndrome, as well as changes in the FF metabolites pattern [17,18]. These abnormalities originate a decrease of MII oocytes, oocyte cleavage, fertilization, implantation, blastocyst conversion, poor egg to follicle ratio, and increased miscarriages [17,18]. Over the years, the focus of research shifted from embryo to oocyte quality to optimize IVF outcomes and to improve pregnancy rates. However, since PCOS is considered a heterogeneous disease, obtaining high-quality embryos is taken into more consideration [17,49].

1.1.2 Endometriosis

Endometriosis is a complex disease regarded as a significant medical and social issue since it is one of the leading causes of female infertility [78,79]. After inflammatory processes and uterine leiomyoma, it is the third most common factor in the pathogenesis of gynecologic diseases [79]. It affects 10–15% of reproductive-age women [80,81], 10–50% of infertile women, and approximately 80% of women suffering from chronic pelvic pain [79]. Despite extensive research, the origin, malignant transformation, and laboratory management of endometriosis are yet not well understood [79].

Endometriosis is a condition in which ectopic tissue with glands and stroma, such as the endometrium, grows outside the uterine cavity [79,80,82–85]. This abnormality is a gynecologic disease associated with estrogen-dependent chronic inflammation [80,86], due to the large production of estrogen [79,82,87]. This will lead to reproductive dysfunction, infertility, and the development of chronic pelvic pain syndrome [79,80,86]. Typically, this pathology is related with oxidative stress, genetic mutations, inflammation, cell invasion, and angiogenesis [78]. The presence of chronic inflammation and oxidative stress in the pelvic cavity can explain the occurrence of infertility [80,88]. In advanced stages altered pelvic anatomy might be observed [78], with latter progression to ovarian cancer [79,89–91]. The disease's negative impact on oocyte quality has also been highlighted, as it can impair embryonic development, lower implantation competence, and reduce clinical pregnancy rates [78,80,92].

This disease has several phenotypes, including superficial endometriosis (SUP), deep infiltrating endometriosis (DIE), and ovarian endometrioma (OMA). The most severe form, OMA, has a negative impact on ovarian physiology [80,88]. All forms of endometriosis are uncommon before menarche and after menopause [79] and the endometrium of women with endometriosis may differ from those of healthy women [79,82]. The abnormal endometria may be able to protect itself from immune system destruction by expressing specific antigens through the accumulation of diverse immune cell populations, as well as the synthesis and secretion of immunosuppressive factors [79,82]. It is capable of peritoneum implantation, aggressive growth, and adhesion into the peritoneum, proliferation and invasion into surrounding tissues, self-defence against physiological apoptosis [79,87], expression of heat shock proteins, and excessive angiogenesis [79,82].

The scientific community is still divided on the origin, etiology, pathogenesis, pathological process, and various manifestations of endometriosis [79,82,93]. Some theories for endometrial implantation relate to the reflux of endometrial cells, altered immune response, coelomic metaplasia, embryonic rest theory, lymphovascular metastasis, molecular alterations, and genetic instability. The most considered approach relies on Sampson's theory of retrograde menstruation, where the fallopian tubes lead to the implantation and proliferation of endometria cells in other areas [79,82,83,85,90,91,94–96]. However, this theory fails to explain the rare cases where the menstrual uterus is absent [79,82,93]. The fetal system regulates and

directs embryogenesis, but the mechanism remains unknown. Abnormalities in this control system may result in detectable immune system abnormalities in the adult immune system [79,97]. These irregularities, in turn, may control the degree of "aggressiveness" of endometriosis and result in different clinical behaviour of endometriosis [79,87,98]. According to a new unifying theory, endometriosis can also be caused by endometrial stem cells that migrate and proliferate during embryogenesis. Small focuses with glands and stroma were found in female fetuses in the cul-de-sac of the peritoneum. These can be remnants or the result of the Müllerian system metaplasia. Endometrial stem cells, which can cause endometriotic foci, may be present in these remnants. Endometrial stem cells differ from bone marrow stem cells since they overexpress immune-related gene pathways [79,99]. According to other theories, the immune system must be compromised for the ectopic endometriotic tissue to grow [79,97].

Some factors are critical to the implantation and proliferation of endometrial cells and the further development of endometriosis. Endometrial debris clearance in the peritoneal cavity is reduced in women with endometriosis due to impaired natural killer cell function, decreased macrophage phagocytosis, and induction of regulatory T cells. These factors may all contribute to endometrial cell tolerance in the peritoneal cavity. Immune response changes detected in the peritoneal fluid and cavity can also present themselves in uterine endometrial tissue, peripheral blood, and FF [83,100]. The immunological profile of FF in endometriosis patients can also reflect immunological changes in the systemic circulation or local inflammation caused by endometriosis lesions in the ovary or peritoneal cavity [83,101].

Endometriosis impairs follicular development in women, resulting in altered protein expression profiles in the FF [102]. Typically, oocyte quality is reduced due to changes in the follicular microenvironment, which affects oocyte development and maturation. The components of FF and the bidirectional intracellular communication between cumulus cells and oocytes play a crucial role in egg development and competence [78]. Therefore, significant differences can be found in FF according to de different types and stages of endometriosis [102]. Lower fecundity in these women is attributed to anatomic changes, such as adhesions, that disrupt folliculogenesis and ovum pick-up mechanisms [83,103]. The trafficking of leukocyte subsets to the eutopic endometrium as a result of inflammatory changes is also involved in the lower fecundity in endometriosis. The chemokines that direct their migration and the inflammatory changes may harm endometrial receptivity, possibly through progesterone resistance and changes in endometrial gene expression [83,104]. Indeed, lower oocyte quality may be the primary cause of poor pregnancy outcomes during IVF or intracytoplasmic sperm injection (ICSI) cycles in endometriosis patients. Although the IVF outcome for endometriosis-related infertility is dependent on the severity of the disease [78].

A thorough understanding of endometriosis pathophysiology is thus required for the development of novel diagnostic and treatment approaches for this debilitating condition [105].

1.1.3 Premature ovarian failure (POF)

Premature ovarian failure (POF), premature ovarian insufficiency (POI) or hypergonadotropic ovarian failure is a clinical syndrome that appears after puberty and before the age of 40 [106–109]. POF occurs when the exhaustion of the number of ovarian follicles is concurrent with autoimmune ovarian damage, along with genetic predisposition [110,111]. In 1939, the hormone profile in women with POF was described as hyper-gonadotropic hypoestrogenism [110,112]. Later, in 1950, Atria and co-workers discussed the clinical features of POF in detail [110]. Finally, in 1967, POF was first described as non-physiological amenorrhoea in non-menopausal women by Moraes-Ruehsen and Jones [110,113,114]. POF develops in about 1% of women [110,111], and the etiology of over 50% of the cases reported remains unknown [113,115]. This pathology is more recurrent in patients from countries with intermediate to low human development index [113].

Female patients are usually diagnosed with this condition due to conceiving difficulties [113,115,116] or when experiencing secondary amenorrhoea [110]. To diagnose young female menopausal symptoms must be determined [110,117]. In some cases, secondary loss of menses shows after stopping contraceptive pills [110,118,119]. Clinically, POF can be evaluated by the patient's general development, mental state, intellectual development, and nutritional status. Laboratories can also resort to pregnancy, drug withdrawal, progesterone, and estrogen tests [106,120]. Since the clinical manifestation of POF does not present a clear profile there is a need to perform tests to eliminate other diseases like PCOS, hypothalamic amenorrhea caused by stress or anorexia, and pathologies like pituitary tumours that influence the hypothalamic-pituitary-gonadal axis [113,116].

POF is characterized by a significant decrease in ovarian function and posterior failure, leading to a substantial reduction in ovarian follicles and infertility [106,108,109,113,116,121]. Due to ovarian disfunction, its main features are oligomenorrhea or frequent menstruation, blood with increased gonadotropin and FSH levels, decreased anti-Müllerian hormone (AMH), antral follicle count (AFC), inhibin B, estradiol, testosterone, and estrogen levels, accompanied by a series of low estrogen symptoms [106,108–110,113,115,122–124].

POF can present itself in two different ways. Up to 10% of the patients have the most severe form of the disease, characterized by a complete absence of pubertal development with primary amenorrhea [113,115]. On the other hand, Idiopathic POF is a kind of secondary amenorrhea with ambiguous causative factors and is the most common type of POF. It usually develops in the reproductive age and starts with gradual or progressive menstruation, rises to amenorrhea accompanied by menopausal symptoms, and leads to an atrophied stage of the internal and external reproductive organs [106,125,126]. Most patients undergo normal pubertal development and eventually show secondary amenorrhoea and menopausal symptoms later in life [113,115].

Although POF negatively impacts fertility, 10% of women with POF can conceive and carry to term, since 25% of patients can ovulate spontaneously due to the ovaries' erratic function [113,115]. Patients may show a resumption of ovarian function, and spontaneous pregnancies can occur. However, being diagnosed with POF can have a mental and physical toll on one's health [110,113,127]. Due to estrogen deficiency, POF women tend to present menopausal symptoms such as hair loss, tachycardia, hot flushes, excessive sweating, reduced sleep quality, mood swings (nervousness, irritability), poor concentration, decreased libido, dyspareunia, as well as skin and mucous membrane dryness [110,113,115,116,128,129]. Furthermore, the deficit in estrogen and testosterone leads to lower bone marrow and bone mineral density, increasing the risk of developing osteopenia, osteoporosis or easily fracturing a bone [110,113,115,124]. An increase in morbidity and mortality is also seen in patients with untreated and uncontrolled POF due to their risk of developing metabolic disorders, thus leading to cardiovascular diseases such as atherosclerosis, hypercholesterolemia, strokes, ischemic heart diseases, as well as urogenital atrophy [110,113,115,128,129].

POF may arise from different factors. About 10% to 20% of these women present a family history of POF [130], while other 20% are usually correlated with other autoimmune diseases [110,128,129]. Some of the autoimmune complications encompass autoimmunity against the adrenal gland [113,115,131], presence of anti-ovarian antibodies, thyroiditis (Hashimoto's disease), adrenal insufficiency (Addison's disease) [110,113,116,128,129], coeliac disease, type 1 diabetes, albinism, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis [110,128,129,132]. Additionally, up to 40% of POF cases account for genetic mutations concerning the X chromosome (Turner syndrome, fragile X syndrome, pseudohypoparathyroidism type 1a) [113,123,133], the AIRE gene, responsible for polyendocrinopathies, and the inhibin codifying gene, which relates to FSH secretion by the pituitary and gametogenesis [134][110]. Congenital enzymatic deficiencies, such as galactosaemia [110], and viral infections like malaria, tuberculosis, Shigella, cytomegalovirus, mumps, and varicella may also lead to POF [110,113,135]. Smoking can also mediate the development of this disease by stimulating the death of oocytes, oocyte depletion, and early menopause [110,113,136]. Lastly, oncologic treatment (radiotherapy and chemotherapy), surgical treatment (oophorectomy) [110,113], and anti-HPV vaccination can cause permanent ovary damage and the development of secondary amenorrhea, leading to POF [110,137].

Up until now, there is no method to restore the function of the ovaries, which generally means psychological intervention, drug therapy (estrogen supplementation therapy and progesterone), and traditional Chinese medicine treatment [106,108,109]. On behalf of the immunologic system, immunomodulation therapy (to induce ovulation) has been used, including high-dose corticosteroid and intravenous immunoglobulin treatment [110,138,139], as well as monoclonal antibodies when treating POF caused by autoimmune ovarian damage [110,140,141]. Recently, melatonin supplementation was described as a treatment modality for perimenopause,

menopause-related depression, fertility, thyroid dysfunction, and decreased gonadotropin levels [110,142–144].

1.2 Assisted reproductive techniques (ART)

Since the first 'test-tube baby' in 1978, ART have grown exponentially. Globally, the field of ART has already seen rapid progress, with over eight million babies conceived [145–148]. Recently, new techniques and changes to laboratory conditions made it possible for a broader group of infertility patients to receive treatment. Pregnancy rates increased, and transferring fewer embryos is now a more effective practice, resulting in a significant decrease in the multiple pregnancy rate in many countries and improved perinatal outcomes [3,145,149].

ART has become an essential part of modern patient care because it is the most effective treatment modality in reproductive medicine. It now refers to family planning, which began with the introduction of the first oral contraceptives in the early 1960s. Birth control pills and other forms of contraception have profoundly changed society. The availability of family planning not only enabled women to pursue professional careers but also aided in delaying reproduction [150]. Nowadays, mature parents have fewer children, but they stimulate the need for high-quality feto-maternal medicine to identify and avoid all potential threads prevalent at the advanced age of the future parents, as well as for optimal obstetrical and neonatal care eliminating all risks to the mother and the baby. These lead to preimplantation embryo genetic testing and future parent genome screening. These societal behaviours, combined with new medical and laboratory advances, have resulted in a thriving industry centred on family planning, reproduction, pregnancy, and birth. The pharmaceutical industry performs its role in this environment by manufacturing sophisticated medications required to control ovarian and uterine functions during all ART procedures. Other industries have taken over the manufacturing of culture media (including quality control), the increasingly sophisticated equipment of the embryology lab, and the manufacturing of surgical devices required for oocyte collection and embryo transfer. The growing complexity of treatment options obliges to frequent collaboration with other medical disciplines, including medical geneticists, oncologists, urologists, and obstetricians [150,151].

ART denominates all interventions, such as the *in vitro* handling of human oocytes, spermatozoa, and embryos for reproduction. Among those interventions are IVF, embryo transfer, ICSI, embryo biopsy, preimplantation genetic testing, assisted hatching and cryopreservation of either gametes or embryos [2,147,150,152,153].

Despite all the advances, ART remains associated with potentially negative obstetric outcomes for mothers and infants. Pregnancy hypertension, preterm delivery [147,152,153], birth defects such as imprinting disorders in children [145,147,148,154,155], and low birth weight [147,152,153] are some examples. Many of these negative outcomes arise from a higher rate of multiple pregnancies following ART [147,150,156]. Multiple pregnancy rates have decreased

significantly as the use of single embryo transfer has increased, but this approach has not yet been adopted by all countries [147,156]. As new ART technologies emerge, it is critical to monitor the safety and health of their offspring [147]. These cause an increase in premature children, who may be born with compromised future health. The growing influence of ART on reproductive choice has sparked political debate, and most countries have enacted legal frameworks. However, ART is constantly expanding, and therapeutic options will continue to fuel political debate [150].

2 Determination of Metabolites in Follicular Fluid

2.1 Follicular Fluid (FF)

Follicular fluid (FF) serves as a complex microenvironment for germ cell–somatic cell communication. It encompasses a variety of metabolites and enables different reactions to take place that are crucial to oocyte growth [18]. It is derived from the diffusion of serum, transudate of plasma, and metabolites synthesized in the follicle wall that will later be altered by granulosa cells (GCs) and theca cells. In addition, compounds that derive from local follicular metabolic processes and the biological activities of ovarian cells are also present. This biological matrix is the only one directly associated with the oocyte since it is where its growth and differentiation occurs *in vivo*. It contains a variety of bioactive molecules that change in quantity and quality during follicle development, as well as specific changes in the follicular microenvironment that lead to follicle and oocyte maturation and development. The biosynthesis and transport of these metabolites are crucial to multiple metabolic reactions; they regulate meiosis, are involved in the synthesis of steroid hormones and glycoproteins by the dominant follicle, and promote follicle and oocyte maturation and development, fertilization, and implantation [157–159]. The follicle wall acts as a highly rough molecular sieve that allows passage to small metabolites while restringing the access to molecules over 100 kDa. There is a bidirectional signal regulation and metabolite transport between GCs and oocytes, such as steroid hormone biosynthesis, oocyte gene transcription, and protein synthesis regulation, showing a deep connection between the oocyte and GCs [158].

A more detailed understanding of FF and its metabolic profile is crucial for further analysis of several pathologies, such as PCOS, endometriosis, and early ovarian failure. FF has become an essential source of information since it is a non-invasive matrix that gathers biological insights about fertility, reflecting the alterations of the patient's microenvironment. Recently, the molecular and biomolecular signature of FF have aroused many interests, leading to several studies that aimed to identify new targets that allow for evaluation of the development of the oocyte. Consequently, an exhaustive characterization and comprehension of FF may help the recognition of metabolites that could potentially disturb normal female function and promote infertility [158–160].

2.2 Metabolomics in the FF

Conventional approaches used to determine and analyse biomarkers related to oocyte development, and to predict its quality and viability, tend to be ineffective, imprecise, and present some analytical limitations. Thus, the quantification of proteins and peptides in biological matrices through metabolomic analysis might predict, with more accuracy, the success of an assisted reproductive procedure [158]. Metabolomics is a high-throughput method for detecting several metabolic contents in diverse biological samples. Untargeted metabolomics, which focuses on the dynamic changes of all small molecules in response to an organismal disturbance, can provide deep insights into etiopathogenesis and the recognition of different biomarkers for a variety of diseases [18]. In general, metabolomics is a recent field of 'omics' technology that arises from genomics, transcriptomics, and proteomics.

Metabolites are described as low-molecular-weight molecules (<1500 Daltons) that derive from a variety of biological and cellular processes. Therefore, they can provide crucial information about the genomic, epigenomic, matrix, and environmental outcomes of a cell, tissue, or organism, creating the perfect association between genes and their respective phenotypes. Since metabolites can be related to specific biological functions and processes in systems, cells, or tissues, a metabolic investigation may prove to be more advantageous than the study of genomics, transcriptomics, and proteomics [157,161–164]. Due to their medical and biological signature, these molecules permit the quantitative measurement of the dynamic chemical reactions that occur in living systems in response to a pathophysiological insult or genetic variation, and they can be accessed via biological matrixes such as blood, urine, plasma, serum, and FF [157,164]. Since the causes of infertility can be traced back to a metabolic imbalance, metabolomics can be used in reproductive medicine to identify and quantify low molecular weight metabolites found in the FF. Indeed, the use of metabolomics made it possible to identify molecules found downstream of gene expression, providing critical information on cellular function [159]. All these factors make them attractive biomarker candidates, perfect for the study of human oocytes and embryos, as well as their development [157,164].

Although many biomarkers have been evaluated in the FF, there are a few consistent results among different works in the literature. Metabolites must be analysed in the FF of the dominant follicle to reflect more accurately the dynamic changes in concentrations that occur during development [165]. The dominant follicle is the largest follicle, making it the clearest and also free from contaminations, such as blood cells [17]. The lack of consensus can be related to the criteria used in the analytical approaches, such as sample preparation of the FF, the presence of contaminants, and mass range. These might derive from the analysis of different follicular sizes, differences in methods for measuring analytes, patient age, number of patients, or number of analysed samples, genetics, BMI, ovarian stimulation, infertility, or other disease diagnoses. Another reason may be linked to the heterogeneity in the IVF protocols, to the human ability to respond to IVF procedures, or even to the psychophysiological characteristics of each woman (or couple). Studies taking place over different periods give rise to FFs with heterogeneous

metabolic profiles, so accuracy also varies from study to study. Many of these issues affect the content of follicle hormones and reflect the oocyte and embryo quality. Likewise, taking into account several external factors, such as environmental pollution, smoking, heavy metals, or pesticides can increase levels of oxidants in the body and act as confounders. In short, most studies are limited, as even daily lifestyle and dietary patterns can influence the pathogenesis of infertility-related diseases and its biomarkers [17,19,49,53,166–168].

Volatilomics, a metabolomic subgroup that reflects biochemical metabolic activity and environmental influences, offers new insights into the physiological processes of several disorders [169–172]. It encompasses all volatile organic compounds (VOCs) that derive from exogenous sources, including nutrition, medications, and environmental exposure, as well as internal sources, like endogenous metabolomic and biochemical processes [169–171]. Multiple pathologies, such as cancer, genetic and metabolic disorders, schizophrenia, and infectious diseases, have already been connected to particular VOC signatures [170,173] and potential biomarkers [174,175]. The volatilomics approach is based on highly sensitive analytical techniques and does not require invasive procedures, since VOCs can be found in readily accessible biofluids [172,176–178], such urine [172,174,177,179–183], exhaled breath [172,174,177,182–188], saliva [172,174,182,183,189–194], skin emanations, breast milk [174,182,183], and tissues [180,195–197]. Volatilomics studies apply to the targeted analysis of a limited number of metabolites connected to a specific biological pathway or the fingerprinting of a significant fraction of metabolites [172,176–178]. In this study, a more untargeted approach was required to detect and identify unexpected changes in the concentrations of specific metabolites [172,198].

2.3 Sample preparation techniques

Due to the complexity of the matrices and the requirement to identify active compounds and impurities at low levels, analysing biological samples is a challenging and complex task [199–204]. A thorough analytical methodological approach involves crucial processes like sample preparation, analytes isolation, and qualitative/quantitative analysis [199].

Specifically, the sample preparation, the first crucial step in a multi-stage instrumental setup, tries to move the target analytes from an unfavourable medium to one that is more palatable. It may also carry out operations including derivatization, analytes concentration/enrichment, and clean-up [199,205–209]. During the last years, several sample preparation methods have been developed, ranging from pressured fluids to microextraction approaches and microwave assistance to completely automated online systems. However, to increase the specificity of this analytical step and facilitate subsequent stages, confirmation methods rather than screening strategies frequently use sorption-based sample preparation procedures [199]. The most straightforward techniques include sample preconcentration [210,211], filtration [199,212], centrifugation [199,213], protein precipitation [214–221], soxhlet extraction [222,223], liquid-liquid extraction [199,224], and support-assisted liquid-liquid extraction [225]. Accurate results

can be achieved by using more sophisticated techniques, such as dispersive solid-phase extraction [199,226], single-drop microextraction [207,227–230], stir-bar sorptive extraction [231–233], solid-phase microextraction (SPME) [234–236], solid-phase extraction [237–239], hydrophobic mechanisms [240–242], electrostatic mechanisms [243], and mixed-mode mechanisms [244–246].

The sample preparation technique performed in this research was SPME since it is one of the most precise and used techniques to isolate biomarkers in liquid biological samples. For this reason, a brief introduction to this technique is described below.

2.3.1 Solid-phase microextraction (SPME)

Microextraction techniques (METs) represent a crucial step in analytical methodologies. They provide samples in appropriate volumes and purification levels required to characterize the target analytes [247,248]. SPME is a simple and powerful sample preparation technique distinguished by its time- and cost-effective analysis, low solvent consumption, high sensitivity, and wide application [247–249]. SPME, the first successful modern MET, was invented in the early 1990s and can be easily automated to achieve high-throughput performance in the clinical setting [247,248]. It addresses diverse challenges of traditional sample preparation by combining several procedures (sampling, extraction, concentration, and loading) in a single step, limiting the possibility of experimental errors [247,249,250].

The basic principle of SPME involves the partition equilibrium of the target analytes between the sample matrix and the stationary phase, where the analytes should be retained [247,249,251]. The analytes can then be thermally desorbed in a GC injector port, removed by solvents for high-performance liquid chromatography (HPLC), or electrophoresed. The combination of these flowcharts enable superior analytical performance for quantifying various substances [247,251]. Figure 1 represents a schematic view of all the components or phases present in a SPME syringe, as well as its external and internal assembly.

There are three types of fused silica-coated fibres highly used. In the direct mode, the coated fibre is immersed directly in the aqueous samples, and the analytes transfer from the sample matrix to the extracting phase. A stirring bar is required for sample agitation to achieve equilibration faster and improve analytes transport from the sample bulk to the fibre vicinity [247,249,252,253]. In the headspace mode (HS-SPME), the extraction of the analytes occurs from the gas phase above a gaseous, aqueous, or solid sample. This procedure shields the fibre from the compromising effects of non-volatile, high molecular-weight substances. It also enables matrix changes [247,249,252]. In the extraction with a membrane protection mode, the separation occurs from the sample with a selective membrane, allowing the passage of analytes while blocking interferences and adverse effects. This technique allows for the analysis of less volatile compounds [247,252].

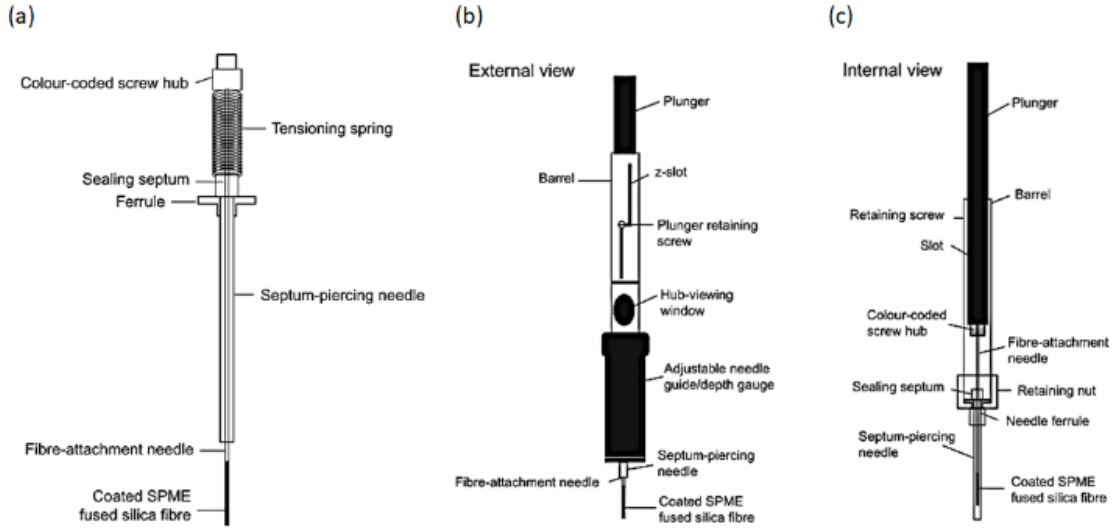


Figure 1 Schematic view of SPME manual fibre assembly (a), and SPME manual fibre assembly holder (external (b) and internal (c) view). Adapted from [362].

The number of analytes extracted by SPME is affected by several experimental parameters, influencing its extraction efficiency. Among these are the nature of the fibre coating, the extraction time and temperature, the ionic strength, and the pH [247,254–257]. Succinctly, non-polar, polar, or semi-polar coating fibres are available. Different polarities increase extraction selectivity while decreasing extracting interferences [247,258].

Extraction time is primarily determined by the agitation rate and the analyte partition coefficient. The maximum sensitivity occurs when the level of analytes extracted remains constant. Small-time variations do not affect the extraction at this point. Furthermore, because of the linear relationship between the amount of analyte adsorbed by the SPME fibre and its initial concentration in the sample matrix in nonequilibrium conditions, complete equilibrium is not required for accurate and precise analysis [247,259].

In thermodynamic terms, exothermic equilibration occurs in SPME extraction. When the extraction temperature rises, so does the extraction rate, leading to a decrease in the distribution constant [247,252]. The headspace-analyte partition coefficient increases with the temperature, resulting in a higher analyte concentration in the headspace and a shorter extraction time [247,260,261].

The addition of salt will change de ionic strength and affect the extraction efficiency in two ways: it can change the properties of the boundary phase or decrease the solubility of hydrophilic compounds in the aqueous phase (salting-out effect) [247,262].

Finally, there is a strong dependence of the extraction efficiency on the pH value of acidic and basic analytes; therefore, the SPME extraction yield can be improved by adjusting the pH of the

samples. Weak acids and bases convert to neutral forms and can be extracted by the SPME fibre [247,263].

2.4 Analytes isolation, and qualitative/quantitative analysis techniques

To separate the target analytes from interferents still present in the sample, the analytes are transported to a high resolution (or high efficiency) chromatographic column. The next stage generally entails identifying and quantifying the separated analytes by combining chromatographic and mass spectrometric methods. The majority of the time, sample preparation must still be done before the number of analytes is introduced into the chromatographic column, even though recent advancements in separation techniques and mass spectrometry have helped to minimize efforts in this area [199].

To date, a wide variety of techniques are used to analyse and measure FF constituents namely, Nuclear Magnetic Resonance (NMR) [102,168,264,265], Gas Chromatography coupled with Mass Spectrometry (GC-MS) [266,267], Liquid Chromatography coupled with Mass Spectrometry (LC-MS) [18,268–271], and Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS) [17,166,272,273]. Some studies have also resorted to Ultra-High Performance Liquid Chromatography Coupled with Mass Spectrometry (UHPLC-MS) [274], Ultra-High Performance Liquid Chromatography with tandem Mass Spectrometry (UHPLC-MS/MS) [77], multiple reaction monitoring profiling [275], and different types of immunoassays [49,276–278].

In the development of this dissertation, GC-MS was used for the isolation, and qualitative and quantitative analysis, once it is highly used to create a profile and quantify biomarkers in liquid biological samples. Therefore, a brief introduction about this technique is described below.

2.4.1 Gas chromatography coupled with mass spectrometry (GC-MS)

Gas chromatography (GC), as other techniques for separating, quantifying, and qualifying multicomponent mixtures, has evolved, making easier to conduct analyses in many applications and fields [279–282]. Although its instrumentation remained unchanged for the last 40 years, its design, materials, and methodology were highly improved. GC is now one of the most widely used techniques, showing the best separation power. This technique's instrumentation consists of four major components: a carrier gas, a column, a detector, and a data system [283–285]. Systems like mass spectrometry (MS) have influenced and improved GC. The combination of the two devices results in improved sensitivity, specificity, and separation of the components to be analysed [283,285,286] It also enables the determination of detailed information on the structure of various compounds, allowing the precise identification and quantification based on their mass-to-charge ratio (m/z) [283,287].

Pollution, forensic, and general trace analysis are the most common applications for GC-MS, as well as purification and determination of thermochemical constants [279,288–290]. GC-MS is also used for metabolite profiling and quantification [291–298]. These include volatile compounds that can be measured directly, as well as non-volatile or semi-volatile metabolites that can be assessed after derivatization [291,298]. It presents well-established libraries of both commercial and 'in house' metabolite databases available [293–298].

As illustrated in figure 2, the main components of a GC-MS encompass an injector, a column, an ion source, a vacuum system, a detector, and a panel for control electronics to monitor the inputs and outputs parameters of the MS [283,299]. In technical terms, a small sample is injected into the GC, vaporized, and directed to the chromatographic column by the carrier gas (typically helium), referred to as the mobile phase [283,291,298,299]. The GC column outlet is connected to the ion source of the MS via a heated transfer line, where the compounds eluting from the column are ionized [291,298,300–303]. The interactions between the carrier gas phase and the stationary phase caused the molecules in the sample to separate. To achieve the highest resolution, a capillary column with good separation should be selected, suitable for the sample [291,298,304–307]. Currently, there is a wide range of instruments that vary in ionization and mass separation. The most common is a single-quadrupole mass spectrometer with electron impact ionization [291,298]. When a sample stream collides with an electron beam, an electron is lost from the sample molecules, and the resulting ions represent the total mass of each analyte. The molecular ion usually fragments due to the large amount of energy imparted, producing smaller ions with characteristic relative abundances that provide a 'fingerprint' for that molecular structure [291,298,300–303].

Some of the disadvantages of this technique rely on the fact that it requires derivatization, the analysis time is long, it does not allow real-time analysis or direct quantitative determinations, and presents a limit of sample capacity [293–298].

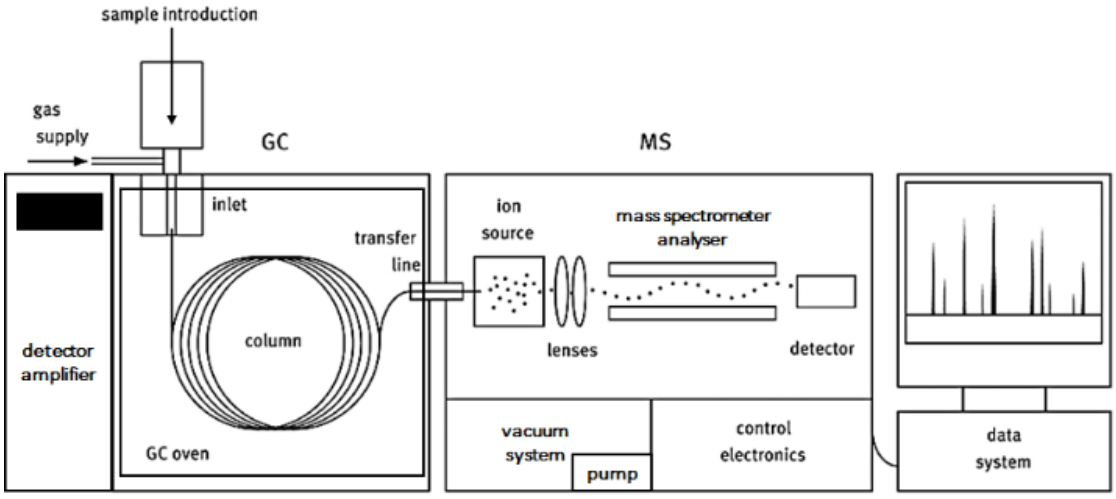


Figure 2 Schematic diagram of GC/MS instrument. Adapted from [363].

Part of the introduction to this dissertation is present in a published. The topics “1.1.1) Polycystic ovary syndrome (PCOS)”, ”2.1) Follicular Fluid (FF) ”, ”2.2) Metabolomics in the FF”, and the second paragraph of the topic ” 2.4) Analytes isolation, and qualitative/quantitative analysis techniques” can be found at “Follicular Fluid: A Powerful Tool for the Understanding and Diagnosis of Polycystic Ovary Syndrome”, by Brinca, A.T.; Ramalhinho, A.C.; Sousa, Â.; Oliani, A.H.; Breitenfeld, L.; Passarinha, L.A.; Gallardo, E., Biomedicines 2022, 10, 1254, 1-27.

The “Abstract”, as well as the last paragraph present in the topic ”2.2) Metabolomics in the FF”, on Volatilomics, are also present in the manuscript entitled: “Volatilomics as an Emerging Strategy to Determine Potential Biomarkers of Female Infertility: A Pilot Study”, by Brinca, A.T.; Anjos, O.; Alves, M.M.C; Sousa, Â.; Oliani, A.H.; Breitenfeld, L.; Passarinha, L.A.; Ramalhinho, A.C.; Gallardo, E., submitted to Biomedicines October 2022.

Chapter II

1 Experimental procedure

1.1 Material and reagents

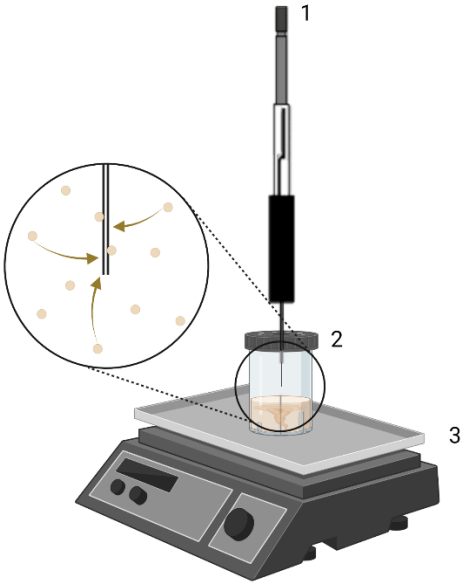
The SPME fibre holder for manual use and the 100 μm polydimethylsiloxane (PDMS) coated fibre were obtained from Supelco (Bellefonte, PA, USA). The SPME fiber was conditioned according to manufacturer's instructions.

1.2 Subjects sample collection

To investigate the metabolomic pattern of FF, 52 samples from women who underwent IVF procedures were analysed. These include 15 patients with PCOS, 8 with endometriosis, 12 with POF, and 17 controls. The 17 controls correspond to women submitted to IVF procedures due to specific conditions that do not affect the FF, such as tubal obstruction, or when the couple's primordial fertility factor was male driven. Women were enrolled between October 2015 and July 2019. All subjects were Caucasian. The samples were stored at −80 ◦C after extraction and kept at 4°C during the experimental procedures and were obtained at the Assisted Reproduction Laboratory of Academic Hospital Center of Cova da Beira in Covilhã, Portugal. All experiments were performed in accordance with the standard guidelines and national requirements, namely Declaration of Helsinki and Portuguese Law 21/2014, and approved by the institutional ethics committee of Academic Hospital Center of Cova da Beira, Covilhã– Portugal (reference number 47/2015, approved on July 15, 2015). An informed consent was obtained from all individuals before inclusion.

1.3 Extraction of metabolites from the follicular fluid (FF)

After placing 2 mL of each FF in a vial, the volatile metabolites were extracted using a 100 μm PDMS, non-bonded SPME fibre exposed in the headspace (HS-SPME) of the flasks for 45 min at 40 °C, in continuous agitation (125 rpm). This procedure is diagrammed in Figure 1. Subsequently, the SPME syringe was injected into the GC injection port for 5 min to allow the desorption of VOCs from the fibre. This methodology was adapted from a previous research carried out by C. Silva and research team [308]. The PDMS fiber was chosen due to its compatibility with volatile analytes that range from 80-500 MW and applicability with manual holder.



Fiber	100 µm PDMS
Sample Volume	2 mL
Extraction Time	45 min
Extraction Temperature	40 °C

Figure 1 Schematic representation of VOCs extraction from FF: 1) SPME syringe; 2) vial with 2 mL of FF; 3) heating mantle with magnetic stirrer. Created with BioRender.com.

1.4 Gas chromatography mass-spectrometry (GC-MS)

VOCs in the headspace were analysed using an HP 7890B gas chromatographic system in conjunction with an Agilent Technologies 5977A mass spectrometer, and Agilent 7693 autosampler. For the separation of the analytes, a capillary column (30 m 0.25-mm I.D., 0.25-m film thickness) with 5% phenylmethylsiloxane (HP-5MS) was provided by J & W Scientific (Folsom, CA, USA). The oven temperature profile was: (a) 5 min at 45 °C; (b) increase temperature until 150 °C, at a rate of 2 °C min^{-1}; (c) 150 °C for 10 min; (d) increase temperature until 220 °C, at a rate of 7 °C min^{-1}; and (e) 220 °C for 10 min. Column flow was constant at 1.0 mL/min using helium (He ultrapure, Nippon gases, Vila Franca de Xira, Portugal) as the carrier gas. The injection port was maintained at 250 °C and operated in the splitless mode (5 min). Regarding MS analyses, the operating temperatures of the transfer line, quadrupole and ionization source were 280, 150 and 230 °C, respectively. The electron impact mass spectra were recorded at 70 eV and the ionization current was 35µ A, and data acquisition was performed in scan mode (50–550 m/z). The identification of metabolites was performed comparing mass spectra with the Agilent MS ChemStation Software (Palo Alto, CA, USA) equipped with the NIST20, Wiley12 and SWGDRUGv8 mass spectral libraries with a similarity threshold higher than 80%, or with commercially standards when available.

2 Results and discussion

A heatmap was performed, representing values for the main variable of interest across two axis variables as a cluster effect. For the heatmap, STATISTICA 7 (StatSoft. Inc. USA) software was used.

Figure 2 represents a heatmap of the different metabolites and their respective tendencies towards each disease. It is, therefore, possible to observe some associations between the samples and their unique metabolomic expressions. The colours range from red to blue according to the comparative abundance of metabolites in the FFs. When comparing the results from each pathology, red relates to a lower presence, while blue indicates a more current presence.

Figure 3 is a percentual representation of all VOCs present along each medical condition. Several VOCs have similar incidences throughout the various health status, such as palmitic acid, tetradecamethylcycloheptasiloxane, cyclotetradecane, and methyl stearate. At the same time, some metabolites are not present in all the samples that concerned a specific pathology. Therefore, some metabolites cannot be related to a particular medical condition.

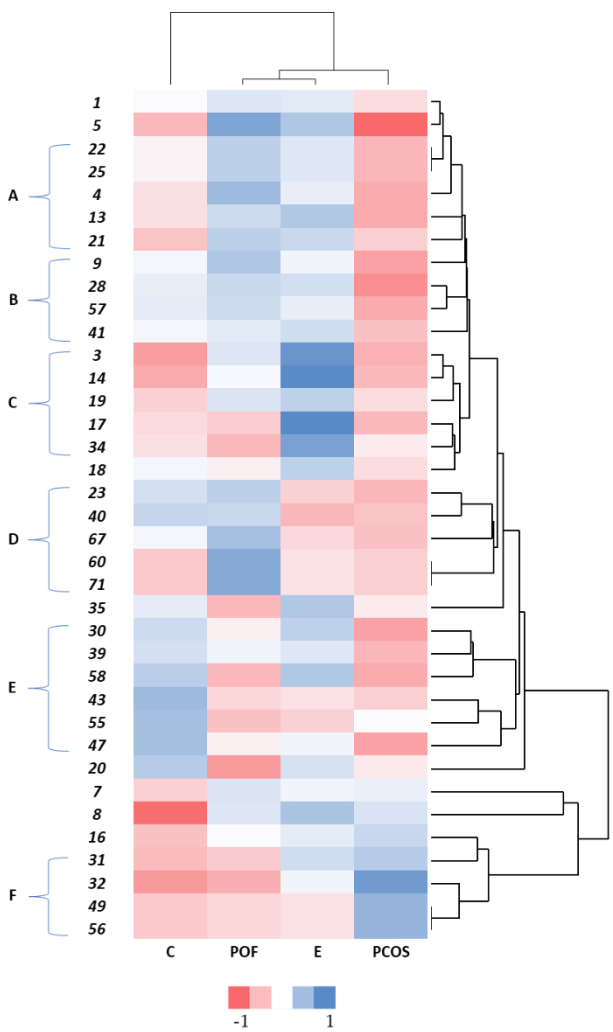


Figure 2 Heatmap of the correlation and tendencies between the FFs of the four medical conditions and respective VOCs. The abscises correspond to: C) controls; POF) premature ovarian failure; E) endometriosis; PCOS) polycystic ovary syndrome. The coordinates represent the several VOCs considered, which have been assigned numbers. They were separated in six Groups according to the distribution throughout the samples. Each number correspondes to a single VOC: 1) palmitic acid; 3) tetradecanal; 4) 2,4-di-tert-butylphenol; 5) diethyl phthalate; 7) 1,2,3,4-tetramethylbenzene; 8) 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene; 9) palmitic acid ME; 13) isopropyl myristate; 14) octadecanal; 16) tetradecamethylcycloheptasiloxane; 17) hexadecanal; 18) gamma-stearolactone; 19) dodecane; 20) dodecamethylcyclohexasiloxane; 21) hexadecyloxirane; 22) octadecane; 23) diisooctylphthalate; 25) 1,2,3,5-tetramethylbenzene; 28) stearyl alcohol; 30) stearic acid; 31) tetradecamethylhexasiloxane; 32) hexadecamethylheptasiloxane; 34) eicosamethylcyclodecasiloxane; 35) octadecan-1-ol trimethylsilvyethe;39) cyclotetradecane; 40) hexadecanoic acid; 41) methyl stearate; 43) heptadecane; 47) butyl-2-methylpropylphthalate; 49) ethyl xylene; 55) tetracosamethyl-cyclododecasiloxane; 56) docosane; 57) hexamethyldisiloxane; 58) 1,3-di-tert-butylbenzene; 60) 1-dodecanol; 67) oleamide; 71) 4,6-dimethyldodecane.

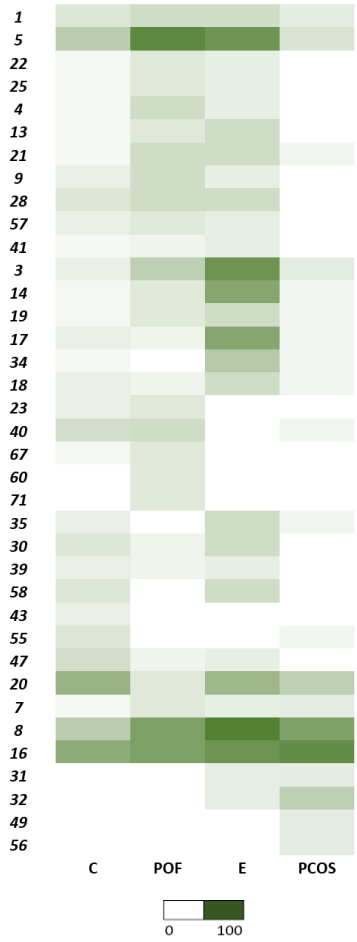


Figure 3 Percentual occurrence of VOCs in all the samples from each medical condition. The abscises correspond to: C) controls; POF) premature ovarian failure; E) endometriosis; PCOS) polycystic ovary syndrome. The coordinates represent the several VOCs considered, which have been assigned numbers: 1) palmitic acid; 3) tetradecanal; 4) 2,4-di-tert-butylphenol; 5) diethyl phthalate; 7) 1,2,3,4-tetramethylbenzene; 8) 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene; 9) palmitic acid ME; 13) isopropyl myristate; 14) octadecanal; 16) tetradecamethylcycloheptasiloxane; 17) hexadecanal; 18) gam-ma-stearolactone; 19) dodecane; 20) dodecamethylcyclohexasiloxane; 21) hexadecyloxirane; 22) octadecane; 23) diisooctylphthalate; 25) 1,2,3,5-tetramethylbenzene; 28) stearyl alcohol; 30) stearic acid; 31) tetradecamethylhexasiloxane; 32) hexadecamethylheptasiloxane; 34) eicosamethyl-cyclodecasiloxane; 35) octadecan-1-ol trimethylsilvyethe; 39) cyclotetradecane; 40) hexadecanoic acid; 41) methyl stearate; 43) heptadecane; 47) bu-tyl-2-methylpropylphthalate; 49) ethyl xylene; 55) tetracosamethyl-cyclododecasiloxane; 56) docosane; 57) hexamethyldisiloxane; 58) 1,3-di-tert-butylbenzene; 60) 1-dodecanol; 67) oleamide; 71) 4,6-dimethyldodecane.

The controls, represented by "C", show a profile that differentiates itself from the infertility complications. The most found metabolites are tetradecamethylcycloheptasiloxane, with an occurrence of 59%, followed by dodecamethylcyclohexasiloxane (53%), 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (35%) and diethyl phthalate (35%). Even though tetradecamethylcycloheptasiloxane was present in several control samples, it is relevant to notice that the remaining clinical conditions had further representability. Additionally, these FFs present a small incidence of several compounds. Metabolites such as 1-dodecanol, 4,6-dimethyldodecane, and all VOCs comprehended in Group F have no representation.

POF and E are the two diseases with more similarities, forming a cluster of their own. Diethyl phthalate, a phthalic acid ester, was found in 83% of endometriosis and in 75% of the POF samples. Both these conditions comprehended twice as more of this metabolite as the controls.

Phthalic acid esters are considered endocrine disrupters [309–312]. Some toxicological studies described phthalates as toxic for human reproduction [313–315]. They decrease fertility rates [316], produce anti-androgenic effects by reducing testosterone and estrogen production at high doses [317–319], affect folliculogenesis [316] by restricting antral follicle growth through inhibition of 17- beta-estradiol production [320–323], generate ovarian dysfunction [320–323], impact oocyte maturation and embryonic development [316]. Du and research team found relevant phthalate concentrations in the FF of women undergoing IVF [316]. These can arise from intoxications, industrial exposures, or ingestions [183,237,324]. In 2013, Upsona and research group released a study on the exposure to select phthalates, showing how pervasive they are among female enrollees. Their research also suggested that phthalates may increase the risk of developing endometriosis, a hormonally-mediated disease, among reproductive-age women [325]. Buck Louis and associates also demonstrated that select phthalates are associated with higher odds of an endometriosis diagnosis [317]. Furthermore, these compounds can be found in distinct biological matrixes and related to diseases such as cancer [183,237,324]. However, there is still a lack of consistency in the findings, reducing the impact of some methodologies [317,325–332].

POF samples present exclusive compounds, such as 1-dodecanol and 4,6-dimethyldodecane. Even though they are only present in 17% of the samples, as shown in Figure 3, Figure 2 describes a relevant correlation considering the controls and remaining diseases. 1-dodecanol appears in para-axillary and nipple–areola regions of pregnant women. Some reports show that this VOC is affected by emotional anomalies [333]. When compared to the controls, urinary samples from specific types of cancer also presented high levels of 1-dodecanol, namely colorectal, leukaemia and lymphoma [334]. However, its effects on the reproductive system are not fully comprehended, and more accurate data is required to formulate a precise mechanism of action for this metabolite [335]. POF FFs also have great representativeness from Groups A and B. On the other hand, Group E is mainly absent, as the VOC dodecamethylcyclohexasiloxane.

Endometriosis has a versatile volatilomic profile, with specific compounds detected in many samples. Since these metabolites are not as present in the remaining ones, they might be suited to characterize the clinical condition. Endometriosis samples correlate the best with metabolites from Group C (tetradecanal (75%), octadecanal (63%), hexadecanal (63%), Eicosamethyl-cyclodecasiloxane (38%)). Group C components are discrepant through all the samples, but the FF from the three pathologies present a slight prevalence of these metabolites compared with the controls. However, according to the heatmap, these may not be suited markers. Octadecanal and tetradecanal are both fatty aldehydes [336,337]. Tetradecanal, also known as myristyl aldehyde, is the reduced form of myristyl acid [337]. Hexadecanal is a volatile straight-chain aldehyde [183] and a final product of glycosphingolipid metabolism [338]. Its metabolization gives origin to phospholipids that can signal within cells [339,340]. Hexadecanal is present in several biological fluids [183], but its levels tend to be low, particularly in the cumulus-oocyte complexes, according to some animal studies [339]. Cordeiro and his team showed that age is

closely related to enhanced glycosphingolipid metabolism [341], demonstrating a negative correlation. Overall, sphingolipids are associated with steroid hormone synthesis, mainly through the modulation of steroidogenic pathways. These molecules may act as second messengers or paracrine regulators for genetic transcription, although the sphingolipid mechanism is still not fully understood [167,342]. The abundance of these compounds in FF may indicate alterations in the proper steroidogenesis process for these patients [167]. Sphingolipid breakdown is also a relevant event during apoptosis [341–343]. Tetradecamethylhexasiloxane and hexadecamethylheptasiloxane, belonging to Group F, and some metabolites from Group A, such as diethyl phthalate, might also help differentiate the FF of endometriosis women. The first two are also siloxanes [344], and tetradecamethylhexasiloxane was already related to male infertility [345,346].

PCOS samples are the ones that present the smallest number of compounds. Even though the prevalent VOC is tetradecamethylcycloheptasiloxane, 1-ethyl-2,3-dimethylbenzene and docosane might be the best predictors once they are only present in the FF of PCOS patients. Tetradecamethylcycloheptasiloxane, also known as cyclomethicone 7, is a cyclic dimethyl polysiloxane compound [347]. These metabolites are known to interfere with fertility and present potential carcinogenic effects (uterine tumours in females). They increase ovarian atrophy and vaginal mucification [348], disturb hormonal function and are reproductive toxicants [349]. 1-Ethyl-2,3-dimethylbenzene, or ethyl xylene, is considered a BTEX (benzene, toluene, ethylbenzene, xylene) member [350]. Exposure to these components generates several health concerns, especially regarding female reproduction and its regulators [351,352]. Human studies have demonstrated alterations in menstrual cycles, normal endocrine function, adverse birth outcomes, and other potential reproductive health risks [353]. Furthermore, still considering image 3, the components from Group F might also help differentiate the PCOS profile. The lack of presence from Groups A and B might also relate to the PCOS profile since these metabolites are relatively present in the controls. However, dodecamethylcyclohexasiloxane (cyclomethicone 6), another cyclic dimethyl polysiloxane compound [347], was majorly found in the FF of controls and endometriosis patients. Its toxicity is confirmed, and some studies reported that odecamethylcyclohexasiloxane causes endometrial tumours. Its mechanism of action, however, remains a mystery [354].

Metabolite 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene is very present in the controls, but its occurrence is even higher in the samples related to the three diseases. This discrepancy may be of high relevance. 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene, or MBP, is also a phthalate metabolite [316]. It is present in 88% of the endometriosis samples and in 67% of both PCOS and POF. It is formed by the liver S9 fractions, and its metabolic activation may occur in the fetal liver, being detect as an *in vivo* metabolite in the fetus [355]. MBP is a very potent estrogenic metabolite of bisphenol A (BPA) [355], an interferent for oocyte development and maturation [309]. However, it presents 1000 times more biological activity than BPA [356]. Endocrine disruption can occur through multiple pathways, including binding to steroid receptors. The androgen receptor (AR) and progesterone receptor (PR) are critical for

reproductive tract growth and function. MBP has the potential to block or interfere in the binding of the endogenous native AR and PR ligands, disrupting AR- and PR-mediated pathways, thus leading to dysfunction [356]. Okuda and co-workers demonstrated that MBP potent estrogenic activity affects uterine weight, myometrial thickness, and luminal epithelial cell height in rats studies [357]. MBP activities were also related to breast cancer [358,359], lung disfunction [360], and pancreatic β-cell death [361].

The results exhibit interest discrimination of FF samples, demonstrating that the volatomic profile can be an advantageous approach to identifying potential infertility biomarkers. These also suggested the possibility of classifying some endogenous metabolites.

3 Conclusions

This work described the application of a HS-SPME/GC–MS methodology to determine the VOCs present in FF samples from women with clinical manifestations related to infertility. The GC-MS analysis determined 136 VOCs in all 52 specimens, these corresponding to 15 PCOS patients, 8 with endometriosis, 12 with POF, and 17 considered controls. Due to their prevalence in all the samples, 37 of all the 136 were studied, and multivariate statistical analysis revealed significant alterations in the levels of certain metabolites according to each pathology. The metabolites that stood out the most are diethyl phthalate, 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene, and tetradecamethylcycloheptasiloxane. POF samples feature metabolites from Groups D, such as 1-dodecanol and 4,6-dimethyldodecane, Groups A and B, accompanied by a lack of Group E. Data related to endometriosis revealed a strong presence of Group C components, as well as tetradecamethylhexasiloxane and hexadecamethylheptasiloxane from Group F. FFs from PCOS women, present pertinent correspondence to tetradecamethylcycloheptasiloxane, 1-ethyl-2,3-dimethylbenzene and docosane. The altered biochemical profiles revealed several compromised metabolomic pathways in the various diseases, with endometriosis and POF presenting several similarities. The high-throughput methodologies employed suggest the possibility of using metabolite identification as a springboard for the search for potential infertility biomarkers. They also benefit the exploration of the linked metabolomic pathways and improve diagnostic clinical tools. However, it is worthwhile to note that this research comprehends a pilot study, and more testing is needed regarding the volatilomic profile of FF in order to improve future prospects.

The “Chapter II” of the present dissertation is present in an article submitted to publication. “Volatilomics as an Emerging Strategy to Determine Potential Biomarkers of Female Infertility: A Pilot Study”, by Brinca, A.T.; Anjos, O.; Alves, M.M.C; Sousa, Â.; Oliani, A.H.; Breitenfeld, L.; Passarinha, L.A.; Ramalhinho, A.C.; Gallardo, E., Biomedicines 2022.

References

1. Chu, K.Y.; Patel, P.; Ramasamy, R. Consideration of gender differences in infertility evaluation. *Curr. Opin. Urol.* **2019**, *29*, 267–271, doi:10.1097/MOU.0000000000000590.
2. Zegers-Hochschild, F.; Adamson, G.D.; Dyer, S.; Racowsky, C.; De Mouzon, J.; Sokol, R.; Rienzi, L.; Sunde, A.; Schmidt, L.; Cooke, I.D.; et al. The international glossary on infertility and fertility care, 2017. *Hum. Reprod.* **2017**, *32*, 1786–1801, doi:10.1093/humrep/dex234.
3. Szamatowicz, M. Assisted reproductive technology in reproductive medicine - Possibilities and limitations. *Ginekol. Pol.* **2016**, *87*, 820–823, doi:10.5603/GP.2016.0095.
4. Boivin, J.; Bunting, L.; Collins, J.A.; Nygren, K.G. Reply: International estimates on infertility prevalence and treatment seeking: Potential need and demand for medical care. *Hum. Reprod.* **2007**, *24*, 2380–2383, doi:10.1093/humrep/dep218.
5. Cunningham, J. Infertility: A primer for primary care providers. *J. Am. Acad. Physician Assist.* **2017**, *30*, 19–25, doi:10.1097/01.JAA.0000522130.01619.b7.
6. Lindsay, T.J.; Vitrikas, K.R. Evaluation and treatment of infertility. *Am. Fam. Physician* **2015**, *91*, 308–314.
7. Inhorn, M.C.; Patrizio, P. Infertility around the globe: New thinking on gender, reproductive technologies and global movements in the 21st century. *Hum. Reprod. Update* **2015**, *21*, 411–426, doi:10.1093/humupd/dmv016.
8. Crawford, N.M.; Steiner, A.Z. Age-related infertility. *Obstet. Gynecol. Clin. North Am.* **2015**, *42*, 15–25, doi:10.1016/j.ogc.2014.09.005.
9. Gleicher, N.; Kushnir, V.A.; Weghofer, A.; Barad, D.H. The " graying" of infertility services: An impending revolution nobody is ready for. *Reprod. Biol. Endocrinol.* **2014**, *12*, 1–12, doi:10.1186/1477-7827-12-63.
10. Halcomb, L. Men and infertility: Insights from the sociology of gender. *Sociol. Compass* **2018**, *12*, 1–12, doi:10.1111/soc4.12624.
11. Stephen, E.H.; Chandra, A. Declining estimates of infertility in the United States: 1982-2002. *Fertil. Steril.* **2006**, *86*, 516–523, doi:10.1016/j.fertnstert.2006.02.129.
12. Mascarenhas, M.N.; Flaxman, S.R.; Boerma, T.; Vanderpoel, S.; Stevens, G.A. National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. *PLoS Med.* **2012**, *9*, 1–12, doi:10.1371/journal.pmed.1001356.
13. Brosens, I.; Gordts, S.; Valkenburg, M.; Puttemans, P.; Campo, R.; Gordts, S. Investigation of the infertile couple: When is the appropriate time to explore female

infertility? *Hum. Reprod.* **2004**, *19*, 1689–1692, doi:10.1093/humrep/deh314.

14. Alchami, A.; O'Donovan, O.; Davies, M. PCOS: Diagnosis and management of related infertility. *Obstet. Gynaecol. Reprod. Med.* **2015**, *25*, 279–282, doi:10.1016/j.ogrm.2015.07.005.

15. Adra, A.; El Zibdeh, M.Y.; Abdul Malek, A.M.M.; Hamrahian, A.H.; Abdelhamid, A.M.S.; Colao, A.; Anastasiades, E.; Ahmed, E.M.A.F.; Ezzeddine, J.I.; El Sattar, M.I.A.; et al. Differential diagnosis and management of abnormal uterine bleeding due to hyperprolactinemia. *Middle East Fertil. Soc. J.* **2016**, *21*, 137–147, doi:10.1016/j.mefs.2016.02.001.

16. Nelson LM NIH Public Access - Primary Ovarian Insufficiency. *N. Engl. J. Med.* **2009**, *360*, 606–614, doi:10.1056/NEJMcp0808697.Primary.

17. Yu, L.; Liu, M.; Wang, Z.; Liu, T.; Liu, S.; Wang, B.; Pan, B.; Dong, X.; Guo, W. Correlation between steroid levels in follicular fluid and hormone synthesis related substances in its exosomes and embryo quality in patients with polycystic ovary syndrome. *Reprod. Biol. Endocrinol.* **2021**, *19*, 1–11, doi:10.1186/s12958-021-00749-6.

18. Ban, Y.; Ran, H.; Chen, Y.; Ma, L. Lipidomics analysis of human follicular fluid form normal-weight patients with polycystic ovary syndrome: a pilot study. *J. Ovarian Res.* **2021**, *14*, 1–11, doi:10.1186/s13048-021-00885-y.

19. Chen, W.; Pang, Y. Metabolic Syndrome and PCOS : Pathogenesis and the Role of Metabolites. *Metabolites* **2021**.

20. Liu, R.; Bai, S.; Zheng, S.; Zhu, X.; Zhang, Y.; Xu, B.; Zhao, W. Identification of the Metabolomics Signature of Human Follicular Fluid from PCOS Women with Insulin Resistance. *Dis. Markers* **2022**, *2022*, 1–10, doi:10.1155/2022/6877541.

21. Broekmans, F.J.; Knauff, E.A.H.; Valkenburg, O.; Laven, J.S.; Eijkemans, M.J.; Fauser, B.C.J.M. PCOS according to the Rotterdam consensus criteria: Change in prevalence among WHO-II anovulation and association with metabolic factors. *BJOG An Int. J. Obstet. Gynaecol.* **2006**, *113*, 1210–1217, doi:10.1111/j.1471-0528.2006.01008.x.

22. Azziz, R. PCOS: A diagnostic challenge. *Reprod. Biomed. Online* **2004**, *8*, 644–648, doi:10.1016/S1472-6483(10)61644-6.

23. Azziz, R.; Hincapie, L.A.; Knochenhauer, E.S.; Dewailly, D.; Fox, L.; Boots, L.R. Screening for 21-hydroxylase-deficient nonclassic adrenal hyperplasia among hyperandrogenic women: A prospective study. *Fertil. Steril.* **1999**, *72*, 915–925, doi:10.1016/S0015-0282(99)00383-0.

24. Azziz, R.; Tarlatzis, R.; Dunaif, A.; Ibanez, L.; Pugeat, M.; Taylor, A.; Fauser, C.J.M.; Medicine, R. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. **2004**, *81*, 19–25, doi:10.1016/j.fertnstert.2003.10.004.

25. Pache, T.D.; Wladimiroff, J.W.; Hop, W.C.J.; Fauser, B.C.J.M. How to discriminate between normal and polycystic ovaries- transvaginal US study. **1992**, 421–423.

26. Jonard, S.; Robert, Y.; Cortet-Rudelli, C.; Pigny, P.; Decanter, C.; Dewailly, D. Ultrasound examination of polycystic ovaries: Is it worth counting the follicles? *Hum. Reprod.* **2003**, *18*, 598–603, doi:10.1093/humrep/deg115.

27. Balen, A. Ovulation induction for polycystic ovary syndrome. *Hum. Fertil.* **2000**, *3*, 106–111, doi:10.1080/1464727002000198791.

28. Van Santbrink, E.J.P.; Hop, W.C.; Fauser, B.C.J.M. Classification of normogonadotropic infertility: Polycystic ovaries diagnosed by ultrasound versus endocrine characteristics of polycystic ovary syndrome. *Fertil. Steril.* **1997**, *67*, 452–458, doi:10.1016/S0015-0282(97)80068-4.

29. Christensen, J.T.; Boldsen, J.; Westergaard, J.G. Ovarian volume in gynecologically healthy women using no contraception, or using IUD or oral contraception. *Acta Obstet. Gynecol. Scand.* **1997**, *76*, 784–789, doi:10.3109/00016349709024348.

30. Adams, J.; Dwpolson, D.; Franks, S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. *Br. Med. J. (Clin. Res. Ed).* **1986**, *293*, 355–359, doi:10.1136/bmj.293.6543.355.

31. FRANKS, S. Polycystic Ovary Syndrome: a Changing Perspective. *Clin. Endocrinol. (Oxf).* **1989**, *31*, 87–120, doi:10.1111/j.1365-2265.1989.tb00457.x.

32. Carmina, E.; Lobo, R.A. Polycystic ovaries in hirsute women with normal menses. *Am. J. Med.* **2001**, *111*, 602–606, doi:10.1016/S0002-9343(01)00979-2.

33. Cleeman, J.I. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *J. Am. Med. Assoc.* **2001**, *285*, 2486–2497, doi:10.1001/jama.285.19.2486.

34. Mohammad, M.B.; Seghinsara, A.M. Polycystic ovary syndrome (PCOS), diagnostic criteria, and AMH. *Asian Pacific J. Cancer Prev.* **2017**, *18*, 17–21, doi:10.22034/APJCP.2017.18.1.17.

35. de Zegher, F.; López-Bermejo, A.; Ibáñez, L. Central Obesity, Faster Maturation, and 'PCOS' in Girls. *Trends Endocrinol. Metab.* **2018**, *29*, 815–818, doi:10.1016/j.tem.2018.09.005.

36. Ehrmann, D.A.; Barnes, R.B.; Rosenfield, R.L.; Cavaghan, M.K.; Imperial, J. Prevalence of Impaired Glucose Tolerance and Diabetes in Women Wi t h Polycystic Ovary Syndro m e. *Diabetes Care* **1999**, *22*, 141–146.

37. Dunaif, A.; Legro, R.S. Prevalence and Predictors of Risk for Type 2 Diabetes Mellitus and Impaired Glucose Tolerance in Polycystic Ovary Syndrome-Authors' Response. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 297–2976, doi:10.1210/jcem.84.8.5939-4.

38. Dunaif, A.; Graf, M.; Mandeli, J.; Laumas, V.; Dobrjansky, A. Characterization of Groups of Hyperaiidrogenic Women with Acanthosis Nigricans, Impaired Glucose Tolerance, and/or Hyperinsulinemia. **1987**, *65*.

39. Robinson, S.; Kiddy, D.; Gelding, S. V.; Willis, D.; Niththyananthan, R.; Bush, A.; Johnston, D.G.; Franks, S. The relationship of insulin insensitivity to menstrual pattern in women with hyperandrogenism and polycystic ovaries. *Clin. Endocrinol. (Oxf).* **1993**, *39*, 351–355, doi:10.1111/j.1365-2265.1993.tb02376.x.

40. Dahlgren, E.; Janson, P.O.; Johansson, S.; Lapidus, L.; Odén, A. Polycystic ovary syndrome and risk for myocardial infarction: Evaluated from a risk factor model based on a prospective population study of women. *Acta Obstet. Gynecol. Scand.* **1992**, *71*, 599–604, doi:10.3109/00016349209006227.

41. Kiddy, D.S.; Hamilton-Fairley, D.; Bush, A.; Short, F.; Anyaoku, V.; Reed, M.J.; Franks, S. Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. *Clin. Endocrinol. (Oxf).* **1992**, *36*, 105–111, doi:10.1111/j.1365-2265.1992.tb02909.x.

42. Clark, A.M.; Ledger, W.; Galletly, C.; Tomlinson, L.; Blaney, F.; Wang, X.; Norman, R.J. Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. *Hum. Reprod.* **1995**, *10*, 2705–2712, doi:10.1093/oxfordjournals.humrep.a135772.

43. Huber-Buchholz, M.M.; Carey, D.G.P.; Norman, R.J. Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: Role of insulin sensitivity and luteinizing hormone. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 1470–1474, doi:10.1210/jc.84.4.1470.

44. Morán, C.; Knochenhauer, E.; Boots, L.R.; Azziz, R. Adrenal androgen excess in hyperandrogenism: Relation to age and body mass. *Fertil. Steril.* **1999**, *71*, 671–674, doi:10.1016/S0015-0282(98)00536-6.

45. Dunaif, A.; Segal, K.R.; Futterweit, W.; Dobrjansky, A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* **1989**, *38*, 1165–1174, doi:10.2337/diab.38.9.1165.

46. Niu, Z.; Lin, N.; Gu, R.; Sun, Y.; Feng, Y. Associations between insulin resistance, free fatty acids, and oocyte quality in polycystic ovary syndrome during in vitro fertilization. *J. Clin. Endocrinol. Metab.* **2014**, *99*, E2269–E2276, doi:10.1210/jc.2013-3942.

47. Teede, H.; Deeks, A.; Moran, L. Polycystic ovary syndrome: A complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med.* **2010**, *8*, doi:10.1186/1741-7015-8-41.

48. Crews, L. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: Executive summary. *Am. J. Clin. Nutr.* **1998**, *68*, 899–917, doi:10.1093/ajcn/68.4.899.

49. Gongadashetti, K.; Gupta, P.; Dada, R.; Malhotra, N. Follicular fluid oxidative stress biomarkers and art outcomes in PCOS women undergoing in vitro fertilization: A cross-sectional study. *Int. J. Reprod. Biomed.* **2021**, *19*, 449–456, doi:10.18502/ijrm.v19i5.9254.

50. Dahlgren, E.; Johansson, S.; Lindstedt, G.; Knutsson, F.; Oden, A.; Janson, P.O.; Mattson, L.A.; Crona, N.; Lundberg, P.A. Women with polycystic ovary syndrome wedge resected in 1956 to 1965: A long-term follow-up focusing on natural history and circulating hormones. *Fertil. Steril.* **1992**, *57*, 505–513, doi:10.1016/s0015-0282(16)54892-4.

51. Wild, S.; Pierpoint, T.; McKeigue, P.; Jacobs, H. Cardiovascular disease in women with polycystic ovary syndrome at long- term follow-up: A retrospective cohort study. *Clin. Endocrinol. (Oxf).* **2000**, *52*, 595–600, doi:10.1046/j.1365-2265.2000.01000.x.

52. Wild, R.A. Long-term health consequences of PCOS. *Hum. Reprod. Update* **2002**, *8*, 231–241, doi:10.1093/humupd/8.3.231.

53. Naessen, T.; Kushnir, M.M.; Chaika, A.; Nosenko, J.; Mogilevkina, I.; Rockwood, A.L.; Carlstrom, K.; Bergquist, J.; Kirilovas, D. Steroid profiles in ovarian follicular fluid in women with and without polycystic ovary syndrome, analyzed by liquid chromatography-tandem mass spectrometry. *Fertil. Steril.* **2010**, *94*, 2228–2233, doi:10.1016/j.fertnstert.2009.12.081.

54. Diamanti-Kandarakis, E.; Kouli, C.R.; Bergiele, A.T.; Filandra, F.A.; Tsianateli, T.C.; Spina, G.G.; Zapanti, E.D.; Bartzis, M.I. A survey of the polycystic ovary syndrome in the Greek Island of Lesbos: Hormonal and metabolic profile. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 4006–4011, doi:10.1210/jcem.84.11.6148.

55. Slayden, S.M.; Moran, C.; Sams, W.M.; Boots, L.R.; Azziz, R. Hyperandrogenemia in patients presenting with acne. *Fertil. Steril.* **2001**, *75*, 889–892, doi:10.1016/S0015-0282(01)01701-0.

56. Futterweit, W.; Dunaif, A.; Yeh, H.C.; Kingsley, P. The prevalence of hyperandrogenism in 109 consecutive female patients with diffuse alopecia. *J. Am. Acad. Dermatol.* **1988**, *19*, 831–836, doi:10.1016/S0190-9622(88)70241-8.

57. Laven, J.S.E.; Imani, B.; Eijkemans, M.J.C.; Fauser, B.C.J.M. New approach to polycystic ovary syndrome and other forms of anovulatory infertility. *Obstet. Gynecol. Surv.* **2002**, *57*, 755–767, doi:10.1097/00006254-200211000-00022.

58. Knochenhauer, E.S.; Key, T.J.; Kahsar-Miller, M.; Waggoner, W.; Boots, L.R.; Azziz, R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the Southeastern United States: A prospective study. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 3078–3082, doi:10.1210/jc.83.9.3078.

59. Pugeat, M.; Nicolas, M.H.; Craves, J.C.; Alvarado-, C.; Fimbel, S.; Dechaud, H.; Lyon, H.C. De Androgens in Polycystic Ovarian Syndrome. **1993**.

60. Balen, A.H.; Conway, G.S.; Kaltsas, G.; Techatraisak, K.; Manning, P.J.; West, C.; Jacobs, H.S. Andrology: Polycystic ovary syndrome: The spectrum of the disorder in 1741 patients. *Hum. Reprod.* **1995**, *10*, 2107–2111, doi:10.1093/oxfordjournals.humrep.a136243.

61. Asunción, M.; Calvo, R.M.; San Millá N, J.L.; Sancho, J.; Avila, S.; Escobar-Morreale, H.F. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *J. Clin. Endocrinol. Metab.* **2000**, *85*, 2434–2438, doi:10.1210/jc.85.7.2434.

62. Legro, R.S.; Driscoll, D.; Strauss, J.F.; Fox, J.; Dunaif, A. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 14956–14960, doi:10.1073/pnas.95.25.14956.

63. Boots, L.R.; Potter, S.; Potter, H.D.; Azziz, R. Measurement of total serum testosterone levels using commercially available kits: High degree of between-kit variability. *Fertil. Steril.* **1998**, *69*, 286–292, doi:10.1016/S0015-0282(97)00464-0.

64. Rosner, W. Errors in the measurement of plasma free testosterone. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 2014–2015, doi:10.1210/jcem.82.6.9999.

65. Vermeulen, A.; Verdonck, L.; Kaufman, J.M. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 3666–3672, doi:10.1210/jcem.84.10.6079.

66. Bili, H.; Laven, J.; Imani, B.; Eijkemans, M.J.C.; Fauser, B.C.J.M. Age-related differences in features associated with polycystic ovary syndrome in normogonadotrophic oligo-amenorrhoeic infertile women of reproductive years. *Eur. J. Endocrinol.* **2001**, *145*, 749–755, doi:10.1530/eje.0.1450749.

67. Fauser, B.C.J.M.; Pache, T.D.; Lamberts, S.W.J.; Hop, W.C.J.; De Jong, F.H.; Dahl, K.D. Serum bioactive and immunoreactive luteinizing hormone and follicle-stimulating hormone levels in women with cycle abnormalities, with or without polycystic ovarian disease. *J. Clin. Endocrinol. Metab.* **1991**, *73*, 811–817, doi:10.1210/jcem-73-4-811.

68. Taylor, A.E.; Mccourt, B.; Martin, K.A.; Anderson, E.J.; Adams, J.M.; Schoenfeld, D.; Hall, J.E. Determinants of Abnormal Gonadotropin Secretion in Clinically Defined Women with Polycystic Ovary Syndrome* Prospective evaluation of consensus criteria for Polycystic Ovary Syndrome: Evidence for subgroups char-acterized by inverse defects of LH and insu. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 2248–2256.

69. Waldstreicher, J.; Santoro, N.F.; Hall, J.E.; Filicori, M.; Crowley, W.F. Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian disease: Indirect evidence for partial gonadotroph desensitization. *J. Clin. Endocrinol. Metab.* **1988**, *66*, 165–172, doi:10.1210/jcem-66-1-165.

70. Balen, A.H.; Tan, S.L.; Macdougall, J.; Jacobs, H.S. Miscarriage rates following in-vitro fertilization are increased in women with polycystic ovaries and reduced by pituitary

desensitization with buserelin. *Hum. Reprod.* **1993**, *8*, 959–964, doi:10.1093/oxfordjournals.humrep.a138174.

71. Tarlatzis, B.C.; Grimbizis, G.; Pournaropoulos, F.; Bontis, J.; Lagos, S.; Spanos, E.; Mantalenakis, S. The prognostic value of basal luteinizing hormone:fOllicle-stimulating hormone ratio in the treatment of patients with polycystic ovarian syndrome by assisted reproduction techniques. *Hum. Reprod.* **1995**, *10*, 2545–2549, doi:10.1093/oxfordjournals.humrep.a135742.

72. Gordon, U.D.; Harrison, R.F.; Fawzy, M.; Hennelly, B.; Gordon, A.C. A randomized prospective assessor-blind evaluation of luteinizing hormone dosage and in vitro fertilization outcome. *Fertil. Steril.* **2001**, *75*, 324–331, doi:10.1016/S0015-0282(00)01701-5.

73. Mendoza, C.; Ruiz-Requena, E.; Ortega, E.; Cremades, N.; Martinez, F.; Bernabeu, R.; Greco, E.; Tesarik, J. Follicular fluid markers of oocyte developmental potential. *Hum. Reprod.* **2002**, *17*, 1017–1022, doi:10.1093/humrep/17.4.1017.

74. Homburg, R.; Levy, T.; Berkovitz, D.; Farchi, J.; Feldberg, D.; Ashkenazi, J.; Ben- Rafael, Z. Gonadotropin-releasing hormone agonist reduces the miscarriage rate for pregnancies achieved in women with polycystic ovarian syndrome. *Fertil. Steril.* **1993**, *59*, 527–531, doi:10.1016/S0015-0282(16)55794-X.

75. Clifford, K.; Rai, R.; Watson, H.; Franks, S.; Regan, L. Does suppressing luteinising hormone secretion reduce the miscarriage rate? Results of a randomised controlled trial. *Bmj* **1996**, *312*, 1508–1511, doi:10.1136/bmj.312.7045.1508.

76. Younglai, E. V.; Foster, W.G.; Hughes, E.G.; Trim, K.; Jarrell, J.F. Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing in vitro fertilization. *Arch. Environ. Contam. Toxicol.* **2002**, *43*, 121–126, doi:10.1007/s00244-001-0048-8.

77. Yang, X.; Wu, R.; Qi, D.; Fu, L.; Song, T.; Wang, Y.; Bian, Y.; Shi, Y. Profile of Bile Acid Metabolomics in the Follicular Fluid of PCOS Patients. *Metabolites* **2021**, *11*, 845, doi:10.3390/metabo11120845.

78. Marianna, S.; Alessia, P.; Susan, C.; Francesca, C.; Angela, S.; Francesca, C.; Antonella, N.; Patrizia, I.; Nicola, C.; Emilio, C. Metabolomic profiling and biochemical evaluation of the follicular fluid of endometriosis patients. *Mol. Biosyst.* **2017**, *13*, 1213–1222, doi:10.1039/c7mb00181a.

79. Mikhaleva, L.M.; Davydov, A.I.; Patsap, O.I.; Mikhaylenko, E. V.; Nikolenko, V.N.; Neganova, M.E.; Klochkov, S.G.; Somasundaram, S.G.; Kirkland, C.E.; Aliev, G. Malignant Transformation and Associated Biomarkers of Ovarian Endometriosis: A Narrative Review. *Adv. Ther.* **2020**, *37*, 2580–2603, doi:10.1007/s12325-020-01363-5.

80. Pocate-Cheriet, K.; Santulli, P.; Kateb, F.; Bourdon, M.; Maignien, C.; Batteux, F.; Chouzenoux, S.; Patrat, C.; Wolf, J.P.; Bertho, G.; et al. The follicular fluid metabolome

differs according to the endometriosis phenotype. *Reprod. Biomed. Online* **2020**, *41*, 1023–1037, doi:10.1016/j.rbmo.2020.09.002.

81. De Ziegler, D.; Gayet, V.; Aubriot, F.X.; Fauque, P.; Streuli, I.; Wolf, J.P.; De Mouzon, J.; Chapron, C. Use of oral contraceptives in women with endometriosis before assisted reproduction treatment improves outcomes. *Fertil. Steril.* **2010**, *94*, 2796–2799, doi:10.1016/j.fertnstert.2010.05.056.

82. Vinatier, D.; Cosson, M.; Dufour, P. Is endometriosis an endometrial disease? *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2000**, *91*, 113–125, doi:10.1016/S0301-2115(99)00263-8.

83. Prins, J.R.; Marissen, L.M.; Scherjon, S.A.; Hoek, A.; Cantineau, A.E.P. Is there an immune modulating role for follicular fluid in endometriosis? A narrative review. *Reproduction* **2020**, *159*, R45–R54, doi:10.1530/REP-19-0050.

84. Kennedy, S.; Bergqvist, A.; Chapron, C.; D'Hooghe, T.; Dunselman, G.; Greb, R.; Hummelshoj, L.; Prentice, A.; Saridogan, E.; Koninckx, P.; et al. ESHRE guideline for the diagnosis and treatment of endometriosis. *Hum. Reprod.* **2005**, *20*, 2698–2704, doi:10.1093/humrep/dei135.

85. Sampson, J.A.; Novak, E. Peritoneal Endometriosis, Due to the Menstrual Dissemination of Endometrial Tissue into the Peritoneal Cavity. *Am. J. Obstet. Gynecol.* **1927**, *15*, 101–110, doi:10.1016/s0002-9378(15)32693-4.

86. Chapron, C.; Marcellin, L.; Borghese, B.; Santulli, P. Rethinking mechanisms, diagnosis and management of endometriosis. *Nat. Rev. Endocrinol.* **2019**, *15*, 666–682, doi:10.1038/s41574-019-0245-z.

87. Adachi, M.; Nasu, K.; Tsuno, A.; Yuge, A.; Kawano, Y.; Narahara, H. Attachment to extracellular matrices is enhanced in human endometriotic stromal cells: A possible mechanism underlying the pathogenesis of endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2011**, *155*, 85–88, doi:10.1016/j.ejogrb.2010.10.026.

88. Santulli, P.; Chouzenoux, S.; Fiorese, M.; Marcellin, L.; Lemarechal, H.; Millischer, A.E.; Batteux, F.; Borderie, D.; Chapron, C. Protein oxidative stress markers in peritoneal fluids of women with deep infiltrating endometriosis are increased. *Hum. Reprod.* **2015**, *30*, 49–60, doi:10.1093/humrep/deu290.

89. Pearce, C.L.; Stram, D.O.; Ness, R.B.; Stram, D.A.; Roman, L.D.; Templeman, C.; Lee, A.W.; Menon, U.; Fasching, P.A.; McAlpine, J.N.; et al. Population distribution of lifetime risk of ovarian cancer in the United States. *Cancer Epidemiol. Biomarkers Prev.* **2015**, *24*, 671–676, doi:10.1158/1055-9965.EPI-14-1128.

90. Ñiguez Sevilla, I.; Machado Linde, F.; Marín Sánchez, M.D.P.; Arense, J.J.; Torroba, A.; Nieto Díaz, A.; Sánchez Ferrer, M.L. Prognostic importance of atypical endometriosis with architectural hyperplasia versus cytologic atypia in endometriosis-associated ovarian cancer. *J. Gynecol. Oncol.* **2019**, *30*, e63, doi:10.3802/jgo.2019.30.e63.

91. Munksgaard, P.S.; Blaakaer, J. The association between endometriosis and ovarian

cancer: A review of histological, genetic and molecular alterations. *Gynecol. Oncol.* **2012**, *124*, 164–169, doi:10.1016/j.ygyno.2011.10.001.

92. Sanchez, A.M.; Viganò, P.; Somigliana, E.; Panina-Bordigno, P.; Vercellini, P.; Candiani, M. The distinguishing cellular and molecular features of the endometriotic ovarian cyst: Frompathophysiology to the potential endometrioma-mediated damage to the ovary. *Hum. Reprod. Update* **2014**, *20*, 217–230, doi:10.1093/humupd/dmt053.

93. Larosa, M.; Facchini, F.; Pozzoli, G.; Leone, M.; Grande, M.; Monica, B.; Urologia, U.O.C.; Ausl, A.; Emilia, R. Endometriosi : le basi eziopatogenetiche. **2010**, *77*, 1–11.

94. Ahn, S.H.; Monsanto, S.P.; Miller, C.; Singh, S.S.; Thomas, R.; Tayade, C. Pathophysiology and immune dysfunction in endometriosis. *Biomed Res. Int.* **2015**, *2015*, doi:10.1155/2015/795976.

95. Izumi, G.; Koga, K.; Takamura, M.; Makabe, T.; Satake, E.; Takeuchi, A.; Taguchi, A.; Urata, Y.; Fujii, T.; Osuga, Y. Involvement of immune cells in the pathogenesis of endometriosis. *J. Obstet. Gynaecol. Res.* 2018, *44*, 191–198.

96. Sasson, I.E.; Taylor, H.S. Stem cells and the pathogenesis of endometriosis. *Ann. N. Y. Acad. Sci.* **2008**, *1127*, 106–115, doi:10.1196/annals.1434.014.

97. Aznaurova, Y.B.; Zhumataev, M.B.; Roberts, T.K.; Aliper, A.M.; AZhavoronkov, A. Molecular aspects of develipment and regulation of endometriosis. *Reprod. Biol. Endocrinol.* 2014, 1–25.

98. Macer, M.L.; Taylor, H.S. Endometriosis and Infertility: A review of the pathogenesis and treatment of endometriosis-associated infertility. *Natl. Institutes Heal.* **2012**, *39*, 535–549, doi:10.1016/j.ogc.2012.10.002.Endometriosis.

99. Laganà, A.S.; Vitale, S.G.; Salmeri, F.M.; Triolo, O.; Ban Frangež, H.; Vrtačnik-Bokal, E.; Stojanovska, L.; Apostolopoulos, V.; Granese, R.; Sofo, V. *Unus pro omnibus, omnes pro uno: A novel, evidence-based, unifying theory for the pathogenesis of endometriosis*; 2017; Vol. 103; ISBN 0306987716.

100. Zhang, T.; De Carolis, C.; Man, G.C.W.; Wang, C.C. The link between immunity, autoimmunity and endometriosis: a literature update. *Autoimmun. Rev.* **2018**, *17*, 945–955, doi:10.1016/j.autrev.2018.03.017.

101. de Barros, I.B.L.; Malvezzi, H.; Gueuvoghlanian-Silva, B.Y.; Piccinato, C.A.; Rizzo, L.V.; Podgaec, S. “What do we know about regulatory T cells and endometriosis? A systematic review.” *J. Reprod. Immunol.* **2017**, *120*, 48–55, doi:10.1016/j.jri.2017.04.003.

102. Karaer, A.; Tuncay, G.; Mumcu, A.; Dogan, B. Metabolomics analysis of follicular fluid in women with ovarian endometriosis undergoing in vitro fertilization. *Syst. Biol. Reprod. Med.* **2019**, *65*, 39–47, doi:10.1080/19396368.2018.1478469.

103. Somigliana, E.; Vigano, P.; Benaglia, L.; Busnelli, A.; Berlanda, N.; Vercellini, P. Management of Endometriosis in the Infertile Patient. *Semin. Reprod. Med.* **2017**, *35*,

031–037, doi:10.1055/s-0036-1597125.

104. Lessey, B.A.; Lebovic, D.I.; Taylor, R.N. Eutopic endometrium in women with endometriosis: Ground zero for the study of implantation defects. *Semin. Reprod. Med.* **2013**, *31*, 109–124, doi:10.1055/s-0032-1333476.

105. Niederberger, C.; Pellicer, A.; Cohen, J.; Ph, D.; Gardner, D.K.; Phil, D.; Palermo, G.D.; Ph, D.; Neill, C.L.O.; Chow, S. Forty years of IVF. **2018**, *110*, doi:10.1016/j.fertnstert.2018.06.005.

106. Yu, L.; Qing, X. Diagnosis of Idiopathic Premature Ovarian Failure by Color Doppler Ultrasound under the Intelligent Segmentation Algorithm. *Hindawi Comput. Math. Methods Med.* **2022**, *2022*, doi:https://doi.org/10.1155/2022/2645607.

107. Jack, C.R.; Bennett, D.A.; Blennow, K.; Carrillo, M.C.; Dunn, B.; Haeberlein, S.B.; Holtzman, D.M.; Jagust, W.; Jessen, F.; Karlawish, J.; et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's Dement.* **2018**, *14*, 535–562, doi:10.1016/j.jalz.2018.02.018.

108. Kouatcheu, S.D.; Marko, J.; Tamura, D.; Khan, S.G.; Lee, C.R.; DiGiovanna, J.J.; Kraemer, K.H. Thyroid nodules in xeroderma pigmentosum patients: a feature of premature aging. *J. Endocrinol. Invest.* **2021**, *44*, 1475–1482, doi:10.1007/s40618-020-01451-x.

109. Zhang, P.; Zhang, H.; Lin, J.; Xiao, T.; Xu, R.; Fu, Y.; Zhang, Y.; Du, Y.; Cheng, J.; Jiang, H. Insulin impedes osteogenesis of BMSCs by inhibiting autophagy and promoting premature senescence via the TGF-β1 pathway. *Aging (Albany. NY).* **2020**, *12*, 2084–2100, doi:10.18632/aging.102723.

110. Jankowska, K. Premature ovarian failure. *Prz. Menopauzalny* **2017**, *16*, 51–56, doi:10.5114/pm.2017.68592.

111. Coulam, C.B.; Adamson, S.C.; Annegers, J.F. Incidence of Premature Ovarian Failure 1986.

112. Heller, C.G.; Heller, E.J. Gonadotropic hormone: Urine Assays of Normally Cycling, Menopausal, Castrated, and Estrin Treated Human Females. **1938**, 171–178.

113. Camilleri, E. A Brief Overview On Premature Ovarian Failure. *Malta Med. Students' Assoc.* **2022**, 1–9.

114. de Moraes-Ruehsen, M.; Jones, G.S. Premature ovarian failure. *Fertil. Steril.* **1967**, *18*, 440–461, doi:10.1016/S0015-0282(16)36362-2.

115. Pal, L.; Torrealday, S.; Kodaman, P. Premature Ovarian Insufficiency - an update on recent advances in understanding and management. *F1000Research* **2017**, *6*, 1–15, doi:10.12688/f1000research.11948.1.

116. Beck-Peccoz, P.; Persani, L. Premature ovarian failure. *Orphanet J. Rare Dis.* **2006**, *1*, 1–5, doi:10.1186/1750-1172-1-9.

117. Starup, J.; Sele, V. Premature Ovarian Failure. *Acta Obstet. Gynecol. Scand.* **1973**, *52*, 259–268, doi:10.3109/00016347309158324.

118. Philip, J.; Sele, V.; Trolle, D. Secondary, Hypergonadotrophic Amenorrhoea. *Acta Obs. Gynecol Scand* **1966**, 142–147.

119. Zárate, A.; Karchmer, S.; Gómez, E.; Castelazo-Ayala, L. Premature menopause. A clinical, histologic, and cytogenetic study. *Am. J. Obstet. Gynecol.* **1970**, *106*, 110–114, doi:10.1016/0002-9378(70)90134-1.

120. Liao, A.H.; Cai, Y.L.; Chuang, H.C.; Lee, C.Y.; Lin, Y.C.; Chiang, C.P. Application of ultrasound-mediated adapalene-coated lysozyme-shelled microbubbles in UVA-induced skin photoaging. *PLoS One* **2020**, *15*, 1–21, doi:10.1371/journal.pone.0232617.

121. Lin, J.; Li, X. lian; Song, H.; Li, Q.; Wang, M. yan; Qiu, X. min; Li, D. jin; Wang, L. A general description for Chinese medicine in treating premature ovarian failure. *Chin. J. Integr. Med.* **2017**, *23*, 91–97, doi:10.1007/s11655-016-2642-7.

122. Paules, C.; Dantas, A.P.; Miranda, J.; Crovetto, F.; Eixarch, E.; Rodriguez-Sureda, V.; Dominguez, C.; Casu, G.; Rovira, C.; Nadal, A.; et al. Premature placental aging in term small-for-gestational-age and growth-restricted fetuses. *Ultrasound Obstet. Gynecol.* **2019**, *53*, 615–622, doi:10.1002/uog.20103.

123. Pankiewicz, K.; Laudański, P.; Issat, T. The role of noncoding rna in the pathophysiology and treatment of premature ovarian insufficiency. *Int. J. Mol. Sci.* **2021**, *22*, doi:10.3390/ijms22179336.

124. Popat, V.B.; Calis, K.A.; Kalantaridou, S.N.; Vanderhoof, V.H.; Koziol, D.; Troendle, J.F.; Reynolds, J.C.; Nelson, L.M. Bone mineral density in young women with primary ovarian insufficiency: Results of a three-year randomized controlled trial of physiological transdermal estradiol and testosterone replacement. *J. Clin. Endocrinol. Metab.* **2014**, *99*, 3418–3426, doi:10.1210/jc.2013-4145.

125. Elouej, S.; Harhouri, K.; Le Mao, M.; Baujat, G.; Nampoothiri, S.; Kayserili, H.U.; Menabawy, N. Al; Selim, L.; Paneque, A.L.; Kubisch, C.; et al. Loss of MTX2 causes mandibuloacral dysplasia and links mitochondrial dysfunction to altered nuclear morphology. *Nat. Commun.* **2020**, *11*, 1–15, doi:10.1038/s41467-020-18146-9.

126. Hong, Z.W.; Feng, Y.M.; Ge, Y.F.; Jing, J.; Hu, X.C.; Shen, J.M.; Peng, L.P.; Yao, B.; Xin, Z.C. Relation of size of seminal vesicles on ultrasound to premature ejaculation. *Asian J. Androl.* **2016**, *18*, 554–560, doi:10.4103/1008-682X.186187.

127. Kalantaridou, S.N.; Nelson, L.M. Premature Ovarian Failure Is Not Premature Menopause SOPHIA. *Ann. New York Acad. Sci.* **2006**, 393–402, doi:10.1016/j.ecl.2015.05.004.

128. Bakalov, V.K.; Vanderhoof, V.H.; Bondy, C.A.; Nelson, L.M. Adrenal antibodies detect asymptomatic auto-immune adrenal insufficiency in young women with spontaneous premature ovarian failure. *Hum. Reprod.* **2002**, *17*, 2096–2100,

doi:10.1093/humrep/17.8.2096.

129. Nelson, L.M. Autoimmune ovarian failure: comparing the mouse model and the human disease. *J. Soc. Gynecol. Investig.* **2001**, *8*, 1–3, doi:10.1016/s1071-5576(00)00110-6.

130. Cordts, E.B.; Christofolini, D.M.; Dos Santos, A.A.; Bianco, B.; Barbosa, C.P. Genetic aspects of premature ovarian failure: A literature review. *Arch. Gynecol. Obstet.* **2011**, *283*, 635–643, doi:10.1007/s00404-010-1815-4.

131. Rossetti, R.; Ferrari, I.; Bonomi, M.; Persani, L. Genetics of primary ovarian insufficiency. *Clin. Genet.* **2017**, *91*, 183–198, doi:10.1111/cge.12921.

132. Betterle, C.; Rossi, A.; Dalla Pria, S.; Artifoni, A.; Pedini, B.; Gavasso, S.; Caretto, A. Premature ovarian failure: Autoimmunity and natural history. *Clin. Endocrinol. (Oxf).* **1993**, *39*, 35–43, doi:10.1111/j.1365-2265.1993.tb01748.x.

133. Sheikhansari, G.; Aghebati-Maleki, L.; Nouri, M.; Jadidi-Niaragh, F.; Yousefi, M. Current approaches for the treatment of premature ovarian failure with stem cell therapy. *Biomed. Pharmacother.* **2018**, *102*, 254–262, doi:10.1016/j.biopha.2018.03.056.

134. Chand, A.L.; Harrison, C.A.; Shelling, A.N. Inhibin and premature ovarian failure. *Hum. Reprod. Update* **2009**, *16*, 39–50, doi:10.1093/humupd/dmp031.

135. Ebrahimi, M.; Asbagh, F.A. Pathogenesis and Causes of Premature Ovarian Failure:An Update. *Nature* 2011, *5 (2)*, 54–65.

136. Kaufman, D.W.; Slone, D.; Rosenberg, L.; Miettinen, O.S.; Shapiro, S. Cigarette smoking and age at natural menopause. *Am. J. Public Health* **1980**, *70*, 420–422, doi:10.2105/AJPH.70.4.420.

137. Colafrancesco, S.; Perricone, C.; Tomljenovic, L.; Shoenfeld, Y. Human Papilloma Virus Vaccine and Primary Ovarian Failure: Another Facet of the Autoimmune/Inflammatory Syndrome Induced by Adjuvants. *Am. J. Reprod. Immunol.* 2013, *70*, 309–316.

138. Cowchock, F.S.; McCabe, J.L.; Montgomery, B.B. Pregnancy after corticosteroid administration in premature ovarian failure (polyglandular endocrinopathy syndrome). *Am. J. Obstet. Gynecol.* **1988**, *158*, 118–119, doi:10.1016/0002-9378(88)90791-0.

139. Blumenfeld, Z.; Halachmi, S.; Peretz, B.A.; Shmuel, Z.; Golan, D.; Makler, A.; Brandes, J.M. Premature ovarian failure - The prognostic application of autoimmunity on conception after ovulation induction. *Fertil. Steril.* **1993**, *59*, 750–755, doi:10.1016/s0015-0282(16)55854-3.

140. Gleicher, N. Some thoughts on the reproductive autoimmune failure syndrome (RAFS) and Th-1 versus Th-2 immune responses. *Am. J. Reprod. Immunol.* **2002**, *48*, 252–254, doi:10.1034/j.1600-0897.2002.01111.x.

141. Simon, A.; Laufer, N. Repeated implantation failure: Clinical approach. *Fertil. Steril.* **2012**, *97*, 1039–1043, doi:10.1016/j.fertnstert.2012.03.010.

142. Bellipanni, G.; Bianchi, P.; Pierpaoli, W.; Bulian, D.; Ilyia, E. Effects of melatonin in

perimenopausal and menopausal women: A randomized and placebo controlled study. *Exp. Gerontol.* **2001**, *36*, 297–310, doi:10.1016/S0531-5565(00)00217-5.

143. Iguchi, H.; Kato, K.I.; Ibayashi, H. Age-dependent reduction in serum melatonin concentrations in healthy human subjects. *J. Clin. Endocrinol. Metab.* **1982**, *55*, 27–29, doi:10.1210/jcem-55-1-27.

144. Cagnacci, A.; Paoletti, A.M.; Soldani, R.; Orru, M.; Maschio, E.; Melis, G.B. Melatonin Enhances the Luteinizing Hormone and Follicle-Stimulating Hormone Responses to Gonadotropin-Releasing Hormone in the Follicular, but not in the Luteal, Menstrual Phase. *J. Clin. Endocrinol. Metab.* **1995**, *80 (4)*, 1095–1099.

145. Hansen, M.; Kurinczuk, J.J.; Milne, E.; de Klerk, N.; Bower, C. Assisted reproductive technology and birth defects: A systematic review and meta-analysis. *Hum. Reprod. Update* **2013**, *19*, 330–353, doi:10.1093/humupd/dmt006.

146. Oosterhout, C. Van; Marcu, D.; Immler, S. Accounting for the genetic load in assisted reproductive technology. *Clin. Transl. Med.* **2022**, 1–4, doi:10.1002/ctm2.864.

147. Wennerholm, U.B.; Bergh, C. Perinatal outcome in children born after assisted reproductive technologies. *Ups. J. Med. Sci.* **2020**, *125*, 158–166, doi:10.1080/03009734.2020.1726534.

148. Owen, C.M.; Segars Jr., J.H. Basic Biostatistics for Clinicians. **2009**, *27*, 417–428, doi:10.1055/s-0029-1237430.Imprinting.

149. Kamel, R.M. Assisted Reproductive Technology after the Birth of Louise Brown. *Nature* 2013, *14(3)*, 96–109.

150. De Geyter, C. Assisted reproductive technology: Impact on society and need for surveillance. *Best Pract. Res. Clin. Endocrinol. Metab.* **2019**, *33*, 1–6, doi:10.1016/j.beem.2019.01.004.

151. Dyer, S.; Chambers, G.M.; De Mouzon, J.; Nygren, K.G.; Zegers-Hochschild, F.; Mansour, R.; Ishihara, O.; Banker, M.; Adamson, G.D. International committee for monitoring assisted reproductive technologies world report: Assisted reproductive technology 2008, 2009 and 2010†. *Hum. Reprod.* **2016**, *31*, 1588–1609, doi:10.1093/humrep/dew082.

152. Qin, J.; Liu, X.; Sheng, X.; Wang, H.; Gao, S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: A meta-analysis of cohort studies. *Fertil. Steril.* **2016**, *105*, 73-85.e6, doi:10.1016/j.fertnstert.2015.09.007.

153. Qin, J.B.; Sheng, X.Q.; Wu, D.; Gao, S.Y.; You, Y.P.; Yang, T.B.; Wang, H. Worldwide prevalence of adverse pregnancy outcomes among singleton pregnancies after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Arch. Gynecol. Obstet.* **2017**, *295*, 285–301, doi:10.1007/s00404-016-4250-3.

154. Huang, J.C.; Lei, Z.L.; Shi, L.H.; Miao, Y.L.; Yang, J.W.; Ouyang, Y.C.; Sun, Q.Y.; Chen, D.Y. Comparison of histone modifications in in vivo and in vitro fertilization mouse embryos. *Biochem. Biophys. Res. Commun.* **2007**, *354*, 77–83, doi:10.1016/j.bbrc.2006.12.163.

155. Pinborg, A.; Henningsen, A.K.A.; Malchau, S.S.; Loft, A. Congenital anomalies after assisted reproductive technology. *Fertil. Steril.* **2013**, *99*, 327–332, doi:10.1016/j.fertnstert.2012.12.001.

156. Sunderam, S.; Kissin, D.M.; Zhang, Y.; Jewett, A.; Boulet, S.L.; Warner, L.; Kroelinger, C.D.; Barfield, W.D. Assisted Reproductive Technology Surveillance — United States, 2018. *MMWR Surveill. Summ.* **2019**, *68*, 1–28, doi:10.15585/MMWR.SS7104A1.

157. O'Gorman, A.; Wallace, M.; Cottell, E.; Gibney, M.J.; McAuliffe, F.M.; Wingfield, M.; Brennan, L. Metabolic profiling of human follicular fluid identifies potential biomarkers of oocyte developmental competence. *Reproduction* **2013**, *146*, 389–395, doi:10.1530/REP-13-0184.

158. Sun, Z.; Wu, H.; Lian, F.; Zhang, X.; Pang, C.; Guo, Y.; Song, J.; Wang, A.; Shi, L.; Han, L. Human Follicular Fluid Metabolomics Study of Follicular Development and Oocyte Quality. *Chromatographia* **2017**, *80*, 901–909, doi:10.1007/s10337-017-3290-6.

159. Luti, S.; Fiaschi, T.; Magherini, F.; Modesti, P.A.; Piomboni, P.; Governini, L.; Luddi, A.; Amoresano, A.; Illiano, A.; Pinto, G.; et al. Relationship between the metabolic and lipid profile in follicular fluid of women undergoing in vitro fertilization. *Mol. Reprod. Dev.* **2020**, *87*, 986–997, doi:10.1002/mrd.23415.

160. Rajska, A.; Buszewska-Forajta, M.; Rachoń, D.; Markuszewski, M.J. Metabolomic insight into polycystic ovary syndrome—An overview. *Int. J. Mol. Sci.* **2020**, *21*, 1–21, doi:10.3390/ijms21144853.

161. Bracewell-Milnes, T.; Saso, S.; Abdalla, H.; Nikolau, D.; Norman-Taylor, J.; Johnson, M.; Holmes, E.; Thum, M.Y. Metabolomics as a tool to identify biomarkers to predict and improve outcomes in reproductive medicine: A systematic review. *Hum. Reprod. Update* **2017**, *23*, 723–736, doi:10.1093/humupd/dmx023.

162. Kalinina, E.A.; Malushko, A. V.; Zubareva, T.M.; Sitkin, S.I.; Dedul, A.G.; Sheveleva, T.S.; Gamzatova, Z.H.; Bejenar, V.F.; Komlichenko, E. V. Metabolomics: The perspective search of methods to overcome infertility. *Gynecol. Endocrinol.* **2015**, *31*, 79–82, doi:10.3109/09513590.2015.1086515.

163. Revelli, A.; Piane, L.D.; Casano, S.; Molinari, E.; Massobrio, M.; Rinaudo, P. Follicular fluid content and oocyte quality: From single biochemical markers to metabolomics. *Reprod. Biol. Endocrinol.* **2009**, *7*, 1–13, doi:10.1186/1477-7827-7-40.

164. Wörheide, M.A.; Krumsiek, J.; Kastenmüller, G.; Arnold, M. Multi-omics integration in biomedical research – A metabolomics-centric review. *Anal. Chim. Acta* **2021**, *1141*, 144–162, doi:10.1016/j.aca.2020.10.038.

165. Li, Z.; Zhu, Y.; Li, H.; Jiang, W.; Liu, H.; Yan, J.; Chen, Z.; Li, W. Leukaemia inhibitory factor in serum and follicular fluid of women with polycystic ovary syndrome and its correlation with IVF outcome. *Reprod. Biomed. Online* **2018**, *36*, 483–489, doi:10.1016/j.rbmo.2017.12.020.

166. Yang, Z.; Zhou, W.; Zhou, C.; Zhou, Y.; Liu, X.; Ding, G.; Hu, Y.; Pan, J.; Sheng, J.; Jin, L.; et al. Steroid metabolome profiling of follicular fluid in normo- and hyperandrogenic women with polycystic ovary syndrome. *J. Steroid Biochem. Mol. Biol.* **2021**, *206*, 105806, doi:10.1016/j.jsbmb.2020.105806.

167. Cordeiro, F.B.; Cataldi, T.R.; de Souza, B.Z.; Rochetti, R.C.; Fraietta, R.; Labate, C.A.; Lo Turco, E.G. Hyper response to ovarian stimulation affects the follicular fluid metabolomic profile of women undergoing IVF similarly to polycystic ovary syndrome. *Metabolomics* **2018**, *14*, 1–11, doi:10.1007/s11306-018-1350-z.

168. Iaccarino, N.; Amato, J.; Pagano, B.; Pagano, A.; D'Oriano, L.; Pelliccia, S.; Giustiniano, M.; Brancaccio, D.; Merlino, F.; Novellino, E.; et al. 1H NMR-based metabolomics study on follicular fluid from patients with Polycystic Ovary Syndrome Nunzia. *Biochim. Clin.* **2018**, doi:10.19186/BC.

169. Janfaza, S.; Khorsand, B.; Nikkhah, M.; Zahiri, J. Digging deeper into volatile organic compounds associated with cancer. *Biol. Methods Protoc.* **2019**, *4*, 1–11, doi:10.1093/biomethods/bpz014.

170. Longo, V.; Forleo, A.; Provenzano, S.P.; Coppola, L.; Zara, V.; Ferramosca, A.; Siciliano, P.; Capone, S. HS-SPME-GC-MS metabolomics approach for sperm quality evaluation by semen volatile organic compounds (VOCs) analysis. *Biomed. Phys. Eng. Express* **2018**, *5*, doi:10.1088/2057-1976/aaeb07.

171. Schmidt, K.; Podmore, I. Current Challenges in Volatile Organic Compounds Analysis as Potential Biomarkers of Cancer. *J. Biomarkers* **2015**, *2015*, 1–16, doi:10.1155/2015/981458.

172. Berenguer, C. V; Pereira, F.; Pereira, J.A.M.; Câmara, J.S. Volatilomics : An Emerging and Promising Avenue for the Detection of Potential Prostate Cancer Biomarkers. *Cancers, mdpi* **2022**, 1–20.

173. Buljubasic, F.; Buchbauer, G. The scent of human diseases: A review on specific volatile organic compounds as diagnostic biomarkers. *Flavour Fragr. J.* **2015**, *30*, 5–25, doi:10.1002/ffj.3219.

174. Longo, V.; Forleo, A.; Ferramosca, A.; Notari, T.; Pappalardo, S.; Siciliano, P.; Capone, S.; Montano, L. Blood, urine and semen Volatile Organic Compound (VOC) pattern analysis for assessing health environmental impact in highly polluted areas in Italy. *Environ. Pollut.* **2021**, *286*, 117410, doi:10.1016/j.envpol.2021.117410.

175. Di Lena, M.; Porcelli, F.; Altomare, D.F. Volatile organic compounds as new biomarkers for colorectal cancer: a review. *Color. Dis.* **2016**, *18*, 654–663, doi:10.1111/codi.13271.

176. Trock, B.J. Application of metabolomics to prostate cancer. *Urol. Oncol. Semin. Orig. Investig.* **2011**, *29*, 572–581, doi:10.1016/j.urolonc.2011.08.002.

177. Gao, Q.; Lee, W.-Y. Urinary metabolites for urological cancer detection: a review on the application of volatile organic compounds for cancers. *Am. J. Clin. Exp. Urol.* 2019, *7*, 232–248.

178. Spratlin, J.L.; Serkova, N.J.; Eckhardt, S.G. Clinical applications of metabolomics in oncology: A review. *Clin. Cancer Res.* **2009**, *15*, 431–440, doi:10.1158/1078-0432.CCR-08-1059.

179. Aggarwal, P.; Baker, J.; Boyd, M.T.; Coyle, S.; Probert, C.; Chapman, E.A. Optimisation of urine sample preparation for headspace-solid phase microextraction gas chromatography-mass spectrometry: Altering sample ph, sulphuric acid concentration and phase ratio. *Metabolites* **2020**, *10*, 1–17, doi:10.3390/metabo10120482.

180. Silva, C.L.; Passos, M.; Câmara, J.S. Solid phase microextraction, mass spectrometry and metabolomic approaches for detection of potential urinary cancer biomarkers - A powerful strategy for breast cancer diagnosis. *Talanta* **2012**, *89*, 360–368, doi:10.1016/j.talanta.2011.12.041.

181. Shirasu, M.; Touhara, K. The scent of disease: Volatile organic compounds of the human body related to disease and disorder. *J. Biochem.* **2011**, *150*, 257–266, doi:10.1093/jb/mvr090.

182. Amann, A.; Costello, B.D.L.; Miekisch, W.; Schubert, J.; Buszewski, B.; Pleil, J.; Ratcliffe, N.; Risby, T. The human volatilome: Volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces and saliva. *J. Breath Res.* **2014**, *8*, doi:10.1088/1752-7155/8/3/034001.

183. De Lacy Costello, B.; Amann, A.; Al-Kateb, H.; Flynn, C.; Filipiak, W.; Khalid, T.; Osborne, D.; Ratcliffe, N.M. A review of the volatiles from the healthy human body. *J. Breath Res.* **2014**, *8*, doi:10.1088/1752-7155/8/1/014001.

184. Rudnicka, J.; Kowalkowski, T.; Buszewski, B. Searching for selected VOCs in human breath samples as potential markers of lung cancer. *Lung Cancer* **2019**, *135*, 123–129, doi:10.1016/j.lungcan.2019.02.012.

185. Kischkel, S.; Miekisch, W.; Patricia, F.; Schubert, J.K. Breath analysis during one-lung ventilation in cancer patients. *Eur. Respir. J.* **2012**, *40*, 706–713, doi:10.1183/09031936.00125411.

186. Pereira, J.; Porto-Figueira, P.; Cavaco, C.; Taunk, K.; Rapole, S.; Dhakne, R.; Nagarajaram, H.; Câmara, J.S. Breath analysis as a potential and non-invasive frontier in disease diagnosis: An overview. *Metabolites* **2015**, *5*, 3–55, doi:10.3390/metabo5010003.

187. Tienpont, B.; David, F.; Bicchi, C.; Sandra, P. High capacity headspace sorptive extraction. *J. Microcolumn Sep.* **2000**, *12*, 577–584, doi:10.1002/1520-

667X(2000)12:11<577::AID-MCS30>3.0.CO;2-Q.

188. Pugliese, G.; Trefz, P.; Brock, B.; Schubert, J.K.; Miekisch, W. Extending PTR based breath analysis to real-time monitoring of reactive volatile organic compounds. *Analyst* **2019**, *144*, 7359–7367, doi:10.1039/c9an01478k.

189. Cavaco, C.; Pereira, J.A.M.; Taunk, K.; Taware, R.; Rapole, S.; Nagarajaram, H.; Câmara, J.S. Screening of salivary volatiles for putative breast cancer discrimination: an exploratory study involving geographically distant populations. *Anal. Bioanal. Chem.* **2018**, *410*, 4459–4468, doi:10.1007/s00216-018-1103-x.

190. Cavaco, C.; Perestrelo, R.; Silva, C.; Aveiro, F.; Pereira, J.; Câmara, J.S. Establishment of the Saliva Volatomic Profi le as an Exploratory and Non-invasive Strategy to Find Potential Breast Cancer Biomarkers. **2014**.

191. Streckfus, C.F.; Brown, R.E.; Bull, J.M. Proteomics, morphoproteomics, saliva and breast cancer: An emerging approach to guide the delivery of individualised thermal therapy, thermochemotherapy and monitor therapy response. *Int. J. Hyperth.* **2010**, *26*, 649–661, doi:10.3109/02656736.2010.506470.

192. Malathi, N.; Mythili, S.; Vasanthi, H.R. Salivary Diagnostics: A Brief Review. *ISRN Dent.* **2014**, *2014*, 1–8, doi:10.1155/2014/158786.

193. Pereira, J.A.M.; Taware, R.; Porto-Figueira, P.; Rapole, S.; Câmara, J.S. The salivary volatome in breast cancer. *Precis. Med. Investig. Pract. Provid.* **2020**, 301–307, doi:10.1016/B978-0-12-819178-1.00029-0.

194. Bobkov, Y. V.; Walker, W.B.; Cattaneo, A.M. Altered functional properties of the codling moth Orco mutagenized in the intracellular loop-3. *Sci. Rep.* **2021**, *11*, 1–16, doi:10.1038/s41598-021-83024-3.

195. Silva, C.; Perestrelo, R.; Silva, P.; Capelinha, F.; Tomás, H.; Câmara, J.S. Volatomic pattern of breast cancer and cancer-free tissues as a powerful strategy to identify potential biomarkers. *Analyst* **2019**, *144*, 4153–4161, doi:10.1039/c9an00263d.

196. Silva, C.L.; Perestrelo, R.; Silva, P.; Tomás, H.; Câmara, J.S. Volatile metabolomic signature of human breast cancer cell lines. *Sci. Rep.* **2017**, *7*, 1–8, doi:10.1038/srep43969.

197. Porto-Figueira, P.; Pereira, J.A.M.; Câmara, J.S. Exploring the potential of needle trap microextraction combined with chromatographic and statistical data to discriminate different types of cancer based on urinary volatomic biosignature. *Anal. Chim. Acta* **2018**, *1023*, 53–63, doi:10.1016/j.aca.2018.04.027.

198. Salciccia, S.; Capriotti, A.L.; Laganà, A.; Fais, S.; Logozzi, M.; De Berardinis, E.; Busetto, G.M.; Di Pierro, G.B.; Ricciuti, G.P.; Del Giudice, F.; et al. Biomarkers in prostate cancer diagnosis: From current knowledge to the role of metabolomics and exosomes. *Int. J. Mol. Sci.* **2021**, *22*, doi:10.3390/ijms22094367.

199. Nazario, C.E.D.; Fumes, B.H.; da Silva, M.R.; Lanças, F.M. New materials for sample preparation techniques in bioanalysis. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2017**, *1043*, 81–95, doi:10.1016/j.jchromb.2016.10.041.

200. Gallo, M.; Ferranti, P. The evolution of analytical chemistry methods in foodomics. *J. Chromatogr. A* **2016**, *1428*, 3–15, doi:10.1016/j.chroma.2015.09.007.

201. Pan, J.; Zhang, C.; Zhang, Z.; Li, G. Review of online coupling of sample preparation techniques with liquid chromatography. *Anal. Chim. Acta* **2014**, *815*, 1–15, doi:10.1016/j.aca.2014.01.017.

202. Huie, C.W. A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. *Anal. Bioanal. Chem.* **2002**, *373*, 23–30, doi:10.1007/s00216-002-1265-3.

203. Chen, J.; Duan, C.; Guan, Y. Sorptive extraction techniques in sample preparation for organophosphorus pesticides in complex matrices. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2010**, *878*, 1216–1225, doi:10.1016/j.jchromb.2010.02.031.

204. Bylda, C.; Thiele, R.; Kobold, U.; Volmer, D.A. Recent advances in sample preparation techniques to overcome difficulties encountered during quantitative analysis of small molecules from biofluids using LC-MS/MS. *Analyst* **2014**, *139*, 2265–2276, doi:10.1039/c4an00094c.

205. Li, P.; Bartlett, M.G. A review of sample preparation methods for quantitation of small-molecule analytes in brain tissue by liquid chromatography tandem mass spectrometry (LC-MS/MS). *Anal. Methods* **2014**, *6*, 6183–6207, doi:10.1039/c4ay00915k.

206. Grimalt, S.; Dehouck, P. Review of analytical methods for the determination of pesticide residues in grapes. *J. Chromatogr. A* **2016**, *1433*, 1–23, doi:10.1016/j.chroma.2015.12.076.

207. Ocaña-González, J.A.; Fernández-Torres, R.; Bello-López, M.Á.; Ramos-Payán, M. New developments in microextraction techniques in bioanalysis. A review. *Anal. Chim. Acta* **2016**, *905*, 8–23, doi:10.1016/j.aca.2015.10.041.

208. Ramos, L. Critical overview of selected contemporary sample preparation techniques. *J. Chromatogr. A* **2012**, *1221*, 84–98, doi:10.1016/j.chroma.2011.11.011.

209. Spietelun, A.; Marcinkowski, Ł.; de la Guardia, M.; Namieśnik, J. Recent developments and future trends in solid phase microextraction techniques towards green analytical chemistry. *J. Chromatogr. A* **2013**, *1321*, 1–13, doi:10.1016/j.chroma.2013.10.030.

210. Alahmad, W.; Sahragard, A.; Varanusupakul, P. Online and offline preconcentration techniques on paper-based analytical devices for ultrasensitive chemical and biochemical analysis: A review. *Biosens. Bioelectron.* **2021**, *194*, 113574, doi:10.1016/j.bios.2021.113574.

211. Kitagawa, F.; Otsuka, K. Recent applications of on-line sample preconcentration

techniques in capillary electrophoresis. *J. Chromatogr. A* **2014**, *1335*, 43–60, doi:10.1016/j.chroma.2013.10.066.

212. Nezhadali, A.; Es'haghi, Z.; Khatibi, A. Selective extraction of progesterone hormones from environmental and biological samples using a polypyrrole molecularly imprinted polymer and determination by gas chromatography. *Anal. Methods* **2016**, *8*, 1813–1827, doi:10.1039/c5ay02174j.

213. Ghazaghi, M.; Mousavi, H.Z.; Rashidi, A.M.; Shirkhanloo, H.; Rahighi, R. Innovative separation and preconcentration technique of coagulating homogenous dispersive micro solid phase extraction exploiting graphene oxide nanosheets. *Anal. Chim. Acta* **2016**, *902*, 33–42, doi:10.1016/j.aca.2015.11.011.

214. Ashri, N.Y.; Abdel-Rehim, M. Sample treatment based on extraction techniques in biological matrices. *Bioanalysis* **2011**, *3*, 2003–2018, doi:10.4155/bio.11.201.

215. Englard, S.; Seifter, S. Precipitation techniques. *Methods Enzymol.* **1990**, *182*, 285–300, doi:10.1016/0076-6879(90)82024-V.

216. Tama, C.I.; Shen, J.X.; Schiller, J.E.; Hayes, R.N.; Clement, R.P. Determination of a novel thrombin receptor antagonist (SCH 530348) in human plasma: Evaluation of Ultra Performance Liquid Chromatography™-tandem mass spectrometry for routine bioanalytical analysis. *J. Pharm. Biomed. Anal.* **2011**, *55*, 349–359, doi:10.1016/j.jpba.2011.01.045.

217. Polson, C.; Sarkar, P.; Incledon, B.; Raguvaran, V.; Grant, R. Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2003**, *785*, 263–275, doi:10.1016/S1570-0232(02)00914-5.

218. Bueters, T.; Dahlström, J.; Kvalvågnaes, K.; Betnér, I.; Briem, S. High-throughput analysis of standardized pharmacokinetic studies in the rat using sample pooling and UPLC-MS/MS. *J. Pharm. Biomed. Anal.* **2011**, *55*, 1120–1126, doi:10.1016/j.jpba.2011.03.042.

219. Biddlecombe, R.A.; Pleasance, S. Automated protein precipitation by filtration in the 96-well format. *J. Chromatogr. B Biomed. Sci. Appl.* **1999**, *734*, 257–265, doi:10.1016/S0378-4347(99)00355-2.

220. Souverain, S.; Rudaz, S.; Veuthey, J.L. Protein precipitation for the analysis of a drug cocktail in plasma by LC-ESI-MS. *J. Pharm. Biomed. Anal.* **2004**, *35*, 913–920, doi:10.1016/j.jpba.2004.03.005.

221. Chang, M.S.; Ji, Q.; Zhang, J.; El-Shourbagy, T.A. Historical Review of Sample Preparation for Chromatographic Bioanalysis: Pros and Cons Min. **2007**, 107–133, doi:10.1002/ddr.20173.

222. López-Bascón-Bascon, M.A.; Luque de Castro, M.D. Soxhlet extraction. *Liq. Extr.* **2019**, 327–354, doi:10.1016/B978-0-12-816911-7.00011-6.

223. Luque de Castro, M.D.; Priego-Capote, F. Soxhlet extraction: Past and present panacea. *J. Chromatogr. A* **2010**, *1217*, 2383–2389, doi:10.1016/j.chroma.2009.11.027.

224. Han, D.; Chen, C.; Zhang, C.; Zhang, Y.; Tang, X. Determination of mangiferin in rat plasma by liquid-liquid extraction with UPLC-MS/MS. *J. Pharm. Biomed. Anal.* **2010**, *51*, 260–263, doi:10.1016/j.jpba.2009.07.021.

225. Cháfer-Pericás, C.; Campíns-Falcó, P.; Herráez-Hernández, R. Application of solid-phase microextraction combined with derivatization to the enantiomeric determination of amphetamines. *J. Pharm. Biomed. Anal.* **2006**, *40*, 1209–1217, doi:10.1016/j.jpba.2005.09.025.

226. Khalilian, F.; Hanzaki, S.A.; Yousefi, M. Synthesis of a graphene-based nanocomposite for the dispersive solid-phase extraction of vancomycin from biological samples. *J. Sep. Sci.* **2015**, *38*, 975–981, doi:10.1002/jssc.201401067.

227. Namera, A.; Saito, T. Recent advances in unique sample preparation techniques for bioanalysis. *Bioanalysis* **2013**, *5*, 915–932, doi:10.4155/bio.13.52.

228. Liu, H.; Dasgupta, P.K. Analytical chemistry in a drop. *TrAC - Trends Anal. Chem.* **1996**, *15*, 468–475, doi:10.1016/S0165-9936(96)00065-9.

229. Jeannot, M.A.; Cantwell, F.F. Solvent microextraction into a single drop. *Anal. Chem.* **1996**, *68*, 2236–2240, doi:10.1021/ac960042z.

230. He, Y.; Lee, H.K. Liquid-Phase Microextraction in a Single Drop of Organic Solvent by Using a Conventional Microsyringe. *Anal. Chem.* **1997**, *69*, 4634–4640, doi:10.1021/ac970242q.

231. David, F.; Ochiai, N.; Sandra, P. Two decades of stir bar sorptive extraction: A retrospective and future outlook. *TrAC - Trends Anal. Chem.* **2019**, *112*, 102–111, doi:10.1016/j.trac.2018.12.006.

232. Camino-Sánchez, F.J.; Rodríguez-Gómez, R.; Zafra-Gómez, A.; Santos-Fandila, A.; Vílchez, J.L. Stir bar sorptive extraction: Recent applications, limitations and future trends. *Talanta* **2014**, *130*, 388–399, doi:10.1016/j.talanta.2014.07.022.

233. He, M.; Chen, B.; Hu, B. Recent developments in stir bar sorptive extraction Microextraction Techniques. *Anal. Bioanal. Chem.* **2014**, *406*, 2001–2026, doi:10.1007/s00216-013-7395-y.

234. Bojko, B.; Reyes-Garcés, N.; Bessonneau, V.; Goryński, K.; Mousavi, F.; Souza Silva, E.A.; Pawliszyn, J. Solid-phase microextraction in metabolomics. *TrAC - Trends Anal. Chem.* **2014**, *61*, 168–180, doi:10.1016/j.trac.2014.07.005.

235. Bojko, B.; Cudjoe, E.; Gómez-Ríos, G.A.; Gorynski, K.; Jiang, R.; Reyes-Garcés, N.; Risticevic, S.; Silva, É.A.S.; Togunde, O.; Vuckovic, D.; et al. SPME - Quo vadis? *Anal. Chim. Acta* **2012**, *750*, 132–151, doi:10.1016/j.aca.2012.06.052.

236. Reyes-Garcés, N.; Gionfriddo, E.; Gómez-Ríos, G.A.; Alam, M.N.; Boyacl, E.; Bojko, B.;

Singh, V.; Grandy, J.; Pawliszyn, J. Advances in Solid Phase Microextraction and Perspective on Future Directions. *Anal. Chem.* **2018**, *90*, 302–360, doi:10.1021/acs.analchem.7b04502.

237. Silva, M.J.; Slakman, A.R.; Reidy, J.A.; Preau, J.L.; Herbert, A.R.; Samandar, E.; Needham, L.L.; Calafat, A.M. Analysis of human urine for fifteen phthalate metabolites using automated solid-phase extraction. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2004**, *805*, 161–167, doi:10.1016/j.jchromb.2004.02.038.

238. Rodil, R.; Quintana, J.B.; Basaglia, G.; Pietrogrande, M.C.; Cela, R. Determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction, silylation and gas chromatography-mass spectrometry. *J. Chromatogr. A* **2010**, *1217*, 6428–6435, doi:10.1016/j.chroma.2010.08.020.

239. Mavumengwana-Khanyile, B.; Katima, Z.; Songa, E.A.; Okonkwo, J.O. Recent advances in sorbents applications and techniques used for solid-phase extraction of atrazine and its metabolites deisopropylatrazine and deethylatrazine: a review. *Int. J. Environ. Anal. Chem.* **2019**, *99*, 1017–1068, doi:10.1080/03067319.2019.1597866.

240. Maikranz, E.; Spengler, C.; Thewes, N.; Thewes, A.; Nolle, F.; Jung, P.; Bischoff, M.; Santen, L.; Jacobs, K. Different binding mechanisms of: Staphylococcus aureus to hydrophobic and hydrophilic surfaces. *Nanoscale* **2020**, *12*, 19267–19275, doi:10.1039/donr03134h.

241. Rudolph, T.; Kumar Allampally, N.; Fernández, G.; Schacher, F.H. Controlling Aqueous Self-Assembly Mechanisms by Hydrophobic Interactions. *Chem. - A Eur. J.* **2014**, *20*, 13871–13875, doi:10.1002/chem.201404141.

242. Shaker, D.S.; Ishak, R.A.H.; Ghoneim, A.; Elhuoni, M.A. Nanoemulsion: A review on mechanisms for the transdermal delivery of hydrophobic and hydrophilic drugs. *Sci. Pharm.* **2019**, *87*, doi:10.3390/scipharm87030017.

243. Lin, J.L.; Huang, C.; Chin, C.J.M.; Pan, J.R. Coagulation dynamics of fractal flocs induced by enmeshment and electrostatic patch mechanisms. *Water Res.* **2008**, *42*, 4457–4466, doi:10.1016/j.watres.2008.07.043.

244. Yang, Y.; Geng, X. Mixed-mode chromatography and its applications to biopolymers. *J. Chromatogr. A* **2011**, *1218*, 8813–8825, doi:10.1016/j.chroma.2011.10.009.

245. Desimone, H.; Beretta, S. Mechanisms of mixed mode fatigue crack propagation at rail butt-welds. *Int. J. Fatigue* **2006**, *28*, 635–642, doi:10.1016/j.ijfatigue.2005.07.044.

246. Guo, T.Y.; Wong, L.N.Y. Cracking mechanisms of a medium-grained granite under mixed-mode I-II loading illuminated by acoustic emission. *Int. J. Rock Mech. Min. Sci.* **2021**, *145*, 104852, doi:10.1016/j.ijrmms.2021.104852.

247. Pereira, J.; Silva, C.L.; Perestrelo, R.; Gonçalves, J.; Alves, V.; Câmara, J.S. Re-exploring the high-throughput potential of microextraction techniques, SPME and MEPS, as powerful strategies for medical diagnostic purposes. Innovative approaches, recent

applications and future trends Microextraction Techniques. *Anal. Bioanal. Chem.* **2014**, *406*, 2101–2122, doi:10.1007/s00216-013-7527-4.

248. Mendes, B.; Gonalves, J.; Câmara, J.S. Effectiveness of high-throughput miniaturized sorbent- and solid phase microextraction techniques combined with gas chromatography-mass spectrometry analysis for a rapid screening of volatile and semi-volatile composition of wines - A comparative study. *Talanta* **2012**, *88*, 79–94, doi:10.1016/j.talanta.2011.10.010.

249. Souza-Silva, É.A.; Jiang, R.; Rodríguez-Lafuente, A.; Gionfriddo, E.; Pawliszyn, J. A critical review of the state of the art of solid-phase microextraction of complex matrices I. Environmental analysis. *TrAC - Trends Anal. Chem.* **2015**, *71*, 224–235, doi:10.1016/j.trac.2015.04.016.

250. Arthur, C.L.; Pawliszyn, J. Solid Phase Microextraction with Thermal Desorption Using Fused Silica Optical Fibers. *Anal. Chem.* **1990**, *62*, 2145–2148, doi:10.1021/ac00218a019.

251. Zambonin, C.G.; Quinto, M.; De Vietro, N.; Palmisano, F. Solid-phase microextraction - Gas chromatography mass spectrometry: A fast and simple screening method for the assessment of organophosphorus pesticides residues in wine and fruit juices. *Food Chem.* **2004**, *86*, 269–274, doi:10.1016/j.foodchem.2003.09.025.

252. Pawliszyn, J. Theory of Solid-Phase Microextraction. **2000**, *38*, 270–278.

253. Vas, G.; Vékey, K. Solid-phase microextraction: A powerful sample preparation tool prior to mass spectrometric analysis. *J. Mass Spectrom.* **2004**, *39*, 233–254, doi:10.1002/jms.606.

254. Spietelun, A.; Kloskowski, A.; Chrzanowski, W.; Namieśnik, J. Understanding solid-phase microextraction: Key factors influencing the extraction process and trends in improving the technique. *Chem. Rev.* **2012**, *113*, 1667–1685, doi:10.1021/cr300148j.

255. Ouyang, G.; Pawliszyn, J. A critical review in calibration methods for solid-phase microextraction. *Anal. Chim. Acta* **2008**, *627*, 184–197, doi:10.1016/j.aca.2008.08.015.

256. Risticevic, S.; Lord, H.; Górecki, T.; Arthur, C.L.; Pawliszyn, J. Protocol for solid-phase microextraction method development. *Nat. Protoc.* **2010**, *5*, 122–139, doi:10.1038/nprot.2009.179.

257. Pawliszyn, J.; Pedersen-Bjergaard, S. Analytical Microextraction: Current Status and Future Trends. *J. Chromatogr. Sci.* **2006**, *44*, 291–307, doi:10.1093/chromsci/44.6.291.

258. Spietelun, A.; Pilarczyk, M.; Kloskowski, A.; Namieśnik, J. Current trends in solid-phase microextraction (SPME) fibre coatings. *Chem. Soc. Rev.* **2010**, *39*, 4524–4537, doi:10.1039/c003335a.

259. Kataoka, H.; Lord;, H.L.; Pawliszyn, J. Applications of solid-phase microextraction in food analysis. *2016 13th Int. Sci. Conf. Actual Probl. Electron. Instrum. Eng. APEIE*

2016 - Proc. **2000**, 35–62, doi:10.1109/APEIE.2016.7806458.

260. Staerk, U.; Külpmann, W.R. High-temperature solid-phase microextraction procedure for the detection of drugs by gas chromatography-mass spectrometry. *J. Chromatogr. B Biomed. Sci. Appl.* **2000**, *745*, 399–411, doi:10.1016/S0378-4347(00)00312-1.

261. Prosen, H.; Zupančič-Kralj, L. Solid-phase microextraction. *TrAC - Trends Anal. Chem.* **1999**, *18*, 272–282, doi:10.1016/S0165-9936(98)00109-5.

262. Perestrelo, R.; Barros, A.S.; Rocha, S.M.; Câmara, J.S. Optimisation of solid-phase microextraction combined with gas chromatography-mass spectrometry based methodology to establish the global volatile signature in pulp and skin of Vitis vinifera L. grape varieties. *Talanta* **2011**, *85*, 1483–1493, doi:10.1016/j.talanta.2011.06.025.

263. Vuckovic, D. In vivo solid-phase microextraction sampling: A promising future. *Bioanalysis* **2011**, *3*, 1305–1308, doi:10.4155/bio.11.109.

264. Zhang, Y.; Liu, L.; Yin, T.L.; Yang, J.; Xiong, C.L. Follicular metabolic changes and effects on oocyte quality in polycystic ovary syndrome patients. *Oncotarget* **2017**, *8*, 80472–80480, doi:10.18632/oncotarget.19058.

265. Castiglione Morelli, M.A.; Iuliano, A.; Schettini, S.C.A.; Petruzzi, D.; Ferri, A.; Colucci, P.; Viggiani, L.; Cuviello, F.; Ostuni, A. NMR metabolic profiling of follicular fluid for investigating the different causes of female infertility: a pilot study. *Metabolomics* **2019**, *15*, 1–10, doi:10.1007/s11306-019-1481-x.

266. Hou, E.; Zhao, Y.; Hang, J.; Qiao, J. Metabolomics and correlation network analysis of follicular fluid reveals associations between l-tryptophan, l-tyrosine and polycystic ovary syndrome. *Biomed. Chromatogr.* **2020**, *35*, 1–14, doi:10.1002/bmc.4993.

267. Zhao, H.; Zhao, Y.; Li, T.; Li, M.; Li, J.; Li, R.; Liu, P.; Yu, Y.; Qiao, J. Metabolism alteration in follicular niche: The nexus among intermediary metabolism, mitochondrial function, and classic polycystic ovary syndrome. *Free Radic. Biol. Med.* **2015**, *86*, 295–307, doi:10.1016/j.freeradbiomed.2015.05.013.

268. Liu, L.; Yin, T. lang; Chen, Y.; Li, Y.; Yin, L.; Ding, J.; Yang, J.; Feng, H.L. Follicular dynamics of glycerophospholipid and sphingolipid metabolisms in polycystic ovary syndrome patients. *J. Steroid Biochem. Mol. Biol.* **2018**, *185*, 142–149, doi:10.1016/j.jsbmb.2018.08.008.

269. Sun, Z.; Chang, H.M.; Wang, A.; Song, J.; Zhang, X.; Guo, J.; Leung, P.C.K.; Lian, F. Identification of potential metabolic biomarkers of polycystic ovary syndrome in follicular fluid by SWATH mass spectrometry. *Reprod. Biol. Endocrinol.* **2019**, *17*, 1–10, doi:10.1186/s12958-019-0490-y.

270. Li, S.; Qi, J.; Tao, Y.; Zhu, Q.; Huang, R.; Liao, Y.; Yue, J.; Liu, W.; Zhao, H.; Yin, H.; et al. Elevated levels of arachidonic acid metabolites in follicular fluid of PCOS patients. **2020**, *159*, 159–169.

271. Jóźwik, M.; Jóźwik, M.; Milewska, A.J.; Battaglia, F.C.; Jóźwik, M. Competitive inhibition of amino acid transport in human preovulatory ovarian follicles. *Syst. Biol. Reprod. Med.* **2017**, *63*, 311–317, doi:10.1080/19396368.2017.1341962.

272. Luti, S.; Fiaschi, T.; Magherini, F.; Modesti, P.A.; Piomboni, P.; Semplici, B.; Morgante, G.; Amoresano, A.; Illiano, A.; Pinto, G.; et al. Follicular microenvironment: Oxidative stress and adiponectin correlated with steroids hormones in women undergoing in vitro fertilization. *Mol. Reprod. Dev.* **2021**, *88*, 175–184, doi:10.1002/mrd.23447.

273. Patil, K.; Yelamanchi, S.; Kumar, M.; Hinduja, I.; Prasad, T.S.K.; Gowda, H.; Mukherjee, S. Quantitative mass spectrometric analysis to unravel glycoproteomic signature of follicular fluid in women with polycystic ovary syndrome. *PLoS One* **2019**, *14*, 1–17, doi:10.1371/journal.pone.0214742.

274. Chen, X.; Lu, T.; Wang, X.; Sun, X.; Zhang, J.; Zhou, K.; Ji, X.; Sun, R.; Wang, X.; Chen, M.; et al. Metabolic alterations associated with polycystic ovary syndrome: A UPLC Q-Exactive based metabolomic study. *Clin. Chim. Acta* **2020**, *502*, 280–286, doi:10.1016/j.cca.2019.11.016.

275. Cordeiro, F.B.; Ferreira, C.R.; Sobreira, T.J.P.; Yannell, K.E.; Jarmusch, A.K.; Cedenho, A.P.; Lo Turco, E.G.; Cooks, R.G. Multiple reaction monitoring (MRM)-profiling for biomarker discovery applied to human polycystic ovarian syndrome. *Rapid Commun. Mass Spectrom.* **2017**, *31*, 1462–1470, doi:10.1002/rcm.7927.

276. Fabjan, T.; Vrtačnik-Bokal, E.; Virant-Klun, I.; Bedenk, J.; Kumer, K.; Osredkar, J. Antimüllerian hormone and oxidative stress biomarkers as predictors of successful pregnancy in polycystic ovary syndrome, endometriosis and tubal infertility factor. *Acta Chim. Slov.* **2020**, *67*, 885–895, doi:10.17344/acsi.2020.5864.

277. Garg, D.; Grazi, R.; Lambert-Messerlian, G.M.; Merhi, Z. Correlation between follicular fluid levels of sRAGE and vitamin D in women with PCOS. *J. Assist. Reprod. Genet.* **2017**, *34*, 1507–1513, doi:10.1007/s10815-017-1011-6.

278. Eskandari, Z.; Sadrkhanlou, R.A.; Nejati, V.; Tizro, G. PCOS women show significantly higher homocysteine level, independent to glucose and E2 level. *Int. J. Reprod. Biomed.* **2016**, *14*, 495–500, doi:10.29252/ijrm.14.8.495.

279. Al-Rubaye, A.F.; Hameed, I.H.; Kadhim, M.J. A Review: Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Natural Compounds of Some Plants. *Int. J. Toxicol. Pharmacol. Res.* **2017**, *9*, 81–85, doi:10.25258/ijtpr.v9i01.9042.

280. Kadhim, M.J.; Sosa, A.A.; Hameed, I.H. Evaluation of anti-bacterial activity and bioactive chemical analysis of Ocimum basilicum using Fourier transform infrared (FT-IR) and gas chromatographymass spectrometry (GC-MS) techniques. *J. Pharmacogn. Phyther.* **2016**, *8*, 127–146, doi:10.5897/JPP2015.0366.

281. Mohammed, G.J.; Kadhim, M.J.; Hussein, H.M. Characterization of bioactive chemical

compounds from aspergillus terreus and evaluation of antibacterial and antifungal activity. *Int. J. Pharmacogn. Phytochem. Res.* **2016**, *8*, 889–905.

282. Vital, P.G.; Rivera, W.L. Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. extracts. *J. Med. Plants Res.* **2009**, *3*, 511–518.

283. Caramelo, D. Determination of antipsychotic drugs in oral fluid samples using dried saliva spots Versão final após defesa, 2019.

284. Jenden, D.J.; Cho, A.K. Applications of Integrated Gas Chromatography/Mass Spectrometry in Pharmacology and Toxicology. **1973**, 371–390.

285. Eiceman, G.A. Instrumentation of Gas Chromatography. *Encycl. Anal. Chem.* **2000**, 10671–10679, doi:10.1002/9780470027318.a5505.

286. Maurer, H.H. Role of gas chromatography-mass spectrometry with negative ion chemical ionization in clinical and forensic toxicology, doping control, and biomonitoring. *Ther. Drug Monit.* **2002**, *24*, 247–254, doi:10.1097/00007691-200204000-00007.

287. Sneddon, J.; Masuram, S.; Richert, J.C. Gas chromatography-mass spectrometry-basic principles, instrumentation and selected applications for detection of organic compounds. *Anal. Lett.* **2007**, *40*, 1003–1012, doi:10.1080/00032710701300648.

288. de Fatima, A.; Modolo, L.; Conegero, L.; Pilli, R.; Ferreira, C.; Kohn, L.; de Carvalho, J. Styryl Lactones and Their Derivatives: Biological Activities, Mechanisms of Action and Potential Leads for Drug Design. *Curr. Med. Chem.* **2006**, *13*, 3371–3384, doi:10.2174/092986706779010298.

289. Marston, A. Role of advances in chromatographic techniques in phytochemistry. *Phytochemistry* **2007**, *68*, 2786–2798, doi:10.1016/j.phytochem.2007.08.004.

290. Milne, A.; Beamish, T. Inhalational and local anesthetics reduce tactile and thermal responses in mimosa pudica. *Can. J. Anesth.* **1999**, 287–289.

291. Wittmann, C. Fluxome analysis using GC-MS. *Microb. Cell Fact.* **2007**, *6*, 1–17, doi:10.1186/1475-2859-6-6.

292. Villas-Bôas, S.G.; Mas, S.; Åkesson, M.; Smedsgaard, J.; Nielsen, J. Mass spectrometry in metabolome analysis. *Mass Spectrom. Rev.* **2005**, *24*, 613–646, doi:10.1002/mas.20032.

293. Sumner, L.W.; Amberg, A.; Barrett, D.; Beale, M.H.; Beger, R.; Daykin, C.A.; Fan, T.W.M.; Fiehn, O.; Goodacre, R.; Griffin, J.L.; et al. Proposed minimum reporting standards for chemical analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* **2007**, *3*, 211–221, doi:10.1007/s11306-007-0082-2.

294. Wishart, D.S. Computational strategies for metabolite identification in metabolomics. *Bioanalysis* **2009**, *1*, 1579–1596, doi:10.4155/bio.09.138.

295. Wishart, D.S. Advances in metabolite identification. *Bioanalysis* **2011**, *3*, 1769–1782, doi:10.4155/bio.11.155.

296. Majchrzak, T.; Wojnowski, W.; Lubinska-Szczygeł, M.; Różańska, A.; Namieśnik, J.; Dymerski, T. PTR-MS and GC-MS as complementary techniques for analysis of volatiles: A tutorial review. *Anal. Chim. Acta* **2018**, *1035*, 1–13, doi:10.1016/j.aca.2018.06.056.

297. Kranenburg, R.F.; Verduin, J.; Stuyver, L.I.; de Ridder, R.; van Beek, A.; Colmsee, E.; van Asten, A.C. Benefits of derivatization in GC–MS-based identification of new psychoactive substances. *Forensic Chem.* **2020**, *20*, 100273, doi:10.1016/j.forc.2020.100273.

298. Brinca, A.T.; Ramalhinho, A.C.; Sousa, Â.; Oliani, A.H.; Breitenfeld, L.; Passarinha, L.A.; Gallardo, E. Follicular Fluid: A Powerful Tool for the Understanding and Diagnosis of Polycystic Ovary Syndrome. *Biomedicines* **2022**, *10*, 1254, doi:10.3390/biomedicines10061254.

299. Pasikanti, K.K.; Ho, P.C.; Chan, E.C.Y. Gas chromatography/mass spectrometry in metabolic profiling of biological fluids. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2008**, *871*, 202–211, doi:10.1016/j.jchromb.2008.04.033.

300. Des Rosiers, C.; Di Donato, L.; Comte, B.; Laplante, A.; Marcoux, C.; David, F.; Fernandez, C.A.; Brunengraber, H. Isotopomer analysis of citric acid cycle and gluconeogenesis in rat liver. Reversibility of isocitrate dehydrogenase and involvement of ATP-citrate lyase in gluconeogenesis. *J. Biol. Chem.* 1995, *270*, 10027–10036.

301. Hellerstein, M.K.; Neese, R.A.; Linfoot, P.; Christiansen, M.; Turner, S.; Letscher, A. Hepatic gluconeogenic fluxes and glycogen turnover during fasting in humans. A stable isotope study. *J. Clin. Invest.* **1997**, *100*, 1305–1319, doi:10.1172/JCI119644.

302. Hellerstein, M.K.; Neese, R.A.; Schwarz, J.M.; Turner, S.; Faix, D.; Wu, K. Altered fluxes responsible for reduced hepatic glucose production and gluconeogenesis by exogenous glucose in rats. *Am. J. Physiol. - Endocrinol. Metab.* **1997**, *272*, doi:10.1152/ajpendo.1997.272.1.e163.

303. Di Donato, L.; Des Rosiers, C.; Montgomery, J.A.; David, F.; Garneau, M.; Brunengraber, H. Rates of gluconeogenesis and citric acid cycle in perfused livers, assessed from the mass spectrometric assay of the 13C labeling pattern of glutamate. *J. Biol. Chem.* **1993**, *268*, 4170–4180, doi:10.1016/s0021-9258(18)53594-8.

304. Chiu, H.H.; Kuo, C.H. Gas chromatography-mass spectrometry-based analytical strategies for fatty acid analysis in biological samples. *J. Food Drug Anal.* **2020**, *28*, 60–73, doi:10.1016/j.jfda.2019.10.003.

305. Masood, A.; Stark, K.D.; Salem, N. A simplified and efficient method for the analysis of fatty acid methyl esters suitable for large clinical studies. *J. Lipid Res.* **2005**, *46*, 2299–2305, doi:10.1194/jlr.D500022-JLR200.

306. Bicalho, B.; David, F.; Rumplel, K.; Kindt, E.; Sandra, P. Creating a fatty acid methyl

ester database for lipid profiling in a single drop of human blood using high resolution capillary gas chromatography and mass spectrometry. *J. Chromatogr. A* **2008**, *1211*, 120–128, doi:10.1016/j.chroma.2008.09.066.

307. Turner, T.D.; Karlsson, L.; Mapiye, C.; Rolland, D.C.; Martinsson, K.; Dugan, M.E.R. Dietary influence on the m. longissimus dorsi fatty acid composition of lambs in relation to protein source. *Meat Sci.* **2012**, *91*, 472–477, doi:10.1016/j.meatsci.2012.02.034.

308. Silva, C.L.; Perestrelo, R.; Silva, P.; Tomás, H.; Câmara, J.S. Implementing a central composite design for the optimization of solid phase microextraction to establish the urinary volatomic expression: a first approach for breast cancer. *Metabolomics* **2019**, *15*, 1–13, doi:10.1007/s11306-019-1525-2.

309. Hart, R.J. Physiological aspects of female fertility: Role of the environment, modern lifestyle, and genetics. *Physiol. Rev.* **2016**, *96*, 873–909, doi:10.1152/physrev.00023.2015.

310. Sifakis, S.; Androutsopoulos, V.P.; Tsatsakis, A.M.; Spandidos, D.A. Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. *Environ. Toxicol. Pharmacol.* **2017**, *51*, 56–70, doi:10.1016/j.etap.2017.02.024.

311. Gore, A.C.; Chappell, V.A.; Fenton, S.E.; Flaws, J.A.; Nadal, A.; Prins, G.S.; Toppari, J.; Zoeller, R.T. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocr. Rev.* **2015**, *36*, 1–150, doi:10.1210/er.2015-1010.

312. Vander Borght, M.; Wyns, C. Fertility and infertility: Definition and epidemiology. *Clin. Biochem.* **2018**, *62*, 2–10, doi:10.1016/j.clinbiochem.2018.03.012.

313. Bang, D.Y.; Lee, I.K.; Lee, B.M. Toxicological characterization of phthalic acid. *Toxicol. Res.* **2011**, *27*, 191–203, doi:10.5487/TR.2011.27.4.191.

314. Przybylińska, P.A.; Wyszkowski, M. Environmental contamination with phthalates and its impact on living organisms. *Ecol. Chem. Eng. S* **2016**, *23*, 347–356, doi:10.1515/eces-2016-0024.

315. Matsumoto, M.; Hirata-Koizumi, M.; Ema, M. Potential adverse effects of phthalic acid esters on human health: A review of recent studies on reproduction. *Regul. Toxicol. Pharmacol.* **2008**, *50*, 37–49, doi:10.1016/j.yrtph.2007.09.004.

316. Du, Y.Y.; Fang, Y.L.; Wang, Y.X.; Zeng, Q.; Guo, N.; Zhao, H.; Li, Y.F. Follicular fluid and urinary concentrations of phthalate metabolites among infertile women and associations with in vitro fertilization parameters. *Reprod. Toxicol.* **2016**, *61*, 142–150, doi:10.1016/j.reprotox.2016.04.005.

317. Buck Louis, G.M.; Peterson, C.M.; Chen, Z.; Croughan, M.; Sundaram, R.; Stanford, J.; Varner, M.W.; Kennedy, A.; Giudice, L.; Fujimoto, V.Y.; et al. Bisphenol A and phthalates and endometriosis: The Endometriosis: Natural History, Diagnosis and Outcomes Study. *Fertil. Steril.* **2013**, *100*, 162–169, doi:10.1016/j.fertnstert.2013.03.026.

318. Coldham, N.G.; Dave, M.; Sivapathasundaram, S.; McDonnell, D.P.; Connor, C.; Sauer, M.J. Evaluation of a recombinant yeast cell estrogen screening assay. *Environ. Health Perspect.* **1997**, *105*, 734–742, doi:10.1289/ehp.97105734.

319. Okubo, T.; Suzuki, T.; Yokoyama, Y.; Kano, K.; Kano, I. Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay in Vitro. *Biol. Pharm. Bull.* **2003**, *26*, 1219–1224, doi:10.1248/bpb.26.1219.

320. Hannon, P.R.; Niermann, S.; Flaws, J.A. Acute Exposure to Di(2-Ethylhexyl) Phthalate in Adulthood Causes Adverse Reproductive Outcomes Later in Life and Accelerates Reproductive Aging in Female Mice. *Toxicol. Sci.* 2016, *150*, 97–108.

321. Davis, B.; Maronpot, R.; Heindel, J. DEHP suppresses estradioland ovuation in cycling rats. *Toxicol. Appl. Pharmacol.* 1994, *128*, 216–223.

322. Craig, Z.R.; Singh, J.; Gupta, R.K.; Flaws, J.A. Co-treatment of mouse antral follicles with 17β-estradiol interferes with mono-2-ethylhexyl phthalate (MEHP)-induced atresia and altered apoptosis gene expression. *Reprod. Toxicol.* **2014**, *45*, 45–51, doi:10.1016/j.reprotox.2014.01.002.

323. Bala, R.; Singh, V.; Rajender, S. Environment, Lifestyle, and Female Infertility. **2021**, 617–638.

324. da Costa, B.R.B.; De Martinis, B.S. Analysis of urinary VOCs using mass spectrometric methods to diagnose cancer: A review. *Clin. Mass Spectrom.* **2020**, *18*, 27–37, doi:10.1016/j.clinms.2020.10.004.

325. Upsona, K.; Sathyanarayana, S.; Roosa, A.J. De; Thompson, M. Lou; Scholes, D.; Dills, R.; Holt, V.L. Phthalates and risk of endometriosis. *NIH Public Access* **2013**, 91–97, doi:10.1016/j.envres.2013.07.003.Phthalates.

326. Cobellis, L.; Latini, G.; DeFelice, C.; Razzi, S.; Paris, I.; Ruggieri, F.; Mazzeo, P.; Petraglia, F. High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis 2003, 1512–1515.

327. Reddy, B.S.; Rozati, R.; Reddy, S.; Kodampur, S.; Reddy, P.; Reddy, R. High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: A prospective case control study. *Fertil. Steril.* **2006**, *85*, 775–779, doi:10.1016/j.fertnstert.2005.08.037.

328. Reddy, B.; Rozati, R.; Reddy, B.; Raman, N. Association of phthalate esters with endometriosis in Indian women. **2006**.

329. Itoh, H.; Iwasaki, M.; Hanaoka, T.; Sasaki, H.; Tanaka, T.; Tsugane, S. Urinary phthalate monoesters and endometriosis in infertile Japanese women. *Sci. Total Environ.* **2009**, *408*, 37–42, doi:10.1016/j.scitotenv.2009.09.012.

330. Weuve, J.; Hauser, R.; Calafat, A.M.; Missmer, S.A.; Wise, L.A. Association of exposure

to phthalates with endometriosis and uterine leiomyomata: Findings from NHANES, 1999-2004. *Environ. Health Perspect.* **2010**, *118*, 825–832, doi:10.1289/ehp.0901543.

331. Huang, P.C.; Tsai, E.M.; Li, W.F.; Liao, P.C.; Chung, M.C.; Wang, Y.H.; Wang, S.L. Association between phthalate exposure and glutathione S-transferase M1 polymorphism in adenomyosis, leiomyoma and endometriosis. *Hum. Reprod.* **2010**, *25*, 986–994, doi:10.1093/humrep/deq015.

332. Kim, S.H.; Chun, S.; Jang, J.Y.; Chae, H.D.; Kim, C.H.; Kang, B.M. Increased plasma levels of phthalate esters in women with advanced-stage endometriosis: A prospective case-control study. *Fertil. Steril.* **2011**, *95*, 357–359, doi:10.1016/j.fertnstert.2010.07.1059.

333. Vaglio, S.; Minicozzi, P.; Bonometti, E.; Mello, G.; Chiarelli, B. Volatile signals during pregnancy: A possible chemical basis for mother-infant recognition. *J. Chem. Ecol.* **2009**, *35*, 131–139, doi:10.1007/s10886-008-9573-5.

334. Silva, C.L.; Passos, M.; Cmara, J.S. Investigation of urinary volatile organic metabolites as potential cancer biomarkers by solid-phase microextraction in combination with gas chromatography-mass spectrometry. *Br. J. Cancer* **2011**, *105*, 1894–1904, doi:10.1038/bjc.2011.437.

335. Health Effects of Selected Chemicals: Volume 4-5 - Nordic Council of Ministers - Google Livros Available online: https://books.google.pt/books?hl=pt-PT&lr=&id=Zi6SLkAmmBQC&oi=fnd&pg=PA211&dq=dodecanol+reproduction&ots=Aqoksj6ITu&sig=XkcmVpq_3jXwd7ndDtnxOdL0Zo0&redir_esc=y#v=snippet&q=1-Dodecanol has not been extensively tested for reproductive&f=false (accessed on Sep 28, 2022).

336. Octadecanal | C18H36O - PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/12533 (accessed on Sep 28, 2022).

337. Tetradecanal | C14H28O - PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/31291 (accessed on Sep 28, 2022).

338. Song, J.; Xiang, S.; Yang, Y.; Sun, Z. Assessment of follicular fluid metabolomics of polycystic ovary syndrome in kidney yang deficiency syndrome. *Eur. J. Integr. Med.* **2019**, *30*, 100944, doi:10.1016/j.eujim.2019.100944.

339. Prates, E.G.; Alves, S.P.; Marques, C.C.; Baptista, M.C.; Horta, A.E.M.; Bessa, R.J.B.; Pereira, R.M. Fatty acid composition of porcine cumulus oocyte complexes (COC) during maturation: Effect of the lipid modulators trans-10, cis-12 conjugated linoleic acid (t10,c12 CLA) and forskolin. *Vitr. Cell. Dev. Biol. - Anim.* **2013**, *49*, 335–345, doi:10.1007/s11626-013-9624-2.

340. Tiper, I. V.; East, J.E.; Subrahmanyam, P.B.; Webb, T.J. Sphingosine 1-phosphate signaling impacts lymphocyte migration, inflammation and infection. *Pathog. Dis.* **2016**, *74*, 1–9, doi:10.1093/femspd/ftw063.

341. Cordeiro, F.B.; Montani, D.A.; Pilau, E.J.; Gozzo, F.C.; Fraietta, R.; Turco, E.G. Lo Ovarian environment aging: follicular fluid lipidomic and related metabolic pathways. *J. Assist. Reprod. Genet.* **2018**, *35*, 1385–1393, doi:10.1007/s10815-018-1259-5.

342. Lucki, N.C.; Sewer, M.B. The interplay between bioactive sphingolipids and steroid hormones. *Steroids* **2010**, *75*, 390–399, doi:10.1016/j.steroids.2010.01.020.

343. Andrieu-Abadie, N.; Levade, T. Sphingomyelin hydrolysis during apoptosis. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **2002**, *1585*, 126–134, doi:10.1016/S1388-1981(02)00332-3.

344. Schmitt, B.G.; Jensen, E.; Laufersweiler, M.C.; Rose, J.L. Threshold of Toxicological Concern: Extending the chemical space by inclusion of a highly curated dataset for organosilicon compounds. *Regul. Toxicol. Pharmacol.* **2021**, *127*, 105074, doi:10.1016/j.yrtph.2021.105074.

345. Neumann, F.; Diallo, F.A.; Hasan, S.H.; Schenck, B.; Traore, I. The Influence of Pharmaceutical Compounds on Male Fertility. *Andrologia* **1976**, *8*, 203–235, doi:10.1111/j.1439-0272.1976.tb02137.x.

346. Kala, S. V.; Lykissa, E.D.; Lebovitz, R.M. Detection and Characterization of Poly(dimethylsiloxane)s in Biological Tissues by GC/AED and GC/MS. *Anal. Chem.* **1997**, *69*, 1267–1272, doi:10.1021/ac961235p.

347. Johnson, W.; Bergfeld, W.F.; Belsito, D. V.; Hill, R.A.; Klaassen, C.D.; Liebler, D.C.; Marks, J.G.; Shank, R.C.; Slaga, T.J.; Snyder, P.W.; et al. Safety Assessment of Cyclomethicone, Cyclotetrasiloxane, Cyclopentasiloxane, Cyclohexasiloxane, and Cycloheptasiloxane. *Int. J. Toxicol.* **2011**, *30*, 149S-227S, doi:10.1177/1091581811428184.

348. Montiel, M.C.; Máximo, F.; Serrano-Arnaldos, M.; Ortega-Requena, S.; Murcia, M.D.; Bastida, J. Biocatalytic solutions to cyclomethicones problem in cosmetics. *Eng. Life Sci.* **2019**, *19*, 370–388, doi:10.1002/elsc.201800194.

349. Dominguez, A.; Fagan, J. Toxins in cosmetics. **2015**, 1–21.

350. Sirotkin, A. V.; Harrath, A.H. Influence of oil-related environmental pollutants on female reproduction. *Reprod. Toxicol.* **2017**, *71*, 142–145, doi:10.1016/j.reprotox.2017.05.007.

351. Sharma, R.P.; Schuhmacher, M.; Kumar, V. Review on crosstalk and common mechanisms of endocrine disruptors: Scaffolding to improve PBPK/PD model of EDC mixture. *Environ. Int.* **2017**, *99*, 1–14, doi:10.1016/j.envint.2016.09.016.

352. Kim, B.M.; Park, E.K.; LeeAn, S.Y.; Ha, M.; Kim, E.J.; Kwon, H.; Hong, Y.C.; Jeong, W.C.; Hur, J.; Cheong, H.K.; et al. BTEX exposure and its health effects in pregnant women following the Hebei Spirit oil spill. *J. Prev. Med. Public Heal.* **2009**, *42*, 96–103, doi:10.3961/jpmph.2009.42.2.96.

353. Suaidi, N.A.; Alshawsh, M.A.; Hoe, S.Z.; Mokhtar, M.H.; Zin, S.R.M. Toxicological

Effects of Technical Xylene Mixtures on the Female Reproductive System: A Systematic Review. *Toxics* **2022**, *10*, 1–20, doi:10.3390/toxics10050235.

354. Etteieb, S.; Cherif, S.; Kawachi, A.; Han, J.; Elayni, F.; Tarhouni, J.; Isoda, H. Combining Biological and Chemical Screenings to Assess Cytotoxicity of Emerging Contaminants in Discharges into Surface Water. *Water. Air. Soil Pollut.* **2016**, *227*, 1–12, doi:10.1007/s11270-016-3049-y.

355. Yoshihara, S.; Mizutare, T.; Makishima, M.; Suzuki, N.; Fujimoto, N.; Igarashi, K.; Ohta, S. Potent estrogenic metabolites of bisphenol A and bisphenol B formed by rat liver S9 fraction: Their structures and estrogenic potency. *Toxicol. Sci.* **2004**, *78*, 50–59, doi:10.1093/toxsci/kfh047.

356. Rehan, M.; Ahmad, E.; Sheikh, I.A.; Abuzenadah, A.M.; Damanhouri, G.A.; Bajouh, O.S.; Al Basri, S.F.; Assiri, M.M.; Beg, M.A. Androgen and progesterone receptors are targets for bisphenol a (BPA), 4-methyl-2,4-bis-(p-hydroxyphenyl)pent-1-ene-A potent metabolite of BPA, and 4-tert-octylphenol: A computational insight. *PLoS One* **2015**, *10*, 1–18, doi:10.1371/journal.pone.0138438.

357. Okuda, K.; Takiguchi, M.; Yoshihara, S. In vivo estrogenic potential of 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene, an active metabolite of bisphenol A, in uterus of ovariectomized rat. *Toxicol. Lett.* **2010**, *197*, 7–11, doi:10.1016/j.toxlet.2010.04.017.

358. Hirao-Suzuki, M.; Nagase, K.; Suemori, T.; Tsutsumi, K.; Shigemori, E.; Tanaka, M.; Takiguchi, M.; Sugihara, N.; Yoshihara, S.; Takeda, S. 4-Methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP) Targets Estrogen Receptor β, to Evoke the Resistance of Human Breast Cancer MCF-7 Cells to G-1, an Agonist for G Protein-Coupled Estrogen Receptor 1. *Biol. Pharm. Bull.* **2021**, *44*, 1524–1529, doi:10.1248/bpb.b21-00417.

359. Hirao-Suzuki, M.; Takeda, S.; Okuda, K.; Takiguchi, M.; Yoshihara, S. Repeated Exposure to 4-Methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP), an Active Metabolite of Bisphenol A, Aggressively Stimulates Breast Cancer Cell Growth in an Estrogen Receptor β (ERβ)–Dependent Manner. *Mol. Pharmacol.* **2019**, *95*, 260–268, doi:10.1124/mol.118.114124.

360. Liu, S.H.; Su, C.C.; Lee, K.I.; Chen, Y.W. Effects of Bisphenol A Metabolite 4-Methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene on Lung Function and Type 2 Pulmonary Alveolar Epithelial Cell Growth. *Sci. Rep.* **2016**, *6*, 1–11, doi:10.1038/srep39254.

361. Huang, C.C.; Yang, C.Y.; Su, C.C.; Fang, K.M.; Yen, C.C.; Lin, C.T.; Liu, J.M.; Lee, K.I.; Chen, Y.W.; Liu, S.H.; et al. 4-Methyl-2,4-Bis(4-Hydroxyphenyl)Pent-1-Ene, a Major Active Metabolite of Bisphenol a, Triggers Pancreatic B-Cell Death Via a Jnk/Ampkα Activation-Regulated Endoplasmic Reticulum Stress-Mediated Apoptotic Pathway. *Int. J. Mol. Sci.* **2021**, *22*, doi:10.3390/ijms22094379.

362. Shirey, R.E. *SPME Commercial Devices and Fibre Coatings*; Elsevier Inc., 2012; ISBN 9780124160170.

363. Sutherland, K. Gas chromatography/mass spectrometry techniques for the characterisation of organic materials in works of art. *Phys. Sci. Rev.* **2018**, *4*, 1–16, doi:10.1515/psr-2018-0010.